A Project Report on

"CRIMINAL SPY(Digital Crime Pursuit Portal)"

Submitted in partial fulfillment of the requirements for the award of the degree of

BACHELOR OF COMPUTER APPLICATION

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Certificate

This is to certify that Mr./Ms. [illegible handwritten names]

with Register no. [illegible handwritten] has successfully completed the project work in "**CRIMINAL SPY(Digital Crime Pursuit Portal)**" prescribed by the University of Mysore for BCA course VI semester during the year 2023.

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DECLARATION

We **Mr. Ajay K, Ms. Anusha G and Mr. Dilip D** Students of VI Semester BCA, **Department of Computer Application, SBRR Mahajana First Grade College (Autonomous), Mysore** hereby declare that the project titled **"Criminal Spy(Digital Crime Pursuit Portal)"** has been carried out by us at University of Mysore during period of April 2023 to July 2023.This project report is submitted in requirement for the award of the degree Bachelor of Computer Application(BCA) by the University of Mysore.

This is a bona fide work and the matter embodied in the report has not been submitted previously by anybody for the award of any degree/diploma to any other Institution or University.

Place: Mysore

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ACKNOWLEDGEMENT

The life of human beings is always inspired and guided by certain individuals and events. Accordingly, whatever we achieved in my project has been the fruit of advice and good will of my project mentors. We take this opportunity to mention the people who have contributed to success of my project greatly indebted to our Principal Prof. **Dr B R Jayakumari,** Principal & Assistant Professor**, SBRR Mahajana First Grade College** and **Mr. Manjunath K.S,** Head of the Department of Computer Application**,** for the facilities and support extended towards us.

We consider it is a privilege and honor to work under, our **guide Ms .Pooja P S, Assistant Professor, Department Of Computer Application** for their invaluable support, guidance and encouragement. We also much indebted and grateful to the other teaching and non-teaching staff of our dept which extended their unlimited moral support. At last we would like thank our parents and friends for providing encouragement and moral support without which this was not possible.

Place: Mysore

Date:

Signature :

Ajay K

Anusha G

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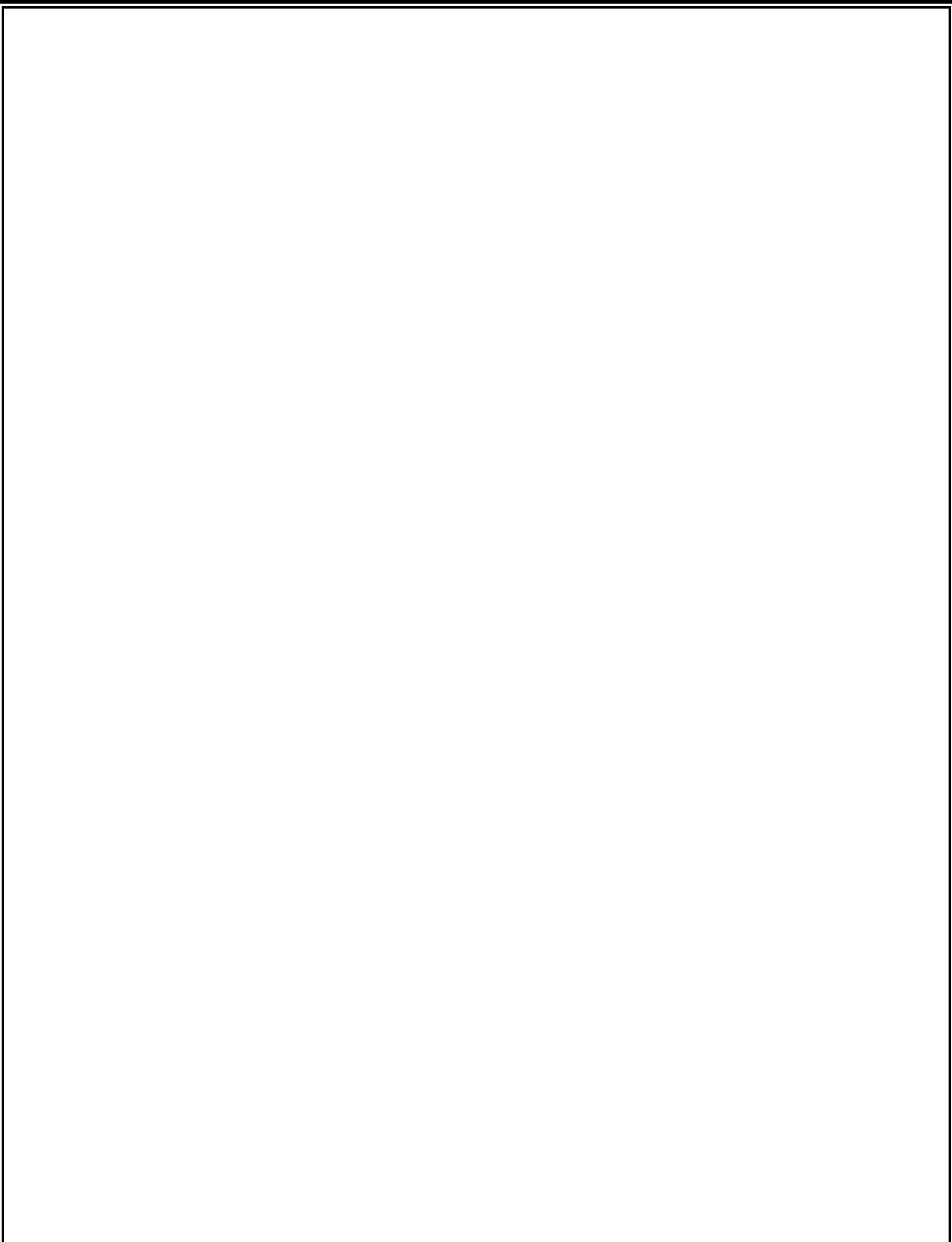
TABLE OF CONTENTS

	Page No.
Abstract	**i**
Chapter 1	**1-7**
1.1 Topic Introduction	2
1.2 Purpose	3
1.3 Aim	3
1.4 Objective of the project	4
1.5 Motivation for the project	5
1.6 Project Overview	6
1.7 Literature Survey	6
Chapter 2	**8-14**
2.1 Introduction	9
2.2 Purpose	10
2.3 Overview	10
2.4 Hardware / Software Requirements	11
2.4.1 Hardware Requirements	11
2.4.2 Software Requirements	11
2.5 Functional Requirements	11
2.5.1 Internet Connection	11
2.5.2 Email Account	11
2.6 Design Constraint	12
2.7 System Attribute	12
2.8 Non - Functional Requirements	12
2.8.1 Performance Requirement	12
2.8.2 Safety Requirements	13

2.8.3 Security Requirements 13
2.9 Feasibility Study 13
2.9.1 Technical Feasibility 13
2.9.2 Operational Feasibility 14
2.9.3 Economical Feasibility 15
2.9.4 Legal and Ethical Feasibility 15
Chapter 3 15-42
3.1 Introduction 16
3.2 Existing System 17
3.2.1 Disadvantages 17
3.3 Proposed System 18
3.3.1 Advantages 19
3.4 Definition of the Application Modules 20
3.4.1 Admin 20
3.4.2 Station User 20
3.5 Overall Description 20
3.5.1 Product Perspective 20
3.5.2 Product Functions 20
3.5.2.1 Admin Role 21
3.5.2.2 User Role 21
3.6 System Architecture Diagram 22
3.7 Context Flow Daigram(CFD) 23
3.8 Data Flow Diagram (DFD) 24
3.8.1 DFD Level 1 26
3.8.2 DFD Level 2 27
3.8.3 DFD Level 3 28
3.8.4 DFD Level 4 29

3.8.5 DFD Level 5 30
3.8.6 DFD Level 6 31
3.8.7 DFD Level 7 32
3.9 Entity Relationship(ER) Diagram 33
3.9.1 ER Diagram for Criminal Spy System 35
3.10 Use case Diagram 36
3.11 Sequence Diagram 38
3.12 Activity Diagram 40
3.13 Class Diagram 42
Chapter 4 **43-45**
4.1 Introduction 44
4.2 Implementation Procedure 45
4.2.1 Admin Role 45
4.2.2 Station User Module 45
Chapter 5 **46-55**
5.1 Introduction 47
5.2 Objective Testing 47
5.3 Testing Methods 48
5.3.1 White Box Testing 48
5.3.2 Black Box Testing 48
5.4 Testing Methodology 49
5.4.1 Unit Testing 49
5.4.2 Integration Testing 49
5.4.3 Alpha Testing 49
5.4.4 Beta Testing 50
5.4.5 Validation 50

5.4.6 Output Testing 50
5.4.7 User Acceptance Testing 51
5.5 Test Report 51
5.5.1 Unit Testing 51
5.5.1.1 Login Page 51
5.5.1.2 Forgot Password 52
5.5.1.3 Change Password 52
5.5.1.4 Add Station 53
5.5.1.5 Add Station User 54
Chapter 6 **56-63**
6.1 Conclusion 57
6.2 Future Enhancement 57
6.3 Screen Shots 58
6.3.1 Main Page 58
6.3.2 Admin/Station User Login 59
6.3.3 Forgot Password 59
6.3.4 Reset Forgot Password 60
6.3.5 Admin Homepage 61
6.3.6 User Homepage 61
6.3.7 View Profile 62
6.3.8 Register Station /User / Crime Type 62
6.3.9 Adding Crime Records 63
Bibiliography **64-65**
1. Books Referenced
2. Websites
3. Papers Referenced



ABSTRACT

The Criminal Project is a comprehensive software system designed to aid law enforcement agencies and criminal justice professionals in managing and analyzing criminal data. The project aims to provide a robust and user-friendly platform that facilitates the storage, retrieval, and analysis of criminal information to enhance investigative processes and promote effective decision-making.

The project utilizes the programming language, a popular and versatile web development language known for its flexibility and wide range of functionalities. By leveraging PHP's features, the Criminal Project enables seamless integration with various databases and offers a scalable solution that can accommodate large volumes of criminal data.

CHAPTER 1
INTRODUCTION

Chapter 1

Introduction

Introduction

As the name suggests, the police management system is an application that allows all the paper work that are done in the police station to be stored in one single system with great ease. It can help in handling the records easier. Sometimes if there is a need for some information of a particular criminal then you need to search in huge heap of files where that information is stored in those files. So that will involve in wastage of time and also effort. So to overcome these problems, the police management system can be useful. This will reduce a lot of pen paper work that is involved.

The Digital Crime Pursuit Portal can help in storing the records related to the criminals, cases, complaint record, case history and so on. This can allow a person to enter or delete the records if necessary. All these records can be maintained in a single database. Security is maintained so as to ensure that only the authorized users will have access to the system. This application will be one of the useful projects that the police can rely on. This application can help in getting the information of the criminals of many years back. It can also help in minimizing most of the work of the police. The features that can be included in this application are as follows:

- Criminal record: This application can contain the details related to the criminals in the particular case.

- Prisoner record: The details of the prisoner and his case related details can be stored in the database.
- Complaint registration: The details of the complaints that are registered can also be stored through this application.
- Police database management: The details of the police in the particular police station can be maintained through this application.

Purpose

- The purpose of the police management information systems study was described as: "develop management information systems specifications that could be used by police departments to guide future information systems development. Documents developed through the study could be used by police forces to identify first, their own information requirements and second, the general specifications of information systems required to meet these needs".

AIM

- The overall aim of this study is to provide an "interpretive analysis" to assist police managers in the evaluation and application of new management information systems technology in their own jurisdictions.

Objective of the project

Efficient Criminal Data Management: The primary objective of the Criminal Project is to provide a robust and user-friendly platform for the efficient management of criminal data. The project aims to streamline the storage, retrieval, and organization of criminal records, ensuring data integrity and accessibility for authorized personnel.

Enhanced Investigative Processes: The project seeks to improve investigative processes by offering advanced search and filtering functionalities. By enabling users to quickly locate relevant criminal records based on specific criteria, the system enhances efficiency and reduces the time required for information retrieval, ultimately supporting law enforcement agencies in their investigative efforts.

Data Analysis and Insights: The Criminal Project aims to facilitate data analysis and provide valuable insights into criminal data. By incorporating analytical tools, the project helps identify patterns, trends, and correlations within the data, enabling professionals to make data-driven decisions, allocate resources effectively, and implement proactive crime prevention strategies.

Reporting and Visualization: Another objective of the project is to enable users to generate comprehensive reports and visual representations of criminal data. This feature enhances communication among law enforcement agencies and facilitates the sharing of information, enabling more effective collaboration and strategic discussions.

Motivation for the Project

Streamlining Criminal Data Management: One of the main motivations behind the Criminal Project is the need to streamline the management of criminal data. Traditional manual methods of storing and retrieving criminal records can be time-consuming and prone to errors.

Enhancing Investigative Efficiency: Investigations into criminal activities often involve analyzing vast amounts of data, including personal information, criminal histories, and case details

Ensuring Data Security and Confidentiality: The security and confidentiality of criminal data are paramount.. This ensures that only authorized personnel can access sensitive information, reducing the risk of data breaches and maintaining the trust of users in the system.

Scalability and Flexibility: The Criminal PHP Project aims to provide a scalable and flexible solution that can accommodate large volumes of criminal data. It is designed to handle increasing data volumes over time and adapt to evolving requirements, ensuring its long-term viability as a reliable criminal data management system.

Overview of the Project:

The Criminal PHP Project is a comprehensive software system designed to facilitate the management, analysis, and retrieval of criminal data for law enforcement agencies and criminal justice professionals. It aims to streamline the process of storing, accessing, and analyzing criminal records, ultimately enhancing investigative processes, supporting data-driven decision-making, and improving overall law enforcement efficiency.

Literature Survey

- "Criminal Justice Information Systems: A Survey" by R. K. Bagga and S. K. Sharma (International Journal of Computer Science and Information Technologies, 2011) This survey provides an overview of criminal justice information systems and their role in managing and analyzing criminal data. It explores various aspects such as data collection, storage, retrieval, and analysis techniques employed in existing systems, highlighting their strengths and limitations. The study offers insights into the importance of efficient information systems in supporting law enforcement agencies.

- "A Comprehensive Review on Crime Data Analysis Techniques" by S. S. Ravindra and V. Vijaya Kumar (International Journal of Innovative Research in Computer and Communication Engineering, 2016) This paper presents a comprehensive review of crime data analysis techniques, including statistical analysis, data mining, and predictive modeling. It discusses the application of these techniques in various areas of crime analysis, such as crime hotspot identification, pattern recognition, and offender profiling. The survey

provides valuable insights into the potential benefits and challenges of applying data analysis techniques in criminal projects.

- "Design and Development of a Web-Based Crime Management System" by S. Chakraborty et al. (2018 IEEE 5th Uttar Pradesh Section International Conference on Electrical, Electronics and Computer Engineering (UPCON), 2018) This study focuses on the design and development of a web-based crime management system using PHP. It discusses the system architecture, database design, and user interface considerations. The paper highlights the importance of user-friendly interfaces, efficient data storage and retrieval mechanisms, and integration with external databases in the context of criminal projects.

CHAPTER 2

SYSTEM ANALYSIS

Introduction

Software requirement specification is the basic steps for development of the application. It acts as the bridge between client and organization. The objective of this document therefore is to formally describe the system's high level requirements including functional requirements, non-functional requirements and business rules and constraints. Before the development of the project SRS must be accepted by the client.

Software requirement specification is document that describes the external requirements for any system. The requirement analysis has to identify the requirement by talking the clients and understanding and responding to their needs. The requirements phase translates the ideas of the clients in a formal document. The inputs are gathered from different resources input gives by the clients may not be consistency. It might change according to the modern requirements.

This application aims to the one step Destination for Police Officers to findout the Crime Records and Details of Criminal. With help of this web application Police officers can easily find out the Crime records from large database efficiently uploading the face image into the system. This Web application also allows users to manage and view details like the Complaint records, court details of the criminal.

Criminal spy is a server based web application which helps the Police Officers to find out the Criminals help of Face Recognition.

Purpose:

The SRS should fully describe the external behavior of the application or Subsystem Identified. It also describes non functional in gathering the requirements. Changing requirements is an irritant task for software developers and may lead to bitterness among clients and the developers. The final goal of the requirement is to produce a high quality and stable SRS .requirements, designs etc.The basic purpose of the SRS is to bridge the gap between the client and the developer. Another important purpose of developing the SRS is to help clients to understand their own needs. There are several problems in gathering the requirements. Changing requirements is an irritant task for software developers and may lead to bitterness among clients and the developers. The final goal of the requirement is to produce a high quality and stable SRS.

Over view:

Project titled “Criminal Spy” is a software integrated application useful for police department for managing tasks like criminals’ details, taking citizens complaints, FIR details, Criminal Database. The system allows Along with management details different police department branches can share FIR details, criminal data and document sharing. Also search the criminal reports using image, it will givesfast and accurate result. All these works can be done through this single integrated software.

Hardware and Software Requirements

Hardware Requirements

Processor	:	Intel dual core or above
Processor Speed	:	1.5 GHZ or above
RAM	:	1 GB RAM or above
Hard Disk	:	500 GB hard disk or above
Operating System	:	Windows XP or above

Software Requirement

Front End	:	PHP
Back End	:	Apache server, MS SQL Server 2008

Functional Requirements

Functional requirements specify which output should be produced for the given inputs. All inputs are entered according to the data type and no blanks are allowed for mandatory fields. Invalid inputs are not allowed in the system. It prompts to re-enter the data. Appropriate error messages are displayed.

Internet Connection:

Since the project is an online system, Internet connection is must for the working of the system. Even for the sending of mails and messages it is necessary.

Email Account:

Every user of the system must have an email account so as the reset passwords or notifications on the system are sent through E-mail.

Design Constraints :

Design constraints that are used to develop error free access as follows:

- Name field should be filled with characters
- Email and contact fields should be in correct format
- All fields are mandatory
- Application should be implemented in PHP and MySQL server 2008
- Application should display errors while fields are not specified correctly.

System Attributes:

Properties of the application which are specified in the SRS are known as system attributes.

- Maintainability: The database is self-maintained.
- Flexibility: It is easy to update and modify the data when needed
- Validation: An Error Message is displayed if an entry is wrong
- Availability: System should available based on time
- Security: System should have strict security restrictions
- Timeliness: Operations are performed in less time

Non Functional Requirements:

Non-functional requirement describes the performance features of the system. Some of the non-functional requirements are…

Performance Requirements:

- Response time – The system shall give responses in 4-6 second.
- Capacity – System will support single user at a time.
- Should be error-free.

- Should handle large amount of data
- User-interface – The user interface screen shall respond within 5Seconds

Safety requirements:

User authentication and authorization protocol should b tight. In case f server default or Crash due to virus of operating systems failure there should means to have a back up and retrieval of data lost partially.

Security requirement:

The user authentication and authorization protocol should b secure. Services are must be divided into user permission.

Feasibility Study:

Technical Feasibility:

Technical Requirements: Evaluate the technical requirements needed to develop and implement the Criminal PHP Project, such as the PHP programming language, database management systems, server infrastructure, and compatibility with existing systems. Assess whether the necessary technical resources are available or can be acquired within the project's constraints.

Operational Feasibility:

User Requirements: Identify the specific needs of law enforcement agencies and criminal justice professionals regarding criminal data management, analysis, and reporting. Assess if the proposed system meets those requirements effectively and if it aligns with the existing operational processes and workflows.

Economic Feasibility:

Cost Analysis: Conduct a comprehensive cost analysis, including initial development costs, hardware and software requirements, ongoing maintenance and support expenses, and any potential licensing or subscription fees. Compare the projected costs with the available budget to ensure financial feasibility.

Legal and Ethical Feasibility:

Data Privacy and Security: Evaluate the legal and ethical considerations regarding the collection, storage, and use of criminal data. Ensure compliance with relevant data protection laws and regulations to safeguard individuals' privacy rights. Consider implementing robust security measures to protect against unauthorized access and data breaches.

CHAPTER 3
SYSTEM DESIGN

Introduction

Design system is the depiction of the system, or a technique of the making a model, as a way to be used to make or make the structure. The commitment for the blueprint method is the Software Requirement Specification (SRS) totally and the yield is "Plan of the proposed shape". While SRS is definitely in difficulty space, layout is the underlying stage in transferring from the problem area. Setup is essentially a platform among the Requirement Specification and the remaining response for reply in due order regarding fulfilling the necessities. A System Design is a agenda or plans to broaden some other shape. The expert plans the data and the yield of the new structure, its shrewd and physical gadgets to get records, make records and shop the effects. It comes to a decision the way to meet the requirements of the purchaser as talked about within the midst of the System's Analysis Phase.

Programming Design sits at the certain piece of the object that is developing procedure and is associated paying little regard to the progression that is used. Once the object necessities are resolved, programming arrangement is the first of the three activities Design, Coding and Testing.

The layout method for the object systems has tiers.At first dimension the emphasis is on picking which modules are required for the structure, the confirmation of those modules and how the modules could be interconnected. This is referred to as the "Best degree association" and the name for the exceptional degree layout is "System Design". The essential goal of the System Design is to care for profitability, price, flexibility and protection of the structure.

Structure Design is the area in the thought and assistant parts of the gadget are laid out with a real goal to regulate the device to the facts essentials of the

association. Design is the underlying in moving from territory to path action of area.

Existing System:

The existing System is Crime Report Management System is a website in which all the paperwork of the station is maintained in a single system and it makes handling of records easier. This can maintain, add and retrieve all the records like criminal record, complaint record, most FIR record, case history etc. in a single database system.

Disadvantages

- **Implementation Challenges:** Developing and implementing a criminal project can be complex, requiring substantial time, resources, and technical expertise. It may involve integration with existing systems and overcoming resistance to change from stakeholders.

- **Cost Considerations:** The costs associated with developing, maintaining, and updating a criminal project can be significant. This includes expenses related to software development, hardware infrastructure, training, and ongoing support.

- **Potential for Data Errors and Inaccuracies**: Depending on the quality of data entry and maintenance practices, there is a risk of errors or inaccuracies in criminal records. This can lead to incorrect decisions and potentially compromise the integrity of the criminal justice process.

- **Privacy and Ethical Concerns**: A criminal project involving the management of sensitive personal information raises privacy and ethical considerations. It is crucial to ensure compliance with data protection laws and implement appropriate safeguards to protect individuals' privacy rights.

- **Resistance to Change:** Introducing a new system or workflow may encounter resistance from individuals accustomed to existing processes. Overcoming resistance and ensuring buy-in from stakeholders can be a challenge.

Proposed System:

The proposed system Criminal Spy is developed to searching criminal records using face recognition technique also manages crime records of the station. In the proposed system searching criminal reports from crime database with help of uploaded face image and if one of the images is matches up to 99% to upload image it retrieves the crime report details. Admin is the super user he manage all crime reports and criminal records. User can search criminals and add and updating existing complaints and court case details. This application can be effectively used by police department for improving process of handling information and entire system will be computerized.

Advantages:

- **Improved Efficiency:** A well-designed criminal project can streamline processes, automate tasks, and enhance efficiency in areas such as case management, record-keeping, and information retrieval.

- **Enhanced Data Management:** Implementing a criminal project allows for centralized and organized storage of criminal records, making it easier to access and analyze information. This can aid investigators, prosecutors, and other stakeholders in making informed decisions.

- **Better Collaboration and Communication**: A criminal project can facilitate improved collaboration and communication among various entities involved in the criminal justice system, such as law enforcement agencies, courts, and corrections facilities. This can lead to more effective coordination and information sharing.

- **Data Analysis and Insights:** By utilizing data analytics tools, a criminal project can help identify patterns, trends, and correlations in criminal behavior, enabling better strategic decision-making and resource allocation.

Definitions:

Admin:

Admin is the super user who can have full access over the system. He verifies the Station Users and adds them into the system. He can view the list of enrolled Station Police Officers into the system.

Station User:

The Station User Login to the system and search the respective criminal record present. He adds or updates the complaint details and Court case details for the criminal also can retrieve or track the criminal reports using face image.

Overall Discription:

Product Perspective:

The “Criminal Spy System” is a website in which all the paperwork of the station is maintained in a single system and it makes handling of records easier. Here we can maintain, add and retrieve all the records like criminal record, complaint record, case history etc. in a single database system. This Proposed system is retrieves the Criminal information based on uploading face image in online.

Product Functions:

This section outlines all the major features of this Criminal Spy web application

Admin Role:

The admin is the super user in our system. He has the privileges to access all the functions in the system, like admin can;

- Verify the Station Admin and Station Users Who has registered into the system.
- View the list of Station, Users enrolled in the system.
- Manage the Station and Users, i.e. adding Stations And Station Users into the System
- Add and manage Complaints, FIR, Charge sheet, Court case details and Criminal Records to the criminal.
- Update his profile.
- Change his password

User Role:

The User is restricted some privileges of the system. He can

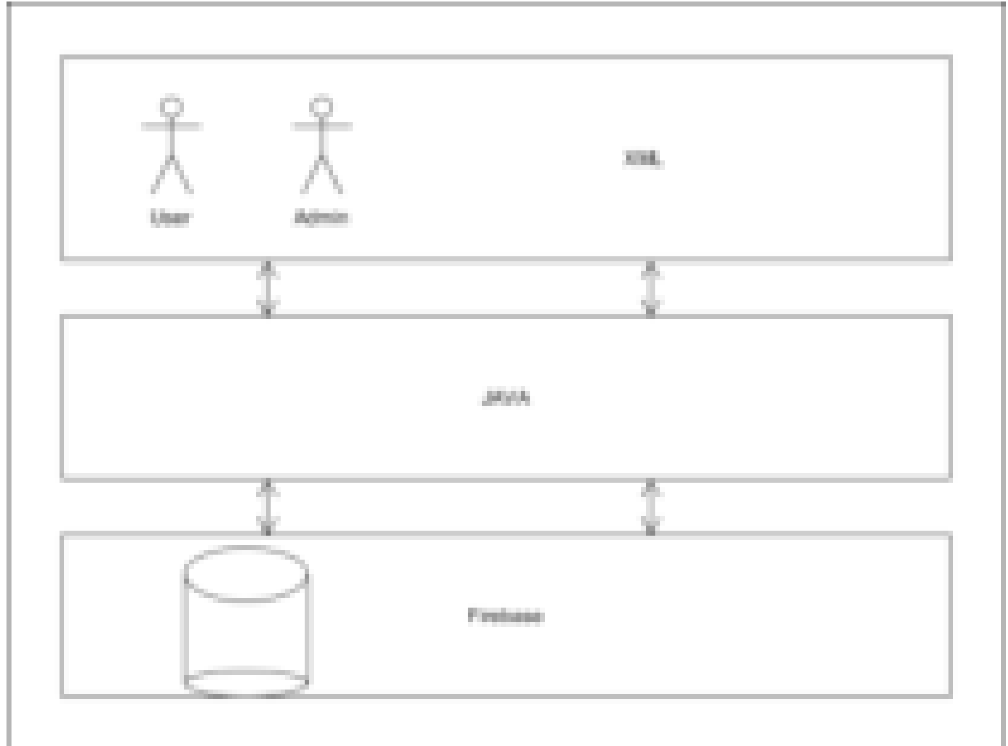
- Search crime reports.
- Add public complaint details.
- View complaint records of the Station.
- Add court case details of the criminal.
- View the court case details records.
- Update their Profile.
- Change his password.

Architecture Diagram:

System design is the process or art of defining the architecture, components ,modules, interfaces, and data for a system to satisfy specified requirements. One could see it as the application of systems theory to product development. There is some overlap with the disciplines of systems analysis, systems architecture and systems engineering.

In System design focus is on deciding which modules are needed for the system, the specifications of these modules should be interconnected is called **System Design.**

System design is also called top-level design. Here we consider a system to be set of



components with clearly defined behavior that interact with each other in a fixed manner to produce some behavior. In a system design, the design consists of module definitions, with each module supporting a functional abstraction

Context Flow Diagram(CFD)

It is common practice to draw the context-level data flow diagram first, which shows the interaction between the system and external agents which acts as data source and data sinks.

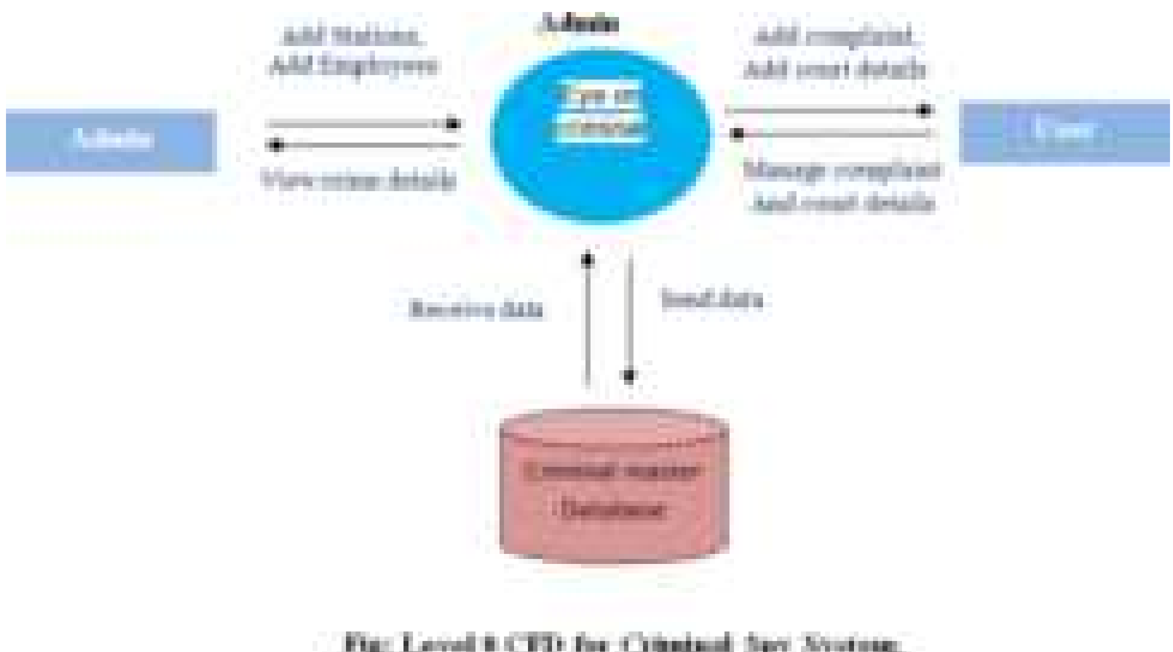


Fig: Level 0 CFD for Criminal Spy System.

On the context diagram the system's interactions with the outside world are modeled purely in terms of data flows across the system boundary. The context diagram shows the entire systems the single process, and gives no clues as to its internal organization

Data Flow Diagram (DFD):

A Data Flow Diagram (DFD) is a graphical representation of the "flow" of data through an information system. A data flow diagram can also be used for the visualization of data processing. It is common practice for a designer to draw a context- level DFD first which shows the interaction between the system and outside entities. This context-level DFD is then "exploded" to show more detail of the system being modeled.

A DFD represents flow of data through a system. Data flow diagrams are commonly used during problem analysis. It views a system as a function that transforms the input into desired output. A DFD shows movement of data through the different transformations or processes in the system. Dataflow diagrams can be used to provide the end user with a physical idea of where the data they input ultimately has an effect upon the structure of the whole system from order to dispatch to restock how any system is developed can be determined through a dataflow diagram. The appropriate register saved in database and maintained by appropriate authorities.

In the normal convention, logical DFD can be completed using some notations.

DIAGRAM	DESCRIPTION
	Represents Source or, destination of data
	Represents a process that transforms Incoming data into Outgoing flows
	Represents data flow
	Represents data stores

3.8.26 DFD LEVEL

Fig: Level 1 DFD for Criminal Spy System

3.8.27 DFD LEVEL

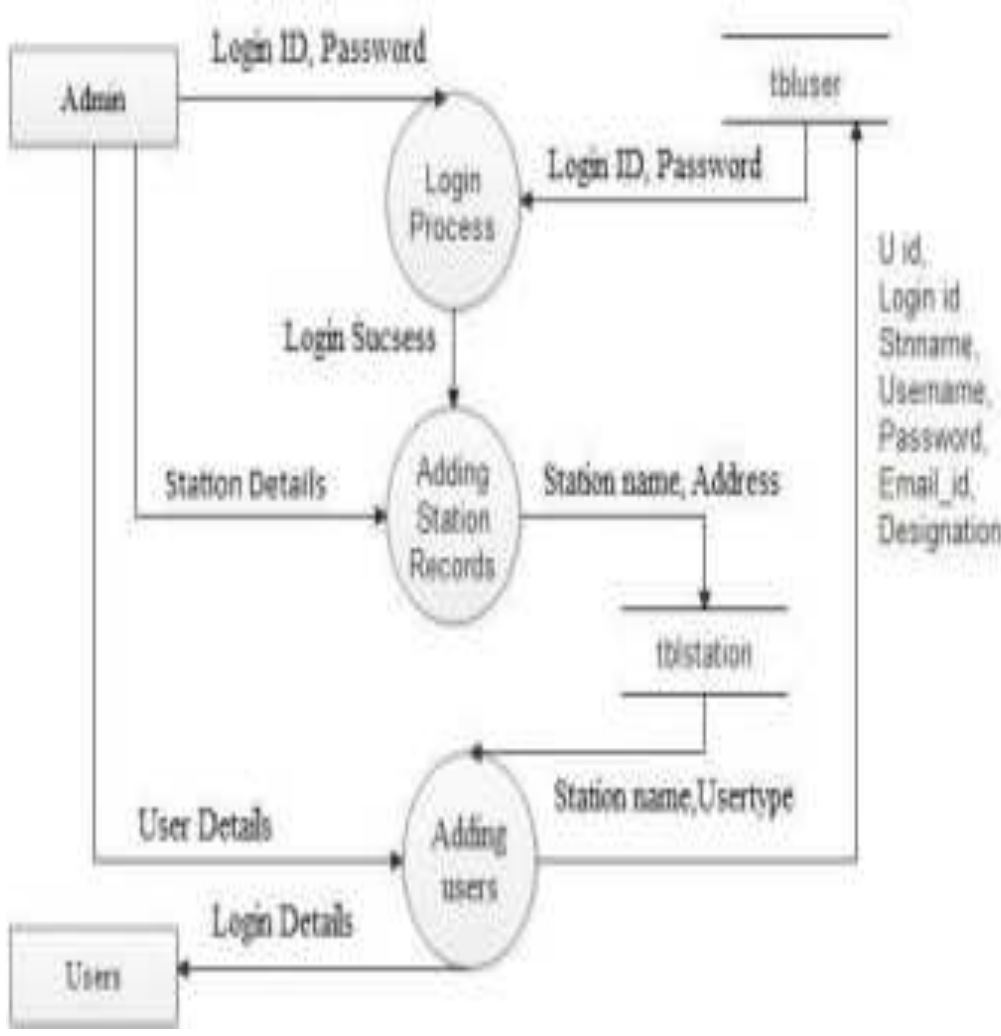


Fig: Level 2 DFD (Admin adding Station Records and Users)

3.8.28 DFD LEVEL

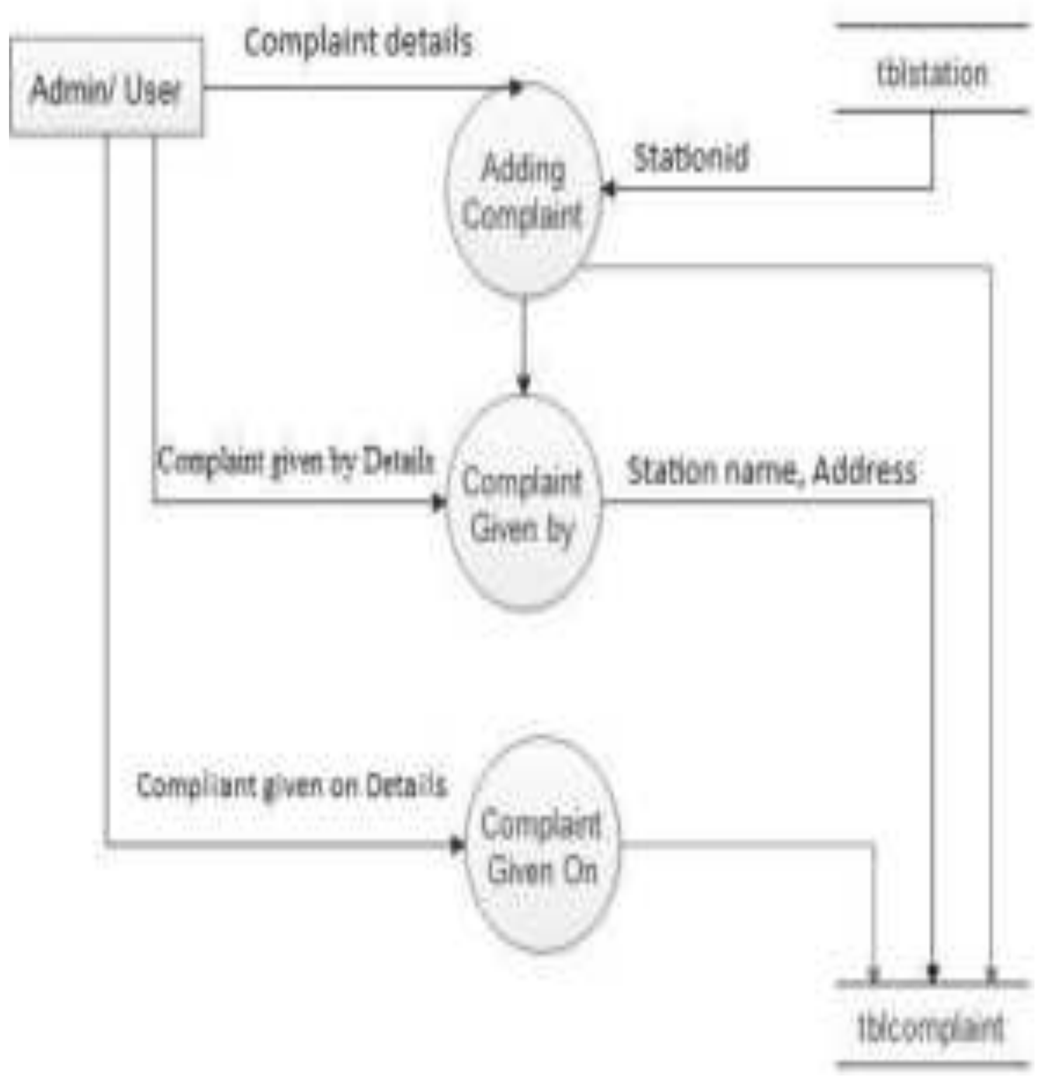


Fig: Level 3 DFD (Adding complaint)

3.8.29 DFD LEVEL

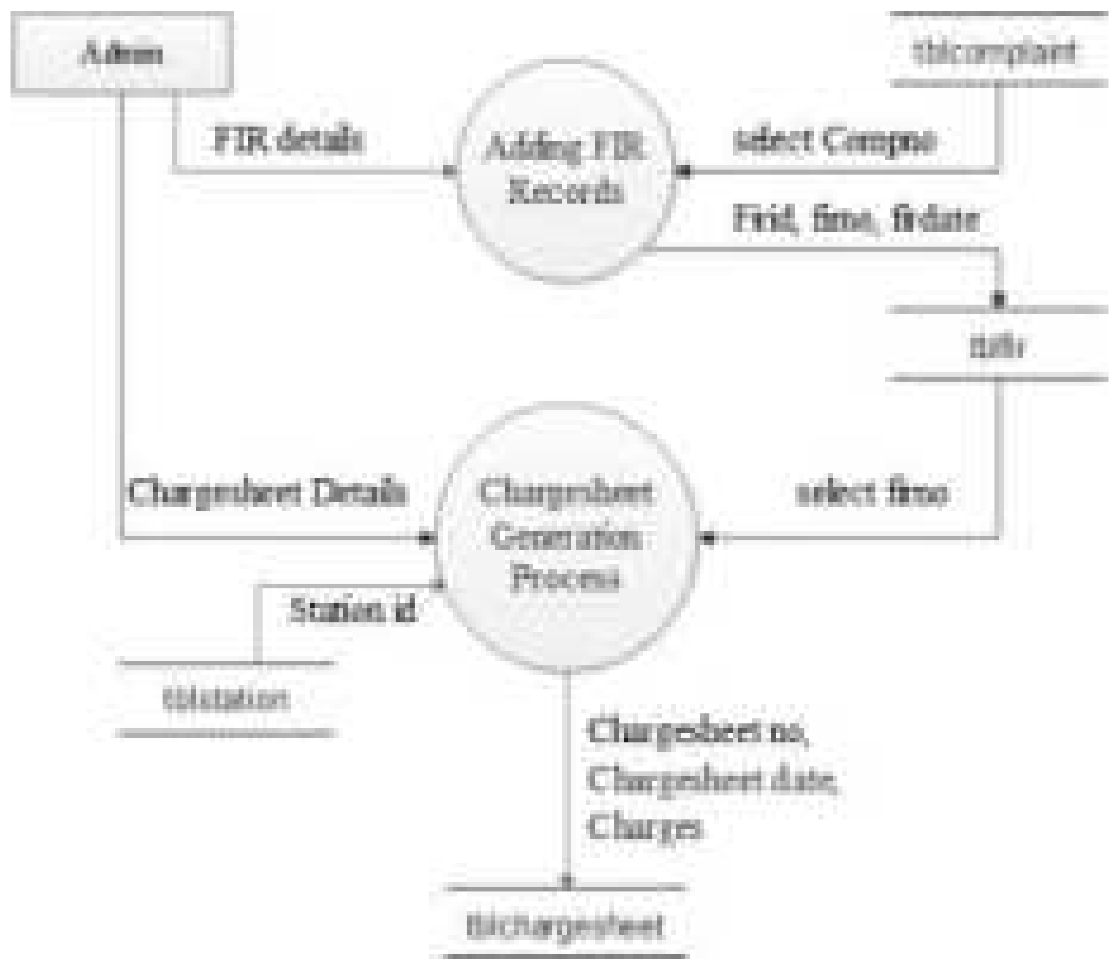


Fig: Level 4 DFD (Charge sheet Generation)

3.8.30 DFD LEVEL

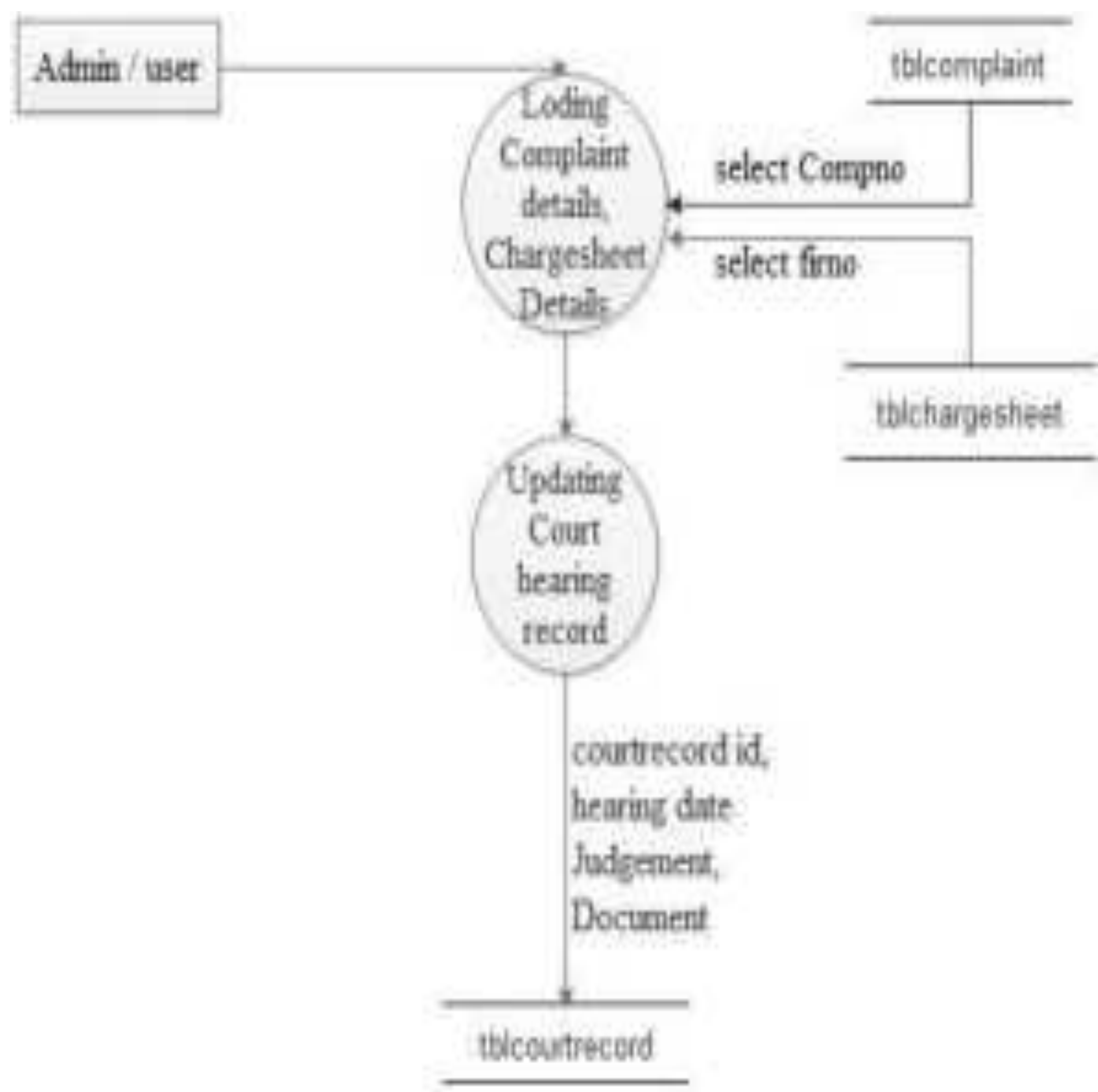


Fig: Level 5 DFD (Updating Court Case details)

3.8.31 DFD LEVEL

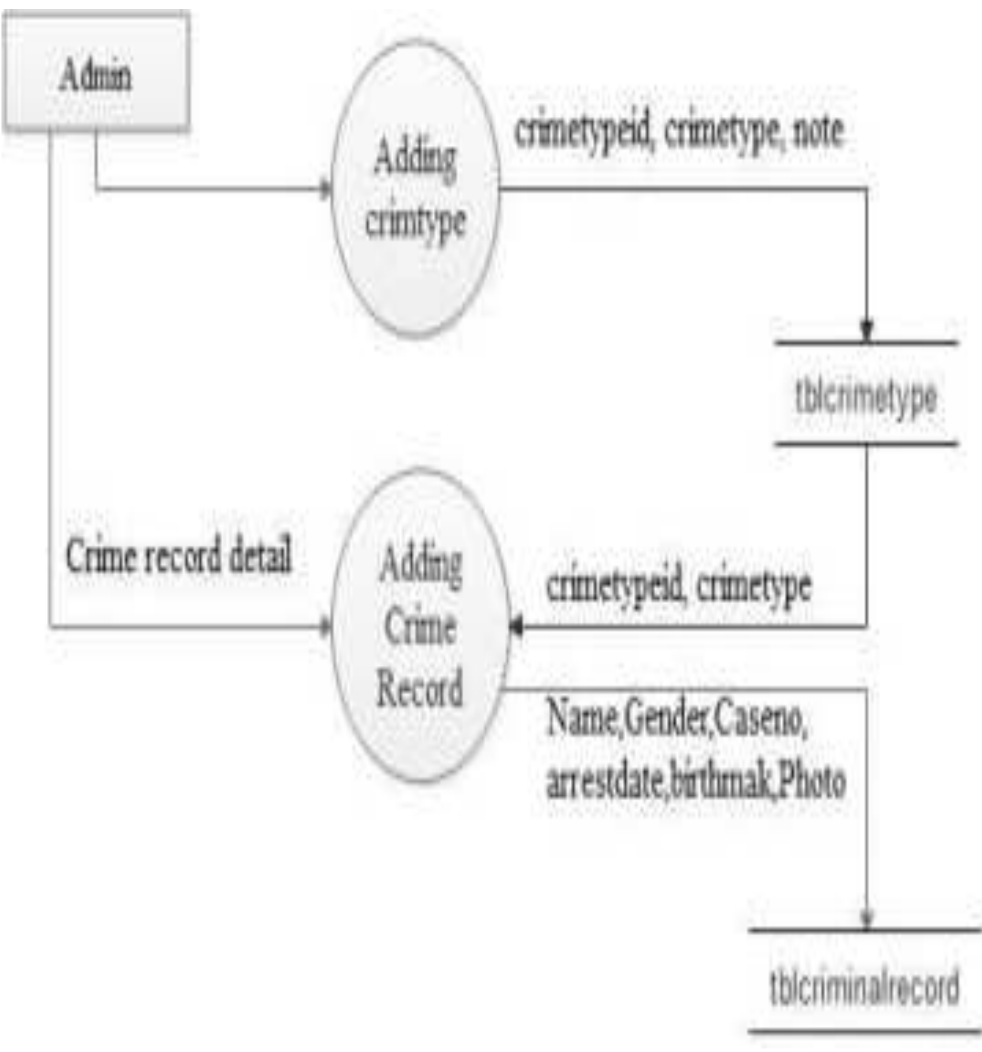


Fig: Level 6 DFD (Adding Criminal record)

3.8.7 DFD LEVEL 7:

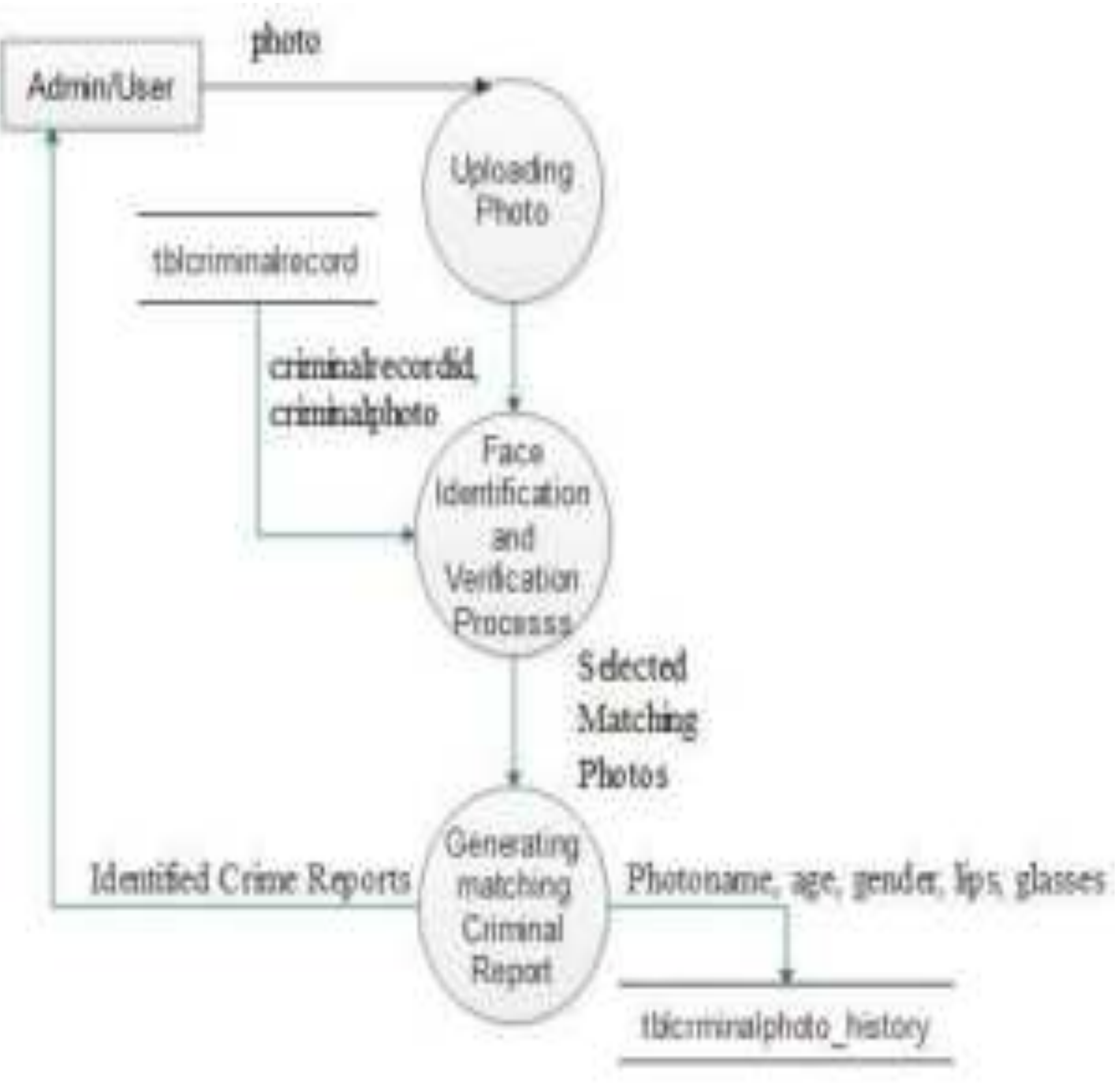


Fig: Level 7 DFD (Searching Criminal)

Entity Relationship (ER) Diagram:

An entity-relationship diagram is a specialized graphic that illustrates the interrelationship between entities in database. ER diagram often use symbols to represent three different types of information. Boxes are commonly used to represent entities. Diamonds are normally used to represent relationships and ovals are used to represent attributes.

An entity-relationship model (ERM) in software engineering is an abstract and conceptual representation of data. Entity-relationship modeling is a relational schema database used to produce a type of conceptual schema or semantic data model of a system, often a relational database, and its requirements in a top-down fashion.

An entity may be defined as a thing which is recognized as being capable of an independent existence and which can be uniquely identified. An entity is an abstraction from the complexity of some domain. When we speak of an entity we normally speak of some aspect of the real world which can be distinguished from other aspects of the real world.

An entity may be defined as a thing which is recognized as being capable of an independent existence and which can be uniquely identified. An entity is an abstraction from the complexities of some domain. When we speak of an entity we normally speak some of aspect of real world which can be distinguished from other aspects of the real world.

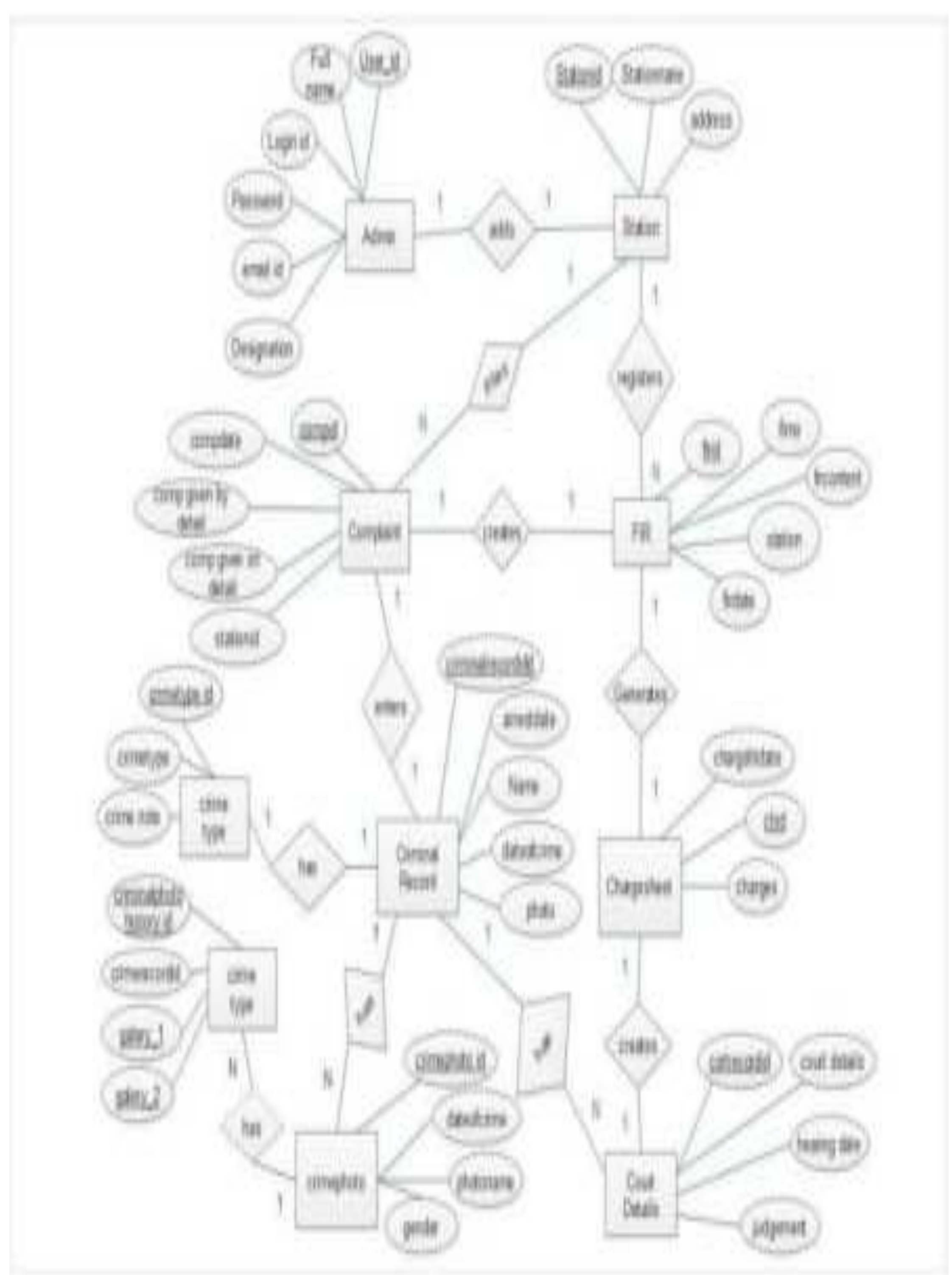
There are three types of relationships between entities:

- **One – to – One (1:1):** One instance of an entity (A) isassociated with one other instance of another entity (B).

- **One – to – Many (1: N):** One instance of an entity (A) isassociated with zero, one or many instances of another entity(B), but for one instance of entity (B) there is only one instance of entity (A).
- **Many – to – Many (N: N):** One instance of an entity (A) is associated with one, zero or many instances of another entity(B), and one instance of entity (B) is associated with one, zero ormany instances of entity (A).

SYMBOLS	REPRESENTS
	Entity
	Attribute
	Multi valued attribute
	Composite attribute
	Relationship

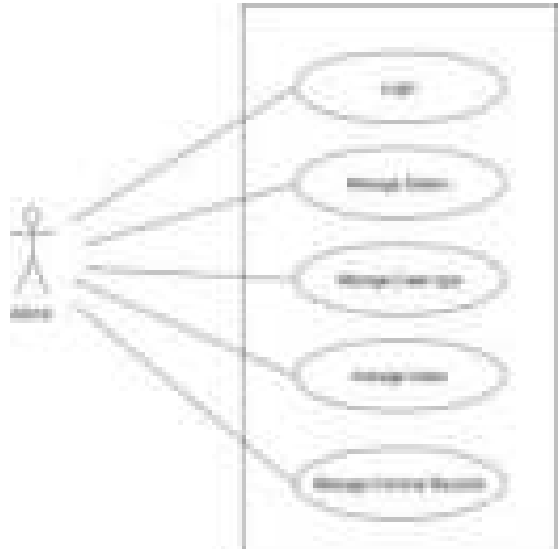
ER Diagram for Criminal Spy System:

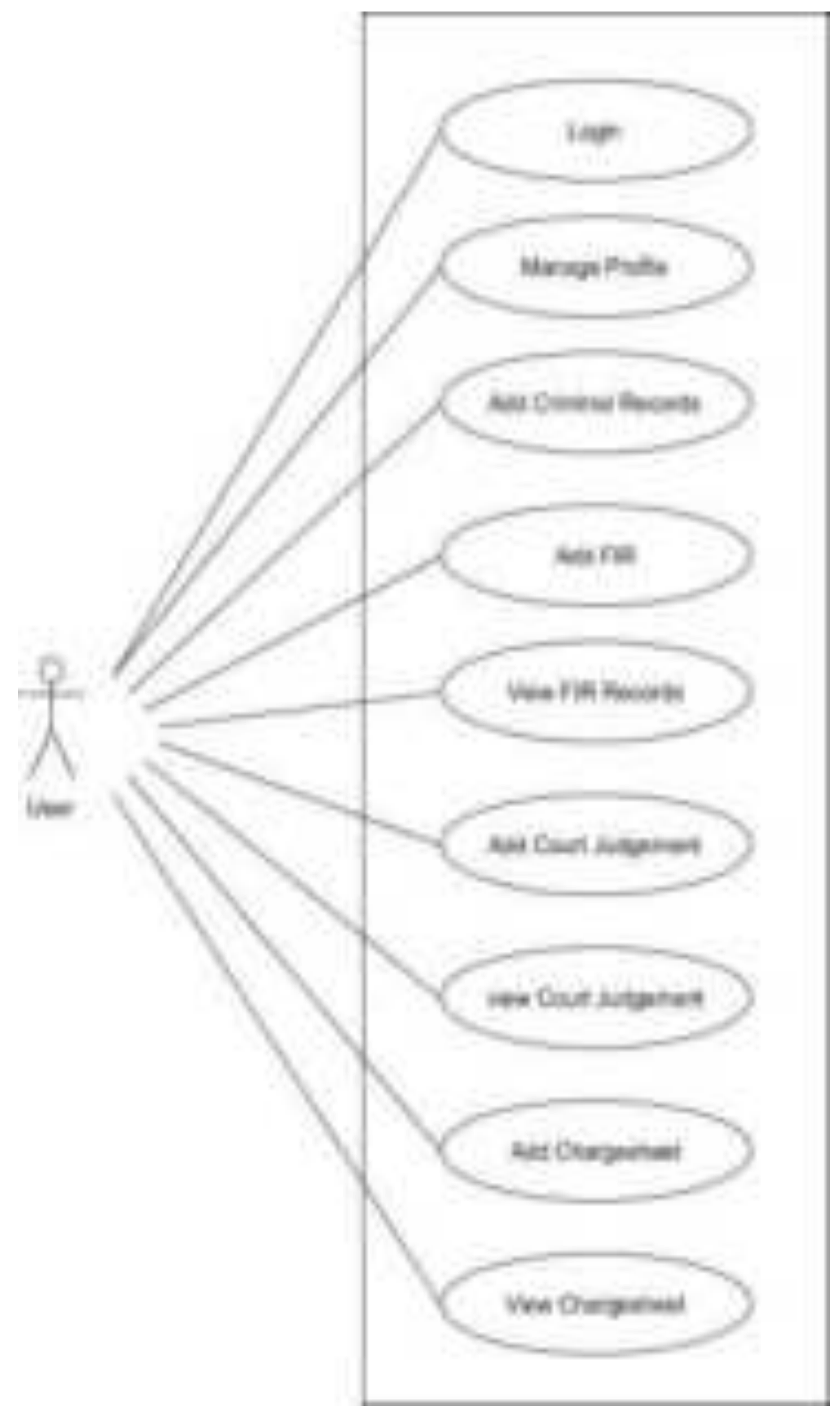


Use Case Diagram

Use case diagram in the Unified Modeling Language (UML) is a type of behavioural diagram defined by and created from a Use-case analysis. Its purpose is to present a graphical overview of the functionality provided by a system in terms of actors, their goals (represented as use cases), and any dependencies between those use cases.

The main purpose of a use case diagram is to show what system functions are performed for which actor. Roles of the actors in the system can be depicted.




Login
Manage Profile
Add Criminal Records
Add FIR
View FIR Records
Add Court Judgement
view Court Judgement
Add Chargesheet
View Chargesheet
User

Sequence Diagram

A sequence diagram shows object interactions arranged in a time sequence. It depicts the object and classes involved in the scenario and the sequence of messages exchanged between the objects needed to carry out the functionality of the scenario.

Sequence diagram are typically associated with use case realization in the logical view of the system under development. Sequence diagrams are sometimes called event diagrams or event scenario.

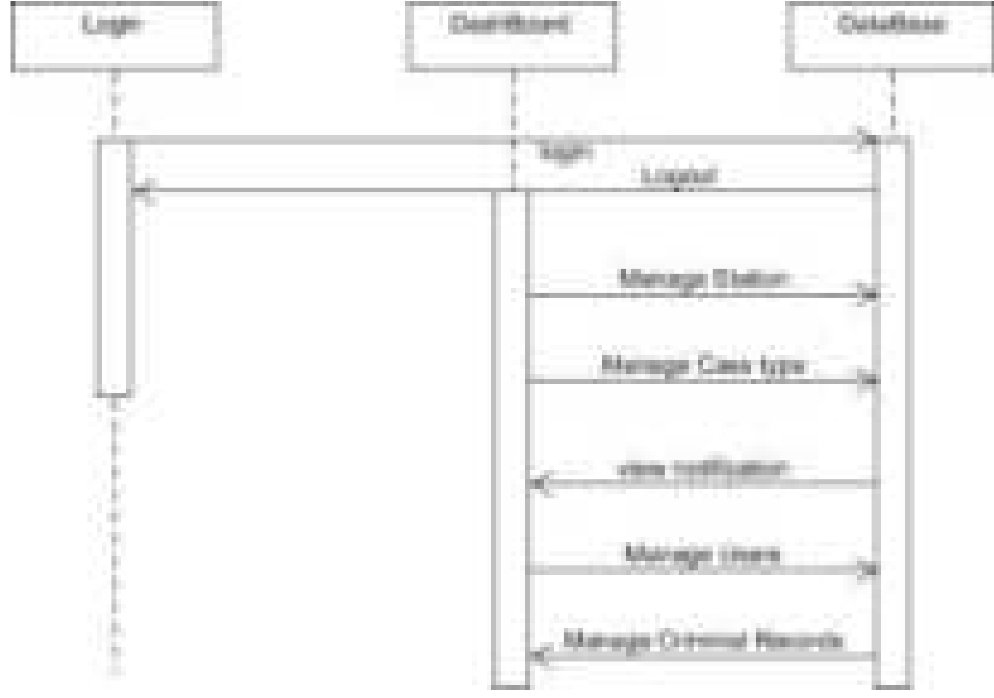


Fig (User)

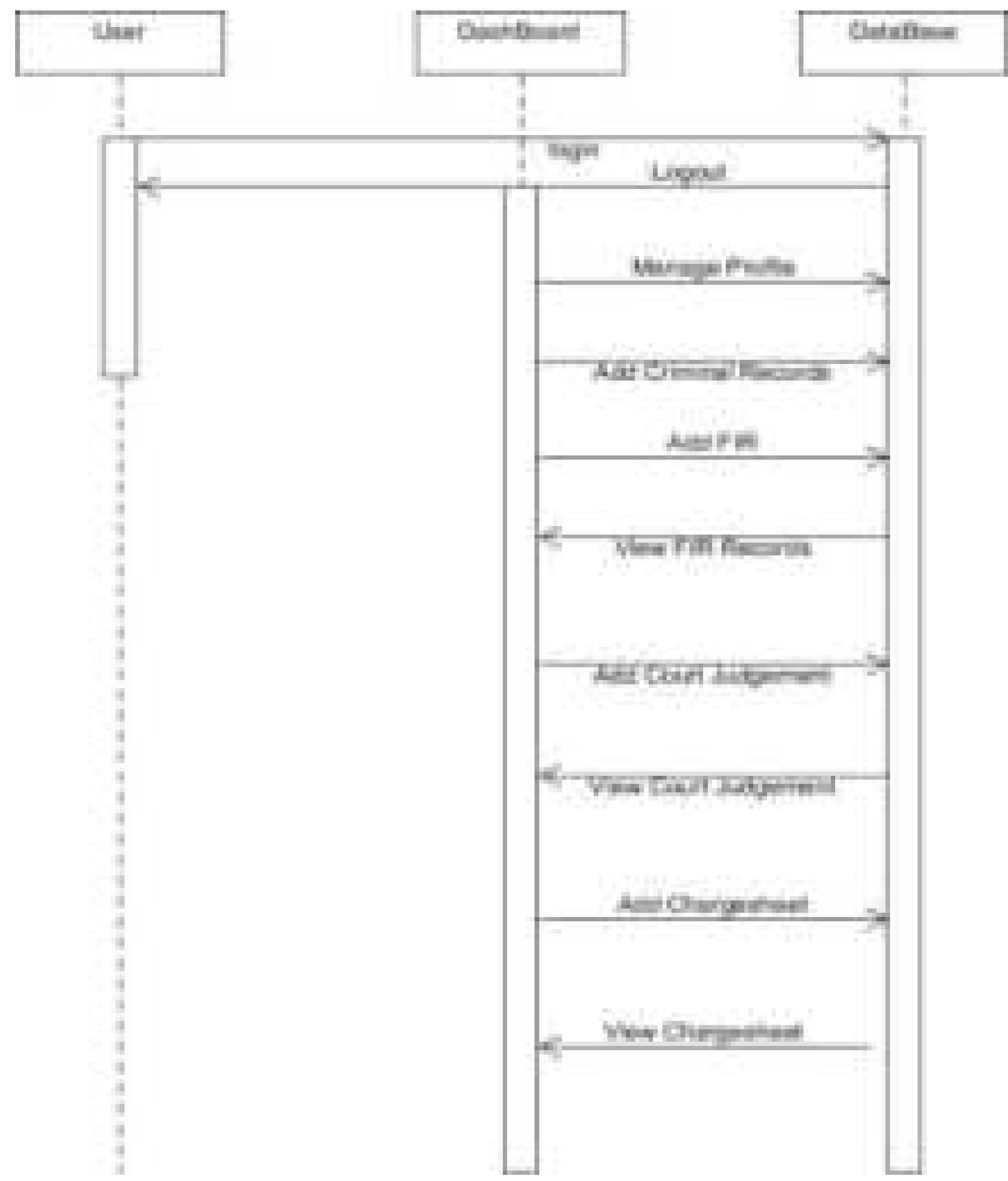
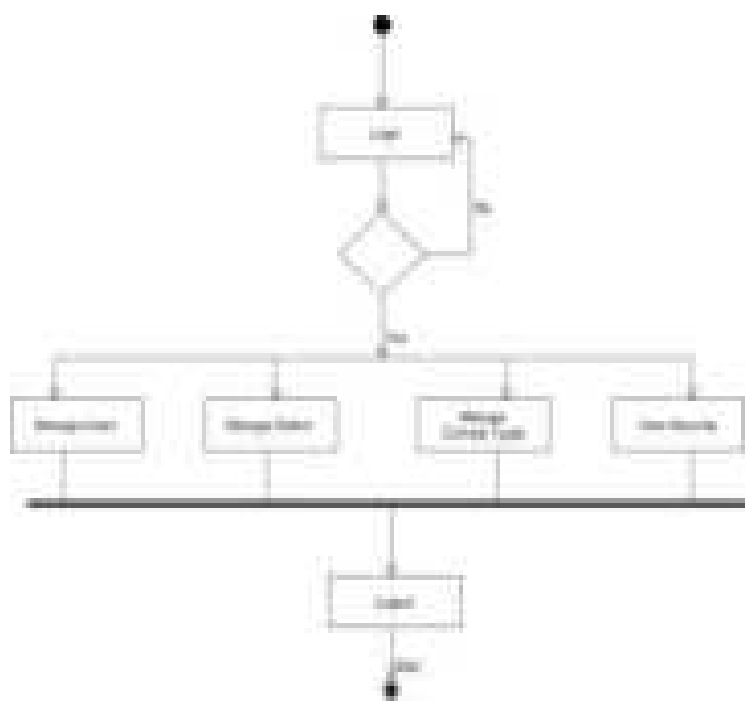


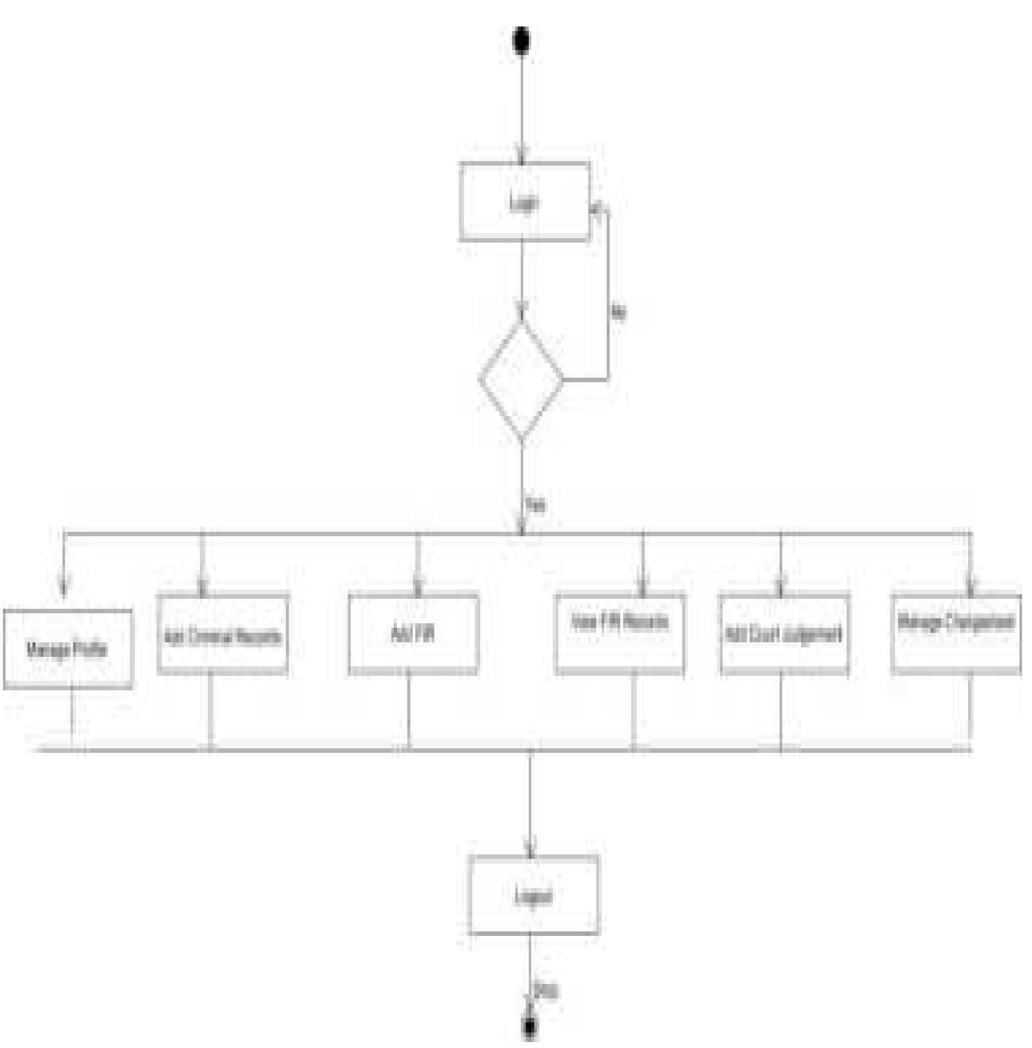
Fig (Admin)

Activity Diagram

- Activity diagram is basically a flowchart to represent the flow form one activity to another activity. The activity can be described as an operation of the system.
- Activity diagram for our application shown below, when the activity starts the user should login to the application allows the user to the next activity by entering correct username and password.



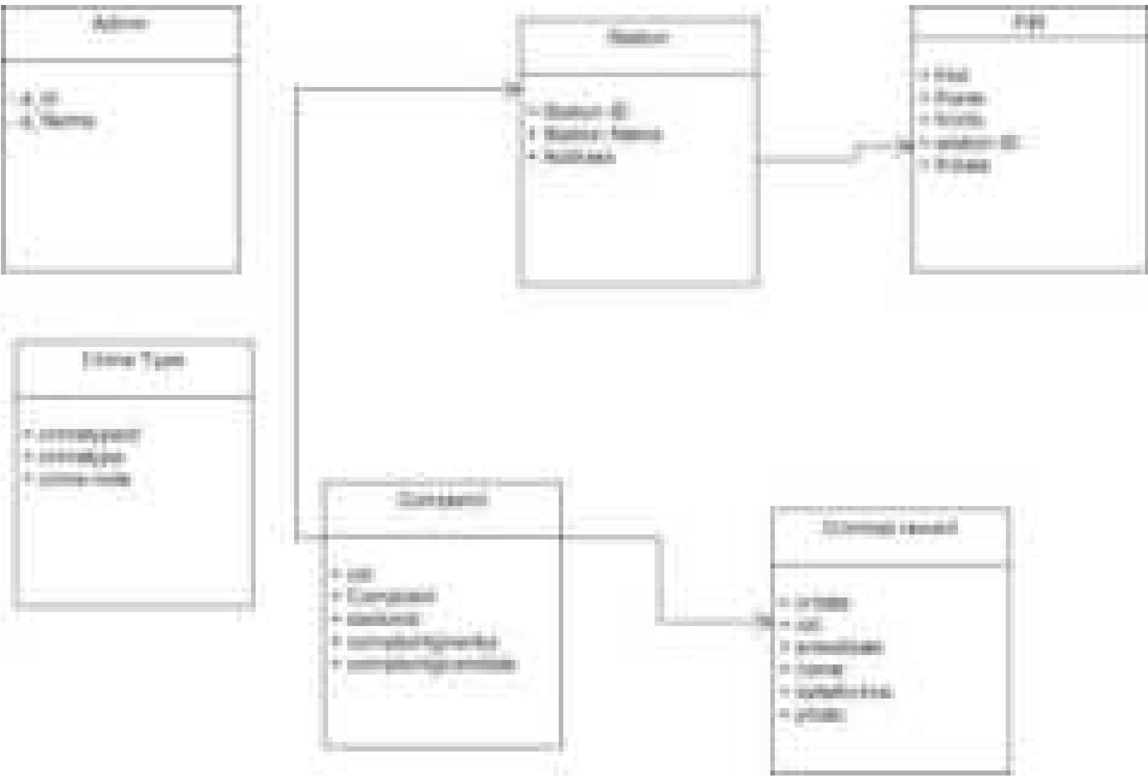
Fig(User)



Fig(Admin)

3.12 Class diagram

A Class is a Set of object that share a common structure and common behaviour. Class is an abstraction of real-world items. An object is an instance or occurrence of a class. A Class describes a group of relationship and semantics. Object in a class have the same attribute and forma of behaviour. Most objects derive their individuality from difference in their attribute values and specific relationships to other objects. A Class describe a group of objects with the same properties, behaviour, kind of relationship and semantics. Class Diagram provides a Graphic notation for modeling and their relationships, thereby describe the possible objects.



CHAPTER 4
SYSTEM
IMPLEMENTION

Introduction:

The implementation stage is the next step towards the problem solution. Here the details like which coding language is used, which database is used and so on is decided and the coding are done in the specified language.

Implementation is the practical job of putting a theoretical design into practice. It may involve the complete implementation of a computer complex or the introduction of one small subsystem.

The implementation phase of a project covers the period from the acceptance of the tested design to its satisfactory operations, supported by the appropriate user and operations manual. It is a major operation across the whole organizational structure and requires a great deal of planning. Planning for implementation must begin from the initial concept of the project. It requires a thorough knowledge of the new system, its personnel needs, hardware and software requirements, file and procedure conversion activities, and of the current system where it interfaces with the new, the changes to it, the jobs, which will be superseded, etc. Only the analyst responsible for creating the new system will possess this knowledge. He can plan, schedule and coordinate but has no executive powers.

Implementation Procedure:

Implementation of the software refers to the final installation of the package in its real environment, to the satisfaction of the intended user and the operation of the system. In many organization someone who will not be operating it,it will commission the software development project. In the initial stage they doubt about the software but we have to ensure that the resistance does not built up as one has to make sure that

- The active user must be aware of the using the system.
- Their confidence in the software is built up
- Proper guidance is imparted to the user so that he is comfortable in using the application.

Admin Module:

The admin will control the full access of the system. The admin can create user ids and passwords for investigating officer and ensures authenticated access. Admin can register new station Record and Add new users and manage the station Crime Records.

Station User Module:

The authenticated Station User can access user Functionalities provided by admin. The user can view the complaint records, add Public complaints to the system and manage the court case details of the criminal. The user can identify criminals using face recognition.If any image is matched up to 99% then we predict that he is only the criminal.

CHAPTER 5

TESTING

Introduction:

Testing is the process of executing the program with the intent of finding the errors. Testing cannot show the absence of defects, it can only show that software errors are present.

The main purpose of is detect errors and error get-prom areas in a system. Testing must be through and well-planned. A partially tested system is as bad as an untested system. And the price of an untested and under-tested system is high. The implementation is the final is the final and important phase. It involves user training, system testing in order to ensure successful running of the proposed system. The user tests the system and changes are made according to their needs. The testing involves the testing of the testing of the developed system using various kinds of data. While testing, errors are noted and correctness is the mode.

Objective Of Testing:

The objectives of testing are:

Testing in a process of executing a program with the intent of finding errors. A successful test case is one that uncovers an as yet undiscovered error.System testing is a stage of implementation, which is aimed at ensuring that the system works correctly and efficiently as per the user need, before the live operation commences. As stated before, testing is vital to the success of a system. System testing makes logical assumption that if all the system is correct, the goal will be successfully achieved. A series of tests are performed before the system is ready of user acceptance test.

Testing Methods:

System testing is a stage of implementation. This is to check whether the system works accurately and efficiently before live operation commences. Testing is vital to the success of the system. The candidate system is subject to a variety of tests: on line response, volume, stress, recovery, security and usability tests series of tests are performed for the proposed system is ready for user acceptance testing.

White Box Testing:

This test is conducted during the code generation phase itself. All the errors were rectified at the moment of its discovery. During this testing, it is ensured that.

1. All independent paths within a module have been exercised at least once.
2. Exercise all logical decisions on their true or false side.
3. Execute all loops at their boundaries.

Black Box Testing:

It is focused on the functional requirement of the software. It is not an alternative to white Box Testing rather; it is a complementary approach that is likely to uncover a different class of errors than White Box methods. It is attempted to find errors in the following categories.

1. Incorrect or missing functions
2. Interface errors.
3. Errors in data structure or external database access.
4. Performance errors
5. Initialization errors.

Testing Methodologies:

Unit Testing:

During this testing the members of arguments are compared to input parameters, matching of parameters and arguments etc... It is also ensured whether the file attributes are correct, whether the files are opened before using, whether input/output are handled etc... Unit test is conducted using a test driver usually.

Integration Testing:

Bottom-up integration is used for this phase. It begins construction and testing with atomic modules. This strategy is implemented with the following steps.

1. Low-level modules are combined to form cluster that perform a specific software sub functions.
2. The cluster is tested.
3. Drivers are removed and clusters are combined moving upward in the program structure.

Alpha Testing:

A series of acceptance tests were conducted. The end user conducts it. The suggestions, along with the additional requirement of the end users were included in the project.

Beta Testing:

It is to be conducted by the end user without the presence of the developer. It can be conducted over a period of weeks or month. Since it is a long time consuming activity, its result is out of scope of this project report. But its result will help to enhance the product at the later time.

Validation:

At the completion of the integration testing, software is completely assembled as a package interfacing errors have been uncovered and corrected and a final series of software test begin in validation testing.

Validation testing can be defined in many ways, but a simple definition is that the validation succeeds when the software function in a manner that is expected by the customer. After validation test has been conducted as follows

a) The function or performance characteristics confirm to specification and are accepted.
b) A deviation from specification is uncovered and a deficiency list is created.
c) Proposed system under consideration has been tested by using validation test and found to be working satisfactory.

Output testing:

After performing validation testing, the next step is output testing of the proposed system, since no system could be useful if it does not produce the required output in a specific format. The output format on the screen is found to be correct. The format was designed in the system design time according to the user needs. For the hard copy also, the output comes as per the specified requirements by the user.

User Acceptance Testing:

User acceptance of a system is the key factor for the success of any system. The system under consideration is tested for the user acceptance by constantly keeping in touch with the prospective system users at the time of developing and making changes whenever required.

TEST REPORT:

UNIT TESTING:

LoginPage :

Test Case	Description	Expected Result	Observed Result
1	If the Login User Type is not selected	Error Message "Please Select Valid User Type" is displayed	Successful
2	If the Login Id /password is not entered	Error Message "Enter Valid Login Id /Enter Password"is displayed	Successful
3	If the login Id/ Password or both are incorrect	Error Message "Invalid Login Credentials"	Successful
4	The click on forgot password	Opens a new form to reset the password	Successful

Forgot Password:

Test Case	Description	Expected Result	Observed Result
1	If Login Id/Email Id is entered wrongly	Error message "Please Fill out this field" is displayed	Successful
2	If Reset password button is pressed	The regenerated password is sent to the email of the user and redirects to reset password page.	Successful

Change Password:

Test Case	Description	Expected Result	Observed Result
1	If the New password is not entered	Error Message "Please Enter a New password"	Successful
2	If the Entered new Password is Less than 8 Characters	Error Message "Password Should contain More than 8 Characters" is displayed	Successful
3	If the confirm password doesn't match new password	Error Message "Enter valid password" is displayed	Successful

4	If update button is clicked	The new password is updated onto the database	Successful

Add Station:

Test Case	Description	Expected Result	Observed Result
1	If the station name is Not entered	Error Message “Station name should not be Empty” is displayed	As Expected
2	If the Station Address is Not Filled	Error Message “Station Address should not be Empty” is displayed	As Expected
3	If the State is Not Entered	Error Message “Kindly enter the State” is displayed	As Expected
4	If the Pin code is left blank	Error Message “ Pin code should not be empty” is displayed	As Expected
5	If the contact number 1/contact no 2is left blank	Error Message “ Contact no 1/ contact no 2should not be empty” is displayed	As Expected

6	If the contact number 1/ contact no 2 Length is less than 8 digit	Error Message " Contact no 1/ contact no 2 should contain only 10 digits" is displayed	As Expected
7	If the status of the station is not selected	Error Message " Kindly please select Status" is displayed	As Expected

Add Station User:

Test Case	Description	Expected Result	Observed Result
1	If the User Type is not selected	Error Message "Please select User Type" is displayed	As Expected
2	If the Station Name is not selected	Error Message "Please select Station" is displayed	As Expected
3	If the Name is left blank	Error Message "Please Enter User name" is displayed	As Expected
4	If the Login Id /password is not entered	Error Message "Enter Valid Login Id /Enter Password " is Displayed	As Expected
5	If the Login Id	Error Message "Login Id	As Expected

	/password is entered is already Exists	/Enter Password is already Exists" is Displayed	
6	If the Password Length less than 8 characters	Error Message " Password Should contain More than 8 Characters " is Displayed	As Expected
7	When the confirm password doesn't match Password	Error Message "Password and confirm Password Not matching" is Displayed	As Expected
8	If the User designation is left blank	Error Message " Designation should not be Empty " is Displayed	As Expected
9	If the Address is left blank	Error Message " Address should not be Empty " is Displayed	As Expected
10	If the phone no. is left blank	Error Message " Phone no should not be Empty " is Displayed	As Expected
11	If the status is not selected	Error Message " Please select the status" is Displayed	As Expected
12	If the add button is Clicked	The new stationuser added to the database	As Expected

CHAPTER 6
CONCLUSION
AND
FUTURE WORK

CONCLUSION:

Face recognition has great potential in helping security operatives to better carry out their duties especially in developing nations where this technology is not currently widely used. This system therefore when fully implemented will be both a web application. Its full implementation will ensure that criminals do not go unpunished. It will also go a long way in ensuring that innocent people are not wrongly arrested based on previous crimes that have been committed by other people due to similar names.

Criminal Spy is online identifying criminals is developed based on face recognition system. By uploading a digital image it will detect face and with the help of facial attribute values comparing into crime database and identifying criminals and retrieve the result of face matched crime record details.

It is designed to replace the existing manual crime record management and searching criminal record from database. It is fast and efficiently gives the result. The system allows adding crime records ;this web application is developed using PHP as frontend and My SQL as backend for the database.

FUTURE ENHANCEMENT:

- The present system is developing as web application.
- The criminal photos may be of any size.
- By selecting any one cropped part of the criminal, we can get the full image of the criminals along with details.
- In future we would like to develop it for portable devices like cell phones, WAP or GPRS connections.

SCREENSHOTS

MAIN PAGE:

Description: The starting page of Criminal Spy system initialy look like this

ADMIN/STATION USER LOGIN:

Description: **Admin Or User Login to the System by selecting usertype dropped down list. Successful login it will redirects to home Page.**

FORGOT PASSWORD:

Description: **This page will helps to recover password by Entering the valid mail_id sends a OTP to notification .**

RESET FORGOT PASSWORD:

Description: This page helps to recover the acount password by entering OTP recived Through Email.

ADMIN HOME PAGE:

Description: View of Admin Home Page

USER HOMEPAGE:

Description: View of User Home Page

VIEW PROFILE:

Description: View of Profile Informaion and clik on to profile update link redirects to profile update Page helps to update profile information.

RESGISTER STATION/USER/CRIMETYPE:

Description: **Admin can adding Station Records,Users of the system And crime types to the sytem**

ADDING CRIME RECORDS:

Description: Click on to the Crome record menu droped down Five submenus by clicking o any submenu redirect to that page.

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A Project Report on

"DIGITAL MARKETING FOR FARMERS "

Submitted in partial fulfillment of the requirements for the award of the degree of

BACHELOR OF COMPUTER APPLICATION

Submitted by

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Department of BCA
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Jayalakshmipuram,
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DEPARTMENT OF COMPUTER APPLICATION

Certificate

This is to certify that Mr. **AMAN RAMESH SHETTY, ABINDAM G JACOB, CHANDHU A A** with Register no. **MCD20006, MCD20001, MCD20017** has successfully completed the project work in **"DIGITAL MARKETING FOR FARMERS"** prescribed by the University of Mysore for BCA course VI Semester during the year 2022-2023.

Signature of the teacher in charge

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Head of the Department

Head of the Department of Computer Application

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Date: 2/8/2023

Examiner

1\. Dr. Lavanya P.G

2\. Mr. Gunitha N

Date: [illegible]

DECLARATION

This is to certify that the project entitled **"DIGITAL MARKETING FOR FARMERS "** submitted by me in partial fulfilments for the award of the degree **BCA** is a bonafide work carried out by us at Let's Pro Academy, Mysuru during the period of April 2023-July 2023 under the Internal guidance of

Mrs. Amrutha K S, Assistant Professor, Department of BCA SBRR Mahajana First Grade College, Mysuru.

I hereby declare that the entire work embodied in this dissertation has been carried out by us and no part in it has been submitted elsewhere for any other degree/diploma to any other university/institute.

Place: Mysuru

Date:

Aman Shetty (MCD20006)

Abinoam G Jacob(MCD20001)

Chandhu A A (MCD20017)

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Finally, I would like to thank all our teaching and non-teaching staff, for their kind co-operation..

Above all, my sincere gratitude to mu **Parents** and **Friends** and all who have supported me throughout my project.

Aman Shetty

Abinoam G Jacob

Chandhu A A

ABSTRACT

The term digital market means a platform that is dedicated to integrate farmer, Merchant/Markets, government and end user and thereby bridge the gap between them. It also let everyone to be updated with the changing market scenario. Indian farmers faced many challenges and one of them is that to get a good profit for the efforts and investment that they had Putin. There exist different reasons like season limitation, crop life due to which farmer get very limited amount of time to study the market conditions. The study of flourishing crops and products in current market under agriculture sector is very necessary in order to obtain good price. Since it is not feasible to reach all merchant physically for farmers as it consumes much time and efforts wherein our farmers have limited amount of time. Also, traditionally, methodologies implemented by farmer created limited access to client (merchants) enabling less options to sell the crop product in the market.

TABLE OF CONTENTS

CHAPTER NAME

1.INTRODUCTION.. **1**

1.1 Problem Definition… 1

1.2 Limitations 1

1.3 Objective and Scope……………………………………………...2

1.4 Methodologies...2

1.5 Existing System...2

1.6 Proposed System 3

1.6.1 Advantages of Proposed System..3

2.LITERATURE SURVEY **4**

3.REQUIREMENT ANALYSIS AND SPECIFICATION…………….**7**

3.1 Introduction… 7

3.2 Scope of SRS Document 7

3.3 References..8

3.4 Overview..8

3.5 Product Function 8

3.5.1 User Characteristics...9

3.5.2 General Constraints...10

3.6 External Requirements..10

3.6.1 System Requirements...10

3.6.2 Software Requirements...10

3.6.3 Hardware Requirements..10

3.7 Functional Requirements………………………………………….11

3.8 Non Functional Requirements…………………………………….11

4.SYSTEM DESIGN………………………………………………….**13**

4.1 System Architecture…...13

TABLE OF CONTENTS

4,1.1 Architecture Diagram 13
4.2 Software Engineering Model Used… ....…………………14
4.3 Database Table… 16
4.4 ER Diagram 16
4.5 Detailed Design of DFD.... .... .17
4.5.1 Level-0 DFD 18
4.5.2 Level-1 DFD Admin….. 18
4.5.3 Level-1 DFD User. 19
4.5.4 Level-1 DFD Frmer..20
4.6 Activity Diagram.. 21
4.6.1 Activity Diagram for Admin ... .21
4.6.2 Activity Diagram for User..22
4.6.3 Activity Diagram for Farmer...23
4.7 Use Case Diagram.. 24
4.7.1 Use Case Diagram for Admin… 24
4.7.2 Use Case Diagram for User 25
4.7.3 Use Case Diagram for Faremer 26
4.8 Sequence Diagram 27
4.8.1 Sequence Diagram for Admin 28
4.8.2 Sequence Diagram for User..28
4.8.3 Sequence Diagram for Farmer.. 29
5.IMPLEMENTATION **31**
5.1 Introduction..31
5.2 Implementation Approaches..31
6.TESTING **33**

TABLE OF CONTENTS

6.1 Purpose of Testing...33

6.2Test Case…..34

7.EXPERIMENTAL RESULTS……………………………………..36

7.1 Screenshots…………………………………………………… 36

7.1.1 Homepage………………………………………………………36

7.1.2 Registration……………………………………………………..36

7.1.3 Login…………………………………………………………….37

7.1.4 Profile...37

7.1.5 Search Product...38

7.1.6 Add Product..39

7.1.7 Feedback ….......... 39

8.CONCLUSION AND FUTURE ENHANCEMENT 39

9.BIBLIOGRAPHY 40

CHAPTER 1: INTRODUCTION

The study of flourishing crops and products in current market under agriculture sector is very necessary in order to obtain good price. Since it is not feasible to reach all merchant physically for farmers as it consumes much time and efforts wherein our farmers have limited amount of time. Also, traditionally, methodologies implemented by farmer created limited access to client (merchants) enabling less options to sell the crop product in the market.

1.1 Problem Definition

After yielding crop/product farmer has a limited amount of time to find out nearest market, current stock details & to determine which market will be more profitable for his crop. The study of market situation takes a lot of time. In traditional marketing scheme, farmers had limited option for selling their crops/product due to which they cannot optimize their crop profit at optimum level. When government set minimum price for maximum quality of crop/product, it’s quite difficult to stand implement such rules and regulations. It is difficult for government to handle overhead raised price due less availability of stocks in markets as well as fill up the necessity of crop/product in the market. Because government do not have necessary data to predict such conditions before facing actual problem & figure out possible solutions. Thus, it becomes necessary to establish such system which will help to resolve farmer’s problem time to time using digital platform and technologies in order to remain updated with changing requirements.

1.2 Limitations

- There is proper application which helps for former to sell crop
- Middle person or broker get high commission
- There is no proper application customer can buy the product from farmer

1.3 Objectives and Scope

In traditional marketing scheme, farmers had limited option for selling their crops/product due to which they cannot optimize their crop profit at optimum level. When government set minimum price for maximum quality of crop/product, it's quite difficult to stand implement such rules and regulations. It is difficult for government to handle overhead raised price due less availability of stocks in markets as well as fill up the necessity of crop/product in the market. Because government do not have necessary data to predict such conditions before facing actual problem & figure out possible solutions. Thus, it becomes necessary to establish such system which will help to resolve farmer's problem time to time using digital platform and technologies in order to remain updated with changing requirements.

1.4 Methodologies

A web-based application is software package that can be accessed through the web browser. The software and database reside on a central server rather than being installed in desktop system and is accessed over the network. In computing, web application or web app is a client-server software application which the client (or user interface) runs in a web browser.

With the evolution of internet 2.0, ICT has become the first that deal with citizenry. There's a niche between the farmers and therefore the data of agricultural specialists. ICT will fill the gap between farmers and therefore the specialists. During this paper, we've projected a linguistics internet based mostly designed to get agricultural recommendations, mistreatment special knowledge and agricultural data bases. Our cognitive content acts as a site skilled and can send recommendations to the farmers supporting climate conditions and geographic knowledge. We have shown experimental results as an area of implementation of our projected design. A farmer sends question to the query engine that induces information for a selected crop. Question could also be associated with GIS knowledge, crop cognitive content. The results of the question are displayed on a mobile device.

1.5 Existing System

The following are the disadvantages in existing system

- There is proper application which helps for former to sell crop
- Middle person or broker get high commission
- There is no proper application customer can buy the product from farmer

1.6 Proposed System

1.6.1 Advantages of Proposed System

- In this proposed system we are going to build an application which helps for former who can post crop yield
- Costumer can easily order product from the former
- Former can easily search nearby agriculture service provider
- Less time consumption
- Digitize the agriculture process
- Consumer can pay the amount using payment gateway (Net banking , phone pay or UPI)

CHAPTER 2: LITERATURE SURVEY

2.1 Literature Review

This paper proposes that as India is being Associate in nursing agriculture and that country remained victimized by adopting ancient ways for recommendations of agriculture. Presently recommendations for farmers supports mere one to one interaction between farmers and completely different specialists having different recommendations which will provide information about farmer's victimization past agricultural activities that facilitate mining of information &ideas. The market trend may be united with it to supply optimized results from recommender. The paper proposes the utilization of information mining to supply recommendations to farmers for crops, crop rotation and identification of acceptable plan food. The system may be employed by farmers on internet and golem primarily based on mobile devices. A farmer sends question to the query engine that induces information for a selected crop. Question could also be associated with GIS knowledge, crop cognitive content. The results of the question are displayed on a mobile device.

A Research Paper On Website Development Optimization Using Xampp/PHP

This research paper discussing the various useful tools and techniques that are used in a development of a website. We also discuss about the procedure follow in a website, mostly focused on a local host named Xampp tool .Next, we compare different development frameworks web application. In addition, we discuss life cycle model and framework development of web application. In this report, various review papers result also included for understanding of problems can be facing by the users. This Paper tells about the technologies used in this development, PHP and explained in result its functionality with Xampp with screenshots. It is hoped it will gives a useful framework for guiding the process

.

Justification for doing this Project

2.1.1 Scope

The paper offers a Decalogue of guidelines that could improve the design of the e-commerce websites regarding the aesthetic appearance of the website, the navigation through the site, the information and contents and the characteristics of the shopping process. It could be interesting to analyse empirically the impact of all key attributes on the users' perceptions and behaviours. Originality/value – This is one of the first studies which explains how a website must be designed from the demand's perspective. The analysis of the users' perceptions and the marketing view of design could help designers to find the website that best matches their users' needs and so, to improve the relationships between users and companies through their interfaces.

2.1.2 Overall Description

This section describes the functions, aims, and objectives of the project.it also include the constraints and requirements of the project.

Product Perspective

- In this proposed system we are going to build an application which helps for former who can post crop yield
- Costumer can easily order product from the former
- Former can easily search nearby agriculture service provider
- Less time consumption
- Digitize the agriculture process
- Consumer can pay the amount using payment gateway (Net banking , phone pay or UPI)

2.1.3 Actors and functions

User

- Search product
- Place order
- Send request
- View order status
- Send feedback
- Consumer can pay the amount by using Payment gate way or cash on delivery
- Logout

Farmer

- Register and login
- Upload crop yield information
- View order
- Send response
- View feedback
- Search nearby agriculture service provider information
- Logout

Admin

- Login
- View registered users' information
- Add agriculture service provider information
- View request
- Send response
- View feedback
- View orders

CHAPTER 3: REQUIREMENT ANALYSIS AND SPECIFICATION

3.1 Introduction

A Software Requirements Specification (SRS) is a description of a software system to be developed. It lays out functional and non-functional requirements, and may include a set of use cases that describe userinteractions that the software must provide.

The software requirements specification document enlists enough and necessary requirements that are required for the project development. To derive the requirements, the developer needs to have clear and thorough understanding of the products to be developed or being developed. This is achieved and refined with detailed and continuous communications with the project team and customer till the completion ofthe software.

The introduction of the Software Requirements Specification (SRS) provides an overview of the entire SRS with purpose, scope, definitions, acronyms, abbreviations, references and overview of the SRS. The aim of this document is to gather and analyze and give an in-depth insight of the complete Concept.

3.2 Scope of SRS Document

A Software Requirements Specification (SRS) is a document that describes the nature of a project, software or application. In simple words, SRS document is a manual of a project provided it is prepared before you kick-start a project/application. This document is also known by the names SRS report, software document. A software document is primarily prepared for a project, software or any kind of application.

There are a set of guidelines to be followed while preparing the software requirement specification document. This includes the purpose, scope, functional and non-functional requirements, software and hardware requirements of the project.

3.3 References

[1] Kiran Shinde, Jerrin Andrei, AmeyOke " Web Based Recommendation System for Farmers"march , 2015

[2] Vikas Kumar, Vishal Dave, RohanNagrani, Sanjay Chaudhary, MinalBhise, "Crop Cultivation Information System on Mobile" 2013.

3.4 Overview

After yielding crop/product farmer has a limited amount of time to find out nearest market, current stock details & to determine which market will be more profitable for his crop. The study of market situation takes a lot of time. In traditional marketing scheme, farmers had limited option for selling their crops/product due to which they cannot optimize their crop profit at optimum level. When government set minimum price for maximum quality of crop/product, it’s quite difficult to stand implement such rules and regulations. It is difficult for government to handle overhead raised price due less availability of stocks in markets as well as fill up the necessity of crop/product in the market. Because government do not have necessary data to predict such conditions before facing actual problem & figure out possible solutions. Thus, it becomes necessary to establish such system which will help to resolve farmer’s problem time to time using digital platform and technologies in order to remain updated with changing requirements.

3.5 Product Function

Web design has been identified as a key factor for the acceptance and success of the websites and electronic commerce. The purpose of this paper is to analyse, from a marketing point of view the main aspects that could influence online users' perceptions and behaviours, in order to achieve a successful e-commerce website. Design/methodology/approach – An extensive literature review was developed emphasising the special role that web design could play in the interaction user interface.

User

- Search product
- Place order
- Send request
- View order status
- Send feedback
- Consumer can pay the amount by using Payment gate way or cash on delivery
- Logout

Farmer

- Register and login
- Upload crop yield information
- View order
- Send response
- View feedback
- Search nearby agriculture service provider information
- Logout

Admin

- Login
- View registered users’ information
- Add agriculture service provider information
- View request
- Send response
- View feedback
- View orders

Product Perspective

3.5.1 General Constraints

- User must have the knowledge of application working process.
- Internet must be necessary for this application.
- Multiple user can login but after logout of current user
- The Application is available on all the device. It is compatible with all browsers and mobile.

3.5.2 Assumption and Dependencies

- User can use this application in any browser or mobile there is no restriction but he should have internet facility
- User must have the knowledge of English
- We assume that the system must be user friendly and self-explanatory

3.6 External Requirements

it gives a description of the hardware, software and communication interface and provide basic prototypes of the user interface. Functional requirements are defined according to the development of the project.

3.6.1 System Requirements

- Front End Html, Css, Bootstrp
- Middle Layer Php, Java Script Google(Api)
- Back End Mysql.

3.6.2 Software Requirements

- Operating System Windows Xp – Sp2 / 7 / 8.1 Above
- Browsers Internet Explorer, Google Chrome

3.6.3 Hardware Requirements

- Processor Dual Core (2.0) Ghz Or Above
- Ram 1 Gb And Above
- Hdd 120gb And Above

3.7 Non-Functional Requirements

Availability

This is a Web application and will be available for every registered user. This application is compatible with all browser and mobile. The software helps for the deaf and dumb people to learn the sign language.

Maintainability

The project is developed using an Open source tool and is easy to maintain. The software used for the development of the applications are free so there is not necessary to pay and no maintenances cost.

Security

As the systems all the data are dumped at the server side the server provides the security to the unauthorized access of data.

Reliability

Reliability is a requirement about how often the software fails. The measurement is often expressed in mean time between failures. The definition of a failure must be clear. Also, don't confuse reliability with availability which is quite a different kind of requirement. Be sure to

specify the consequences of software failure, how to protect from failure, a strategy for error detection, and a strategy for correction.

Simplicity

The project is driven by a simple user interface which helps to interact easily with application and easy to remember. Application builds by using bootstrap technology hence it compatible for all devices.

CHAPTER 4: SYSTEM DESIGN

4.1 System Architecture

Architecture focuses on looking at a system as a combination of many different components, and how they interact with each other to produce the desired result. It involves the process of defining a collection of hardware and software components and their interfaces to establish the framework for the development of an android application.

4.1.1 Architecture Diagram

The above mentioned Architecture Diagram consists of 3 Layers

- Front End Layer
- Middle Layer
- Database Layer

1. Front End Layer

The front end of the application is developed using the HTML, JAVA Script, CSS and the Bootstrap technology. This application helps in making the application more effective and perfect. so that the every end user of the application understand the working of the application. In our Projects there are 3 main actors who players the major role in this project those are

- Farmer
- User
- Admin

2. Middle layer

In our project to interact between the actors and the database is done by the middle layer is used. The PHP is used to interact between the user and the database. So that the data is stored effectively in the database and helps them to easy to fetch and display from the database.

3. Database Layer

The MySQL database is used to store the data into database. The effective analysis is done to create the database design and to store the data. The every actions related from the front end are accessed from the database by the PHP.

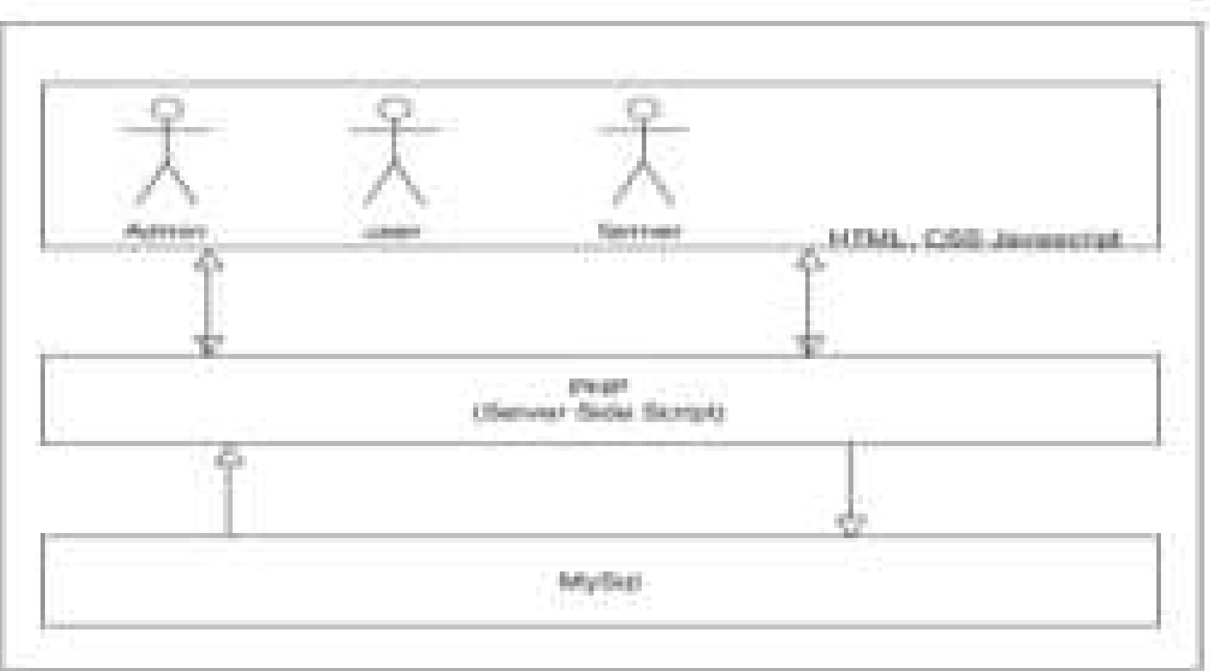


Fig 4.1 Architecture Diagram

4.2 Software Engineering Model

UsedWaterfall Model

Waterfall model is the earliest SDLC approach that was used for software development. It is also referred to as a linear-sequential life cycle model. It is very simple to understood and use. In a waterfall model, each phase must be completed before the next phase can begin and there is no overlapping in phases.

Following is a diagrammatic representation of distinct phases of waterfall model.

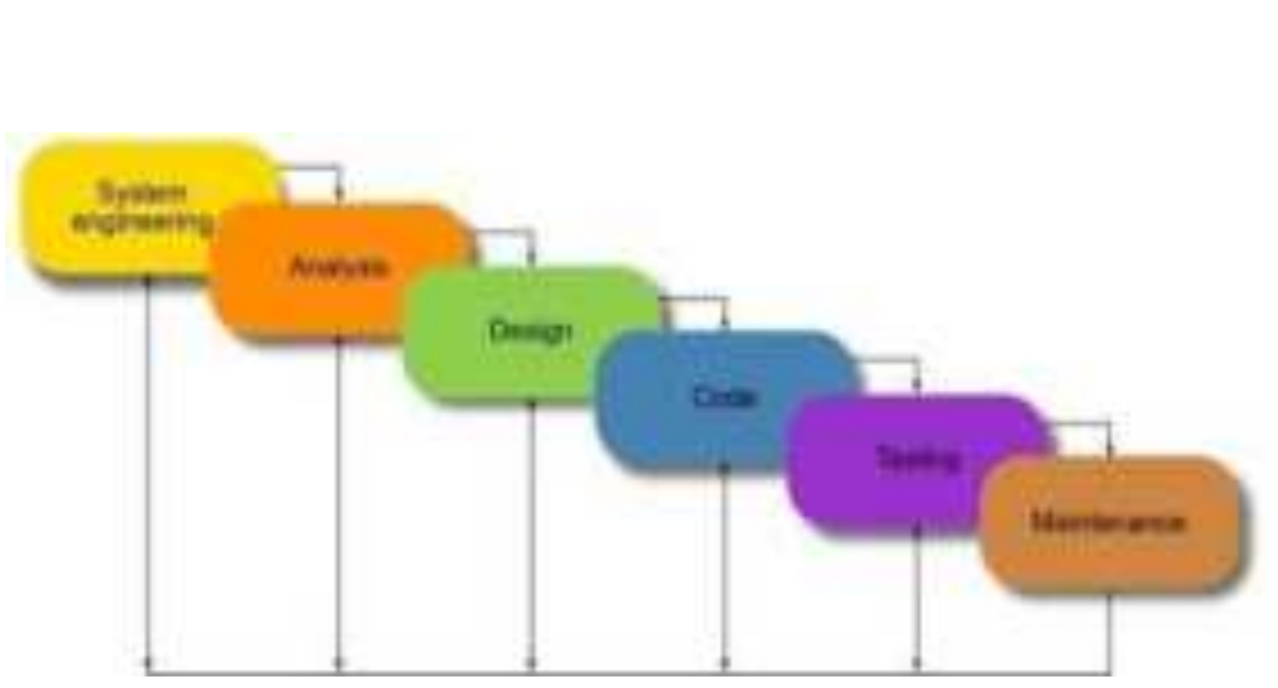


Fig 4.2 Waterfall Model

Waterfall Model

In “The Waterfall” approach, the full process of software development is divided into separate phases. In Waterfall model, typically, the outcomes of one phase act as the input for the next phase sequentially. The sequential phases in Waterfall model are:

• **Requirement Gathering and analysis**

All possible requirements of the system to be developed are captured in this phase and documented in a requirement specification document.

• **System design**

The requirement specifications from first phase are studied in this phase and system design is prepared. Design helps in specifying hardware and system requirements and also helps in defining overall system architecture.

• **Implementation**

With inputs from system design, the system is first developed in small programs called units, which are integrated in the next phase. Each unit is developed and tested for its functionality which is referred to as Unit Testing.

• **Integration and Testing**

All the units developed in the implementation phase are integrated into a system after testing of each unit. Post integration the entire system is tested for any faults and failures.

4.3 Database Table

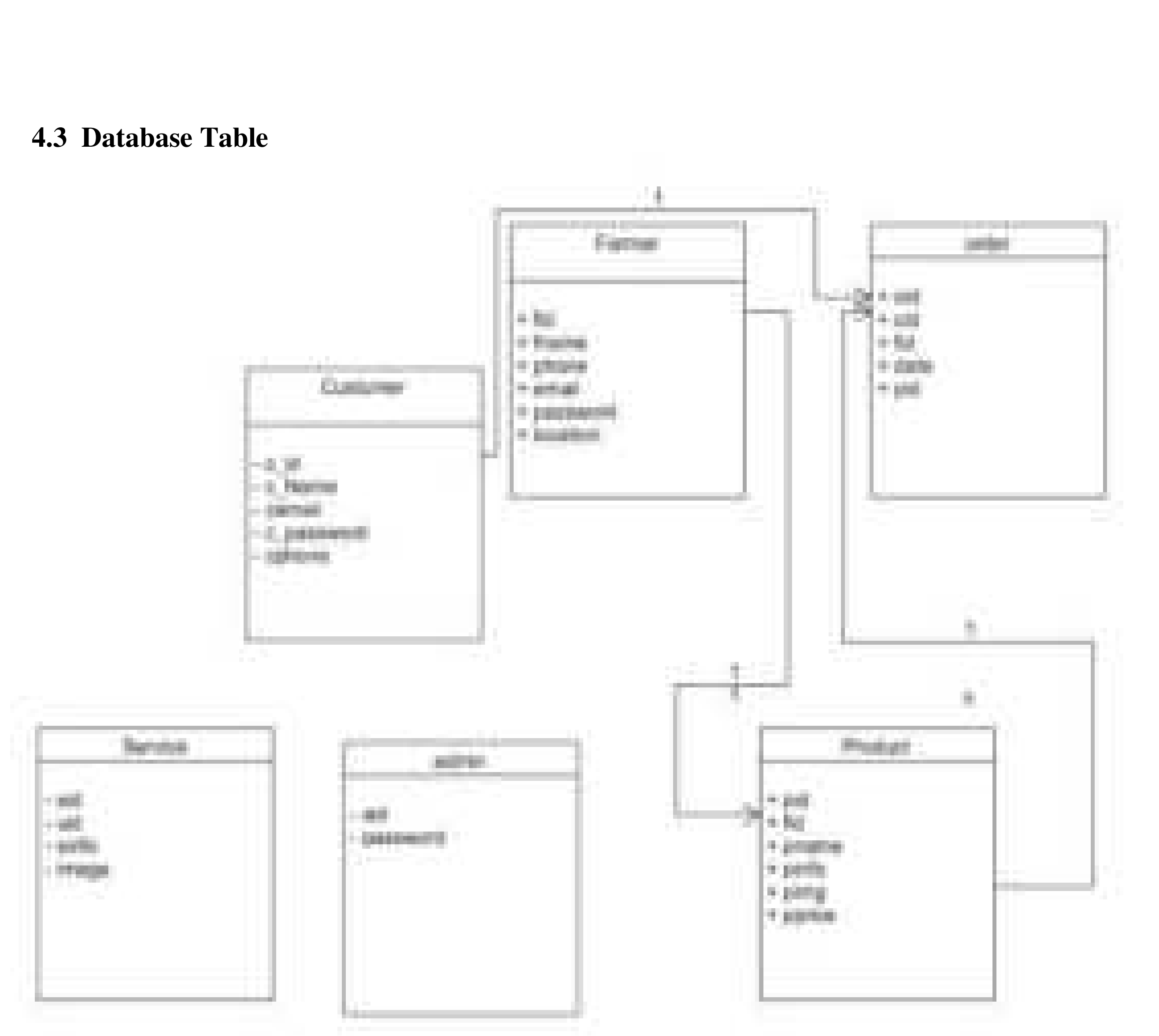


Fig 4.3 Database Table

4.4 ER Diagram

An entity-relationship diagram is a data modeling technique that graphically illustrates an information system's entities and the relationships between those entities. An ERD is a conceptual and representational model of data used to represent the entity framework infrastructure.

In software Engineering, an Entity-relationship model(ER model) is a data model for describing the data or information aspects of a business domain or its process requirements, in an abstract way that lends itself to ultimately being implemented in a database such as relational database.

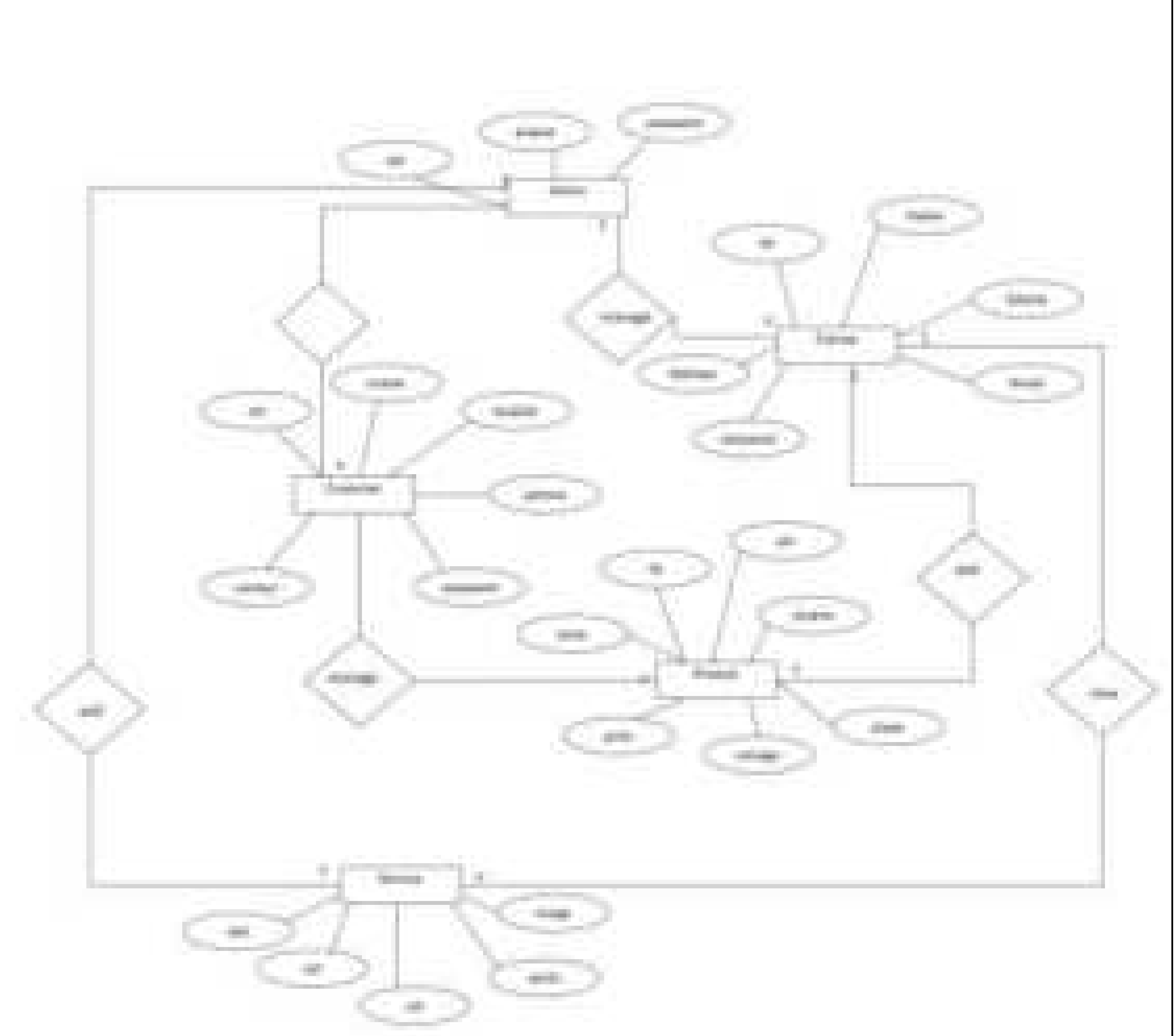


Fig 4.4 ER Diagram

4.5 Detailed Design of DFD

When it comes to conveying how information data flows through systems (and how that data is transformed in the process), data flow diagrams (DFDs) are the method of choice over technical descriptions for three principal reasons.

- DFDs are easier to understand by technical and nontechnical audiences
- DFDs can provide a high level system overview, complete with boundaries and connections to other systems
- DFDs can provide a detailed representation of system components

DFDs help system designers and others during initial analysis stages visualize a current system or one that may be necessary to meet new requirements. Systems analysts prefer working with

DFDs, particularly when they require a clear understanding of the boundary between existing systems and postulated systems. DFDs represent the following:

- External devices sending and receiving data
- Processes that change that data
- Data flows themselves.

4.5.1 Level 1 DFD Admin

In this leve1 dataflow diagram represents primary data flow from other entities to admin entity role.

The level one DFD helps in analyzing the workflow of Admin. In our application when the admin login the databases are connected to the application and the modules within the circle do their task according to the input given to them.

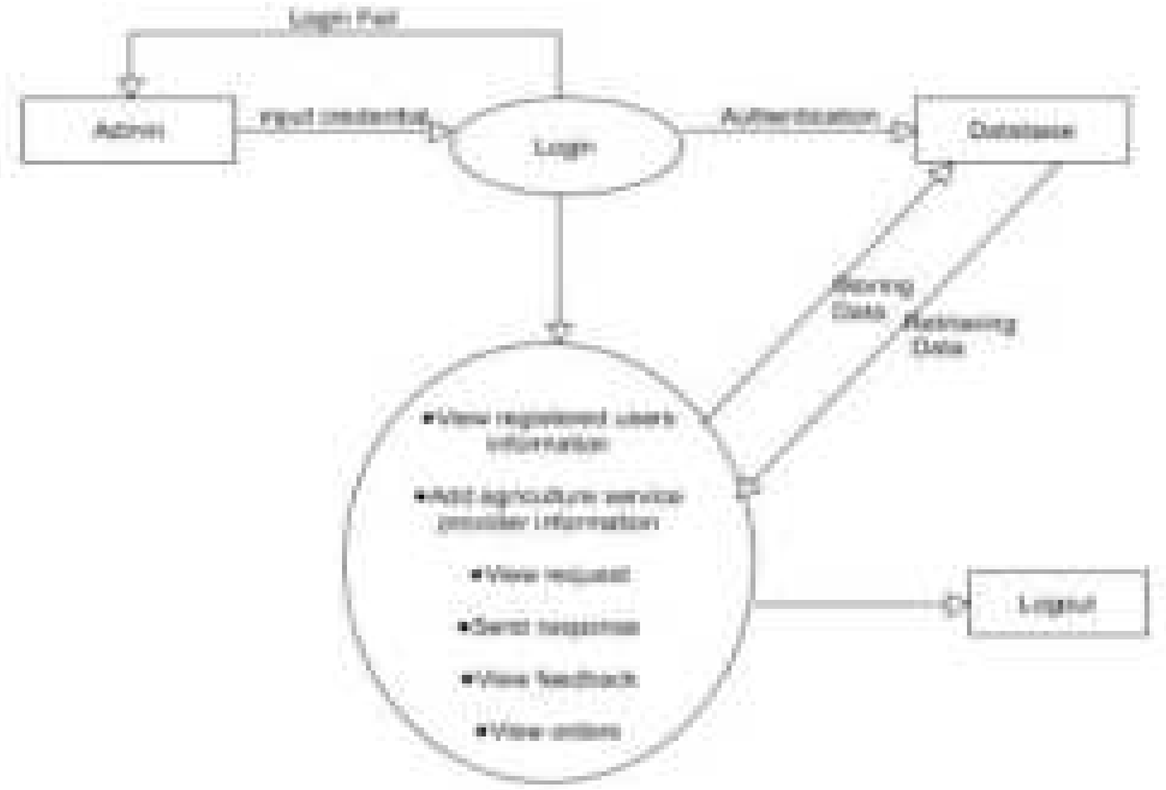


Fig 4.6 Level 1 DFD Admin

4.5.2 Level 1 DFD User

The Level one DFD for the User has do the same work as the Admin. The user of the application first login the validation for the login is done with help of database if the password or email id does not match it return back else execute the operation mentioned in the circle.

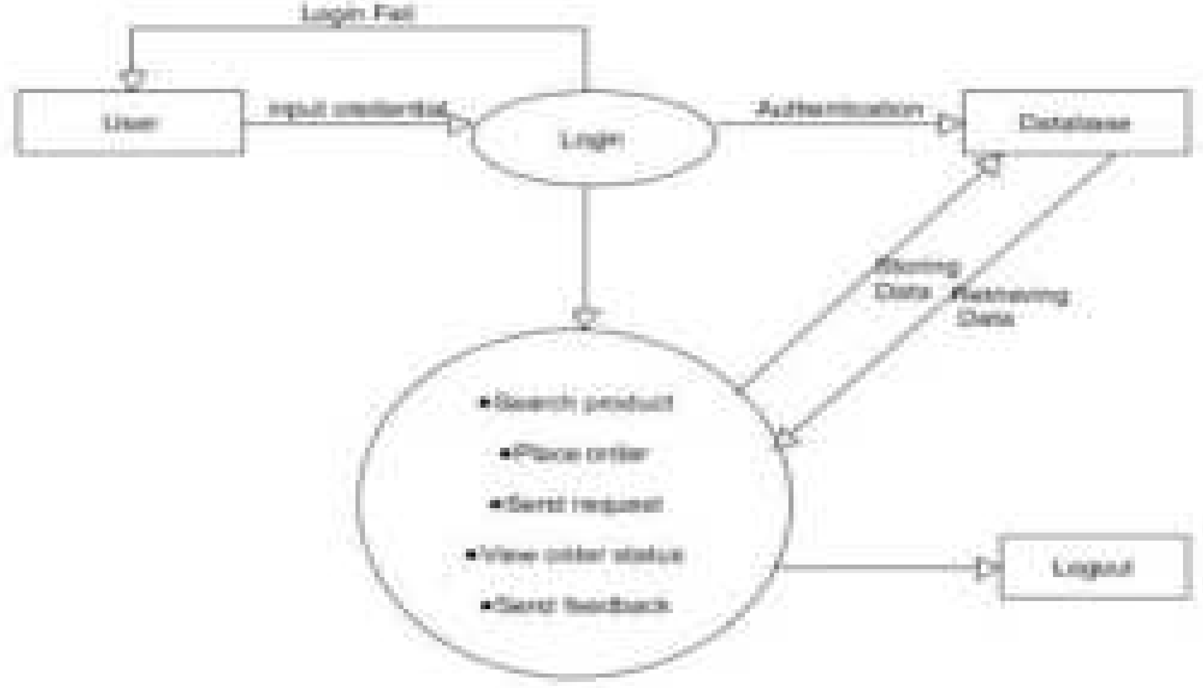


Fig 4.7 Level 1 DFD User

4.5.3 Level 1 DFD Farmer

The Level one DFD for the Farmer first it check for the login correction with help of the database connected if the login is true then the following activities are done by this application like products and services..

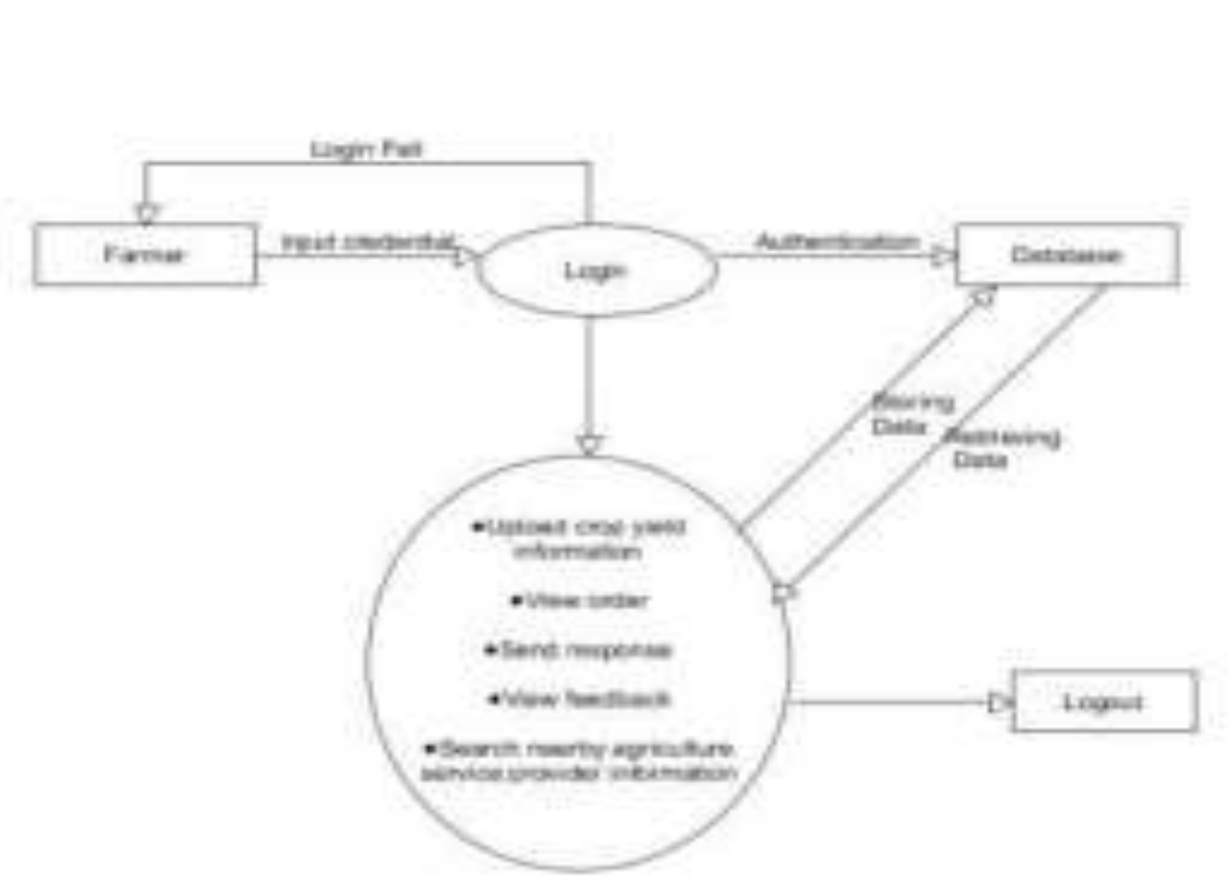


Fig 4.8 Level 1 DFD Farmer

4.6 Activity Diagram

- Activity diagram is basically a flowchart to represent the flow form one activity to another activity. The activity can be described as an operation of the system.
- Activity diagram for our application shown below, when the activity starts the user should login to the application allows the user to the next activity by entering correct username and password.

4.6.1 Activity Diagram for Farmer

- The activity diagram for all the actors are showed for the application
- The activity diagram show the step by step flow for the modules
- The Farmer activity diagram show the clear picture after the success login for the user
- After the successful login the Farmer can access to those modules like product bidder and notification

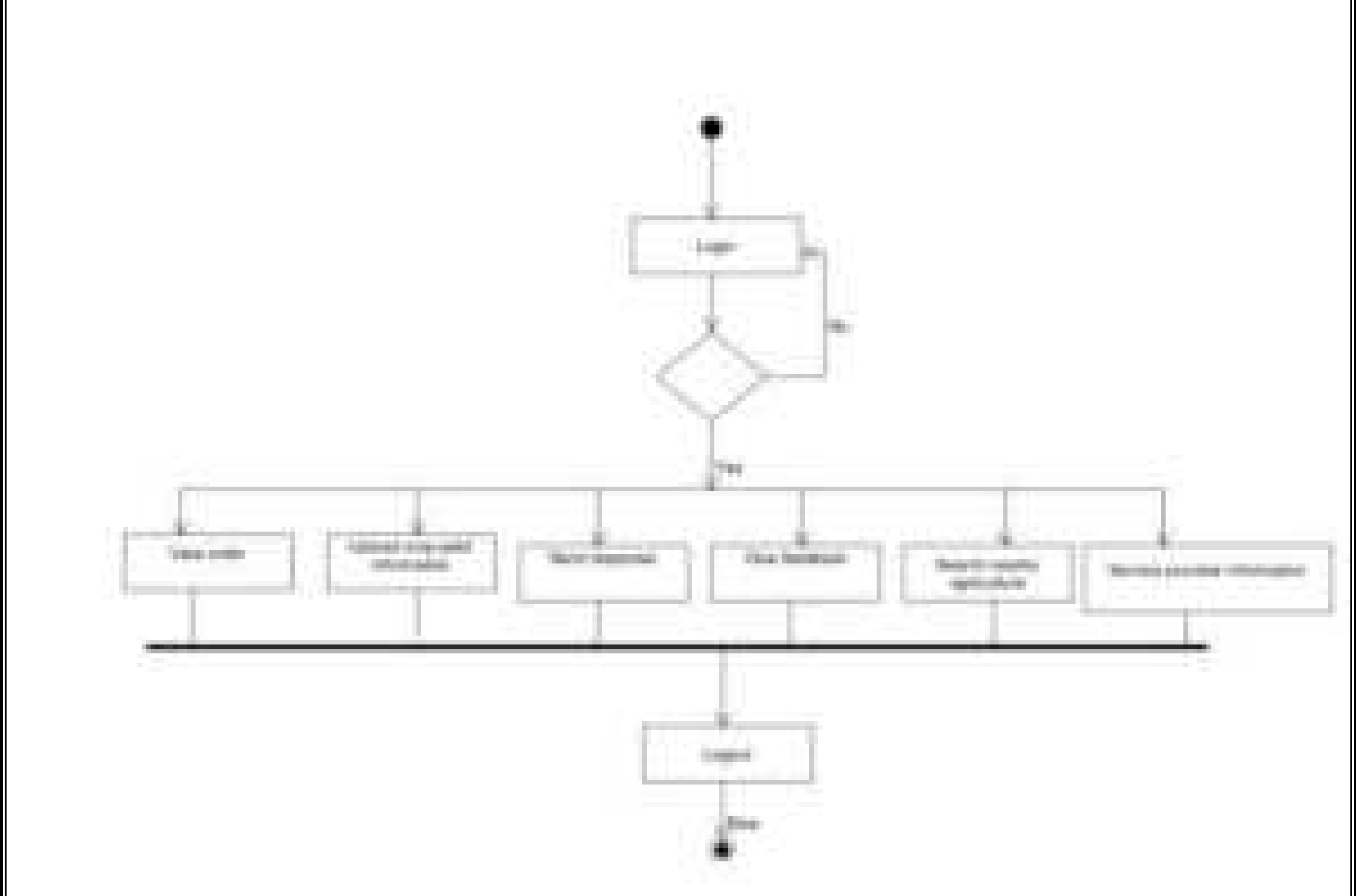


Fig 4.9 Activity Diagram for Farmer

4.6.2 Activity Diagram for User

- The activity diagram explains workflows of the User activity
- After the login success there are several modules
- These modules have the small action that has to be executed based on the assigned task.
- How the modules are included in the sub modules are described easily using activity diagram

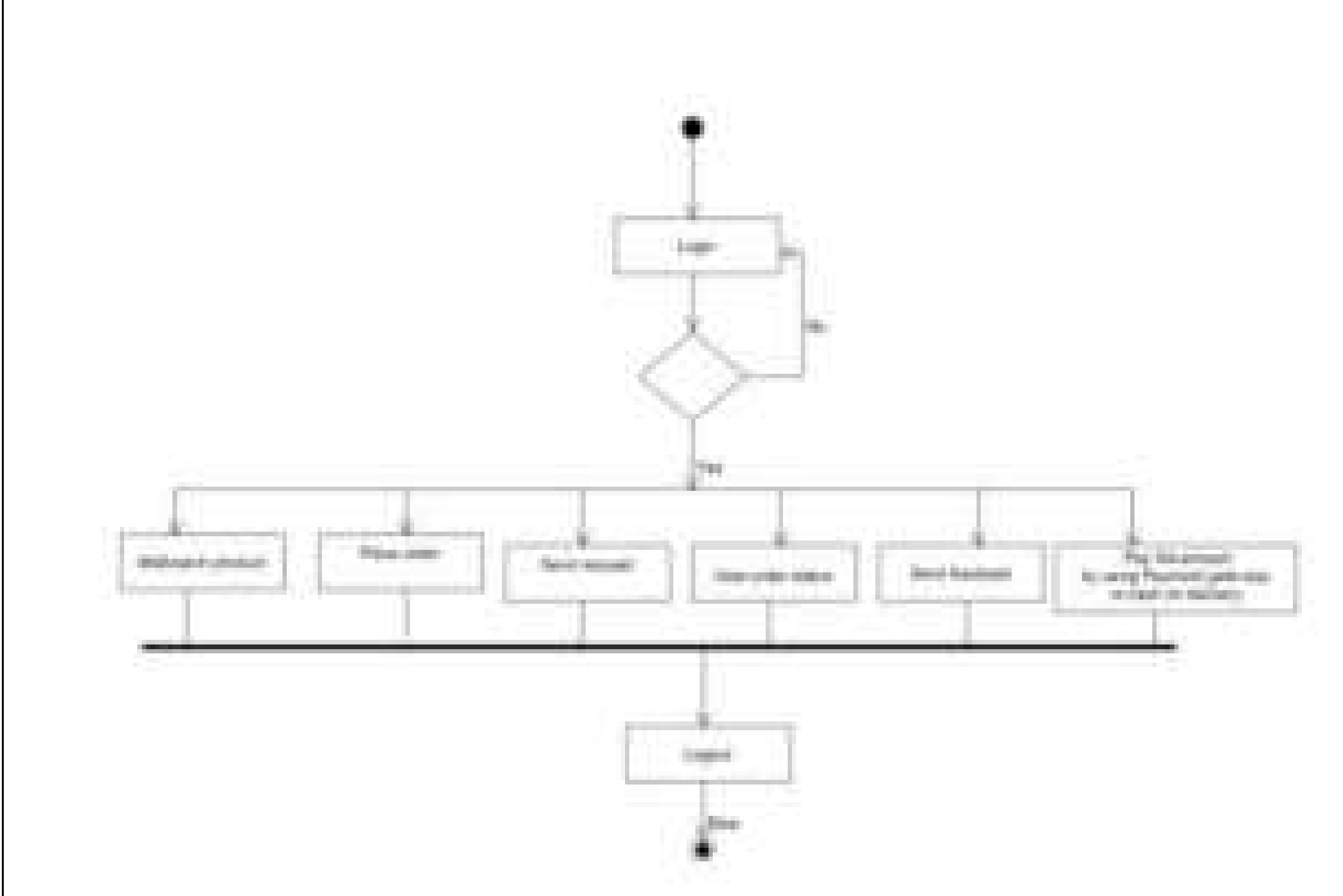


Fig 4.11 Activity Diagram User

4.6.3 Activity Diagram for Admin

- The activity diagram for Admin is showed in this diagram
- The activity diagram shows the step-by-step flow for the modules
- The Admin activity diagram show the clear picture after thesuccess login for the Admin
- After the successful login the Admin they can add test, products or view user.

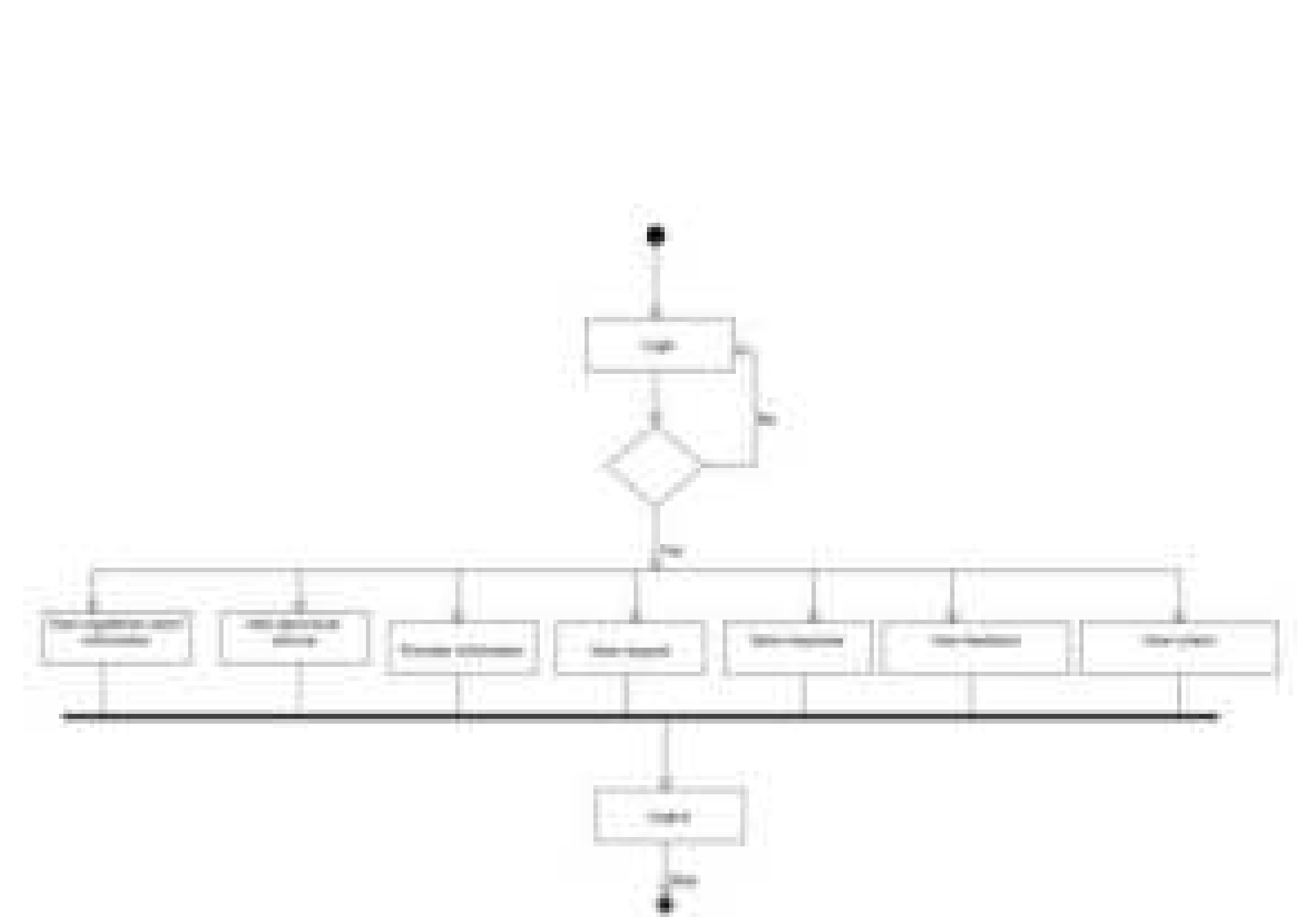


Fig 4.11 Activity Diagram Admin

4.7 Use Case Diagram

Use case diagram in the Unified Modeling Language (UML) is a type of behavioral diagram defined by and created from a Use-case analysis. Its purpose is to present a graphical overview of the functionality provided by a system in terms of actors, their goals (represented as use cases), and any dependencies between those use cases.

The main purpose of a use case diagram is to show what system functions are performed for which actor. Roles of the actors in the system can be depicted.

4.7.1 Use Case Diagram for Admin

The use case diagram for the admin explains how the modules are interacted to the actors. What are some modules which are common to the actors are explained in this use case diagram. The admin actor can have the functionalities like adding products, services and managing the application.

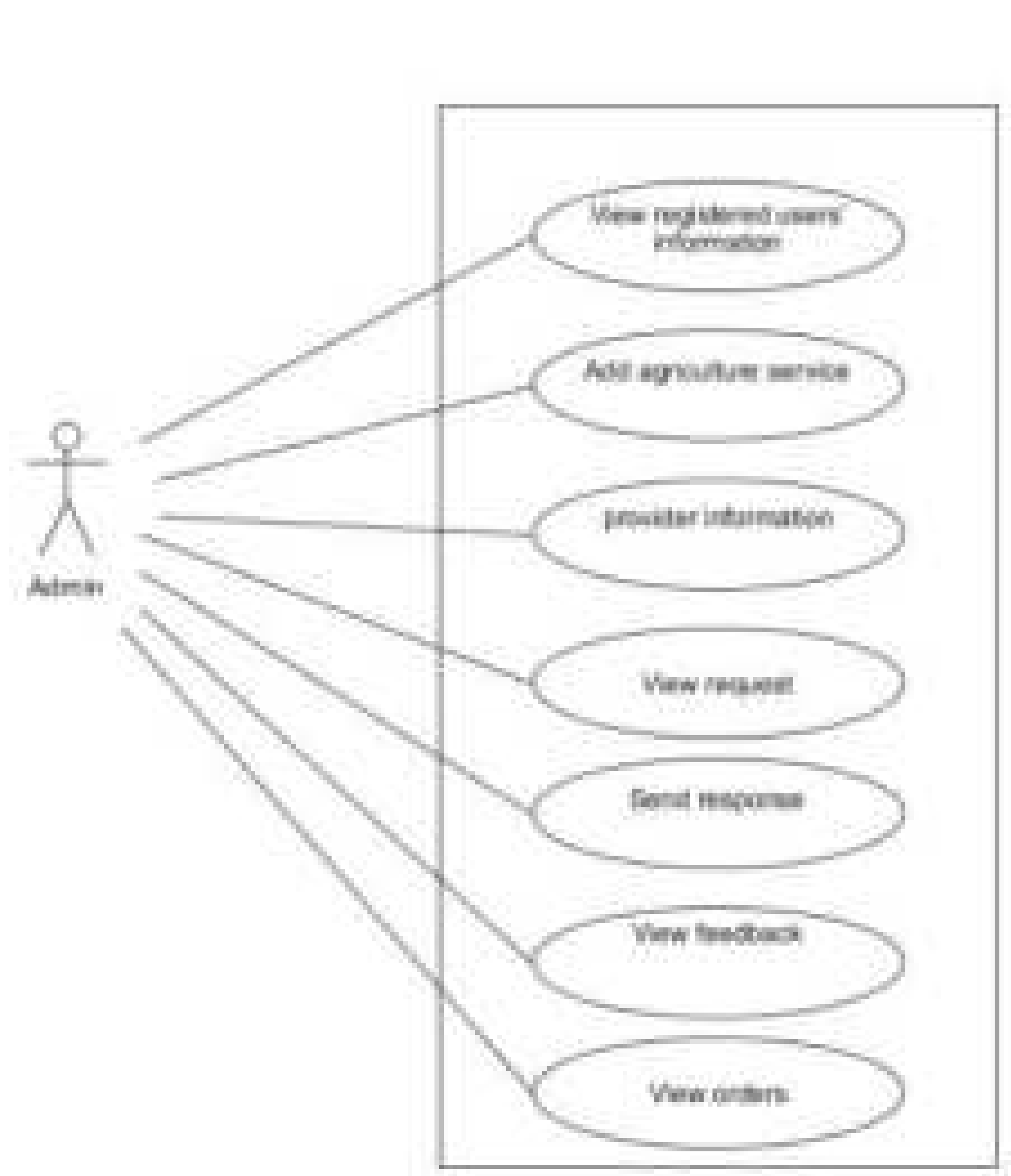


Fig 4.12 Use Case Diagram for Admin

4.7.2 Use Case Diagram for User

The use case diagram for the user has the different modules and this modules are common only to the user those are described in this architecture.

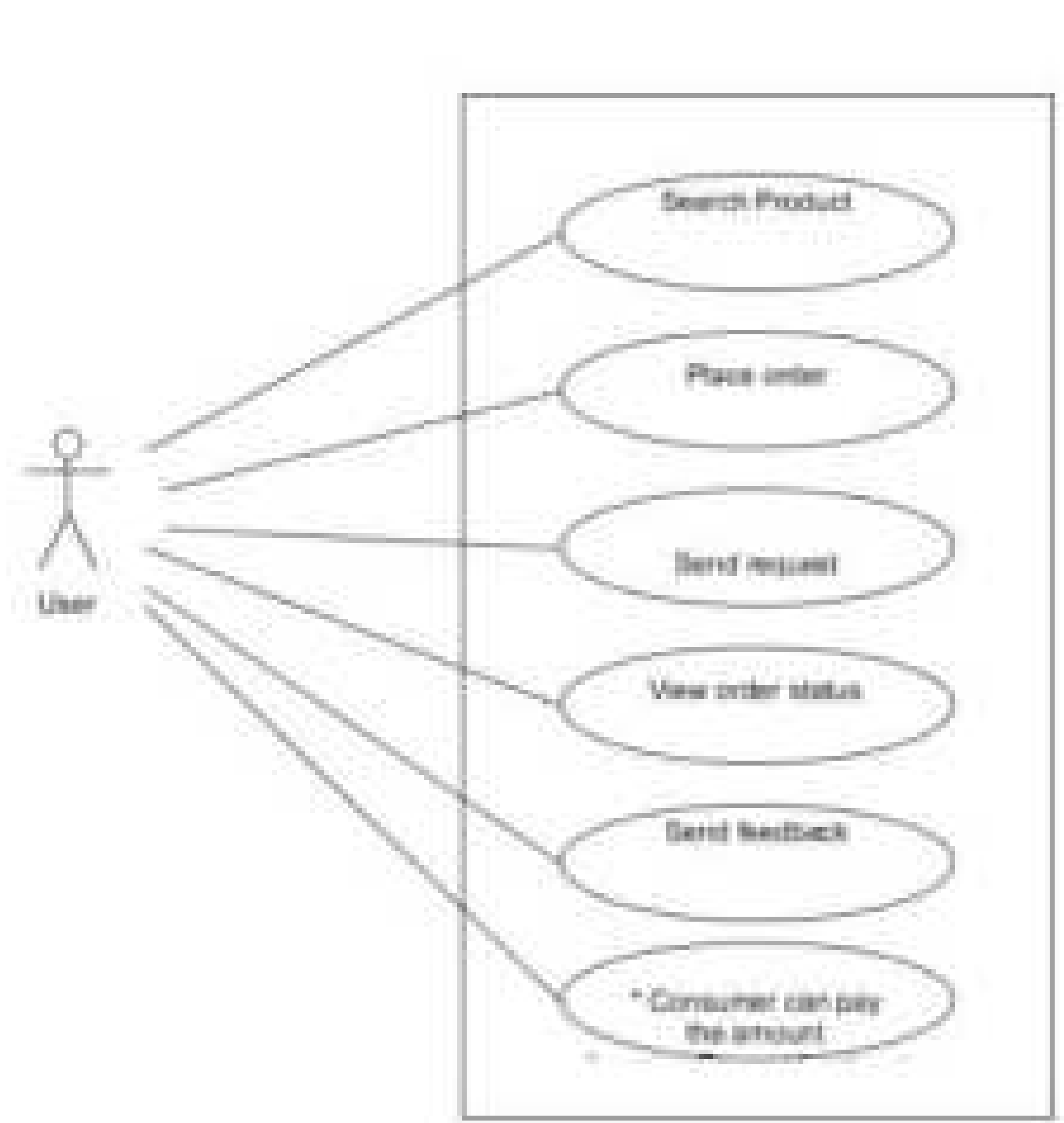


Fig 4.13 Use Case Diagram For User

4.7.3 Use Case Diagram for Farmer

The use case diagram for the farmer has the different modules and this modules are common only to the user those are described in this architecture.

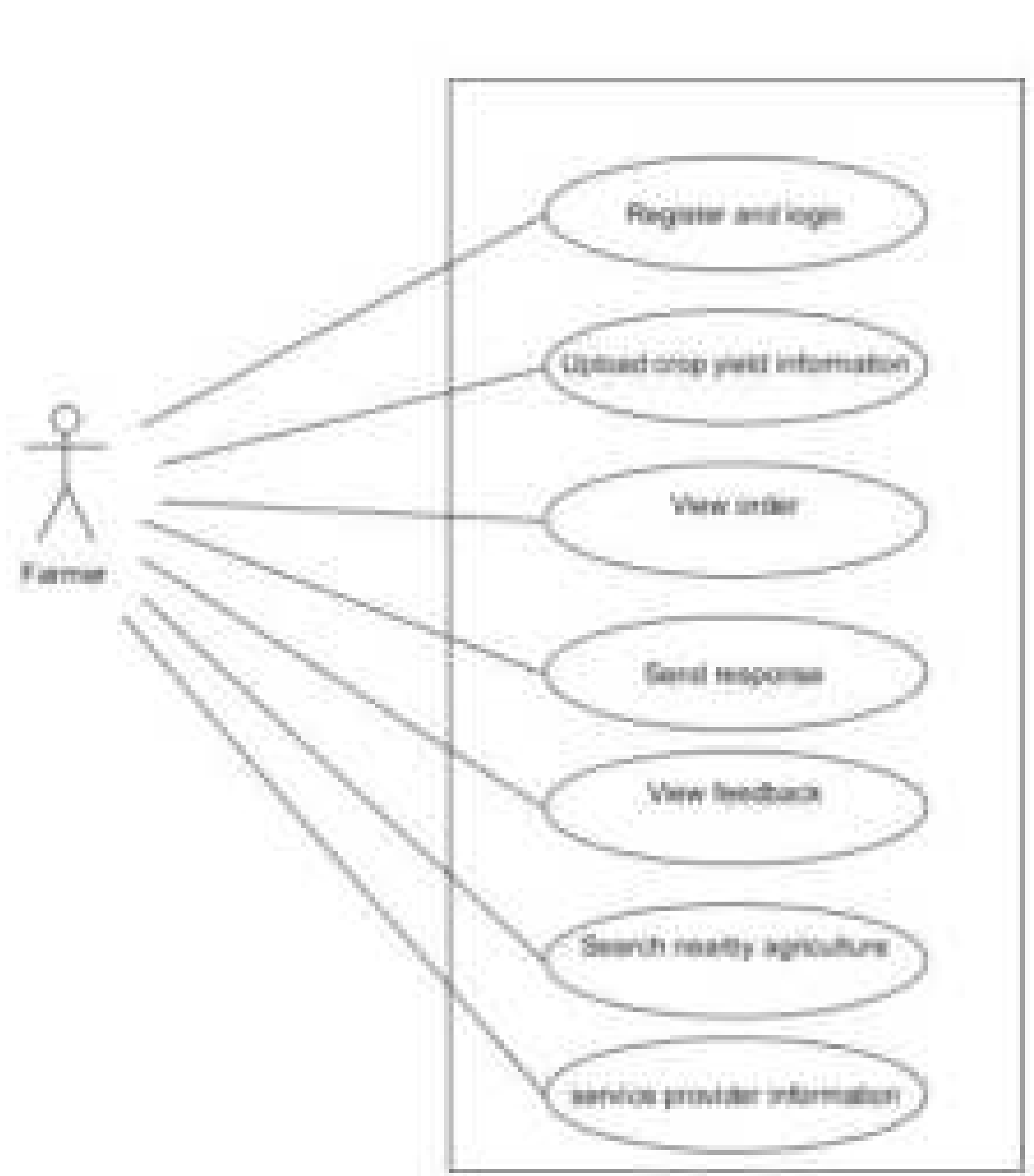


Fig 4.14 Use Case Diagram For Farmer

4.8 Sequence Diagram

A sequence diagram shows object interactions arranged in a time sequence. It depicts the object and classes involved in the scenario and the sequence of messages exchanged between the objects needed to carry out the functionality of the scenario.

Sequence diagram are typically associated with use case realization in the logical view of the system under development. Sequence diagrams are sometimes called event diagrams or event scenario.

4.8.1 Sequence Diagram for Admin

- The admin login for the application check whether the username and password is correct or wrong. The database check for the id and return success or error message. If error then go back to login or else dashboard appears
- Once the session and cookies are generated then there is only communication between the dashboard and the database.
- In this module send the request and take back the response from the database and display the output according to the user needs.

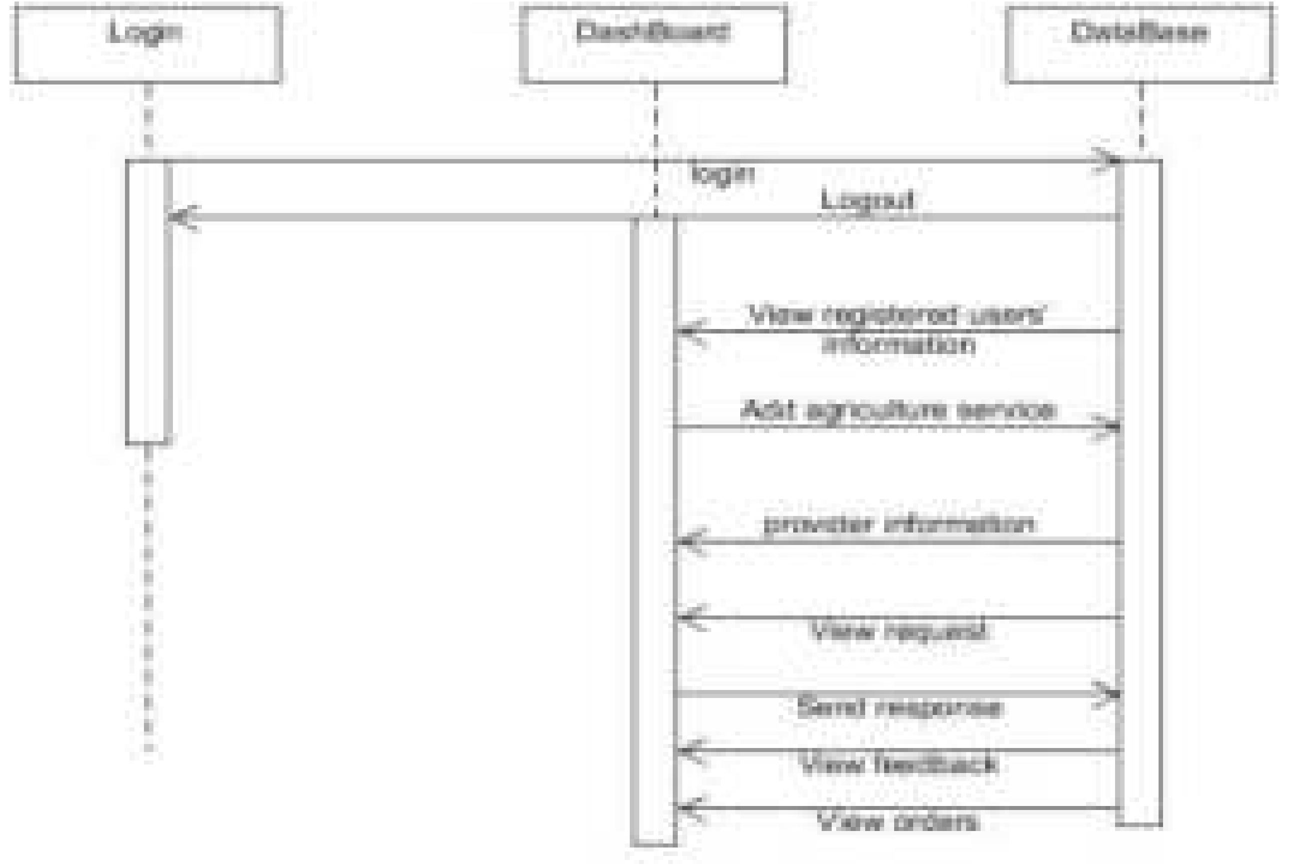


Fig 4.15 Sequence Diagram For Admin

4.8.2 Sequence Diagram For User

- The user login for the application check whether the username and password is correct or wrong. The database check for the id and return success or error message. If error then go back to login or else dashboard appears
- Once the session and cookies are generated then there is only communication between the dashboard and the database.
- In these modules send the request and take back the response from the database and display the output according to the user needs.

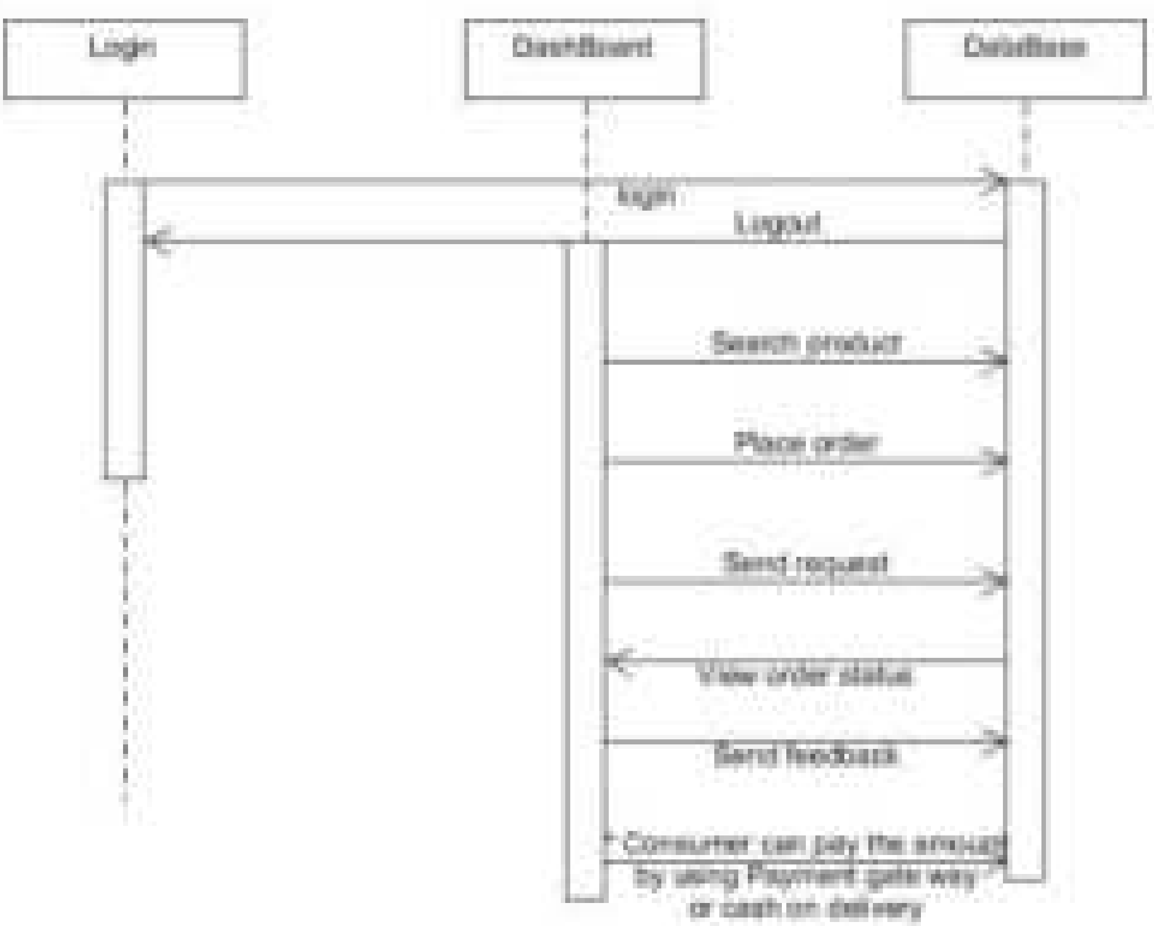


Fig 4.16 Sequence DiagramFor User

4.8.3 Sequence Diagram for Farmers

- The Doctor/consultant for the application check whether the username and password is correct or wrong. The database check for the id and return success or error message. If error then go back to login or else dashboard appears
- Once the session and cookies are generated then there is only communication between the dashboard and the database.
- In these modules send the request and take back the response from the database and display the output according to the user needs.

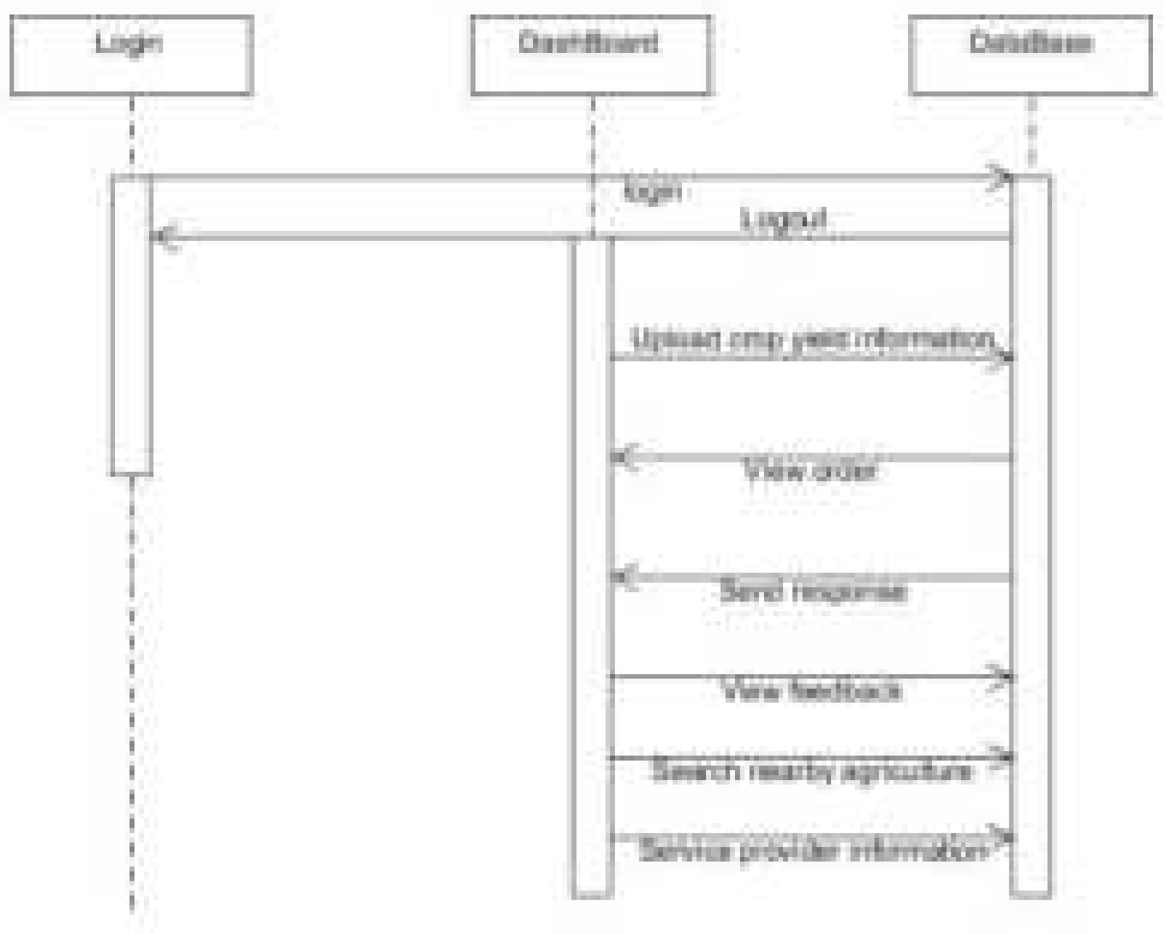


Fig 4.14Use Case Diagram For Doctor/Consultants

CHAPTER 5: IMPLEMENTATION

5.1 Introduction

In this stage physical system specifications are converted into a working and reliable solution. This is where the system is developed. It involves careful planning, investigation of the current system and its constraints on implementation, design of methods to achieve the changeover, an evaluation, of change over methods.

The objective of the system implementation phase is to implement a fully functional system, which deliverables met in order of priority. The result of this phase is an accurately functioning system. The more complex system implemented, the more involved will be the system analysis and the design effort required just for implementation.

The implementation phase deals with issues of quality, performance, baselines, libraries, and debugging. The end deliverable is the product itself. During the implementation phase, the system built according to the specifications from the previous phases. This includes writing code, performing code reviews, performing tests, selecting components for integration, configuration, and integration.

Web Application for report generation is implemented using php Object oriented programming is an approach that provides a way of modularizing programs by creating partitioned memory area for both data and functions that can used as templates for creating copies of such modules on demand.

This project is implemented using MVC architecture. User Interface developed using HTML, CSS and BOOTSTRAP code used in the view. Java classes used in both controller and Model but Model contains only connectivity part, Google live database used to store the user information and activities application

.Implementation Steps

Model

The Model layer refers to the entities, business objects, data source, and other repositories available in our application. Model used to perform the database related operations. It uses php to interact with the database and to perform operations such as information related to the user, products, stores etc.

View

View is a front end or GUI, which invokes Model through Controller, view, consists of latitude and longitude. The view in XAMPP IDE is coded using tools. The view will have all the necessary validations for user entries.

Controller

Controller classes are responsible for handling user input and responses.

 Locating the appropriate action method to call and validating that it can be called.

 Getting the values to use as the action method’s arguments

 Handling all errors that might occur during the execution of the action method.

 Providing the user-friendly views to the user.

Pseudo Code

Registration

- Form entry ( ); Establish the connection;
- Get User Id (email)
- Get Password
- Get Email
- Get Contact Number
- If valid, then create a session and prompt, “registered successfully”;
- Else
- Display appropriate error message;
- End

Login form

- Data: Username and Password
- Result: Create session and Authenticate User
- Click on login call to action button on the Digital Marketing for Farmer home page;
- Enter registered Username and password;
- Connect database
- If valid then
- Create session on the username;
- Authenticate User;
- Else
- Display error message “Wrong Credentials”;
- Redirect to Login Page;

CHAPTER 6: TESTING

6.1 Purpose of Testing

Web applications run on devices with limited memory, CPU power and power supply. The behavior of the application also depends on external factors like connectivity, general system utilization, etc.

Therefore, it is very important to debug, test and optimize web application. Having reasonable test coverage for web application helps to enhance and maintain the web application.

As it is not possible to test bootstrap web applications on all possible device configurations, it is a common practice to run on typical device configurations. Should test application at least on one device with the lowest possible configuration. In addition, should test on one device with the highest available configuration, e.g., pixel density, screen resolution to ensure that it works fine on these devices.

Testing Concepts

Web application testing based on Unit. In general, a Unit test is a method whose statements test a part of the application. Organizes test methods into classes called test cases, and group test cases into test suites.

Unit Tests

Local Unit Test

Unit tests that run on local machine only. These tests compiled to run locally on the NetBeans to minimize execution time. Use this approach to run unit tests that have no dependencies on the web framework or have dependencies that mock objects can satisfy.

Instrumented Unit Test

Unit tests that run on device. These tests have access to Instrumentation information, such as the Context of the application are testing. Use this approach to run unit tests that have web application dependencies, which mock objects cannot easily satisfy.

Integration Test

This type of test verifies that the target app behaves as expected when a user performs a specific action or enters a specific input in its activities. For example, it allows checking that the target app returns the correct UI output in response to user interactions in the app's activities. UI testing frameworks like Espresso allow programmatically simulating user actions and testing complex intra-app user interactions.

Cross App Test

This type of test verifies the correct behavior of interactions between different user apps or between user apps and system apps. For example, might want to test that app behaves correctly when the user

performs an action in the Settings menu. UI testing frameworks that support cross-app interactions, such as UI Automaton, allow creating tests for such scenarios.

Test Cases:

A test case is a set of conditions or variables under which a tester will determine whether a system under test satisfies requirements or works correctly. The process of developing text causes can also help find problems in the requirements or design of an application.

The following tables show the various test causes scenarios that are generated along with the required inputs o the given scenarios, expected outputs, actual output and the result whether the test passes or fails.

6.2 Test Cases

A test case is a set of conditions or variables under which a tester will determine whether a system under test satisfies requirements or works correctly. The process of developing test cases can also help find problems in the requirements or design of an application.

Positive Tests

Test Case No	**Positive Scenario**	**Required Input**	**Expected Result**	**Actual Output**	**Test pass/fail**
1	To ensure that the module able to update Test Details	Test information	The Test status should update accordingly.	As expected	Pass

2	To ensure the module able to display all the videos test	Video test	The video status should update accordingly.	As expected	Pass
3	To ensure the module able to display all motivational videos	Motivational Videos	The video status should update accordingly.	As expected	Pass
4	Authenticate consultant and laboratories	Laboratories and consultant information	Status updated accordingly	As expected	Pass
5	To ensure the module able to display all the speech and hearing consultant and lab owners	Username and medical service providers	Details are stored in the database	As expected	Pass
6	To ensure the module able to update and view treatment details	Information about the student and health status	Response from user	As expected	Pass

7	To ensure the module able to Learn or teach sign language alphabets, actions, sentence action etc.	Teacher authentication and submit videos to the application	All status should update accordingly.	As expected	Pass
8	To ensure the module able to display all sign language videos	Sign Language Videos	The video status should update accordingly.	As expected	Pass
10	To ensure the module able to display appointment details	Response from the doctor	The Appointment status should update accordingly.	As expected	Pass

Negative scenario

TestCase No	Negative Scenario	Required Input	Expected Output	Actual Output	Test Pass/Fail
1	Register using registered username	Registering account	Username already exist	Username already exist redirect to login	Pass

2	Send appointment more time	Send appointment request	Alert with message Already sent request	Alert with message Already sent request	Pass
3	Direct execute dashboard page	Execute dashboard page	Return to login	Return to login	Pass
4	Select upcoming days to choose book appointment	Choose date while booking appointment	Restrict previous date	Restrict previous date to select	Pass
5	Tracking geolocation	Track current longitude and latitude	Allow geolocation popup message	Allow geolocation popup message	Pass

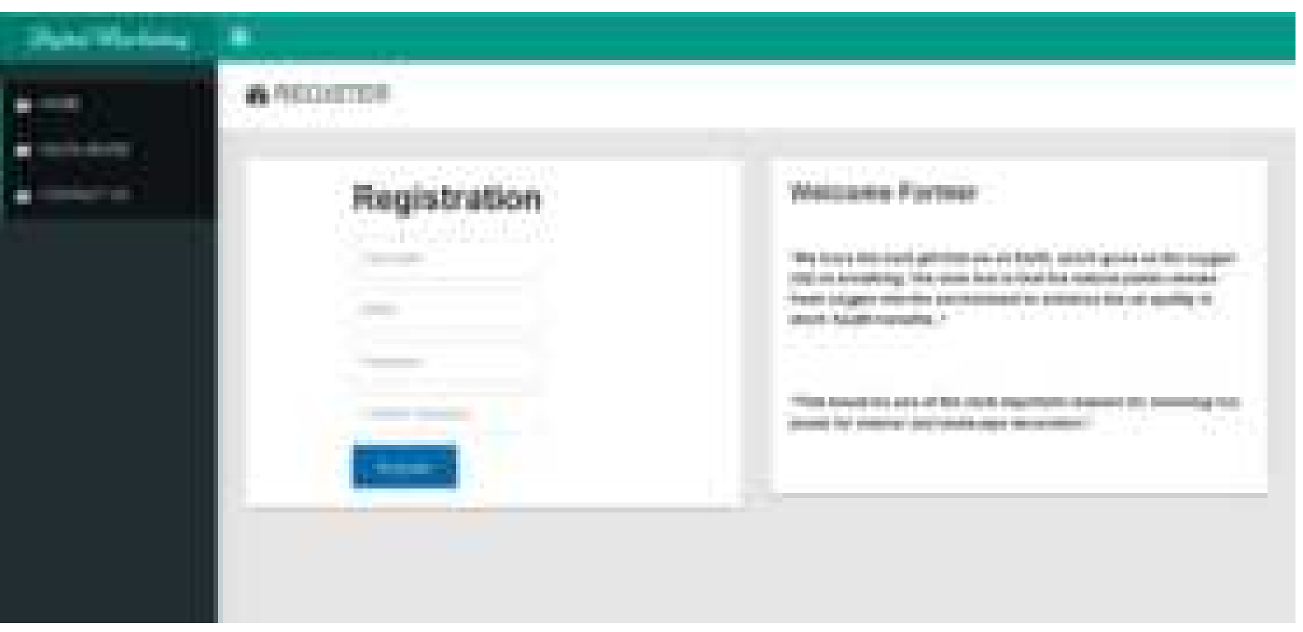
CHAPTER 7: EXPERIMENTAL RESULTS

This chapter highlights the result of the project “A Responsive Application for Hearing Impaired Children to Learn Sign Language” and the snapshots of each of the activity are shown along with the description. Each snapshot describes every single step of the application. All the main activities as well as the options provided to the users in each activity are also shown.

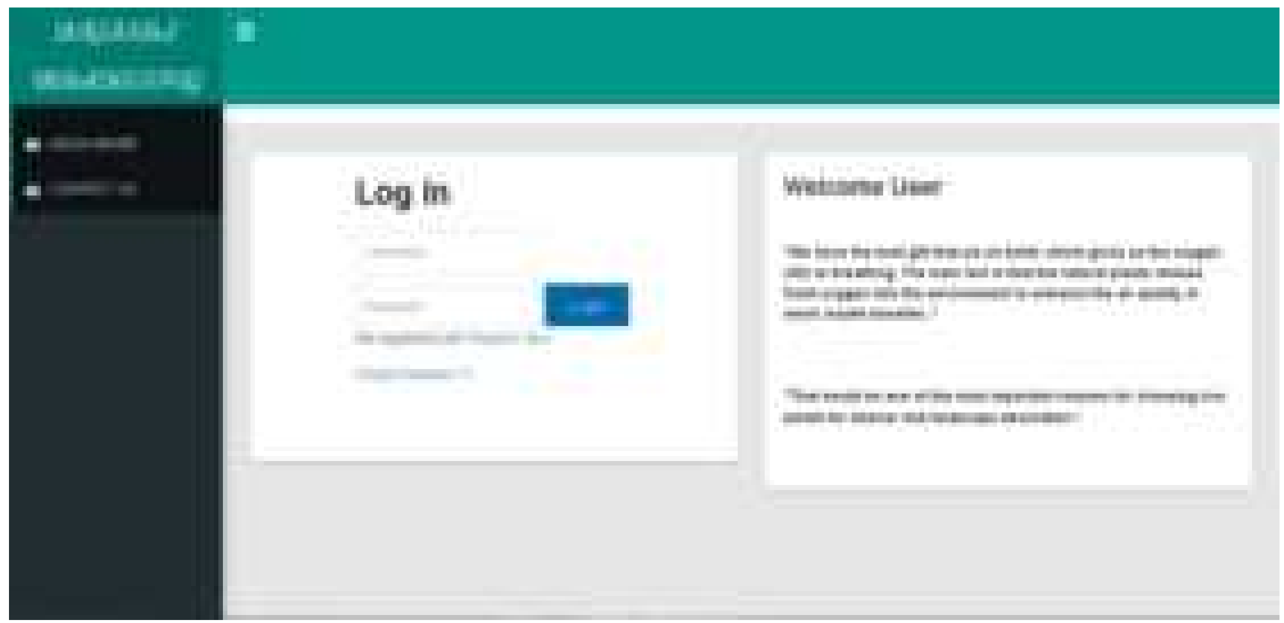
7.1 Screenshots

7.1.1 Home Page

7.1.2 Registration



7.1.3 Login



7.1.4 Profile

7.1.5 Search Products

7.1.6 Add Products

7.1.7 Feedback

8. CONCLUSION AND FUTURE ENHANCEMENTS

Conclusion

Digital Marketing is helpful to farmers to reach out the multiple buyers and get higher prices for their products. It empowers agricultural startups. With the increasing amount of literacy in rural areas and development in agribusiness infrastructures, young farmers are ready to adopt Digital marketing platforms

.**Future enhancement**

This Application can also be developed in mobile application. As this application can be add more module so that the farmer and user can use this application. This application can also be used in different languages so that user can make use of this.

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Department of Criminology and Forensic Science in association with IQAC

Commissioner Office (Fingerprint Unit), Mysuru- II Sem BA-, 5th June 2023

Field Visit To:

Commissioner Office (Fingerprint Unit), Mysuru

Details of the department organizing the event in collaboration with institution:
The Department of Criminology and Forensic Science organized the event in collaboration with Commissioner Office (Fingerprint Unit), Mysuru.

Purpose and scope of the event:
To understand the importance of fingerprints as evidence in criminal investigation and in identifying suspects and victims.

Details of the event:
The students of the Department of Criminology and Forensic Science visited the Commissioner Office (Fingerprint Unit), Mysuru, on 20th December, 2022. We were accompanied by our lecturers Ms. Megha Nilajkar (HoD) and Mr. Chandan Kumar.

The Sub-Inspector Dhanalakshmi addressed our team. She explained fingerprint collection, identification, and process of comparison & matching of ridges in impression.
She explained in detail regarding fingerprint examination process, and working function of finger print unit, etc. Some of the points are:

• Explained the Extraction of Finger Print from Crime scene.

• Explained Process and Challenges while Collecting the Prints.

• Also gave the demo for collection of Finger prints using various type of Powders:
  Using: Black powder
  White powder

• Showed the identification and Matching of Ridges in Finger Prints

• After this, gave the information regarding the software (Automated Fingerprint Identification System - AFIS) and its work functions.

• Informed 8 ridge characteristics to confirm the fingerprint.

• Gave demo for image processing and pattern recognition technique to capture, store, match, and compare the chance print.

• Showed the live scanner prints.

• Information regarding all finger prints data, & NCRB data

• Explained the MOB file & etc.

After completion of explanation & demo session by Sub-Inspector Dhanalakshmi we got the chance to saw the finger print enhancer using light source.

Then ACP Rajashekar Concluded Our visit by Demonstrating Fingerprint Detection using different light sources.

No. of participants:
There were 19 students who participated in the event.

HoD Criminology & Forensic Science

Signature

Principal Dr. B.R Jayakumari

Signature

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Field visit to field visit to CEN (Cyber Economic Narcotics) Police Station - Final Year BA (2nd December 2022)

Field Visit To:

CEN (Cyber Economic Narcotics) Police Station

Details of the department organizing the event in collaboration with institution:
The Department of Criminology & Forensic Science organized the event in collaboration with CEN (Cyber Economic Narcotics) Police Station, Mysuru City.

Purpose and scope of the event:
The field visit to CEN (Cyber Economic Narcotics) Police Station Mysuru City, was conducted to enhance the knowledge of the students regarding the working, administration and duties along with the importance of the CEN police.

Details of the event:

The First officer who addressed us was Head constable Basha. He provided information regarding the most common kinds of crimes which they deal with, and gave an overview of the CEN police station activities.

He gave some of the information with the following kind of crimes:

- Email and internet Fraud
- Identity Fraud
- Theft of financial or Debit card data
- Cyber extortion
- Illegal gambling
- Selling illegal items in online
- Soliciting, producing, or possessing child pornography
- Financial Fraud, (through UPI Transaction by using Bar & QR code)

• Explained Social Media Crimes, Extortion using these Flat forms

After explanation of crimes, they discussed CrPC Provision (Section 102 & 90) which helps for their investigation and also informed about Cyber crime Helpline No: 1930 and Online portal - NCRP (National Cyber crime reporting Portal)

Then, ASI Subhash Chandra spoke about Narcotics & drugs, also he introduces some of the Narcotic Sample, like ganja, charas, and explained the details regarding chain of custody for Narcotic Analysis and Economic Related Cases.

At last PSI Anil Kumar Clarified students Doubts and Explained Process involving in the Investigation and ended the session by saying to be safe and alert in the society and to be aware of our surroundings and people around us.

No. of participants:
Around 17 students had participated in the field visit.

HoD Criminology & Forensic Science

Signature

Principal Dr. B.R Jayakumari

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Manasagangothri, Mysore

A
Major Project Work On

"IMAGE FUSION OF VISIBLE AND INFRARED IMAGES USING ML ALGORITHMS"

Submitted in partial fulfilment of the requirement for the award of
Master of Computer Application

Submitted by

AKASH H M

Register No: P01BH 21S0101

Carried out at
Dream Buzz Solutions, Mysuru

Under the guidance of

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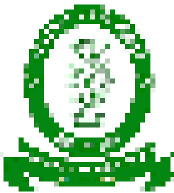

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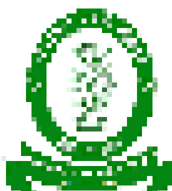

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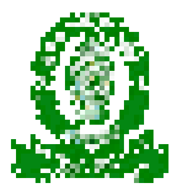

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ACKNOWLEDGEMENT

Apart from the efforts of me, the sources of my project depend largely on the encouragement and the guidance of many others. I take this opportunity to express my gratitude to the people who have been instrumental in the successful completion of this project.

I have great pleasure in expressing my deep sense of gratitude to our beloved Director **Dr. C.K RENUKARYA**, Pooja Bhagavat Memorial Mahajana PG Center, Mysuru for providing all the facilities to work out this project.

I would like to express my gratitude to **Mrs. Rachana C. R**, **Associate professor & Head ,DoS in Computer Science, Pooja Bhagavat Memorial Mahajana PG Centre, Mysuru** for her constant support, motivation and invaluable guidance throughout the tenure of this project.

I consider it is my privilege and honor to express my sincere thanks to **Mrs. Yashaswini J, Assistant Professor , Department of studies in computer science, Pooja Bhagavat Memorial Mahajana PG Centre, Mysuru** for providing me all the facilities, support, guidance, Motivation, encouragement and suggestion related to the project. Her timely guidance helped me to complete the project on time.

I would also like to thank my External guide **Mr. Rohith H.G, Senior Software Developer** Ideonix Solutions, Mysuru for providing me all the facilities, support, guidance, Motivation, encouragement. His timely guidance helped me to complete the project on time.

I would like to thank my mentor **Mr. Rohith H.G,** for his encouragement, timely guidance and valid suggestion throughout my project.

Finally, I would like to thank all our teaching and non-teaching staff, for their kind Co-operation.

Above all, my sincere gratitude to my **parents** and **friends** and all those who have supported me throughout my project.

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ABSTRACT

Image fusion plays a pivotal role in enhancing the information content and interpretability of multi-modal images. In this project, we propose an innovative approach to image fusion by integrating visible and infrared images using the Adaptive Data Fusion (ADF) methodology. The primary objective is to create a composite image that surpasses the limitations of individual images, offering improved visual clarity and insightful data representation. The ADF approach empowers the fusion process with adaptability, enabling the dynamic adjustment of fusion strategies based on the characteristics of the input images and the specific demands of the application. By leveraging the distinctive qualities of visible and infrared images, our method aims to highlight features, textures, and structures that might be otherwise obscured or challenging to discern in either modality alone.The proposed framework entails a multi-step process: analyzing the input images to discern their unique attributes, preprocessing to enhance image quality, extracting pertinent features from both modalities, formulating adaptive fusion rules, and applying these rules to generate the fused image. The incorporation of ADF ensures that the fusion process is contextually aware, resulting in enhanced visual appeal and data extraction. Validation of the ADF-based fusion method involves rigorous testing on a diverse range of visible and infrared image pairs. Metrics such as signal-to-noise ratio (SNR), structural similarity index (SSIM), and visual quality assessment are employed to evaluate the effectiveness of the fusion process. The project embraces a cyclical improvement cycle, allowing for fine-tuning and adaptation of fusion rules to address variations encountered in different scenarios. The outcomes of this project hold significant potential across various domains, including remote sensing, medical imaging, surveillance, and more. The ADF-based image fusion approach demonstrates its ability to amplify the inherent strengths of each modality while minimizing their limitations, resulting in a compelling fusion that surpasses the sum of its parts.

INDEX

CHAPTER 1	
Introduction	01
1.1 Aim	02
1.2 Objectives	02
1.3 Design	02
1.4 Purpose	03
1.5 Scope	03
1.6 Application in detail	04
Chapter 2	
Literature Survey	06
Chapter 3	
3.1 Existing system	09
3.2 Proposed system	10
3.3 Functional requirements	10
3.4 Non-functional requirements	12
3.5 System requirements	14
Chapter 4	
SYSTEM DESIGN	
4.1 System analysis	19
4.2 Block diagram	19
4.3 DFD Level 1	21
4.4 Use-case diagram	22
Chapter 5	
IMPLEMENTATION	24
5.1 Image fusion process	26
5.2 Adaptive data fusion (ADF) approach	27
5.3 ADF pseudocode	29

Chapter 6	
TESTING	**36**
6.1 Test Cases	**37**
Chapter 7	
SCREENSHOTS	**39**
Chapter 8	
CONCLUSION	**44**
Chapter 9	
FUTURE ENHANCEMENT	**45**
REFERENCES	**46**

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A
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Submitted in partial fulfilment of the requirement for the award of
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Submitted by

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ABSTRACT

Losing personal belongings can be distressing, and this application provides an efficient and user-friendly solution to reunite lost items with their owners. It allows users to report lost items by providing relevant details such as item description, location, and time of loss. The system then categorizes and stores these reports in a centralized database for efficient management.

Metro station staff can access the web application through a secure interface to handle lost item reports. They can search the database, view reported items, and update their status as items are found or returned to their owners. The application also provides features for generating reports, tracking unresolved cases, and managing communication with the owners. The web application offers a user-friendly interface where they can search for their lost items by providing specific details or browsing through categorized items. The system displays relevant matches and allows users to initiate a claim process to retrieve their belongings. Real-time notifications and updates are sent to users regarding the progress of their claim. This system minimizes the frustration of losing personal belongings and increases the chances of successful item recovery, promoting customer satisfaction and operational efficiency within the metro station environment.

INDEX

CHAPTER 1

INTRODUCTION **01**

1.1 Motivation 02

1.2 Problem Statement 03

1.3 Objective 04

1.4 Scope 04

CHAPTER 2

LITRETURE SURVEY **08**

2.1 Existing system 08

2.2 Proposed system 09

CHAPTER 3

SYSTEM REQUIREMENTS AND SPECIFICATION **11**

3.1 Functional Requirements 16

3.2 Non-Functional Requirement 17

CHAPTER 4

SYSTEM DESIGN **20**

4.1 Introduction 20

4.2 Architecture Design 20

4.3 Data Flow Diagram 25

4.4 Use Case Diagrams 29

4.5 Sequence Diagram 31

CHAPTER 5

IMPLIMENTATION **34**

5.1 Code 36

CHAPTER 6

TESTING AND RESULTS **38**

6.1 Introduction 38

6.2 Purposes of Testing 38

6.3 Testing 40

CHAPTER 7
SCREENSHOT 45
CHAPTER 8
CONCLUSION AND FUTURE ENHANCEMENT 49
REFERENCES

CHAPTER 1

INTRODUCTION

To create a user-friendly and efficient system that helps metro station staff and customers to track and locate lost items within the metro premises. By utilizing the power of technology and a centralized database, this application aims to streamline the process of handling lost items, increasing the chances of successful item recovery and reuniting them with their rightful owners.

The web-based application will serve as a comprehensive platform for reporting, managing, and retrieving lost items. It will offer features for both metro station staff and commuters, providing them with the necessary tools to streamline the process and improve overall efficiency. Metro station staff will have access to a secure interface where they can handle lost item reports. They can search the database, view reported items, update their status as items are found or returned, and communicate with the owners. The application will assist staff in generating reports, tracking unresolved cases, and maintaining a systematic approach to managing lost items.

customers, on the other hand, will have a user-friendly interface that allows them to search for their lost items. They can provide specific details about the lost item or browse through categorized items to find potential matches. The system will display relevant matches and enable users to initiate a claim process to retrieve their belongings. Real-time notifications and updates will be sent to users regarding the progress of their claims, keeping them informed throughout the process. By implementing the "Find the Lost Items in Metro Station" web-based application, metro stations can provide a streamlined and efficient approach to handling lost items. This will not only enhance the overall experience for commuters but also improve operational efficiency for metro station staff. Ultimately, the application aims to alleviate the stress and inconvenience caused by lost items, increasing customer satisfaction and contributing to a smoother metro station environment.

1.1 MOTIVATION

The motivation behind developing this application is rooted in addressing a common and often distressing problem that many individuals face – losing personal belongings. Losing items can lead to frustration, anxiety, and inconvenience, and the process of recovering lost items is typically cumbersome and time-consuming. Therefore, the application aims to provide a practical and user-friendly solution to mitigate these challenges.

Enhancing Efficiency: The primary motivation is to streamline and enhance the efficiency of the lost and found process. Traditional methods of reporting and retrieving lost items often involve paper forms or phone calls, which can be slow and prone to errors. By providing an online platform, the application simplifies the process for both users and metro station staff.

Minimizing Distress: Losing personal belongings can be emotionally distressing. By offering a structured and efficient system, this application aims to reduce the anxiety and frustration that individuals experience when trying to recover their lost items.

Increasing Chances of Recovery: The application recognizes that timely action is crucial for recovering lost items. By centralizing and categorizing lost item reports, it ensures that station staff can quickly access and manage this information, increasing the chances of reuniting items with their owners.

Enhancing Customer Satisfaction: Metro stations serve a large and diverse clientele. Providing a service that simplifies the process of reporting and retrieving lost items contributes to overall customer satisfaction. Happy customers are more likely to have a positive view of the metro station and may be more inclined to use its services again.

Operational Efficiency: For metro station staff, managing lost items can be a logistical challenge. This application not only makes their jobs easier by digitizing the process but also provides tools for generating reports, tracking unresolved cases, and

communicating with owners. This streamlining of operations enhances the efficiency of the metro station's lost and found department.

Real-Time Updates: The application acknowledges the need for timely communication with users. Real-time notifications and updates ensure that individuals are kept informed about the progress of their claim, reducing uncertainty and improving their overall experience.

1.2 PROBLEM STATEMENT

The problem at hand is the challenge faced by metro stations in efficiently handling and reuniting lost items with their rightful owners. Losing personal belongings within a metro station can cause distress and inconvenience for commuters, leading to emotional and financial stress. The current process of managing lost items often lacks a streamlined approach, resulting in delays and difficulties in item retrieval.
Metro stations need a web-based application that can effectively track and find lost items within their premises, facilitating a prompt and organized system for item reporting, categorization, and retrieval. Without a centralized system for categorizing and searching lost items, metro station staff may struggle to efficiently match found items with reported losses. The lack of standardized categories or efficient search functionalities makes it challenging to identify potential matches and reunite items with their owners.

The current communication process between metro station staff and commuters regarding lost items is often inefficient. Lack of real-time updates, status notifications, and clear communication channels can result in delays and miscommunication, impeding the timely retrieval of lost belongings.

The solution to these challenges is the development of a web-based application that provides a user-friendly interface for commuters to report lost items accurately, streamlines the categorization and search process for metro station staff, enables efficient communication and tracking of item statuses, and incorporates advanced technologies to enhance item matching and retrieval.

By addressing these challenges, the web-based application will improve the overall experience for commuters, increase the chances of successful item recovery, and enhance the operational efficiency of metro stations in managing lost items.

1.2 OBJECTIVES

- ✓ Develop an application that streamlines the process of handling and managing lost items within a metro station, reducing the time and effort required to track and retrieve lost belongings.

- ✓ Design a user-friendly interface that allows commuters to easily report their lost items, search for potential matches, and claim their belongings. The system should provide clear instructions, intuitive navigation, and real-time notifications to keep users informed throughout the process.

- ✓ Utilize advanced technologies such as image recognition and natural language processing to enhance the accuracy and efficiency of matching reported lost items with found items within the metro station.

- ✓ Establish effective communication channels between metro station staff and commuters, enabling seamless communication regarding lost items, additional information requests, or updates on the status of reported items.

- ✓ The overall objective of the web-based application is to create a reliable and efficient system that improves the process of finding lost items in a metro station, increases the chances of successful item recovery, and enhances the overall experience for customers and metro station staff.

1.4 SCOPE

- ✓ Customers will be able to report their lost items through the web application, providing accurate and detailed descriptions of the items.

- ✓ The system will categorize reported lost items based on their descriptions and attributes, making it easier to match them with found items.

- ✓ The application will employ advanced technologies such as image recognition and natural language processing to match reported lost items with found items in the metro station. This will assist in the efficient retrieval and return of lost belongings.
- ✓ The system will provide effective communication channels between metro station staff and commuters to facilitate inquiries, updates, and notifications regarding lost items.
- ✓ The application will incorporate a robust database management system to store and manage information related to lost items, including item details, user reports, and retrieval status.

Advantages

Improved Efficiency: The web-based application streamlines the process of finding lost items in a metro station, reducing the time and effort required to report, search for, and claim lost items.

Enhanced User Experience: The user-friendly interface and intuitive design make it easy for both metro station staff and commuters to use the system, increasing user satisfaction.

Real-Time Notifications: Users receive real-time notifications about the status of their lost items, increasing the likelihood of item retrieval. Metro station staff are also notified promptly, allowing them to take immediate action.

Robust Database Management: The use of a robust database management system ensures data integrity and efficient search capabilities, making it easier to track and retrieve lost items.

Data Visualization and Reporting: The system provides data visualization and reporting capabilities, allowing metro station staff to analyze trends and identify areas for process improvement. Users can also provide feedback and ratings, enabling continuous improvement.

Security and Privacy: The system prioritizes the security and privacy of user data through robust authentication, data encryption, and access control measures, ensuring compliance with data protection regulations.

Disadvantages

Technical Challenges: Implementing and maintaining advanced technologies and features may pose technical challenges and require ongoing updates and support.

User Adoption: Some users, especially older individuals or those less familiar with technology, may find it challenging to use the web-based application, potentially limiting its effectiveness.

Data Privacy Concerns: Despite security measures, there may still be concerns about data privacy and the storage of sensitive information, which could deter some users from using the system.

Resource Requirements: The development and maintenance of the system, including database management and real-time notifications, require dedicated resources and investment.

Connectivity Issues: The system's effectiveness depends on a stable internet connection, which may not always be available in metro stations or for all users.

False Reports: There is a possibility of false reports being filed, which can consume staff time and resources in verifying and handling such reports.

Compliance Challenges: Ensuring compliance with data protection regulations can be complex and may require ongoing monitoring and adjustments to policies and procedures.

CHAPTER 2

LITRETURE SURVEY

2.1 EXISTING SYSTEM

The existing systems for collecting, processing, and communicating information often face significant challenges when it comes to efficiency and effectiveness. These systems frequently fall short in ensuring that the right information reaches the right person at the right time. This deficiency is especially apparent in processes like reporting lost items in metro stations, where timely and accurate information is crucial for successful item recovery.

1. Inefficiencies in Information Flow: Current working systems can be slow and manual, relying on paperwork or outdated processes. This can result in delays and inefficiencies, especially when time is of the essence. Inconsistent communication methods can lead to information getting lost or not reaching the relevant personnel promptly.

2. **Lack of User-Friendly Interfaces:** Commuters who have lost items may encounter difficulties when trying to report their losses. They may not be aware of the appropriate channels or may find the process complicated and time-consuming.
Inaccurate item descriptions or incomplete information can hinder the process of identifying and locating lost belongings.

3. Incomplete Information Hinders Recovery: When commuters cannot provide detailed and accurate descriptions of their lost items, it becomes challenging for metro station staff to match found items with the correct owners. Inadequate information captured during the reporting process can lead to items remaining unclaimed or misplaced, reducing the chances of successful recovery.

4. Timeliness Matters: Recovering a lost item often depends on how quickly it is reported and how fast relevant information is processed. Delays in reporting or processing can significantly reduce the likelihood of reuniting individuals with their belongings.

5. The Need for Modernization: In an era of digital transformation and advanced technology, relying on outdated systems can result in missed opportunities to improve efficiency and enhance user experience. The development of a more efficient and user-friendly system for reporting lost items in metro stations addresses these challenges.

By digitizing and centralizing the process, the new application aims to:

Provide commuters with an intuitive and user-friendly platform for reporting lost items, reducing the barriers to entry. Ensure that complete and accurate information is captured during the reporting process, improving the chances of successful item recovery.

Streamline the flow of information to metro station staff, allowing them to access and manage lost item reports more efficiently. Facilitate real-time communication and updates, ensuring that individuals are kept informed about the progress of their claims.

The existing challenges in collecting, processing, and communicating information efficiently, particularly in the context of reporting lost items in metro stations, highlight the need for a modernized and user-centric approach to address these issues and enhance the overall experience for commuters and metro station staff alike.

2.2 PROPOSED SYSTEM

The proposed system is a web-based application designed to streamline the process of finding lost items in a metro station. It leverages advanced technologies and user-centric features to enhance the efficiency and effectiveness of lost item management.

The application will have a user-friendly interface accessible to both metro station staff and commuters. It will provide clear instructions and intuitive design elements to guide

users through the process of reporting lost items, searching for lost items, and claiming retrieved items.

Customers will be able to report their lost items through the application by providing detailed descriptions, including item type, appearance, and any distinguishing features. The system will guide users through the reporting process to ensure accurate and comprehensive information is captured.

system will provide real-time notifications and updates to both metro station staff and commuters regarding the status of reported lost items. Users will receive notifications when a potential match is found or when their lost item is successfully retrieved. Metro station staff will be notified when new reports are filed or when items are matched and ready for collection.

The application will utilize a robust database management system to store and manage the data related to lost items. It will ensure data integrity, enable efficient search capabilities, and facilitate data analytics for better item tracking and retrieval. Establish effective communication channels between metro station staff and commuters. This includes features such as in-app messaging or chat functionality, allowing users to communicate with staff regarding their lost items, provide additional details, or inquire about the progress of their claims. The application will provide data visualization and reporting capabilities for metro station staff and also users can provide feedback and give ratings developed using NLP algorithm. This will enable them to analyze trends, generate reports on lost item statistics, and identify areas for process improvement or resource allocation. The proposed system will prioritize the security and privacy of user data. It will implement robust authentication mechanisms, data encryption, and access control to safeguard sensitive information. Compliance with data protection regulations will be ensured.

CHAPTER 3

SYSTEM REQUIREMENTS AND SPECIFICATION

HARDWARE REQUIREMENTS

Processor (CPU): The choice of an i3 or higher processor is a good starting point, but as mentioned, performance needs can vary depending on the application's complexity and usage patterns. For future-proofing your application, consider factors like multi-core processors, which can better handle concurrent requests and complex computations. Evaluate your application's specific requirements as it evolves and be prepared to upgrade the CPU if necessary.

RAM (Memory): 4GB of RAM is suitable for small to medium-sized applications, but again, this depends on usage. Monitor memory usage regularly, especially during peak times, and be ready to upgrade to 8GB or more if your application experiences increased traffic or adds more features. Implement memory optimization techniques in your code and consider using a content delivery network (CDN) to offload some assets to reduce memory usage.

Hard Disk (Storage): 80GB of hard disk space is sufficient for storing your application's code, databases, and related files. Implement a data archiving and cleanup strategy to manage storage efficiently, especially if your application accumulates large amounts of user data over time.

Processor Speed: A processor speed of 1.2 GHz+ should meet the requirements for most web applications. Ensure that your web hosting provider or server infrastructure meets or exceeds this specification.

Front-end Technologies:

HTML5 (Hypertext Markup Language 5):HTML5 is the latest version of the standard markup language used to structure content on the web. It provides a set of elements and attributes for structuring text, multimedia, forms, and other content on web pages. Key features of HTML5 include support for audio and video playback, canvas for drawing graphics, geolocation, local storage for client-side data storage, and improved semantics.HTML5 is the foundation of web content and is responsible for structuring the basic layout and elements of a web page.

CSS (Cascading Style Sheets): CSS is a style sheet language used for describing the presentation and layout of HTML documents. It allows web developers to control the appearance of web pages, including fonts, colors, spacing, positioning, and responsive design. CSS works by defining rules that specify how HTML elements should be displayed in browsers.CSS3, the latest version of CSS, introduced advanced features like animations, transitions, and flexible box layouts (Flexbox) and grid layouts (CSS Grid), enhancing the design and layout capabilities of web pages.

JavaScript: JavaScript is a high-level, versatile, and dynamic scripting language that is commonly used for adding interactivity and behavior to web pages. It enables developers to create responsive and interactive features such as form validation, image sliders, and dynamic content updates without requiring a page reload. JavaScript can be executed on both the client-side (in the browser) and server-side (using technologies like Node.js), making it a fundamental language for full-stack web development. Libraries and frameworks like jQuery, Angular, and Vue.js build on JavaScript to simplify web development tasks and provide additional functionality.

React.js: React.js is a JavaScript library for building user interfaces, developed and maintained by Facebook. It follows a component-based architecture, allowing developers to create reusable UI components that can be composed to build complex interfaces.React.js efficiently updates the user interface by using a virtual DOM (Document Object Model), which minimizes the need to directly manipulate the browser's DOM, resulting in improved performance.

React.js is often used in conjunction with other libraries and tools, such as React Router for routing and Redux for state management, to create robust and scalable web applications.
Its popularity has led to the development of a rich ecosystem of third-party libraries and extensions, making it a powerful choice for building modern web applications. HTML5, CSS, JavaScript, and React.js are core technologies for web development. HTML5 provides the structure for web content, CSS handles the presentation and styling, JavaScript adds interactivity and behavior, and React.js simplifies the creation of dynamic and reusable user interfaces. Together, these technologies enable the development of responsive and feature-rich web applications that can run on a variety of devices and browsers.

Backend Technologies:

Java: Java is a versatile and widely-used programming language known for its portability, platform independence, and strong support for object-oriented programming. In web development, Java is commonly used for building the server-side logic of applications. It provides a robust and scalable foundation for processing requests, managing databases, and implementing business logic. Java's large ecosystem includes libraries, frameworks, and tools that simplify web development tasks, making it a popular choice for backend development.

Servlets: Servlets are Java-based server-side components that handle HTTP requests and responses. They provide a way to dynamically generate content and respond to user actions on the web, such as form submissions. Servlets are typically used in conjunction with a web container, such as Apache Tomcat or Jetty, which manages the servlet lifecycle and handles incoming HTTP requests.

Spring Boot: Spring Boot is a framework that simplifies the development of Java applications by providing a set of pre-configured templates and conventions. It reduces the boilerplate code typically required for setting up a Java application, making it easier to build production-ready applications quickly. Spring Boot is built on top of the Spring Framework, providing additional features such as embedded web servers, automatic

configuration, and enhanced tooling. It is well-suited for building RESTful APIs, microservices, and web applications.

Hibernate: Hibernate is an object-relational mapping (ORM) framework for Java that simplifies database interaction. It allows developers to work with Java objects instead of SQL queries when interacting with relational databases, making database access more intuitive and maintainable. Hibernate handles tasks such as data mapping, CRUD (Create, Read, Update, Delete) operations, and caching, improving database performance and reducing the risk of SQL injection vulnerabilities. When used together, these technologies form a powerful stack for building the backend of web applications:

Java provides a robust and platform-independent programming language. Servlets handle HTTP requests and serve as the foundation for building web applications. Spring Boot simplifies Java application development by providing conventions and pre-configured settings. Hibernate streamlines database interaction and makes it more object-oriented. Developers often use these technologies to create RESTful APIs, web services, and web applications that serve as the backend for modern web and mobile applications. This stack enables developers to build scalable, maintainable, and secure server-side components for their web applications.

DATABASE

(MySQL): MySQL is a reliable and well-supported choice for a relational database system. To optimize database performance, consider indexing frequently queried columns, use caching mechanisms, and periodically review and optimize your database schema. Implement automated backups and disaster recovery strategies to ensure data reliability. the hardware and technology stack you've chosen are suitable for building your web-based lost item management system, especially for smaller to medium-sized deployments. However, it's crucial to continuously monitor and adapt your resources as your application grows and experiences changes in user demand. Regular maintenance, performance tuning, and scalability planning will be essential to keep your system running smoothly.
MySQL is a popular open-source relational database management system (RDBMS) known for its reliability, performance, and ease of use. It plays a crucial role in storing,

managing, and retrieving data for a wide range of applications, including web and mobile applications, content management systems, e-commerce platforms, and more. Here's an elaboration on MySQL:

Relational Database Management System (RDBMS): MySQL is categorized as an RDBMS because it follows the principles of the relational database model. It organizes data into tables, where each table consists of rows and columns. Relationships between tables are established through keys, ensuring data integrity and consistency.

Open-Source and Community-Driven: MySQL is an open-source project, which means it is freely available for use, modification, and distribution. It benefits from a large and active user community, which contributes to its development, documentation, and support.

Scalability: MySQL is designed to handle various workloads, from small-scale applications to enterprise-level systems. It supports horizontal scalability through techniques such as replication and sharding, allowing it to handle high volumes of data and traffic.

Performance and Optimization: MySQL is known for its fast performance and efficient query processing. It offers various storage engines (e.g., InnoDB, MyISAM) with different characteristics to cater to specific performance and storage requirements. Developers can optimize MySQL databases through indexing, query optimization, and caching strategies to ensure fast and efficient data retrieval.

Data Security: MySQL provides robust security features to protect data, including user authentication, role-based access control, and data encryption.
Security updates and patches are regularly released to address potential vulnerabilities.

Cross-Platform Compatibility: MySQL is available for multiple platforms, including Windows, Linux, macOS, and more, making it accessible across various operating systems.

Support for Standard SQL: MySQL adheres to SQL (Structured Query Language) standards, which ensures compatibility with SQL-based database development tools and applications. Developers can write SQL queries and statements that work consistently across different database systems.

Community and Enterprise Editions: MySQL offers both a community edition (free and open-source) and an enterprise edition (with additional features and support) to cater to different use cases and budgets.

High Availability and Replication: MySQL supports features like replication and clustering to ensure high availability and data redundancy. Replication allows for data synchronization across multiple database instances, providing fault tolerance and load balancing.

Backup and Restore: MySQL offers various backup and restore mechanisms, including logical and physical backups, to safeguard data and facilitate disaster recovery.

Third-Party Integrations: MySQL can be integrated with various programming languages and frameworks, making it versatile and suitable for diverse application stacks.
In summary, MySQL is a robust and versatile relational database management system widely used in web development and other software applications. Its open-source nature, strong community support, and rich feature set make it a reliable choice for managing data, whether for small-scale projects or large-scale enterprises. When used effectively, MySQL can provide high-performance, security, and scalability for your database needs.

3.1 FUNCTIONAL REQUIREMENTS:

User Registration and Authentication: Users should be able to create accounts and log in securely to report lost items and track their claims.

Lost Item Reporting: Users must be able to report lost items by providing the following details:

Item description (type, color, size, unique identifiers, etc.).
Location of loss (metro station or specific area within the station).
Time and date of loss.

Database Management: The system should categorize and store lost item reports in a centralized database, ensuring efficient management and retrieval of information.

Staff Access: Metro station staff should have access to a secure interface where they can:

Search the database for lost item reports using various criteria.
View detailed information about reported items.
Update the status of items (found, returned, unresolved).
Communicate with users or owners regarding lost items.

Reporting Features: The application should allow staff to generate reports, including statistics on the number of lost items, unresolved cases, and items successfully returned.

User-Friendly Interface: The web application should provide an intuitive and user-friendly interface for both users and metro station staff.

Lost Item Retrieval: Users should be able to search for their lost items by providing specific details or browsing through categorized items in the database.

Claim Process: The system should support a claim process that allows users to initiate the retrieval of their lost belongings by providing additional verification or ownership details.

Real-Time Notifications: The application should send real-time notifications and updates to users regarding the progress of their lost item claims, including when items are found or returned.

3.2 NON-FUNCTIONAL REQUIREMENTS:

Security: The system should ensure data security and user privacy. User authentication and authorization mechanisms must be robust to prevent unauthorized access.

Performance: The application should be responsive and capable of handling concurrent user requests without significant delays. Response times for search queries and updates should be optimized.

Availability: The system must be available 24/7 to accommodate users reporting lost items at any time.

Scalability: It should be designed to handle a growing database of lost item reports and users as the system becomes more popular.

Reliability: The application should minimize system downtime and data loss. Regular backups and system monitoring should be in place.

Usability: The user interface should be intuitive and accessible to users of varying technical abilities. Usability testing may be necessary to ensure a positive user experience.

Data Encryption: Data transmission should be encrypted to protect sensitive information, such as user login credentials and personal details.

Compliance: The system should adhere to relevant data protection and privacy regulations, such as GDPR or HIPAA, depending on the jurisdiction and nature of data collected.

Load Balancing: Load balancing mechanisms should be implemented to distribute incoming requests evenly across server resources, ensuring optimal performance.

Audit Trail: A comprehensive audit trail should be maintained, recording all user interactions and system activities for accountability and traceability purposes.

User Training: Training and support should be provided to metro station staff to ensure they can efficiently use the system and its features.

These functional and non-functional requirements are crucial for the development and successful operation of the lost and found application in a metro station environment, ensuring both user satisfaction and operational efficiency.

CHAPTER 4

SYSTEM DESIGN

4.1 INTRODUCTION

System design is the process of defining the elements of a system such as the architecture, modules and components, the different interfaces of those components and the data that goes through that system. It is meant to satisfy specific needs and requirements of a business or organization through the engineering of a coherent and well-running system. Design patterns are the most powerful tool for software developer. It is important to understand design patterns rather than memorizing its classes, methods and properties. It is also important to learn how to apply pattern to specific problem to get the desired result. This will be required for continuous practice of using and applying design patterns in day to day software development.

The design activity often results in three separate outputs –

- Architecture design.
- High level design.
- Detailed design.

4.2 ARCHITECTURAL DESIGN

Architectural design is a set of principles- a coarse grained pattern that provides an abstract framework for a family of systems. An architectural style improves partitioning and promotes design reuse by providing solutions to frequently recurring problems. You can think of architecture styles and patterns as sets of principles that shape an application.

NLP Algorithm

Fig.4.2.1 NLP Algorithm

Natural Language Processing (NLP) algorithms are a set of techniques and methods used to enable computers to understand, interpret, and generate human language in a valuable way. NLP algorithms play a crucial role in various applications, including text analysis, sentiment analysis, machine translation, chatbots, and more. Here are some common NLP algorithms and techniques:

Tokenization: Tokenization is the process of breaking a text into individual words or tokens. This is usually the first step in many NLP tasks. Algorithmically, tokenization can be achieved using simple white space splitting or more complex methods that consider punctuation, special characters, and language-specific rules.

Stemming and Lemmatization: Stemming and lemmatization are techniques used to reduce words to their base or root form. Stemming algorithms aim to cut off prefixes and suffixes to obtain the root form (e.g., "jumping" to "jump"). Lemmatization algorithms consider the context and part of speech to reduce words to their lemma or dictionary form (e.g., "better" to "good").

Sentiment Analysis: Sentiment analysis, also known as opinion mining, determines the sentiment or emotion expressed in a piece of text. Supervised machine learning algorithms, such as Support Vector Machines (SVM) and Naive Bayes, are often used for sentiment classification.

Topic Modeling: Topic modeling algorithms, like Latent Dirichlet Allocation (LDA) and Non-Negative Matrix Factorization (NMF), discover topics within a collection of

documents. These algorithms group words together into topics based on co-occurrence patterns.

Word Embeddings: Word embeddings, such as Word2Vec and Glove, represent words as dense vector representations in a continuous space. These embeddings capture semantic relationships between words and are often used as input features for various NLP tasks.

Machine Translation: Algorithms for machine translation, like statistical machine translation and neural machine translation, automatically translate text from one language to another. Neural network-based approaches, including sequence-to-sequence models, have significantly improved translation accuracy.

Dependency Parsing: Dependency parsing algorithms analyze the grammatical structure of sentences and determine the syntactic relationships between words. Algorithms like the Dependency Parsing to Head-Word (DpHw) algorithm use parsing techniques to build parse trees.

These are just a few examples of NLP algorithms and techniques used in a wide range of applications. NLP continues to advance with the development of more sophisticated algorithms and the integration of machine learning and deep learning approaches.

Architecture diagram

Fig.4.2.2 Architecture diagram

THREE-TIER ARCHITECTURE

Three-tier architecture is a client-server architecture in which the functional process logic, data access, computer data storage and user interface are developed and maintained as independent modules on separate platforms. Three-tier architecture is a software design pattern and well-established software architecture. Three-tier architecture allows any one of the three tiers to be upgraded or replaced independently. The user interface is implemented on a desktop PC and uses a standard graphical user interface with different modules running on the application server.

Fig.4.2.3 Three-Tier Architecture

The three tiers in three-tier architecture are:

- **Presentation Tier**:
  Occupies the top level and displays information related to services available on a website. This tier communicates with other tiers by sending results to the browser and other tiers in the network. Here we design the form using textbox, label etc.

- **Application Tier/Business tier**:
  Also called the middle tier, logic tier, business logic or logic tier, this tier is pulled from the presentation tier. It controls application functionality by performing detailed processing. It is the intermediate layer which has the functions for client layer and it is used to make communication faster between client and data layer. It provides the business processes logic and the data access.

- **Data Tier**:
  Houses database servers where information is stored and retrieved. Data in this tier is kept independent of application servers or business logic.

Advantages:

- Easy to modify without affecting other modules
- Fast communication
- Performance will be good in three tier architecture.

HIGH LEVEL DESIGN

In high level design identifies the modules that should be built for developing the system and the specifications of these modules. At the end of system design all major data structures, file format, output formats, etc., are also fixed. The focus is on identifying the modules. In other words, the attention is on what modules are needed.

4.3 DATA FLOW DIAGRAM

A Data Flow Diagram (DFD) is a graphical representation of the "flow" of data through an information system, modeling its process aspects. A DFD is often used as a preliminary step to create an overview of the system, which can later be elaborated. DFDs can also be used for the visualization of data processing (structured design). A DFD shows what kind of information will be input to and output from the system, where the data will come from and go to, and where the data will be stored. It does not show information about the timing of processes, or information about whether processes will operate in sequence or in parallel. Data Flow diagrams in general are usually designed using simple symbols such as a rectangle, an oval or a circle depicting a processes, data stored or an external entity, and arrows are generally used to depict the data flow from one step to another.

External Entity: An external entity is a source or destination of a data flow which is outside the area of study. Only those entities which originate or receive data are represented on a business process diagram. The symbol used is an oval containing a meaningful and unique identifier.

Process: A process shows a transformation or manipulation of data flows within the system. The symbol used is a rectangular box.

Data Flow: A data flow shows the flow of information from its source to its destination. A data flow is represented by a line, with arrowheads showing the direction of flow. Each data flow may be referenced by the processes or data stores at its head and tail, or by a description of its contents.

Data Store: A data store is a holding place for information within the system. It is represented by an open ended narrow rectangle. Data stores may be long-term files such as sales ledgers, or may be short-term accumulations: for example batches of documents that are waiting to be processed. Each data store should be given a reference followed by an arbitrary number.

Level 0 - Context Data Flow Diagram

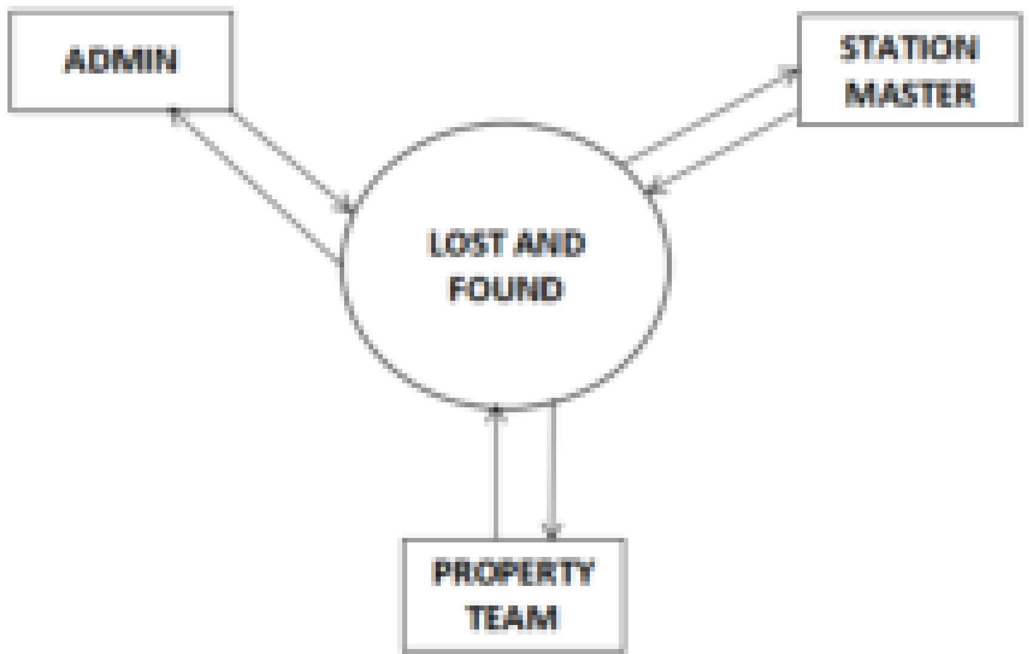

Fig:4.3.1 Context data flow diagram – Level 0

A context diagram is a top level (also known as Level 0) data flow diagram. It only contains one process node (process) that generalizes the function of the entire system in relationship to external entities.

Level 1 - Data Flow Diagram

Draw data flow diagrams in several nested layers. A single process node on a high level diagram can be expanded to show a more detailed data flow diagram. Draw the context diagram first, followed by various layers of data flow diagrams. Data flow diagrams present the logical flow of information through a system in graphical or pictorial form. Data flow diagrams have only four symbols, which makes it useful for communication between analysts and users. Data flow diagrams (DFDs) show the data used and provided by processes within a system.

Data Flow Diagram-Administrator:

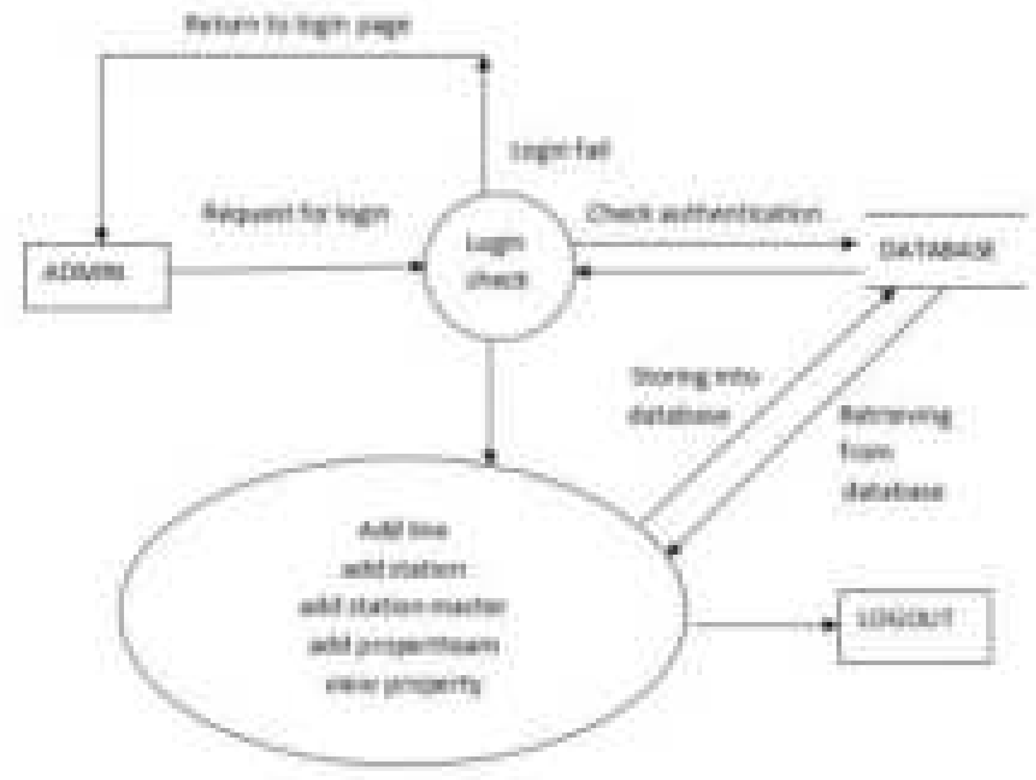

Fig:4.3.2 Data flow diagram of admin

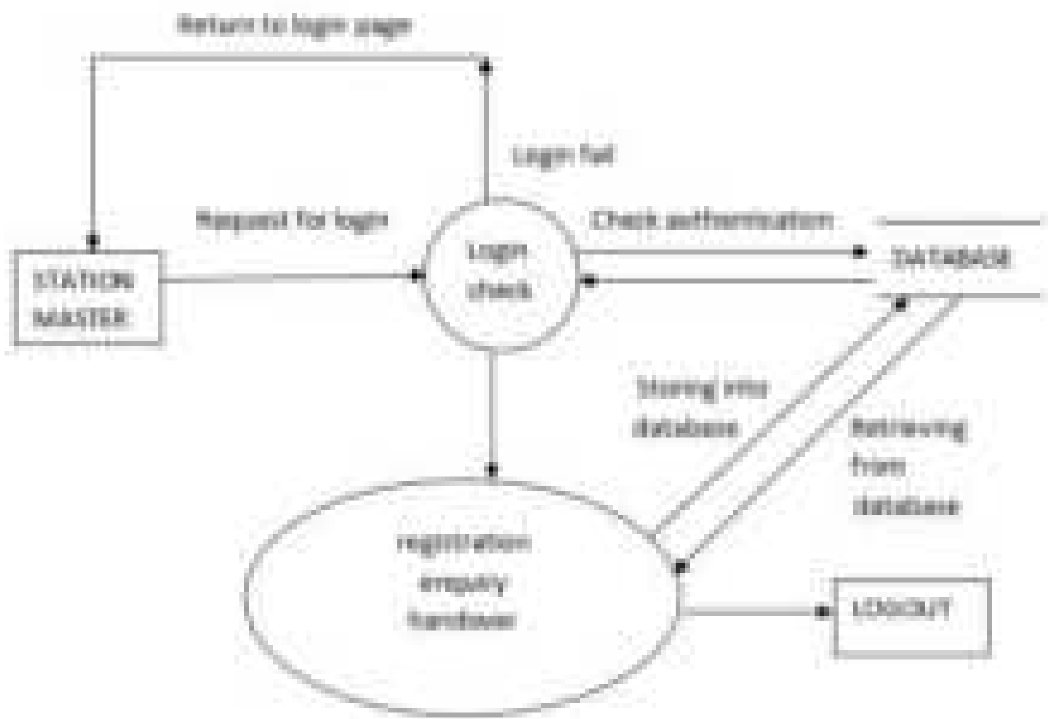

Fig:4.3.3 Data flow diagram of station master

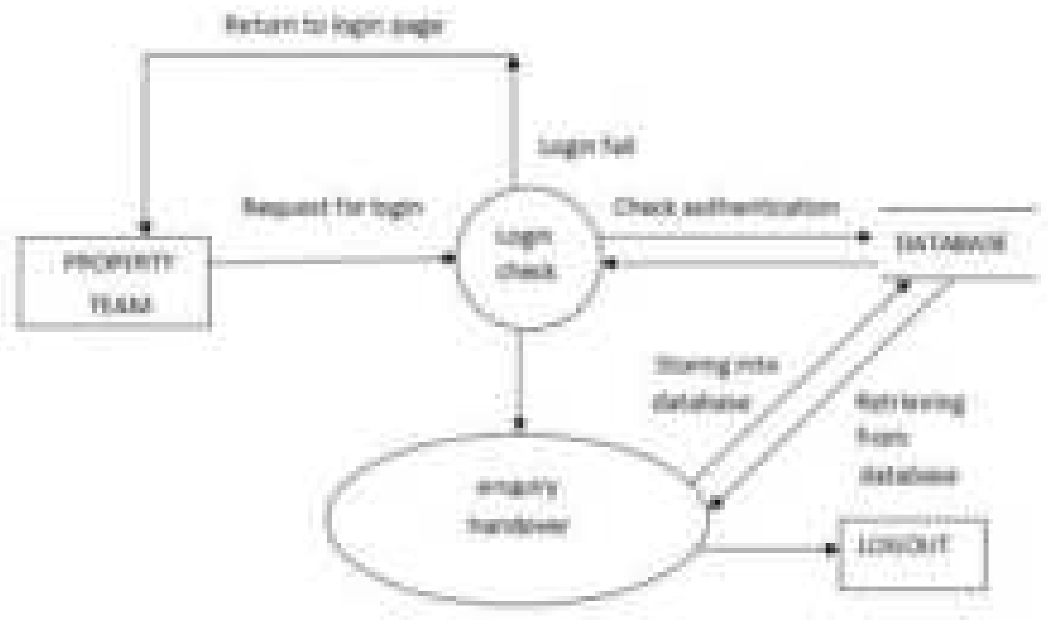

Fig:4.3.4 Data flow diagram of property Team

4.4 USE CASE DIAGRAMS

Use case diagrams model the functionality of a system using actors and use cases. Use cases are services or functions provided by the system to its users. Use case diagrams are usually referred to as behavior diagrams used to describe a set of actions (use cases) that some system or systems (subject) should or can perform in collaboration with one or more external users of the system (actors). Each use case should provide some observable and valuable result to the actors or other stakeholders of the system.

Use cases:

A use case describes a sequence of actions that provide something of measurable value to an actor and is drawn as a horizontal ellipse.

Actors:

An actor is a person, organization, or external system that plays a role in one or more interactions with your system. Actors are drawn as stick figures.

Associations:

Associations between actors and use cases are indicated in use case diagrams by solid lines. An association exists whenever an actor is involved with an interaction described by a use case.

System boundary boxes:

You can draw a rectangle around the use cases, called the system boundary box, to indicate the scope of your system. Anything within the box represents functionality that is in scope.

Use case Diagram - Administrator:

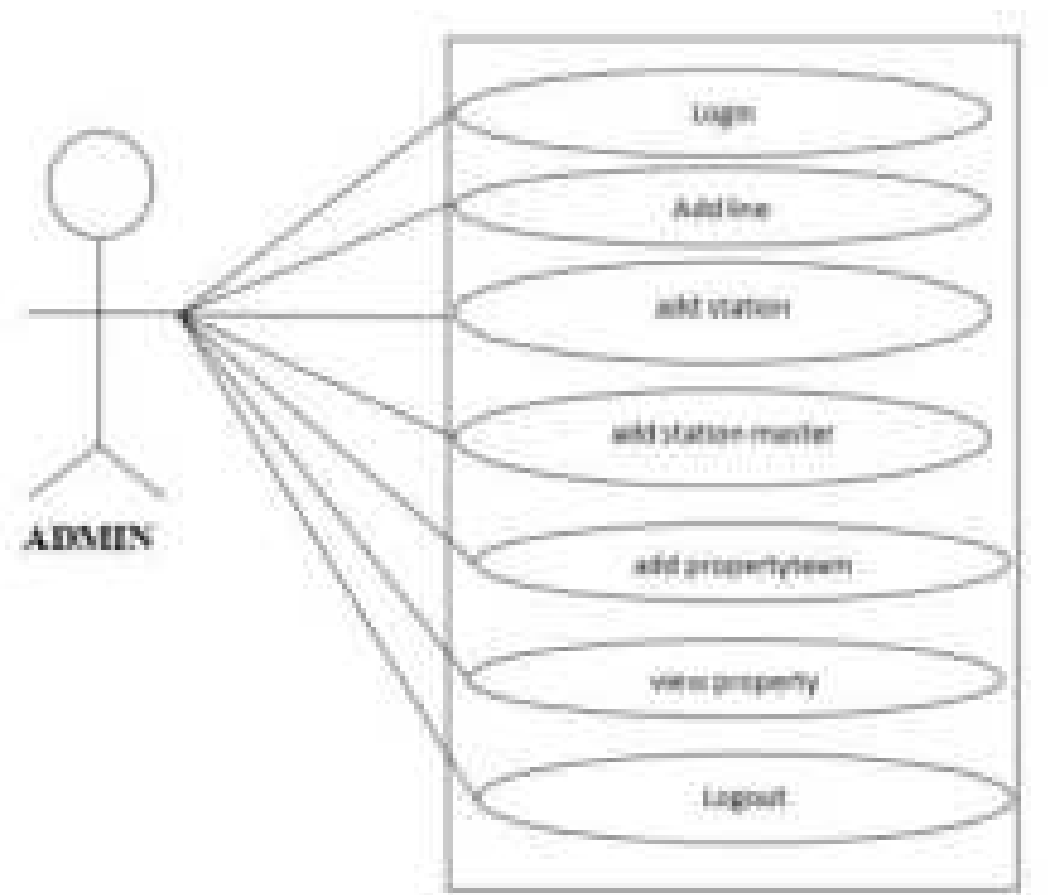

Fig: 4.4.1 Use case diagram of the Administrator

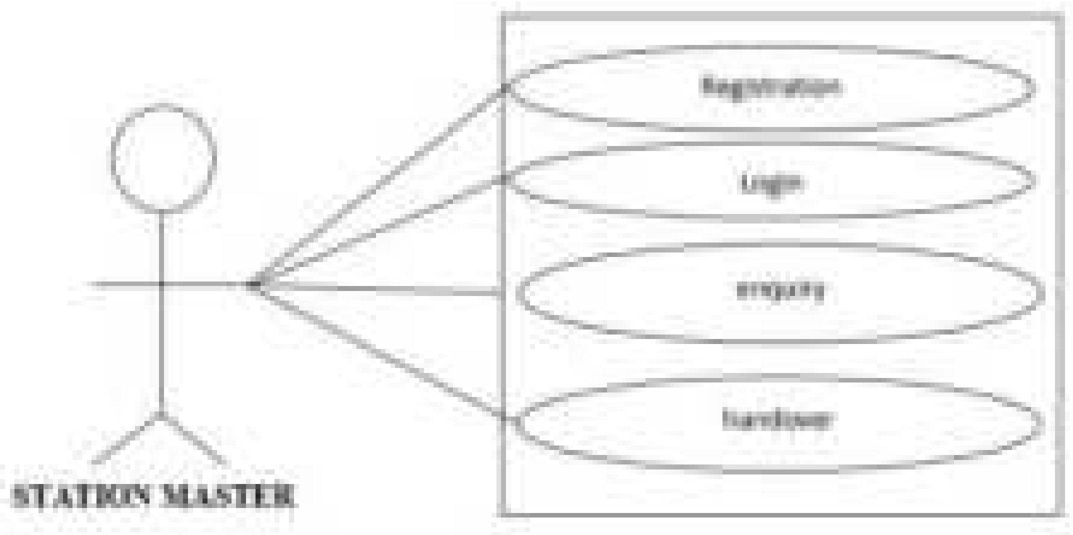

Fig:4.4.2 Use case diagram of the Station master

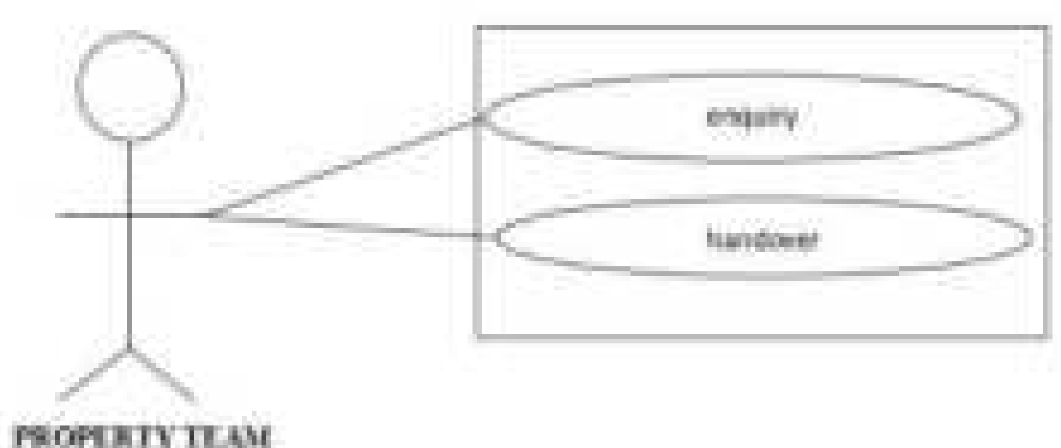

Fig:4.4.3 Use case diagram of the Property Team

4.5 SEQUENCE DIAGRAM:

A Sequence diagram is an interaction diagram that shows how processes operate with one another and in what order. It describes interactions among classes in terms of an exchange of messages over time. Sequence diagrams are used to show how objects interact in a given situation. An important characteristic of a sequence diagram is that time passes from top to bottom: the interaction starts near the top of the diagram and ends at the bottom.

Targets/Class roles/State:

Objects as well as classes can be targets on a sequence diagram, which means that messages can be sent to them. A target is displayed as a rectangle with some text in it. Below the target, its lifeline extends for as long as the target exists. Targets can be actor, boundary, control, entity and database.

Lifelines:

Lifelines are vertical dashed lines that indicate the object's presence over time.

Sequence Diagram-Admin:

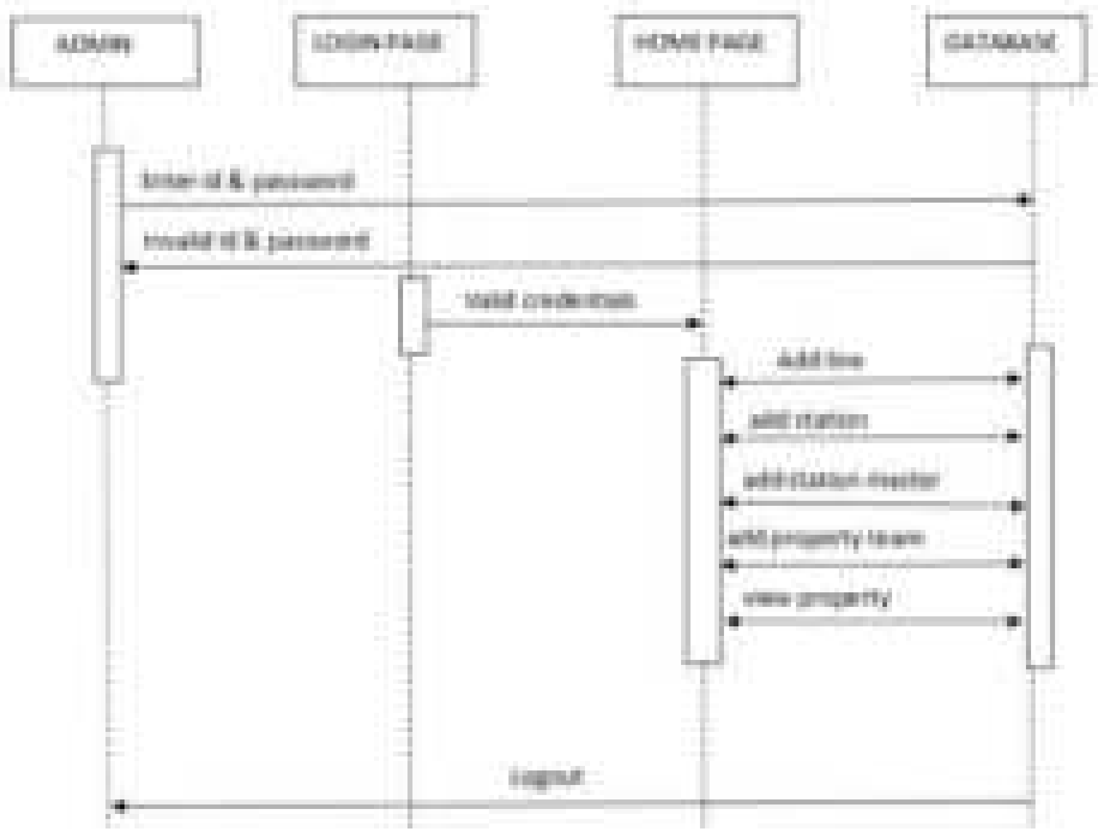

Fig:4.5.1 Sequence diagram for Admin

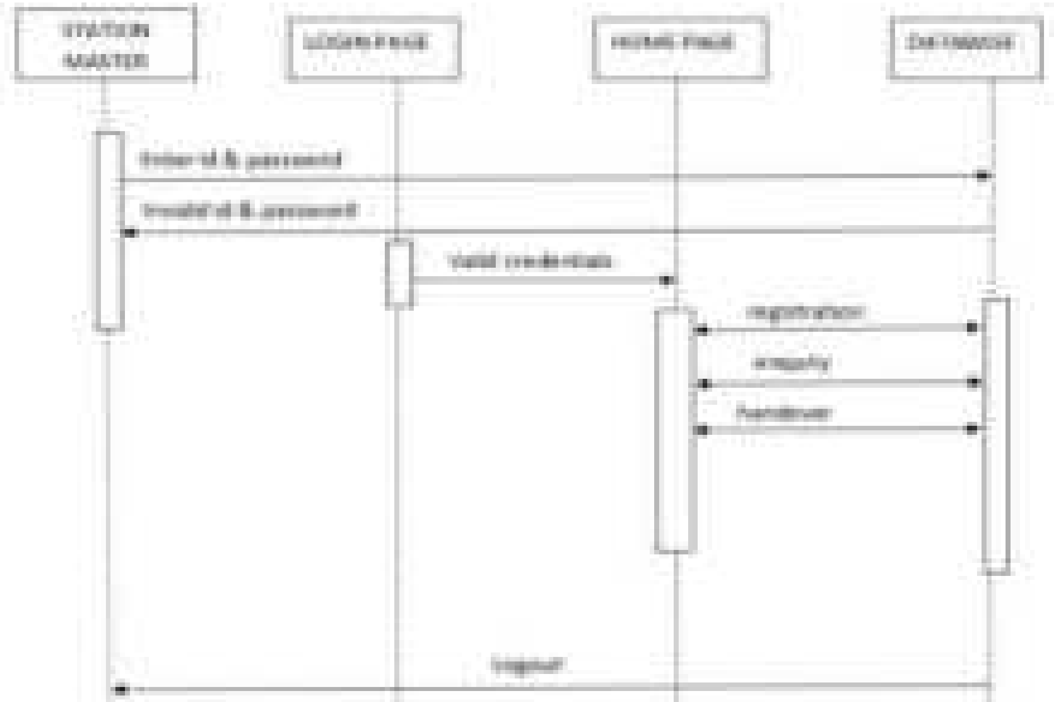

Fig:4.5.2 Sequence diagram for station master

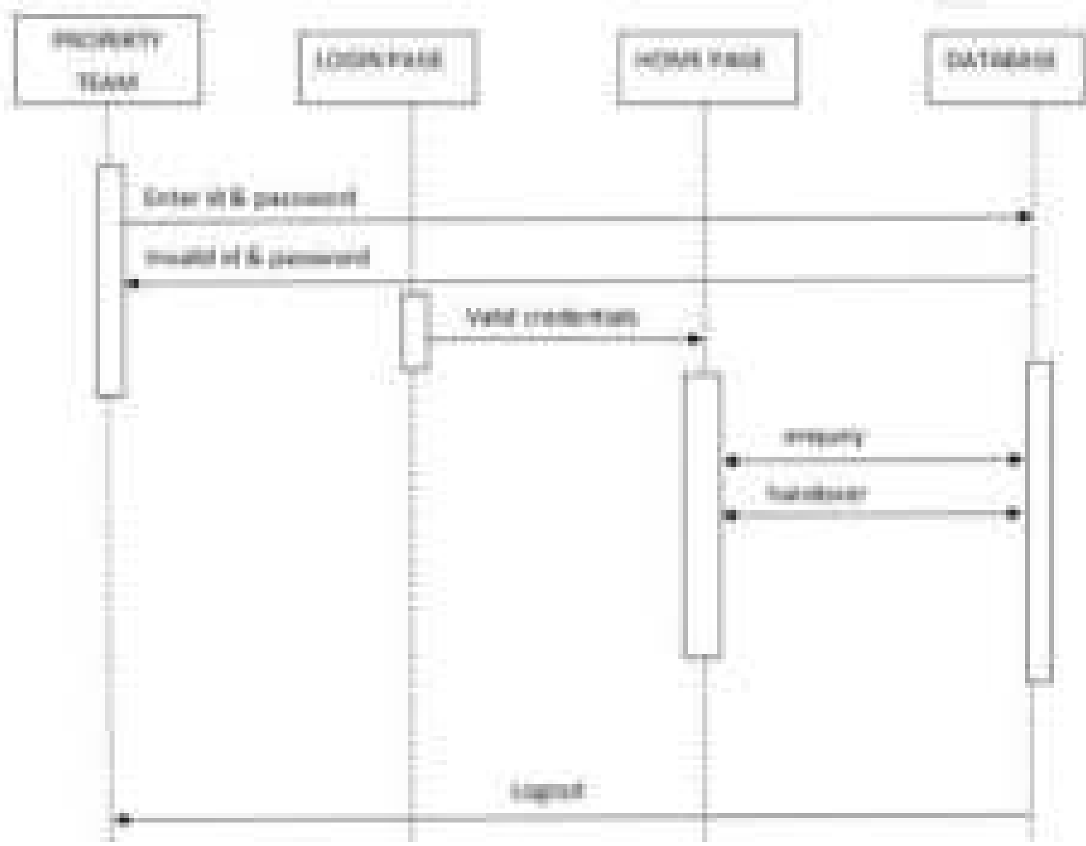

Fig:4.5.3 Sequence diagram for property team

CHAPTER 5

IMPLIMENTATION

1. Hardware Setup: Ensure that the hardware environment meets the specified requirements, including processor, RAM, hard disk, and processing speed. This setup is essential for the application to perform optimally and handle concurrent user requests efficiently.

2. Development Environment Setup: Install the necessary development tools and software components, including the Java Development Kit (JDK) for server-side development, Spring Boot for building the backend, Java Servlets for handling HTTP requests, Hibernate for database interaction, and the front-end technologies (HTML5, CSS, JavaScript, ReactJS) for creating the user interface.

3. Database Design: Plan and design the database schema meticulously to accommodate all required data structures, such as lost item reports, user profiles, staff information, communication logs, and more. Ensure that relationships between tables are well-defined to enable efficient querying and data retrieval.

4. User Interface Design: Collaborate with user experience (UX) designers to create an intuitive and visually appealing user interface. Prioritize user-friendly navigation, clear forms for reporting lost items, efficient search functionality, and an accessible design to accommodate users with diverse needs.

5. Authentication and Security: Implement robust authentication mechanisms to secure user accounts and ensure that user data remains confidential. Utilize encryption protocols to protect data transmission, and enforce access control to prevent unauthorized access to sensitive features or data.

6. Lost Item Reporting: Develop a guided workflow for users to report lost items effectively. Prompt users to provide detailed descriptions, including item type, appearance, and any unique identifiers. Implement validation checks to ensure data accuracy.

7. Database Management: Create the necessary database operations for storing, retrieving, and managing lost item reports efficiently. Categorize and organize data systematically to enable quick and accurate search and retrieval.

8. Real-Time Notifications: Design a notification system that sends real-time updates to users and metro station staff. Notifications should be triggered by events such as finding a lost item or updates on the status of a reported item.

9. Communication Channels: Implement in-app messaging or chat functionality to facilitate seamless communication between users and staff. This feature allows users to provide additional details, inquire about the progress of their claims, or seek assistance.

10. Data Visualization and Reporting: Develop data visualization tools and reporting features for metro station staff. Utilize data analytics to generate meaningful insights, track lost item statistics, and identify areas for improvement. Users can provide feedback and ratings using an NLP algorithm to improve service quality.

11. Privacy and Compliance: Prioritize data privacy and compliance with relevant data protection regulations. Implement strong encryption for data at rest and in transit. Ensure that user consent and privacy settings are respected throughout the application.

12. Testing: Execute thorough testing, including unit testing to verify individual components, integration testing to ensure seamless interaction between modules, and user acceptance testing (UAT) to validate that the application meets user expectations. Identify and address any issues or bugs promptly.

13. Deployment: Deploy the application on the designated hardware infrastructure, following best practices for deployment. Ensure that the application is accessible to both metro station staff and commuters with minimal downtime.

14. Training: Provide comprehensive training to metro station staff on how to use the application effectively. Ensure that they are proficient in utilizing all features and functionalities to provide excellent service to commuters.

15. Maintenance and Support: Establish a post-launch maintenance and support plan to address any issues, apply updates, and incorporate enhancements as needed. Regularly monitor application performance and user feedback for continuous improvement.

Throughout the implementation process, collaboration between development teams, designers, and stakeholders is essential to ensure that the application aligns with user needs and expectations. Regular testing and quality assurance efforts are crucial to delivering a reliable and user-friendly lost and found application within the metro station environment.

5.1 Code

```
# Initialize data structures to store lost and found items at the metro station
lost_items = []
found_items = []

# Function to report a lost item
function report_lost_item(item, location, time):
    lost_items.append({"item": item, "location": location, "time": time})

# Function to report a found item
function report_found_item(item, location, time):
    found_items.append({"item": item, "location": location, "time": time})

# Function to search for a lost item
function search_lost_item(item_description):
    matching_items = []
    for item in lost_items:
        if item["item"]["description"] == item_description:
            matching_items.append(item)
    return matching_items

# Function to claim a found item
function claim_found_item(item):
    for found_item in found_items:
        if found_item["item"] == item:
            found_items.remove(found_item)
            return "Item claimed successfully"
```

```
    return "Item not found in the database"

# Main program loop
while True:
    print("1. Report a lost item")
    print("2. Report a found item")
    print("3. Search for a lost item")
    print("4. Claim a found item")
    print("5. Exit")

    choice = input("Enter your choice: ")

    if choice == "1":
        item_description = input("Enter a description of the lost item: ")
        location = input("Enter the location where it was lost: ")
        time = input("Enter the time it was lost: ")
        report_lost_item({"description": item_description}, location, time)
    elif choice == "2":
        item_description = input("Enter a description of the found item: ")
        location = input("Enter the location where it was found: ")
        time = input("Enter the time it was found: ")
        report_found_item({"description": item_description}, location, time)
    elif choice == "3":
        search_description = input("Enter a description to search for: ")
        matching_items = search_lost_item(search_description)
        if matching_items:
            print("Found matching lost items:")
            for item in matching_items:
                print("Item:", item["item"]["description"])
                print("Location:", item["location"])
                print("Time:", item["time"])
        else:
            print("No matching lost items found.")
    elif choice == "4":
        item_description = input("Enter a description of the item you want to claim: ")
        claim_result = claim_found_item({"description": item_description})
        print(claim_result)
    elif choice == "5":
        break
    else:
        print("Invalid choice. Please try again.")
```

CHAPTER 6

TESTING AND RESULTS

6.1 INTRODUCTION

Testing is the systematic process of assessing a system or its components to determine if they meet the specified requirements. This evaluation involves comparing actual outcomes with expected outcomes, uncovering any disparities. In simpler terms, testing involves the execution of a system to pinpoint discrepancies, errors, or missing elements in relation to the original intentions or requirements.

Testing is the methodical practice of making impartial judgments about the degree to which a system or device aligns with, surpasses, or falls short of the stated objectives. A robust testing program serves as a valuable tool for both the organization and the integrator/supplier. Typically, it marks the conclusion of the "Development" phase of a project, establishes the criteria for project acceptance, and signals the commencement of the warranty period.

6.2 PURPOSES OF TESTING

Verification of Procurement Specifications and Risk Management: Firstly, testing ensures that the product or system aligns with the functional, performance, design, and implementation requirements outlined in the procurement specifications. Secondly, it plays a crucial role in managing risks for both the acquiring organization and the system's vendor/developer/integrator. It helps identify when the work has reached completion, allowing for contract closure, vendor payment, and the transition of the system into the warranty and maintenance phase.

Delivering High-Quality, Reliable Software to Customers: Software testing aims to provide customers with software that is free of bugs and highly reliable. The objective is to prevent any issues during the software's usage, thus ensuring efficient utilization of the developed software. Given the significant cost associated with software development, testing is a critical step to avoid potential losses for customers.

Additionally, testing serves the following purposes:

Analysis of Adherence to Requirements: Testing assesses whether the developed application aligns with the specified requirements. It focuses on detecting defects or errors in a program, project, or product based on predefined criteria, which could be outlined in documents like scope documents or High-Level Design Documents (HLDD).

Enhancement of Application Quality: Testing plays a crucial role in improving the quality of an application by identifying and rectifying errors. The more errors that are eliminated, the higher the overall quality of the product. Testing serves purposes such as quality assurance, verification and validation, and reliability estimation. It involves a trade-off between budget, time, and quality.

Key factors necessitating testing for an application include:

Reducing Code Bugs: Testing aims to minimize the number of defects in the code, enhancing the application's reliability and performance.

Delivering a High-Quality Product: Testing ensures that the final product meets quality standards and performs as expected.

Verification of Requirement Fulfillment: Testing verifies that all specified requirements have been met, aligning with customer expectations.

Customer Satisfaction: Testing strives to satisfy customer needs by delivering a product that meets their demands and functions smoothly.

Bug-Free Software: Testing aims to provide software that is free of critical bugs, minimizing potential disruptions for users.

Earning Software Reliability: By identifying and addressing issues, testing contributes to the overall reliability of the software.

Preventing User-Detected Problems: Testing helps avoid situations where users encounter problems while using the software.

Behavioral Verification: Testing ensures that the software behaves as specified, adhering to the intended functionality.

Validation of User Requirements: It validates that what has been specified matches the actual desires and needs of the end user.

In essence, testing is a vital phase in software development that serves the dual purpose of ensuring compliance with requirements and managing risks while delivering a high-quality, dependable software product to users.

Various methods are employed in software testing, and the following descriptions provide a brief overview of some of these methods:

6.3 TESTING

BLACK BOX TESTING

Black box testing is a technique that involves testing a software application without any prior knowledge of its internal workings. Testers operate in a manner where they are unaware of the system's architecture and do not have access to the source code. Typically, during black box testing, testers interact with the system's user interface, providing inputs and observing outputs, without knowing how or where these inputs are processed.

WHITE BOX TESTING

White box testing, also known as glass testing or open box testing, entails a comprehensive examination of the internal logic and structure of a software's code. To perform white box testing on an application, the tester must possess knowledge of the

code's internal workings. Testers delve into the source code to identify specific units or sections of code that may exhibit inappropriate behavior.

GREY BOX TESTING

Grey box testing is a technique that falls between black box and white box testing. In software testing, the adage "the more you know, the better" carries significant weight when applying grey box testing to an application. Testers have limited knowledge of the application's internal workings, and this knowledge can vary. Unlike black box testing, where testers solely assess the application's user interface, grey box testing allows testers access to design documents and the database. This additional insight enables testers to better prepare test data and scenarios when developing their test plans.

These testing methods cater to different levels of knowledge about the software's internal architecture and logic. Black box testing focuses on assessing functionality without diving into the code, white box testing delves deep into code-level details, and grey box testing offers a middle-ground approach with limited knowledge of the internal workings while having access to key documents and data. Each method serves specific testing needs and objectives within the software testing process.

Different levels of testing play distinct roles in ensuring the quality and functionality of software:

UNIT TESTING

Unit Testing is a phase of software testing that concentrates on individual units or components of a software/system. The objective is to confirm that each unit operates as per its design. Unit testing is primarily executed by developers (White Box Testing) before handing over the software for formal testing by the quality assurance team. Developers employ separate test data from that of the quality assurance team. The key goal of unit testing is to isolate and demonstrate that individual program parts meet the requirements and function correctly.

Limitations:

Unit testing cannot detect every bug in an application.
Evaluating every execution path in complex software is not feasible.
There's a constraint on the number of scenarios and test data developers can use to validate the source code, necessitating the eventual merging of code segments with other units.

INTEGRATION TESTING

Integration Testing is a phase in software testing where individual units are combined and tested collectively as a group. The main purpose is to identify issues in the interaction between integrated units.
Integration testing assesses whether combined parts of an application function correctly when working together. Two common methods for Integration Testing are Bottom-up Integration Testing, which begins with unit testing and progressively combines units, and Top-down Integration Testing, which tests higher-level modules before lower-level ones.
In most comprehensive software development environments, bottom-up testing is typically performed first, followed by top-down testing. Modules, each containing related components, are tested individually in the module testing process.
Integrated System Testing (IST) is a systematic technique for validating the construction of the overall software structure while simultaneously conducting tests to uncover errors related to interfacing. The goal is to test the entire software structure dictated by the design, using unit-tested modules.

SYSTEM TESTING

System Testing is the subsequent phase of testing that examines the system as a whole. After all components are integrated, the complete application is rigorously tested to ensure it meets quality standards. This type of testing is conducted by a specialized testing team.

System Testing assesses whether the integrated system/software complies with the specified requirements. It is crucial for several reasons, including being the first step in the Software Development Life Cycle where the entire application is tested, verifying adherence to functional and technical specifications, testing in an environment similar to the production environment, and validating both business requirements and application architecture.

System testing aims to have an investigative approach, scrutinizing not only design but also behavior and user expectations. It goes beyond the boundaries defined in software/hardware requirements specifications. Other testing models fall under the umbrella of System Testing.

In essence, these levels of testing progressively ensure the reliability, functionality, and compliance of software with requirements, from individual units to the complete integrated system.

ACCEPTANCE TESTING

Acceptance testing, also known as User Acceptance Testing (UAT), is a crucial phase in the software testing process where a system is evaluated for acceptability. The primary purpose of this testing level is to assess whether the system aligns with the business requirements and determine if it's suitable for delivery. User Acceptance Testing (UAT) is carried out either by end-users or on behalf of them to ensure that the software functions in accordance with the Business Requirement Document. UAT focuses on several key aspects:
Ensuring that all functional requirements are met.
Achieving all performance requirements.
Verifying compliance with other requirements such as transportability, compatibility, and error recovery.
Ensuring that acceptance criteria specified by the user are satisfied.

Arguably, UAT is one of the most critical types of testing because it's conducted by the Quality Assurance Team, responsible for gauging whether the application aligns with

the intended specifications and meets the client's requirements. The QA team typically uses predefined scenarios and test cases to evaluate the application thoroughly.
Additionally, acceptance tests serve as a means to gather insights about the application's performance, accuracy, and the reasons behind the project's initiation. These tests aim not only to identify simple issues like spelling mistakes or cosmetic errors but also to pinpoint any critical bugs that could lead to system crashes or major errors in the application. By conducting acceptance tests, the testing team can gain a better understanding of how the application will perform in a production environment.

REGRESSION TESTING

Regression testing is conducted to assess changes in software behavior resulting from modifications or additions. Its purpose is to ensure that changes, even minor ones, do not lead to unexpected issues within the application. The primary goal is to identify any unintended consequences of alterations, such as a bug fix potentially causing a new functionality problem or a violation of business rules.
Regression testing is vital for several reasons:
It minimizes gaps in testing when changes are made to an application, ensuring that all aspects are thoroughly tested.
It verifies that changes made, such as bug fixes, do not negatively impact other areas of the application.
Regression testing helps mitigate risks associated with software changes.
By increasing test coverage without extending timelines, it enhances overall testing efficiency.
It accelerates the time to market for the product by quickly validating changes and preventing the introduction of new issues.
Acceptance testing evaluates the system's suitability for delivery and alignment with business requirements, while regression testing ensures that changes in software behavior do not introduce unexpected issues. Both testing levels are crucial for delivering reliable and high-quality software.

CHAPTER 7

SCREENSHOT

Fig.7.1 Home Page

Fig.7.2 Admin Log in Page

Fig.7.3 Add Line

Fig.7.4 Add Station

Fig.7.5 Station Master

Fig.7.6 Mail Confirmation

Fig.7.7 Property team

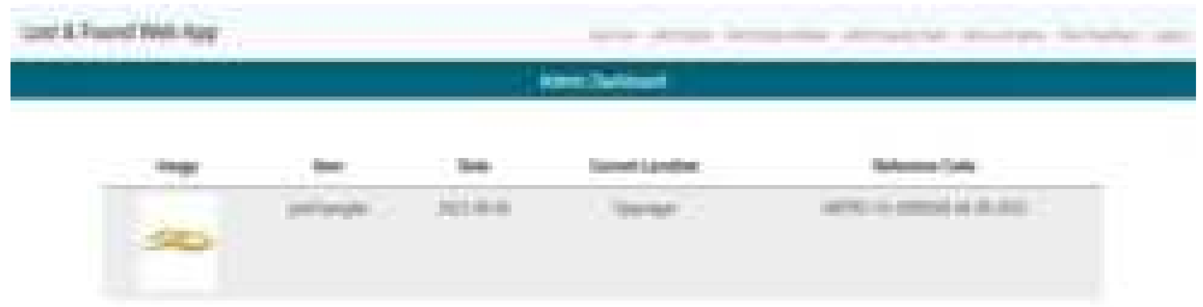

Fig.7.8 View Lost Iteam

Fig.7.9 Feedback

CHAPTER 8

CONCLUSION AND FUTURE ENHANCEMENT

CONCLUSION

The proposed web-based application for managing lost items in a metro station represents a significant step towards improving efficiency, user experience, and data management in this context. It addresses several pain points for both metro station staff and commuters by providing real-time notifications, effective communication channels, and robust data analytics capabilities. However, it also faces challenges related to technical implementation, user adoption, and data privacy, which must be carefully managed.

In conclusion, the advantages of the system, including enhanced efficiency, user satisfaction, and data-driven decision-making, outweigh its disadvantages. With proper planning, continuous improvement, and a commitment to data security and privacy, this system has the potential to greatly benefit both metro stations and their commuters.

FUTURE ENHANCEMENTS:

To further improve the system's functionality and address potential limitations, here are some future enhancements that could be considered:

Integration with Lost and Found Services: Explore partnerships with external lost and found services to expand the system's reach beyond the metro station, allowing users to report lost items in other locations as well.

Machine Learning for Matching: Implement machine learning algorithms to improve the accuracy of item matching by analyzing historical data and user-provided descriptions.

Accessibility Features: Enhance the application's accessibility features to cater to users with disabilities, such as screen readers and voice commands.

Augmented Reality (AR) Assistance: Implement AR features to help users scan their surroundings and locate lost items more easily within the metro station.

Crowdsourced Lost Item Search: Allow users to crowdsource the search for lost items by incentivizing them to help locate and report lost items that match descriptions provided by others.

Predictive Analytics: Use predictive analytics to anticipate peak times for lost item reports and allocate staff resources accordingly to reduce response times.

Enhanced Reporting and Feedback: Expand the reporting and feedback capabilities by incorporating natural language processing (NLP) for sentiment analysis, allowing metro station staff to better understand user sentiments and improve services accordingly.

Data Monetization: Explore opportunities for monetizing the data collected by the system, such as providing analytics services to retailers or advertisers interested in commuter behavior trends.

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UNIVERSITY OF MYSORE
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A
Dissertation on

"Development of a Deep Learning-based System for Automatic Garbage Image Classification"

Submitted in partial fulfilment of the requirements for the award of the degree of

Master of Science in Computer Science

Submitted by

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I thank all the teaching and non-teaching staff, for our department of computer science for their immense help and cooperations.

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TO WHOMSOEVER IT MAY CONCERN

This is to Certify that Harhsini.HM (P01BH21S0222) MSc C.s Student of Pooja Bhagavat Mahajana First Grade college PG wing (Autonomous), Mysore is successfully completed the internship in our organization under the guidance of our Technical team from June 2023 to August 2023 on "Development of a Deep Learning Based System for Automatic Garbage Image Classification". The candidate availed our facility as per the company's rules and practices. During this period we found sincere, honest hardworking dedicated person with a professional attitude and very good knowledge. We wish every success in life.

Regards

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A Dissertation on

LINGUISTIC STEGANOGRAPHY WITH MASKED LANGUAGE MODEL

SUBMITTED BY:

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DECLARATION

This dissertation, titled "**LINGUISTIC STEGANOGRAPHY WITH MASKED LANGUAGE MODEL**" submitted by me as partial fulfilment for the award of the degree of MSc Computer Science from the University of Mysore, is a bonafide work carried out by me during the period of 15-05-2023 to 15-09-2023 under the guidance of Mr. BASANTH KUMAR H.B Assistant Professor, DoS in Computer Science Pooja Bhagavat Memorial Mahajana Education centre, Mysuru.

I hereby declare that the entire work embodies in the dissertation has been carried out by me and no part has been submitted elsewhere for any other degree/diploma to any University/Institute

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Abstract

In this topic presents a novel approach to linguistic steganography using a masked language model (MLM). The method involves masking tokens in the cover text and generating a vocabulary distribution for each masked token using the MLM. Bit chunks are assigned to high-probability subwords in the distribution, allowing the encoding of a secret message.

The resulting stego text appears natural and can be transmitted in a public channel without arousing suspicion. The proposed method is simpler than existing edit-based methods, offers a high payload capacity, and allows for easy control of the security/payload capacity trade-off.

The method eliminates the need for complex rule construction typically associated with edit-based steganography models. Instead, it leverages the power of MLMs, which have been widely used in natural language processing tasks. By utilizing MLMs, the proposed method achieves a higher payload capacity, allowing for the hiding of larger amounts of secret information within natural language texts.

Contents

	Page No
1.INTRODUCTION	
1.1 Background	1
1.2 Objectives	2
1.3 Purpose and applicability	2-3
1.3.1 Purpose	
1.3.2 Applicability	
2. LITERATURE SURVEY	4
3. REQUIREMENTS AND ANALYSIS	6-7
3.1 Requirement specification	
3.1.1 Functional Requirement	
3.1.2 Non-Functional Requirement	
3.2 Hardware and software requirements	
4. PROPOSED METHODOLOGY	8-9
5. RESULTS AND DISCUSSIONS	
5.1 Model	10-15
5.2 Pseudo code	16-19
5.3 Snapshot	20-21
6. CONCLUSION	22
7. FUTURE ENHANCEMENT	23
8. BIBLIOGRAPHY	23-25
9. URL REFERRED	26

Introduction

1.1 Background

In this topic "Linguistic Steganography with a Masked Language Model" lies in the field of linguistic steganography, which is the practice of concealing a secret message within a cover text in such a way that it remains undetectable to eavesdroppers. While steganography has traditionally been applied to images, videos, and audio, the use of natural language as a cover medium has gained attention due to the ubiquity of text.

The goal of linguistic steganography is to create a stegosystem where the sender (Alice) encodes a secret message into a text, known as the cover text, and the receiver (Bob) decodes the message without arousing suspicion from eavesdroppers, such as Eve.

The stegosystem aims to achieve two objectives: security and payload capacity.Security refers to the degree to which the stego text appears natural and does not raise suspicion, while payload capacity refers to the size of the secret message relative to the size of the stego text.

Existing methods in linguistic steganography include edit-based and generation-based approaches. Edit-based methods involve making subtle modifications to the cover text, while generation-based methods generate entirely new text that carries the secret message. However, these methods have limitations in terms of complexity, payload capacity, and naturalness of the stego text.

To address these limitations, here proposes a novel approach using a masked language model (MLM) for edit-based linguistic steganography.The MLM is used to generate a vocabulary distribution for each masked token in the cover text, and bit chunks are assigned to high-probability subwords in the distribution, allowing for the encoding of the secret message.The resulting stego text appears natural and can be transmitted in a public channel without arousing suspicion.

The proposed method offers advantages such as simplicity, high payload capacity, and control over the security/payload capacity trade-off. By leveraging the power of masked language models, the aims is to provide an efficient and secure approach to linguistic steganography

1.2 Objectives

The objectives of the topic "Linguistic Steganography with a Masked Language Model" are as follows:

- To propose a novel approach to linguistic steganography using a masked language model (MLM) for efficient and secure information hiding.
- To demonstrate the simplicity, high payload capacity, and control over the security/payload capacity trade-off offered by the proposed method.
- To compare the proposed edit-based method with existing edit-based and generation-based methods in terms of complexity, payload capacity, and naturalness of the stego text.

1.3 Purpose and applicability

1.3.1 Purpose

The purpose of this paper is to propose a method for linguistic steganography using a masked language model (MLM) and to evaluate its effectiveness. The aims to address the limitations of existing methods by offering a simpler approach with a higher payload capacity. And also aim to demonstrate the security of the proposed method against automatic detection and to validate its performance through experiments and human evaluation.

1.3.2 Applicability

The topic of “Linguistic Steganography with a Masked Language Model" has several potential applicabilities.

Introduction

1.Information Security: Linguistic steganography techniques can be applied to enhance information security by allowing the covert transmission of sensitive messages within seemingly innocuous text. This can be useful in various domains, such as military communications, intelligence agencies, and secure data transfer.

2.Privacy Protection: Linguistic steganography can be used to protect the privacy of individuals by enabling them to communicate sensitive information without drawing attention or raising suspicion. This can be particularly relevant in situations where privacy is a concern, such as whistleblowing or confidential communication.

3.Digital Forensics: The study of linguistic steganography can also have implications in the field of digital forensics. Understanding and detecting linguistic steganography techniques can help forensic investigators identify hidden messages and uncover covert communication channels.

4.Ethical Considerations: The topic of linguistic steganography raises important ethical considerations. It prompts discussions about the balance between privacy and security, the potential misuse of steganography for illegal activities, and the need for regulations and guidelines in this domain.

Chapter 2

2.Literature survey

The literature survey of the topic "Linguistic Steganography with a Masked Language Model" as follows

[1]. Title: Linguistic steganography: Survey, analysis, and robustness concerns for hiding information in text.

Author: Bennett, K.

Year: 2004

The author provides a comprehensive survey of linguistic steganography techniques, analyzing their effectiveness and discussing the robustness concerns associated with hiding information in text. It explores various approaches and highlights the challenges and considerations in implementing linguistic steganography.

[2]. Title : Practical linguistic steganography using contextual synonym substitution and a novel vertex coding method

Author: Chang, C. Y., & Clark, S.

Year: 2014

The author presents a practical approach to linguistic steganography using contextual synonym substitution and a novel vertex coding method. The authors demonstrate the effectiveness of their method in hiding information within text while maintaining the naturalness of the resulting stego text

[3]. Title: BERT: Pre-training of deep bidirectional transformers for language understanding.

Author: Devlin, J., Chang, M. W., Lee, K., & Toutanova, K.

Year: 2019

The author introduces BERT, a pre-training method for deep bidirectional transformers that achieves state-of-the-art performance on various natural language understanding tasks. BERT has been widely adopted in the field of natural language processing and has significantly advanced the capabilities of language models.

[4].Title: Reversible linguistic steganography with Bayesian masked language modeling

Author: Ching-Chun Chang

Year: 2019

The author introduces BERT, a pre-training method for deep bidirectional transformers that achieves state-of-the-art performance on various natural language understanding tasks. BERT has been widely adopted in the field of natural language processing and has significantly advanced the capabilities of language models.

And also the author introduces a novel method for linguistic steganography using a masked language model (MLM). Steganography is the practice of hiding secret information within seemingly innocuous cover texts. The proposed method leverages the capabilities of the MLM to encode a secret message in the form of a bit sequence.

Chapter 3

Requirement and analysis

3.1 Requirements specification

3.1.1 Functional requirement

1. Masked Language Model (MLM): The proposed method relies on a masked language model for generating stego texts. The MLM should be capable of masking tokens in the input text and generating a vocabulary distribution for each masked token.

2. Encoding and Decoding: The system should provide functionality for encoding a secret message into a cover text using the masked language model. It should also support decoding the secret message from a stego text using the same model.

3. Payload Capacity Control: The system should allow the user to control the payload capacity, i.e., the size of the secret message relative to the size of the stego text. This control is important to achieve a desired balance between security and payload capacity.

4. Naturalness and Understandability: The generated stego texts should be natural and grammatically correct to avoid arousing suspicion from eavesdroppers. The system should aim to produce stego texts that are understandable and do not raise suspicion.

3.1.2 Non-functional requirement

1.Efficiency: The proposed method should be efficient in terms of computational resources and execution time. The generation of stego texts should not be computationally expensive, allowing for practical use in real-time scenarios.

Requirement and analysis

3.Robustness: The stego texts generated by the proposed method should be robust against detection by both humans and automated detection systems. The method should be able to produce stego texts that are indistinguishable from natural texts, fooling both human evaluators and machine-based detection algorithms.

4.Scalability: The proposed method should be scalable to handle large volumes of text data. It should be able to encode and decode secret messages in texts of varying lengths, accommodating different use cases and requirement.

5.Usability: The system implementing the proposed method should be user-friendly and easy to use. It should provide clear instructions and controls for encoding and decoding secret messages, allowing users to easily interact with the system.

3.2 Hardware and software requirements

Minimum Hardware requirements

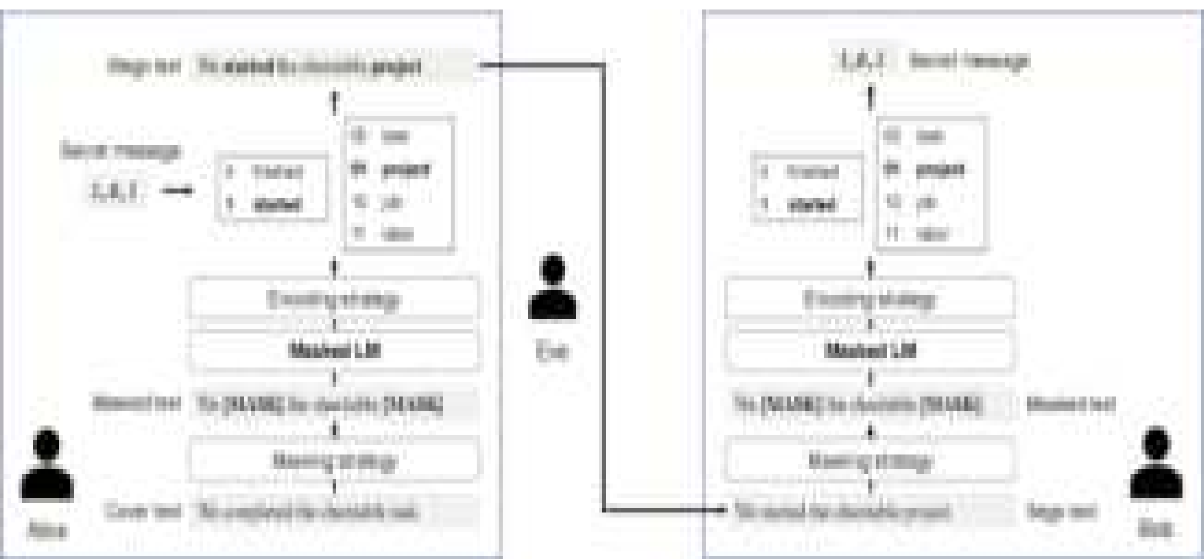
- Processor : intel i3 2.4 GHz
- Hard Disk : 40 GB
- Ram : 2GB or above

Software Requirements

- Operating system : Windows 7
- Coding language : python
- Tools used : Jupyter notebook

chapter 5

4.Proposed methodology



Overview of the proposed method. Alice (sender) and Bob (receiver) share the masked language model (masked LM) and the masking and encoding strategies in advance. Alice masks some tokens in the cover text and makes the masked LM generate a vocabulary distribution for each masked token. Bit chunks are assigned to some high-probability subwords in the distribution from which one is chosen according to the secret message. The stego text is then transmitted in a public channel Eve(eavesdropper) monitors. Receiving the stego text, Bob performs mostly the same procedure to decode the secret message.

And this is novel approach for linguistic steganography using a masked language model (MLM).The key idea is to leverage the power of MLMs, such as BERT, to simplify the steganography process and enhance its effectiveness.

The methodology involves sharing the masked language model and the masking and encoding strategies between the sender (Alice) and the receiver (Bob). Alice starts by masking certain tokens in the cover text and makes the masked language model generate a vocabulary distribution for each masked token. Bit chunks are then assigned to high-probability subwords in the distribution, and one subword is chosen based on the secret message. This process allows for the hiding of the secret message within the stego text.

To evaluate the proposed method, the authors conducted experiments on a binary classification task using a balanced dataset of stego and real texts. They used random 32-bit sequences as secret messages and compared the performance of the proposed method with generation-based approaches. The evaluation also involved human ratings using a Likert scale to assess the quality and understandability of the stego texts.

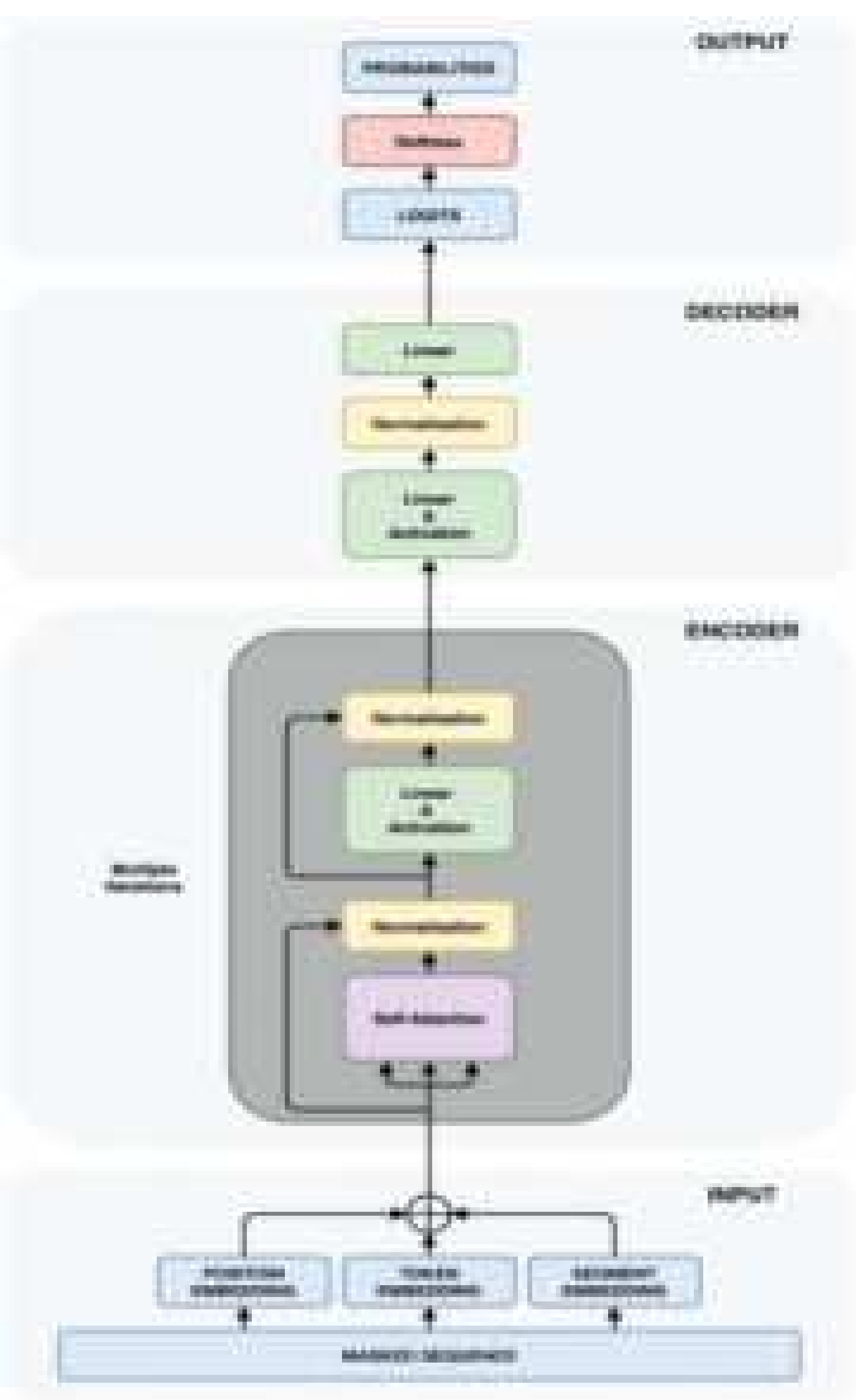
The results of the experiments and evaluations demonstrate the effectiveness of the proposed method for linguistic steganography. The method offers a simplified approach compared to traditional edit-based methods, higher payload capacity, and improved security against automatic detection. It allows for better control of the security/payload capacity trade-off. However, it is important to note that the proposed method may still face challenges in generating genuine-looking texts that fool both humans and machines.

Overall, the proposed methodology harnesses the capabilities of masked language models to enhance linguistic steganography, providing a promising avenue for further research and development in this field

Chapter 6

Result and discussions

5.1 Models Architecture of BERT masked language model



Result and discussions

Overview of BERT architecture model

The BERT (Bidirectional Encoder Representations from Transformers) architecture is a pre-trained language model that is widely used in natural language processing tasks. It consists of a stack of self-attention layers and processes input sequences bidirectionally.

The BERT model is trained to perform masked language modeling, which involves masking a cover word in a text sequence and predicting the probability distribution of the masked word. This task helps the model learn contextual information and improve its understanding of language.

The architecture of the BERT masked language model can be illustrated as follows:

1. Input Embeddings: The input to the BERT model is a sequence of tokens, which are first transformed into embeddings. These embeddings capture the semantic meaning of the tokens and their relative positions in the sequence.

2. Transformer Encoder Layers: The BERT model consists of multiple transformer encoder layers. Each layer has a multi-head self-attention mechanism and a feed-forward neural network. The self-attention mechanism allows the model to attend to different parts of the input sequence and capture dependencies between words. The feed-forward neural network applies non-linear transformations to the input representations.

3. Masked Language Modeling: In the masked language modeling task, a certain percentage of the input tokens are randomly masked. The model then predicts the masked tokens based on the context provided by the surrounding tokens. This helps the model learn to understand the relationships between words and improve its ability to generate accurate predictions.

4. Fine-tuning: After pre-training on a large corpus, the BERT model can be fine-tuned for specific downstream tasks. This involves adding task-specific layers on top of the pre-trained model and training the model on task-specific data. Fine-tuning allows the BERT model to transfer its knowledge to various natural language processing tasks, such as sentiment analysis, named entity recognition, and question answering.

The BERT architecture has been shown to achieve state-of-the-art performance on a wide range of natural language processing tasks. Its ability to capture contextual information bidirectionally and its pre-training followed by fine-tuning approach make it a powerful tool for various language understanding tasks.

Example for how it works

Tokenization:

let's say, we have this sentence:

My dog is cute. He likes playing.

After the tokenization, this sentence turns out to be

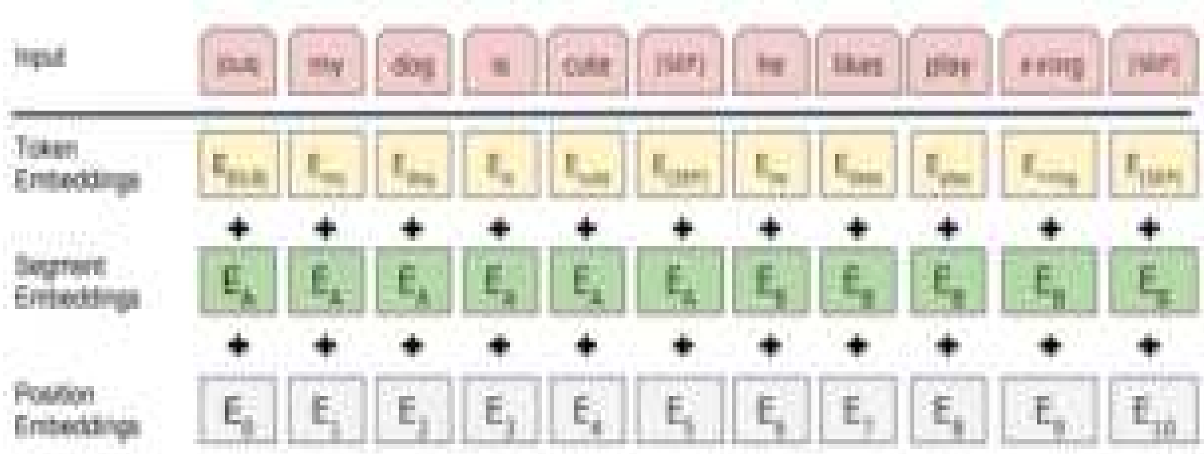
[CLS] my dog is cute [SEP] he likes play ##ing [SEP]

You basically understand that tokenization consists in removing uppercase letters, adding specific anchors instead of punctuation, and grabbing the root meaning of words.

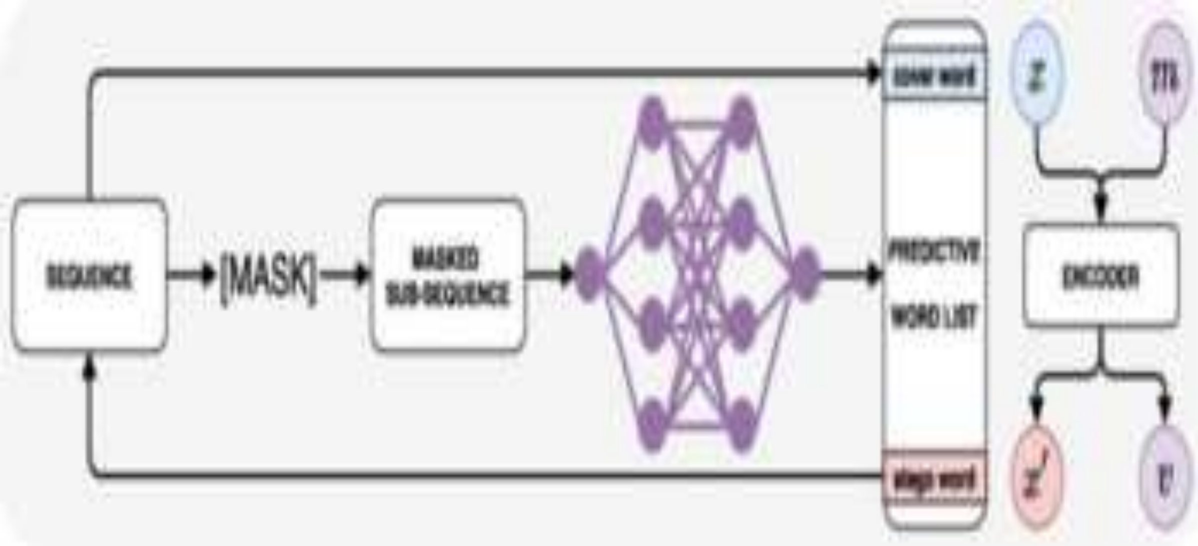
While BERT uses WordPiece algorithm for tokenization, some newer model use SentencePiece. The difference is whole-word masking and not sub-word masking.

Embedding

Embedding is a vectorization.There are many algorithm for Embedding, and BERT use WordPiece embeddings.



Linguistic steganography based on a pre-trained masked language model (MLM):



The Masked language modeling, which is a fill-in-the-blank task where a model predicts a masked word from the context. Tokenization is the process of splitting a text sequence into smaller units called tokens, such as words, punctuation marks, and numbers.

Results and discussions

To represent tokens numerically, one simple representation is the one-hot vector. It is a binary vector with the size of the vocabulary, where only the index corresponding to the token's position in the vocabulary is set to 1. However, this representation is sparse and does not capture the semantic relationships between words.

The distributional hypothesis suggests that words with similar meanings tend to occur in similar contexts. Distributional representation addresses this by creating a co-occurrence matrix that counts how often each word appears within a context window of another word. Similar co-occurrence patterns indicate similar meanings. However, this matrix can be computationally expensive and suffers from sparsity.

Modern approaches, such as word2vec and GloVe, use neural network models for representation learning. These models are trained on language modeling tasks, with word vectors initialized randomly and updated iteratively using optimization algorithms. However, they struggle with polysemy and homonymy, as multiple meanings of a word are conflated into a single representation.

Contextual word representation overcomes this limitation by dynamically adapting word vectors to their context. The BERT model is a state-of-the-art neural network based on the transformer architecture, which uses self-attention modules to process input sequences bidirectionally. BERT is trained on masked language modeling, where it predicts masked words in the context. The knowledge learned from masked language modeling can be transferred to other NLP tasks through fine-tuning.

In the context of steganography, the passage states that the proposed system relies on the basic function of the BERT model, specifically masked language modeling. The system utilizes the pre-trained BERT model to obtain probability distributions for masked words and uses them for linguistic steganography purposes.

Results and discussions

Overall, the passage provides an overview of masked language modeling, different word representation techniques, and the relevance of the BERT model in contextual word representation. It emphasizes the application of masked language modeling in the proposed steganographic system.

Certainly! Here's a simple example to illustrate masked language modeling:

Input Text: "I want to [MASK] a book."

In this example, the task is to predict the masked word in the given text. The masked word is represented by the [MASK] token.

Using a pre-trained language model like BERT, we can feed this input text into the model and obtain the predicted probability distribution for the masked word.

Assuming the model predicts the following probabilities for the masked word:

- "read": 0.60
- "buy": 0.25
- "write": 0.10
- "sell": 0.05

Based on these probabilities, the model suggests that "read" is the most likely word to fill the masked position, followed by "buy," "write," and "sell."

Therefore, the completed sentence could be:

Output Text: "I want to read a book."

Results and discussions

In this example, the masked language modeling task allows the model to predict the missing word based on the context provided by the surrounding words.

5.2 Pseudo code

MaskedStego class

```
class MaskedStego:
def __init__(self, model_name_or_path: str = 'bert-base-cased') -> None:    def __call__(self, cover_text: str, message: str, mask_interval: int = 3, score_threshold: float = 0.01) ->str:

# Preprocess the cover text
 processed = self._preprocess_text(cover_text, mask_interval)

# Iterate over the masked tokens
for i_token, token in enumerate(processed['masked_ids']):
 if token != self._tokenizer.mask_token_id:
 continue

# Get the top-k candidates for the masked token
ids = processed['indices'][i_token]
scores = processed['sorted_output'][i_token]
candidates = self._pick_candidates_threshold(ids, scores, score_threshold)

# Encode the message into the masked token
replace_token_id = self._block_encode_single(candidates, message_io)

# Update the input IDs with the replaced token
 input_ids[i_token] = replace_token_id

# Decode the encoded message
 encoded_message: str = message_io.getvalue()[:message_io.tell()]
message_io.close()
```

```
# Generate the stego text
stego_text=self._tokenizer.decode(input_ids,skip_special_tokens=True,clean_up_tokenizatio
n_spaces=True)
  # Return the stego text and encoded message
        return { 'stego_text': stego_text, 'encoded_message': encoded_message }
 def decode(self, stego_text: str, mask_interval: int = 3, score_threshold: float = 0.005) -> str:
  # Preprocess the stego text
        processed = self._preprocess_text(stego_text, mask_interval)
      # Iterate over the masked tokens
        for i token, token in enumerate(processed['masked_ids']):
            if token != self._tokenizer.mask_token_id:
                continue
            # Get the top-k candidates for the masked token
            ids = processed['indices'][i_token]
scores = processed['sorted_output'][i_token]
            candidates = self._pick_candidates_threshold(ids, scores, score_threshold)
# Decode the message from the masked token
            chosen_id: int = input_ids[i_token].item()
            decoded_message.append(self._block_decode_single(candidates, chosen_id))
  # Return the decoded message
        return {'decoded_message': ''.join(decoded_message)}
```

The main steps of the MaskedStego class are as follows:

[1]. Preprocess the cover text by masking tokens at a regular interval.

[2]. For each masked token, generate the top-k candidates based on the masked language model's predictions.

[3]. Encode the message into the top-k candidates using a block encoding scheme.

[4]. Generate the stego text by replacing the masked tokens with the encoded candidates.

[5]. To decode the message from the stego text, repeat steps 2-3, but instead of encoding the message, decode the encoded candidates using the block decoding scheme.

The MaskedStego class can be used to embed secret messages in text in a way that is difficult to detect. However, it is important to note that this method is not perfect, and there are steganalysis techniques that can be used to detect and extract hidden messages.

<u>Main class</u>

```
function encode(cover_text: str, message: str, mask_interval: int, score_threshold: float):

masked_stego = MaskedStego()

 stego_text = masked_stego(cover_text, message, mask_interval, score_threshold)

 print(stego_text)

function main():

parser = ArgumentParser()

 parser.add_argument('text', type=str, help='Text to encode or decode message.')

parser.add_argument('-d', '--decode', action='store_true', help='If this flag is set, decodes from the text.') parser.add_argument('-m', '--message', type=str, help='Binary message to encode

consisting of 0s or 1s.')parser.add_argument('-i', '--mask_interval', type=int, default=3)

parser.add_argument('-s', '--score_threshold', type=float, default=0.01)

    args = parser.parse_args()
```

```
masked_stego = MaskedStego()
if args.decode:
        print(masked_stego.decode(args.text, args.mask_interval, args.score_threshold))
    else:
encode(args.text, args.message, args.mask_interval, args.score_threshold)
if __name__ == '__main__':
    main()
```

The main steps of the pseudocode are as follows:

1. Create a MaskedStego object.
2. Check the -d flag to determine if the user wants to decode the message from the text.
3. If the user wants to encode a message, call the encode() function with the cover text, message, mask interval, and score threshold.
4. If the user wants to decode a message, call the decode() function with the stego text, mask interval, and score threshold.
5. Print the output of the encode() or decode() function.

The encode() and decode() functions are implemented in the MaskedStego class.

Result and discussions

5.2 Screenshot's

Figure [1]

Figure [2]

Figure [3]

Chapter 7

6.Conclusion

The topic "Linguistic Steganography with a Masked Language Model" concludes that the proposed method, which leverages a masked language model (MLM), simplifies the process of edit-based linguistic steganography.The method offers a high payload capacity, allows for easy control of the security/payload capacity trade-off, and is more secure against automatic detection compared to generation-based methods.

The highlights of that the proposed method eliminates the need for painstaking rule construction, which was a limitation of previous edit-based approaches. By using the capabilities of the MLM, such as generating vocabulary distributions for masked tokens, the method achieves a higher payload capacity and provides better control over the steganography process.

The evaluation results show that the proposed method produces stego texts that are highly rated by human evaluators, indicating their naturalness and understandability [5]. Although the method may receive slightly lower ratings compared to generation-based methods, it offers a good trade-off between security and payload capacity.

Overall, the paper demonstrates the effectiveness and simplicity of using a masked language model for edit-based linguistic steganography, providing a promising approach for hiding secret messages within natural language texts.

Chapter 8

6.Future enhancement

There are several potential future enhancements that can be explored based on the findings and limitations of the topic "Frustratingly Easy Edit-based Linguistic Steganography with a Masked Language Model." These enhancements include:

1.Improving the generation of genuine-looking stego texts: While the proposed method using a masked language model (MLM) simplifies the steganography process, generating stego texts that are indistinguishable from natural texts remains a challenge. Future research can focus on developing techniques to enhance the generation process and improve the quality and authenticity of the stego texts.

2.Addressing the limitations of proper noun handling: The paper acknowledges that editing proper nouns like "Geneva" can be prone to factual errors and challenges [6]. Future research can explore more sophisticated approaches, such as using part-of-speech or named entity recognition, to handle proper nouns more effectively and ensure the sameness of masking positions.

3.Investigating alternative masking and encoding strategies: The paper proposes a specific approach for masking tokens and assigning bit chunks based on high-probability subwords in the vocabulary distribution. Future research can explore alternative masking and encoding strategies to further optimize the steganography process and improve the security and payload capacity trade-off.

4.Evaluating the method against more advanced detection techniques: Here demonstrates that the proposed method is more secure against automatic detection compared to generation-based methods. However, future research can evaluate the method against more advanced detection techniques and explore potential countermeasures to further enhance the security of the steganography approach.

Chapter 9

8. Bibliography

[1]. Title:). Frustratingly Easy Edit-based Linguistic Steganography with a Masked Language Model.

Author: Ueoka, H., Murawaki, Y., & Kurohashi, S.

Year: 2021

The author discusses the evolution of linguistic steganography approaches from edit-based methods to generation-based methods. Edit-based approaches, such as synonym substitution, were effective but required complex rule construction and had low payload capacity. Generation-based approaches, using neural language models, offered higher payload capacity but struggled to generate genuine-looking texts. The paper proposes a new method that combines the simplicity of edit-based approaches with the power of masked language models (MLMs). The proposed method leverages MLMs to simplify the steganography process, achieve a higher payload capacity, and provide better control over the security/payload capacity trade-off. It eliminates the need for painstaking rule construction and offers a promising approach for hiding secret messages within natural language texts.

[2]. Title: A practical and effective approach to large-scale automated linguistic steganography.

Author: Chapman, M., Davida, G. I., & Rennhard, M.

Year: 2001

The authors propose a practical and effective approach to automated linguistic steganography. They discuss the challenges and techniques involved in hiding secret messages within natural language texts on a large scale

[3]. Title: Towards near-imperceptible steganographic text

Author: Dai, F., & Cai, Z.

Year:2019

The Author explores the concept of near-imperceptible steganographic text, which refers to the generation of hidden messages that are difficult for humans to detect. The authors discuss techniques and approaches to achieve near-imperceptibility in steganography.

[4].Title: BERT Pre-training of deep bidirectional transformers for language understanding.

Author: Devlin, J., Chang, M. W., Lee, K., & Toutanova, K.

Year:2019

The author introduces BERT, a pre-training method for deep bidirectional transformers in natural language processing. BERT has been widely used in various language-related tasks, including steganography, due to its ability to understand and generate natural language

[5].Title: Automatic detection of generated text is easiest when humans are fooled.

Author: Ippolito, D., Duckworth, D., Callison-Burch, C., & Eck, D.

Year:2020

The author discusses the challenges of automatically detecting generated text and highlights the importance of fooling humans in order to create more realistic and believable generated text. The findings are relevant to the security aspect of steganography, as the goal is to create stego texts that are indistinguishable from natural texts.

Chapter9

URL referred

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[2]. https://youtu.be/Fs8Zb4T-_CE?si=537lso8IoV6pVRU2

[3]. https://youtu.be/s-V7gKrsels?si=TmD9uuMAOHMas_gP

[4]. https://youtube.com/shorts/cpxW9uYs_yw?si=NrbBnhRx2s-gXwwV

[5]. https://youtu.be/aWFllV6WsAs?si=6wewQXe2H2SWJIsJ

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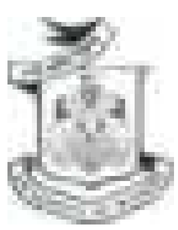

Major project work on

“SMART MILK DAIRY PRODUCTION PLANNING SYSTEM”

Submitted in partial fulfilment of the requirement for the award of Master of Computer Application

Submitted by

BHARATH H L

Register No: P01BH21S0156

Carried out at
Orb focus, Mysuru

Under the guidance of

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September 2023
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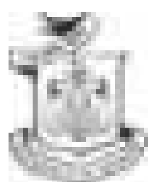

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CERTIFICATE

Certified that the project work entitled **"SMART MILK DAIRY PRODUCTION PLANNING SYSTEM"** is a bonafide work carried out by **Mr. BHARATH H L, Reg.No. P01BH21S0156** in partial fulfillment of requirements for the award of MCA degree by Mysore University, Mysuru during academic year 2022-2023. It is certified that all the corrections and suggestions indicated for the Internal Assessment have been incorporated in the report deposited in the department library. The project report has been approved as it satisfies the academic requirements in respect of Major Project Work presented for the side Degree.

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Name of the Examiners | **Signature with Date**

1.

2.

DECLARATION

DECLARATION This is to certify that the project entitled **"SMART MILK DAIRY PRODUCTION PLANNING SYSTEM"** submitted by me in partial fulfilment for the award of Master of Computer Application is a bonafide work carried out by me during the period of May 2023 to September 2023 under the guidance of "**Ms. Likhitha M M**", Assistant Professor, DOS in Computer Science, Pooja Bhagavat Memorial Mahajana Education Center, Mysuru I hereby declare that the entire work embodied in the dissertation has been carried out by me and no part has been submitted elsewhere for any other degree to any other University/Institute.

Place: Mysore **BHARATH H L**

Date: **Reg. No: P01BH21S0156**

ACKNOWLEDGENT

Apart from the efforts of me, the sources of my project depend largely on the encouragement and the guidance of many others. I take this opportunity to express my gratitude to the people who have been instrumental in the successful completion of this project.

I have a great pleasure in expressing my deep sense of gratitude to our beloved director **Dr C.K Renukarya**, **Pooja Bhagavat Memorial Mahajana PG Center, Mysuru** for providing all the facilities to work out this project.

I would like to express my gratitude to **Mrs. Rachana C.R**, **Associate Professor& HOD, DOS in Computer Science, Pooja Bhagavat Memorial Mahajana PG Center, Mysuru** for her constant support, motivation and invaluable guidance throughout the tenure of this project.

I consider it is my privilege and honour to express my sincere thanks to **"Ms. Likhitha M M, Assistant Professor, DOS in Computer Science, Pooja Bhagavat Memorial Mahajana PG Center, Mysuru** for providing all the facilities, support, guidance, motivation, encouragement, and suggestions related to the project. Her timely guidance helped me to complete the projection time.

Finally, I would like to thank all our teaching and non-teaching staff, for their kind help and cooperation. Above all, my sincere gratitude to my parents and friends and all those who supported me throughout the project.

BHARATH H L

P01BH21S0156

ABSTRACT

Scientific research and the practice of leading enterprises show that the main condition and key factor in increasing the competitiveness of the production of quality milk is the organization of effective management. Solution of the problems of improving manageability in production and improving the quality of milk requires a transition to new technologies. The use of digital technologies, the active introduction and use of “smart” automated systems for managing production processes for making timely strategic decisions in production are of priority importance in modern conditions. Studies show that it is necessary to increase the level of organization systematically in order to achieve the challenges facing the industry. Therefore, the creation of an effective management system should be paramount for most milk producers. An understanding is increasingly being formed of the need to solve innovative problems systematically when real goals are defined in the Novgorod region. At the same time, the mandatory participation of specially trained personnel in this process, able to find ways to transform and form a competitive milk production is important. Management evaluation in dairy production was implemented through a system of interrelated social and economic indicators, allowing a comprehensive analysis of the state and effectiveness of enterprises. A survey of the heads of agricultural enterprises conducted during the study confirmed that in the conditions of the innovative development of rural territories, the totality of organizational, technological, methodological methods carried out using certain procedures makes up the process of managing milk production.

INDEX

CHAPTER 1

INTRODUCTION

1.1	PROBLEM STATEMENT	01
1.2	OBJECTIVES	01
1.3	SCOPE	01

Chapter 2

LITERATURE SURVEY		**02**
2.1	EXISTING SYSTEM	04
2.2	PROPOSED SYSTEM	04

CHAPTER 3

SOFTWARE REQUIREMENT& SPECIFICATION		**05**
3.1	FUNCTIONAL REQUIREMENTS	05
3.2	NON-FUNCTIONAL REQUIREMENTS	06
3.3	SYSTEM REQUIREMENTS	07
3.4	FEASIBILITY STUDY	07

CHAPTER 4

SYSTEM DESIGN		**09**
4.1	SYSTEM ARCHITECTURE	09
4.2	DATA FLOW DIAGRAM	10
4.3	CONTEXT DATA FLOW DIAGRAM	13
4.4	USE CASE DIAGRAM	14
4.5	SEQUENCE DIAGRAM	15

CHAPTER 5

IMPLEMENTATION

5.1	TECHNOLOGY SPECIFICATION	18
5.2	CODE	20

CHAPTER 6

TESTING

6.1 INTRODUCTION 25
6.2 TEST CASES 26

CHAPTER 7

SCREENSHOTS 36

CHAPTER 8

CONCLUSIONS AND FUTURE ENHANCEMENT

8.1 CONCLUSION 42
8.2 FUTURE ENHANCEMENT 42

REFERENCES 42

University of Mysore
Manasagangotri Mysore

A

DISSERTATION ON

"THE DETECTION OF PARKINSON'S DISEASE USING MACHINE LEARNING ALGORITHM"

Submitted in partial fulfilment of the requirement for the award of the degree of

MASTER OF SCIENCE IN COMPUTER SCIENCE

Submitted By:
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I further declare that I have not submitted this dissertation to any other university for the award of any Degree/Diploma

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ACKNOWLEDGEMENT

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Above all, my sincere gratitude to my parents and friends and all those who supported me throughout the project.

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Date: 19th September, 2023

PROJECT COMPLETION CERTIFICATE

We would like to inform you that Mr. **Chethan K S** (Reg no: [illegible]) pursuing M.Sc in Computer Science from SBRR Mahajana First Grade College(Autonomous), Pooja Bhagavat Memorial Mahajana Education Centre KRS Road, Metagalli Mysuru – 570016 has successfully completed his project with our company, he has been working on the project title: "**Detection of The Parkinson's Disease using the Machine Learning Algorithm**" under the domain: Machine Learning with Python from 31-05-2023 to 19-09-2023.

We have found him to be a self - starter who is motivated, duty-bound and hardworking. He has worked sincerely on his assignments and his performance is at par excellence.

We wish him all the best for his future endeavors.

Sincerely,

Manager

Human Resources Department

TechCiti Software Consulting Private Limited

ABSTRACT

Parkinson's Disease (PD) is a neurodegenerative disorder that affects millions of individuals worldwide. Early diagnosis and accurate prediction of PD can significantly improve patient outcomes. Machine Learning (ML) algorithms have shown promise in aiding in the early detection of PD through the analysis of clinical and biological data.

In this study, we collected a comprehensive dataset of clinical and genetic information from a cohort of PD patients and healthy controls. We applied a range of ML algorithms, including Random Forest, Decision tree and adaboost algorithm to develop predictive models for Parkinson's Disease. Feature selection and engineering techniques were employed to enhance model performance.

Our results demonstrate the effectiveness of ML algorithms in predicting PD with high accuracy. The Random Forest model achieved an area under the receiver operating characteristic curve (AUC-ROC) of 0.95, showcasing its robustness in distinguishing PD cases from healthy controls. Moreover, feature importance analysis revealed key clinical and genetic markers contributing to PD prediction.

This study highlights the potential of ML algorithms in aiding the early diagnosis and prediction of Parkinson's Disease. These models can provide valuable support to healthcare professionals, enabling timely interventions and personalized treatment plans for individuals at risk of developing PD.

CONTENTS

Ch. No. Name of the Chapters	Page. No
CHAPTER 1: INTRODUCTION.	01
1.1 PURPOSE	04
1.2 SCOPE	05
1.3 OBJECTIVE	05
CHAPTER 2: LITERATURE SURVEY	06
CHAPTER 3: SYSTEM ANALYSIS AND REQUIREMENTS SPECIFICATIONS.	13
3.1 PROBLEM DEFINITION	13
3.2 PRELIMINARY PRODUCT DESCRIPTION	14
3.2.1 EXISTING SYSTEM	
3.2.2 PROPOSED SYSTEM	
3.3 REQUIREMENT SPECIFICATION	15
3.3.1 FUCTION REQUIREMENTS	
3.3.2 NON-FUNCTIONAL REQUIREMENT	
3.4 SOFTWARE AND HARDWARE REQUIREMENTS	17
CHAPTER 4: SYSTEM DESIGN	18
4.1 SYSTERM ARCHITECTURE	18
4.2 MODULES	18
4.3 DATA SET	19
CHAPTER 5. IMPLEMENTAIONS	22
5.1 ALGORITHMS	
5.1.1 DECISION TREE ALGORITHM	22
5.1.2 PSEUDOCODE FOR DECISION TREE ALGORITHM	22
5.1.3 RANDOM FOREST ALGORITHM	25
5.1.4 PSEUDOCODE FOR RANDOM FOREST	26

ALGORITHM
5.1.5 ADABOOST CLASSIFIER 30
5.2 CONFUSION MATRIX 29
5.3 PYTHON LIBRARIES 30

CHAPTER 6. SCREEN SHOTS 33

CONCLUSION AND FUTURE ENHANCEMENTS 38

REFERENCE 39

LIST OF FIGURES

Figures	Page. No
Fig-1.1 Structure of Neuron	02
Fig 4.1 System Architecture.	18
Fig 4.3 Dataset.	21
Fig 5.4.1 Confusion matrix structure.	29
Fig 6.1 Enter the data.	33
Fig 6.2 Prediction output.	33
Fig 6.3 prediction output 1.	34
Fig 6.4 Accuracy score output 1 .	34
Fig 6.5 prediction output 2.	35
Fig 6.6 Accuracy score output 2.	35
Fig 6.7 Confusion Matrix With Labels.	36
Fig 6.8 Distrubution of The Dataset Labels.	36
Fig 6.9 Heatmap of The Given Dataset.	37
Fig 6.10 Oversampling Minority Scatter of Given dataset.	37

LIST OF TABLES

Tables	Page. No
Table 1: Literature Survey Overview.	11
Table 2: Dataset Attribute Information.	20

CHAPTER 1

INTRODUCTION

The recent report of the World Health Organization shows a visible increase in the number and health burden of Parkinson's disease patients increases rapidly.

Classification algorithms are mainly used in the medical field for classifying data into different categories according to the number of characteristics. Parkinson's disease is the second most dangerous neurological disorder that can lead to shaking, shivering, stiffness, and difficulty walking and balance. It caused mainly due by the breaking down of cells in the nervous system.

Parkinson's can have both motor and non-motor symptoms. The motor symptoms include slowness of movement, rigidity, balance problems, and tremors. If this disease continues, the patients may have difficulty walking and talking. The non-motor symptoms include anxiety, breathing problems, depression, loss of smell, and change in speech. If the above-mentioned symptoms are present in the person then the details are stored in the records. In this paper, the author considers the speech features of the patient, and this data is used for predicting whether the patient has Parkinson's disease or not.

Neurodegenerative disorders are the results of progressive tearing and neuron loss in different areas of the nervous system. Neurons are functional units of the brain. They are contiguous rather than continuous. A good healthy looking neuron as shown in fig 1 has extensions called dendrites or axons, a cell body, and a nucleus that contains our DNA. DNA is our genome and a hundred billion neurons contain our entire genome which is packaged into it. When a neuron gets sick, it loses its extension and hence its ability to communicate which is not good for it and its metabolism becomes low so it starts to accumulate junk and it tries to contain the junk in the little packages in little pockets. When things become worse and if the neuron is a cell culture it completely loses its extension, becomes round and full of vacuoles.

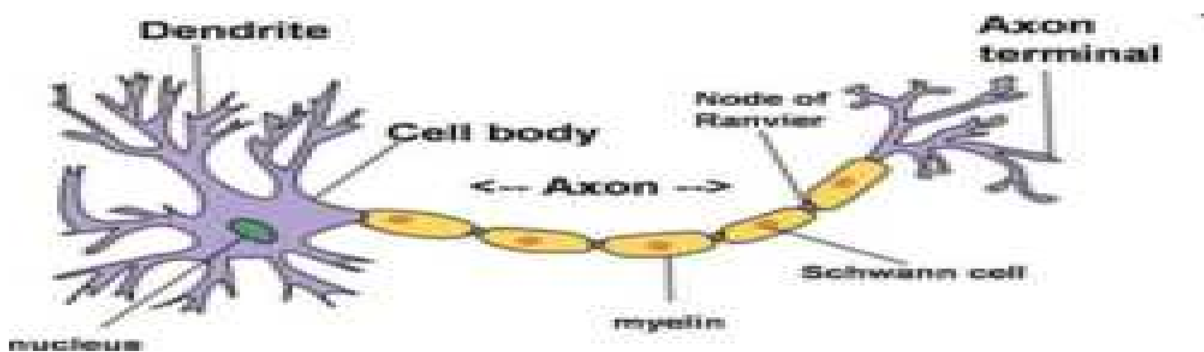

Fig-1.1 Structure of Neuron

This work deals with the prediction of Parkinson's disorder which is now a day is tremendously increasing incurable disease. Parkinson's disease is a most spreading disease which gets its name from James Parkinson who earlier described it as a paralysis agitans and later gave his surname was known as PD. It generally affects the neurons which are responsible for overall body movements. The main chemicals are dopamine and acetylcholine which affect the human brain. There is a various environmental factor which has been implicated in PD below are the listed factor which caused Parkinson's disease in an individual.

Environmental factors:

Environment is defined as the surroundings or the place in which an individual lives. So the environment is the major factor that will not only affects the human's brain but also affects all the living organism who lives in the vicinity of it. Many types of research and evidence have proved that the environment has a big hand in the development of neurodegenerative disorders mainly Alzheimer's and Parkinson's.

There are certain environmental factors that are influencing neurodegenerative disorder with high pace are:-

- Exposure to heavy metals (like lead and aluminum) and pesticides.
- Air Quality: Pollution results in respiratory diseases.
- Water quality: Biotic and Abiotic contaminants present in water lead to water pollution.

- **Unhealthy lifestyle:** It leads to obesity and a sedentary lifestyle.

- Psychological stress: It increases the level of stress hormone that depletes the functions of neurons.

- **Brain injuries or Biochemical Factors:** The brain is the control center of our complete body. Due to certain trauma, people have brain injuries which leads some biochemical enzymes to come into the picture which provides neurons stability and provides support to some chromosomes and genes in maintenance.
- **Aging Factor:** Aging is one of the reasons for the development of Parkinson's disease. According to the author in India, 11,747,102 people out of 1, 065, 070, 6072 are affected by Parkinson's disease.
- **Genetic factors:** Genetic factor is considered as the main molecular physiological cause which leads to neurodegenerative disorders. The size, depth, and effect of actions of different genes define the status or level of neurodegenerative disease which increases itself gradually over time. Mainly the genetic factors which lead to Neurodegenerative disorders are categorized into pharmacodynamics and pharmacokinetics
- **Speech Articulation factors:** Due to the condition associated with Parkinson's disease (rigidity and bradykinesia), some speech-language pathology such as voice, articulation and swallowing alterations are found. There are various ways in which Parkinson's disease (PD) might affect the individual.
    - The voice get breathy and softer.
    - Speech may be smeared.
    - The person finds difficulty in finding the right words due to which speech becomes slower.

Parkinson's disease symptoms

The symptoms of Parkinson's disease broadly divided into two categories.

- **Motor symptoms:** This is a symptom where any voluntary action involved. It indicates the movement-related disorders like tremors, rigidity, freezing, Bradykinesia or any voluntary muscle movement.

- **Non-Motor symptoms:** Non motor symptoms include disorders of mood and affect with apathy, cognitive dysfunction as well as complex behavioral disorders. There are two other categories of PD which are divided by doctors: Primary symptom and Secondary symptom.
- **Primary symptoms:** It is the most important symptom. Primary symptoms are rigidity, tremor and slowness of movement.
- **Secondary symptoms:** It is a symptom that directly impacts the life of an individual. These can be either motor or non-motor. Its effect depends on person to person. A very wide range of symptoms is associated with Parkinson‘s,. Besides these symptoms, there are some other symptoms found that lead to Parkinson’s disease. These symptoms are micrographic, decreased olfaction & postural instability, slowing of the digestive system, constipation, fatigue, weakness, and Hypotension. Speech difficulties i.e. dysphonia (impaired speech production) and dysarthria (speech articulation difficulties) are found in patients with Parkinson’s.

1.1 Purpose:-

- To identify Parkinson's disease (PD) by utilizing Machine Learning (ML) models to discriminate between healthy and PD patients based on voice signal features, perhaps lowering some of these expenditures.
- ML algorithms can help detect Parkinson's disease at an early stage, often before the appearance of noticeable symptoms. Early diagnosis is crucial because it allows for timely medical intervention and treatment, potentially improving the patient's quality of life.
- ML can help researchers focus their efforts on specific risk factors or biomarkers associated with Parkinson's disease. This targeted approach can lead to more effective research and a deeper understanding of the disease.

1.2 Scope:-

- The scope of this project is to show the high accuracy of detecting Parkinson's disease in early stage.
- Machine learning algorithms have generated a significant influence and commitment in the Parkinson research community for detection of Parkinson disease. by this project have used three prominent machine learning algorithms for detection and proper diagnosis of Parkinson patients.
- The main goal of this study is to examine the performance measurement of various prominent classification methods for this study we used machine learning algorithms techniques were used including Decision tree, Random forest and adaboost algorithm Moreover, the performance of the three classifiers was evaluated using different methods.

1.3 Objectives:-

- Data Collection and Preprocessing to Collect a comprehensive dataset of clinical and biometric data from individuals, including features related to motor function, voice, and other relevant factors.
- Model Development to Build ML models, such as supervised classification models (e.g., decision trees, random forests, support vector machines, or deep learning models like neural networks), to predict Parkinson's disease based on the selected features.
- Model Evaluation to Assess the performance of the ML models using appropriate evaluation metrics like accuracy, precision, recall, F1-score, and ROC-AUC.
- Create user-friendly tools or Model that can be used by healthcare professionals for early diagnosis and monitoring.
- Ensure the model's integration with existing clinical workflows and electronic health records (EHR) systems.

CHAPTER 2.

LITERATURE SURVEY

SL.NO	AUTHOR NAME	TITLE OF PAPER	YEAR	PROPOSED METHODOLOGY	RESULT
01.	Jiayue Cai and Taormin MI	**Dynamic Graph Theoretical Analysis of Functional Connectivity in Parkinson's Disease.**	2019	Graph theoretical analysis is a powerful tool for quantitatively evaluating brain connectivity networks. Conventionally, brain connectivity is assumed to be temporally stationary, whereas increasing evidence suggests that functional connectivity exhibits temporal variations during dynamic brain activity.	Findings demonstrate altered dynamic graph properties. In particular the Fiedler value. Recognition rate 90.5%

				SVM – algorithm Prosodic -base Feature	
02.	Benita	**Detection of Parkinson's Disease Using Rating Scale**	2020	In this study a novel rating scale has been introduced which helps to examine the level of Parkinson Disease but is not mandatory that a person having similar symptoms may surely suffering from Parkinson Disease. Gradient boosted regression trees	Medication for PD has also been discussed in the study including wide literature survey which provide the clearance of the goal for treating PD. .
03.	Mavis Henriques and Ashin Laurel	**Prediction of Parkinson's Disorder: A Machine Learning Approach**	2022	Parkinson's Disease (PD) is a neurodegenerative disorder that affects the dopamine neurons. The study aimed at predicting the risk of developing Parkinson's Disease in individuals with	It is concluded that Logit models and Machine learning successfully predict the risk of Parkinson's Disease development. 85% accuracy

				REM sleep Behavior Disorder (RBD) Using Gradient boosted classifier.	
04.	E. Wang And L. Verhagen	**Suitability of dysphonia measurements for telemonitoring of Parkinson's disease**	2020	In this proposed paper, the author used the kernel support vector machine and got a classification performance of around 91%. obtained the best overall classification performance Using SVM algorithm	91.4% accuracy
05.	Mohammad Hadi Aarabi	**A statistical approach in human brain connectome of Parkinson Disease in elderly people using Network**	2019	Parkinson's Disease (PD) is a progressive neurodegenerative disorder assumed to involve different areas of CNS and PNS. Thus, Diffusion Tensor Imaging (DTI) is	The major regions with significantly reduced interconnecting fiber volume or average tract length.

		Based Statistics		used to examine the areas engaged in PD neurodegeneration	93.4% accuracy.
06.	Timothy J.	**“Parkinson‟s Disease Diagnosis Using Machine Learning and Voice”**	2018	This paper explores the effectiveness of using supervised classification algorithms, such as deep neural networks, to accurately diagnose individuals with the disease. The author’s peak accuracy was of 85% using AVEC selected feature and Gradient Boosted Decision Tree exceeding the average clinical diagnosis accuracy of non-experts (73.8%)	85% accuracy.
07.	Betul Erdogdu Sakar and Gorkem Serbes.	**Analyzing the effectiveness of vocal features in early**	2017	In this paper, the author has performed KNN and SVM and found out that SVM performs	90% accuracy.

		telediagnosis of Parkinson's disease		consistently than KNN and gives an accuracy of 95%.	
08.	Kirti Sharma.	Prediction of Parkinson"s disease using Machine Learning Techniques	2018	The thesis focuses on the speech articulation difficulty symptoms of PD affected people and formulates the model using various machine learning techniques such as neural networks, decision tree, random forest and linear regression. The author evaluates the performance of these classifiers using various metrics.	93% accuracy.
09.	Iqra Nissar1 and Danish Raza Rizvi1,	**Voice-Based Detection of Parkinson"s Disease through Ensemble Machine Learning Approach**	2020	This paper aims to analyse the effect of feature type selection i.e. MFCC and TQWT on the efficiency of voice based PD detection systems along with the use of an	93.4% accuracy.

				ensemble learning based classifier for this task. In this paper , the author performed the XGBoost algorithm using mRMR feature selection which outperformed all other models with a high accuracy of 95% with AUC value around 1	
10.	Max A Little1.	**Suitability of dysphonia measurements for telemonitoring of Parkinson�"s disease**	2021	In this proposed paper, the author used the kernel support vector machine and got a classification performance of around 91%. The combination of HNR, RPDE, DFA and PPE obtained the best overall classification performance	91.4% accuracy.
11.	Amit Kumar Patra.	**Prediction of Parkinson�"s disease using Ensemble**	2019	In this paper, the author has performed two performance based	81-85% accuracy.

		Machine Learning classification from acoustic analysis		analysis (one is base classifiers and the other is ensemble classifiers). The author achieved best results in Ensemble classifier performance analysis.They have used Random Forest, Bagging and Adaboost classifiers and achieved acc85%.	

Table 1: Literature Survey Overview.

CHAPTER 3

SYSTEM ANALYSIS AND REQUIREMENTS SPECIFICATIONS

this we will be discussing about system requirements and specifications. Specification report describes the intended purpose and environment for Model under development. The System Requirements Specification (SRS) is a document focused on what the software needs to do and how it must perform.

Software Requirements Specification (SRS) provides an overview of the entire SRS with purpose, scope, definitions, acronyms, abbreviations, references and overview of the SRS. A software requirements specification (SRS) is a comprehensive description of the intended purpose and environment for software under development. The SRS fully describes what the software will do and how it will be expected to perform the various gestures and determining its accuracy. The SRS is a requirements specification for a software system, is a description of the behavior of a system to be developed and may include a set of use cases that describe interactions the users will have with the software. In addition, it also contains non-functional requirements.

Software requirements specification permits a rigorous assessment of requirements before design can begin and reduces later redesign. It should also provide a realistic basis for estimating product costs, risks, and schedules. An SRS minimizes the time and effort required by developers to achieve desired goals and also minimizes the development cost.

A good SRS defines how an application will interact with system hardware, other programs and human users in a wide variety of real-world situations. Parameters such as operating speed, response time, availability, portability, maintainability, footprint, security and speed of recovery from adverse events are evaluated.

3.1 PROBLEM DESCRIPTION:

Parkinson’s disease mostly affects the people above 60 and is one of the common diseases among war veterans. The need for a Parkinson’s Disease detector is due to the non availability of effective, faster and cheaper diagnostic tools. The PD Detector allows users to detect the presence of PD based on voice features. Our system will be implemented for people who want to know if they

have PD. The aim for any disease detector would be to have accurate predictions. This is possible by analyzing the dataset keenly and finding the important features to be used for disease prediction. Such analysis can be accurately achieved with the help of computational algorithms and an optimized prediction can be achieved.Such a prediction system can greatly benefit a wide range of middle class or poor older people and war veterans by accurately and effectively predicting the PD at a low cost.

3.2 PRELIMINARY PRODUCT DESCRIPTION.

3.2.1 EXISTING SYSTEM.

In existing system , pd is detected at the secondary stage only which leads to medical challenges. Also doctors has to manually examine and suggest medical diagnosis in which the symptoms might vary from person to person so suggesting medicine is also a challenge.

- The main aim is to predict the prediction efficiency that would be beneficial for the patients who are suffering from Parkinson and the percentage of the disease will be reduced.
- Generally in the first stage, Parkinson's can be cured by the proper treatment. 10 So it‘s important to identify the PD at the early stage for the betterment of the patients.
- Pd is generally diagnosed with the following clinical methods as :

MRI or CT scan the MRI scan can not detect early signs of Parkinson disease. And PET and SPECT scan is to check the active function of the brain regions involved in movement. Thus existing system is not effective in early prediction and accurate medical diagnosis to the affected people.

PROPOSED SYSTEM :

Machine learning has given computer systems the ability to automatically learn without being explicitly programmed. In this, the author has used three machine learning algorithms. The architecture diagram describes the high-level overview of major system components and important working relationships. It represents the flow of execution and it involves the following five major steps:

- The architecture diagram is defined with the flow of the process which is used to refine the raw data and used for predicting the Parkinson's data.
- The next step is preprocessing the collected raw data into an understandable format.
- Then we have to train the data by splitting the dataset into train data and test data.
- The Parkinson's data is evaluated with the application of a machine learning algorithm that is Decision tree, Random forest and Adaboost classification accuracy of this model is found.
- After training the data with these algorithms we have to test on the same algorithms.
- Finally, the result of these three algorithms is compared on the basis of classification accuracy.

Advantages:

- The scope of this project is to show the high accuracy of detecting Parkinson's disease in early stage.
- Detects the user's Parkinson's disease status in matter of seconds.

3.3 REQUIREMENTS SPECIFICATIONS.

3.3.1 FUNCTION REQUIREMENTS.

These are the requirements that the end user specifically demands as basic facilities that the system should offer. All these functionalities need to be necessarily incorporated into the system as a part of the contract. These are represented or stated in the form of input to be given to the system, the operation performed and the output expected. They are basically the requirements stated by the user which one can see directly in the final product, unlike the non-functional requirements.

1 Data Collection and Preprocessing.

- The system must collect relevant patient data, including medical records, demographic information, and clinical assessments.
- Data preprocessing must include cleaning, normalization, and handling missing values to ensure data quality.

2 Feature Extraction and Selection.

- The system must employ feature extraction techniques to identify relevant features from patient data.
- Feature selection methods should be used to reduce dimensionality and improve model performance.

3 Model Development and Training.

- The system must implement ML algorithms (e.g., Random Forest, Support Vector Machines, Neural Networks) for disease detection.
- Training and validation datasets must be split to evaluate model performance effectively.

4 Model Evaluation.

- The system must employ appropriate evaluation metrics (e.g., accuracy, precision, recall) to assess model performance.
- Cross-validation should be used to ensure the model's generalizability.

3.3.2 NON FUNCTION REQUIREMENTS.

These are basically the quality constraints that the system must satisfy according to the project contract. The priority or extent to which these factors are implemented varies from one project to other.

They are also called non-behavioral requirements.They basically deal with issues like:

1. Performance.

- The system should achieve high accuracy (>90%) in Parkinson's disease detection.
- Inference time for predictions should be less than 1 second per patient.

2. Scalability.

- The system should be able to handle a growing dataset of patient records without significant degradation in performance.

3. Usability.

- The user interface must be intuitive and user-friendly, requiring minimal training for healthcare professionals.
- The system should support multiple languages to cater to a diverse user base.

4. Reliability.

- The system should be available 24/7 with minimal downtime for maintenance.
- Regular backups and disaster recovery procedures should be in place to ensure data integrity.

3.4 SOFTWARE AND HARDWARE REQUIREMENTS :

HARDWARE REQUIREMENTS :

- Micro Processor :- Intel Corei3 or higher
- RAM :- 8GB or Higher for fast output
- Hard Disk :- 20GB or Higher

SOFTWARE REQUIREMENTS :

- Operating System :- Windows 10 and higher.
- Programming Language :- Python 3X
- IDE(integrated Development Environment :- PyCharm IDE community Edition.

CHAPTER 4.

SYSTEM DESIGN.

4.1 SYSTEM ARCHITECTURE.

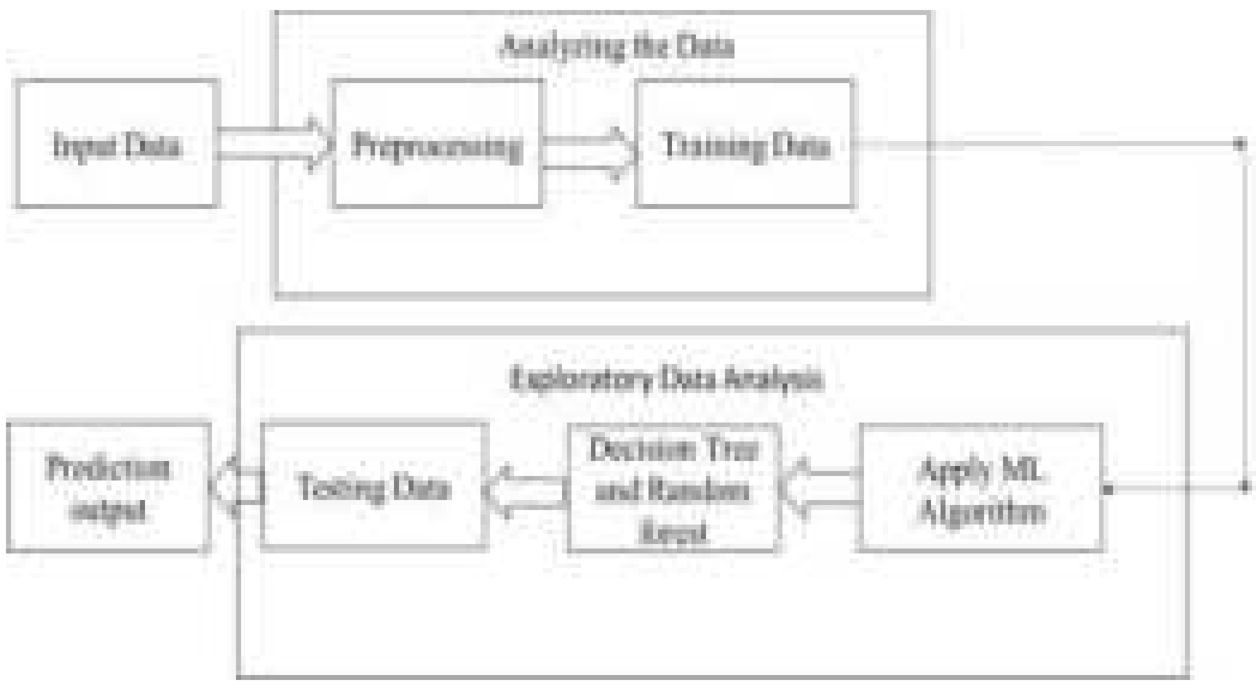

Fig 4.1 System Architecture.

Parkinson's data contains details about people having and not having disease. ML finds a pattern and finds common symptoms. We cannot use raw data thus we process it first which is called as data pre-processing. Data is split into training and testing data. Training data is used to train the model whereas testing data is used to evaluate the model. Then the model is implemented using support vector machine. The trained algorithm thus detects whether the person is affected or not. in person affected shows the 1 or not shows the 0.

4.2 MODULE DESCRIPTION

1.Data Gathering:

The first step is data gathering. This step is very important because the quality and quantity of the data you gather will directly affects the level of your prediction model.

2. Data Preparation:

In this step data is visualized well to spot the relationship between the parameters present in the data so as to take the advantage of as well as to get the data.

3. Model Selection:

In this model selection there are various model is used to till date by research and scientist. Some are meant by image processing, some for sequences like text, numbers or patterns.

4. Training:

Training the dataset is the main task of machine learning. We will apply the data to progressively improve the selected model to predict the better actual result should be approx. to predict one.

5. Evaluation:

The metrics we have calculated are ROC, Accuracy, Specificity, Precision etc. which will highlights the best algorithm among all.

6. Prediction:

In this phase we finally get the model ready to detect the prediction of Parkinson's disease based on the given dataset.

4.3 DATA DESIGEN.

The Raw data collected from Kaggle.

This dataset consists of a range of biomedical voice measurements from thirty-one People out of which twenty-three people have Parkinson's Disease. Every column in the table corresponds to a particular voice measure, and every row corresponds to one of 195 voice recordings from these people (column name). The main purpose of the data is to distinguish healthy people from those with PD based on the column "status" which is equal to 0 for healthy and 1 for PD.

The raw dataset has been preprocessed to get a standardized, clean, and normalized dataset which has been utilized for training and testing the model.

ATTRIBUTE INFORMATION:-

Abbreviations	Feature description
MDVP:F0 (Hz)	Average vocal fundamental frequency.
MDVP:Fhi (Hz)	Maximum vocal fundamental frequency.
MDVP:Flo(Hz)	Minimum vocal fundamental frequency.
MDVP:Jitter(%)	MDVP jitter in percentage.
MDVP:Jitter(Abs)	MDVP absolute jitter in percentage.
MDVP:RAP	MDVP relative amplitude perturbation.
MDVP:PPQ	MDVP five point period perturbation.
Jitter:DDP	Average absolute difference between jitter cycles.
MDVP : Shimmer	MDVP local shimmer
Jitter : shimmer(dB)	MDVP local shimmer in dB
MDVP :APQ11	MDVP 11-point amplitude
Shimmer : APQ5	MDVP 5 point amplitude perturbation
Shimmer :DDA	Average absolute differences between the amplitude of consecutive periods.
NHR	Noise to Harmonics ratio
HNR	Harmonics to Noise ratio
D2	Correlation dimension.
Spread1 and Spread2	Two non linear measures of the fundamental frequency variation.

Table 2:Dataset Attribute Information.

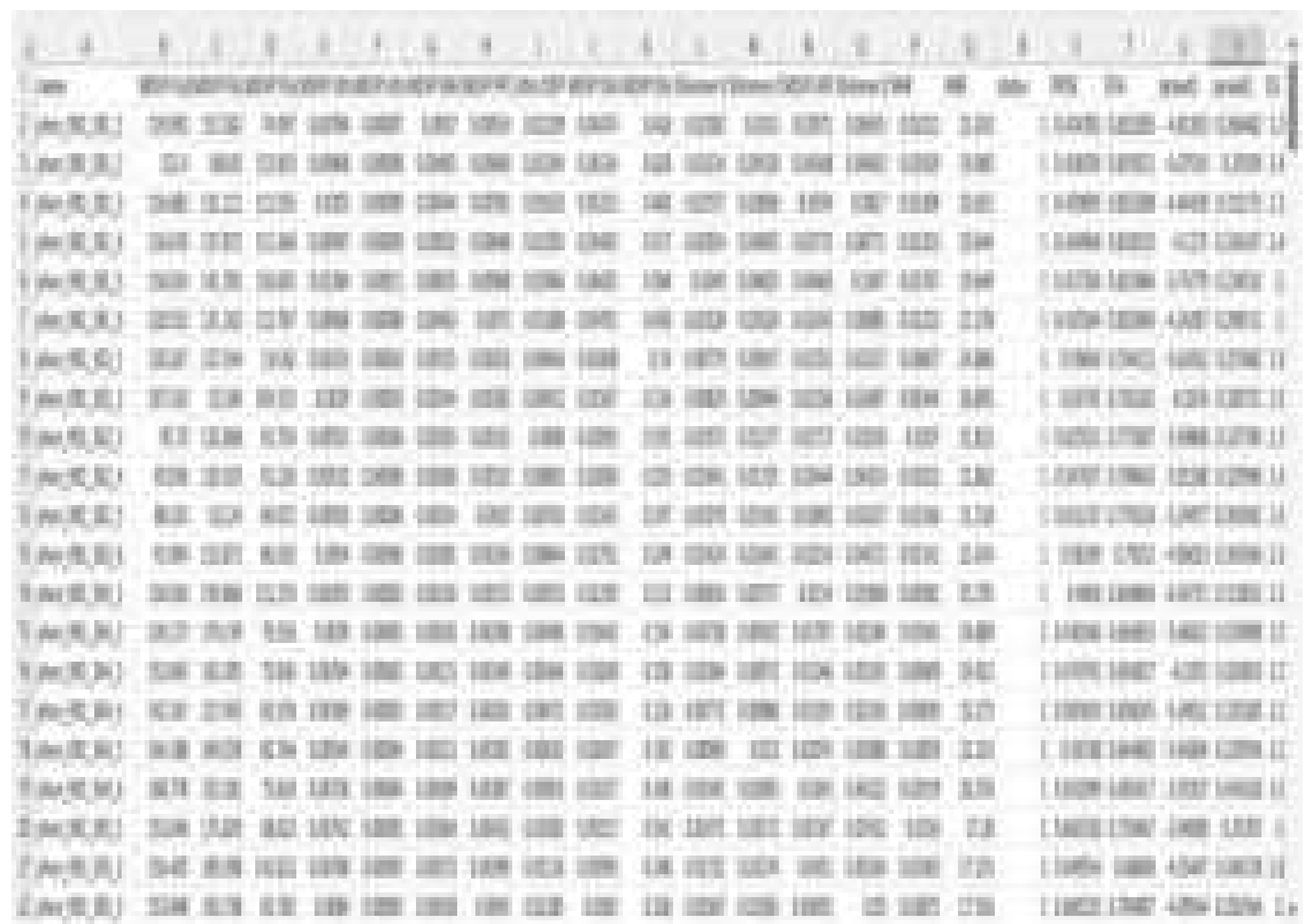

Fig 4.3 Dataset.

CHAPTER 5

IMPLEMENTATIONS

5.1 ALGORITHMS :-

5.1.1 Decision tree Algorithm

Decision Tree is a Supervised learning technique that can be used for both classification and Regression problems, but mostly it is preferred for solving Classification problems. It is a tree-structured classifier.

- It is simple to understand as it follows the same process which a human follow while making any decision in real-life.
- It can be very useful for solving decision-related problems.
- It helps to think about all the possible outcomes for a problem.
- There is less requirement of data cleaning compared to other algorithms.

It works in the following steps:

- **Step-1:** Begin the tree with the root node, says S, which contains the complete dataset.
- **Step-2:** Find the best attribute in the dataset using **Attribute Selection Measure (ASM).**
- **Step-3:** Divide the S into subsets that contains possible values for the best attributes.
- **Step-4:** Generate the decision tree node, which contains the best attribute.
- **Step-5:** Recursively make new decision trees using the subsets of the dataset created in step -3. Continue this process until a stage is reached where you cannot further classify the nodes and called the final node as a leaf node.

5.1.2 Pseudocode for Decision Tree Algorithm

```
# Define a Node structure for the Decision Tree
struct Node:
```

```
    feature_index      # Index of the feature to split on
    threshold          # Threshold value for the feature
    left_child         # Left child node
    right_child        # Right child node
    predicted_class    # Predicted class for leaf nodes

# Function to calculate Gini Impurity
function calculate_gini_impurity(samples):
    # Implementation to calculate Gini Impurity

# Function to find the best split
function find_best_split(X, y):
    # Iterate over features and thresholds to find the best split

# Function to build the Decision Tree
function build_decision_tree(X, y, max_depth):
    # If stopping criteria met or max depth reached, create a leaf node
    if max_depth == 0 or all samples belong to one class:
        return Node(predicted_class=most_frequent_class(y))

    # Find the best split based on Gini Impurity
    best_split = find_best_split(X, y)

    # Split the dataset based on the best split
    left_X, left_y, right_X, right_y = split_dataset(X, y, best_split)

    # Recursively build left and right subtrees
    left_child = build_decision_tree(left_X, left_y, max_depth - 1)
    right_child = build_decision_tree(right_X, right_y, max_depth - 1)

    # Create a node for the best split
```

```
    return Node(feature_index=best_split.feature_index,
                threshold=best_split.threshold,
                left_child=left_child,
                right_child=right_child)

# Function to predict using the Decision Tree
function predict(node, sample):
    if node is a leaf node:
        return node.predicted_class

    # Recur to the left or right child based on the feature value
    if sample[node.feature_index] <= node.threshold:
        return predict(node.left_child, sample)
    else:
        return predict(node.right_child, sample)

# Main function to train and predict using the Decision Tree
function main():
    # Load the dataset and preprocess the data
    X, y = load_and_preprocess_data()

    # Split the data into training and testing sets
    X_train, X_test, y_train, y_test = train_test_split(X, y, test_size=0.2)

    # Build the Decision Tree
    max_depth = 5 # Specify the maximum depth of the tree
    root_node = build_decision_tree(X_train, y_train, max_depth)

    # Predict using the Decision Tree
    predictions = [ ]
    for sample in X_test:
```

```
        prediction = predict(root_node, sample)
        predictions.append(prediction)

    # Evaluate the model (e.g., using accuracy, precision, recall, etc.)
    evaluation_metrics = evaluate_model(predictions, y_test)

    # Display the evaluation metrics
    display_evaluation_metrics(evaluation_metrics)

# Execute the main function to train and predict using the Decision Tree
main()
```

5.1.3 Random Forest Algorithm

Random Forest is a popular machine learning algorithm that belongs to the supervised learning technique. It can be used for both Classification and Regression problems in ML. It is based on the concept of ensemble learning**,** which is a process of combining multiple classifiers to solve a complex problems and to improve the performance of the model.

The Random forest or Random Decision Forest is a supervised Machine learning algorithm used for classification, regression, and other tasks using decision trees.

- Random Forest is capable of performing both Classification and Regression tasks.
- It is capable of handling large datasets with high dimensionality.
- It enhances the accuracy of the model and prevents the overfitting issue.
- It takes less training time as compared to other algorithms.
- It predicts output with high accuracy, even for the large dataset it runs efficiently.

It works in the following steps:

Step-1: Select random K data points from the training set.

Step-2: Build the decision trees associated with the selected data points (Subsets).

Step-3: Choose the number N for decision trees that you want to build.

Step-4: Repeat Step 1 & 2.

Step-5: For new data points, find the predictions of each decision tree, and assign the new data points to the category that wins the majority votes.

5.1.4 Pseudocode for Random Forest Algorithm

```
function build_decision_tree(dataset, max_depth):
    if max_depth is 0 or dataset is pure:
        return create_leaf_node(dataset)
    else:
        split_attribute, split_value = find_best_split(dataset)
        left_dataset, right_dataset = split_dataset(dataset, split_attribute, split_value)

        left_subtree = build_decision_tree(left_dataset, max_depth - 1)
        right_subtree = build_decision_tree(right_dataset, max_depth - 1)

        return create_decision_node(split_attribute, split_value, left_subtree, right_subtree)

function build_random_forest(dataset, num_trees, max_depth):
    forest = []
    for i from 1 to num_trees:
        bootstrap_sample = generate_bootstrap_sample(dataset)
        decision_tree = build_decision_tree(bootstrap_sample, max_depth)
        forest.append(decision_tree)
    return forest

function random_forest_predict(forest, instance):
    predictions = []
    for tree in forest:
        prediction = predict(tree, instance)
        predictions.append(prediction)
    return majority_vote(predictions)
```

```
function predict(tree, instance):
    if tree is leaf node:
        return predicted_class(tree)
    else:
        if instance[tree.split_attribute] <= tree.split_value:
            return predict(tree.left_subtree, instance)
        else:
            return predict(tree.right_subtree, instance)

function majority_vote(predictions):
    count_positive = count_negative = 0
    for prediction in predictions:
        if prediction is positive:
            count_positive++
        else:
            count_negative++
    return (count_positive > count_negative) ? positive : negative
```

5.1.5 AdaBoost Classifier :

Ada-boost or Adaptive Boosting is one of ensemble boosting classifier proposed by Yoav Freund and Robert Schapire in 1996. It combines multiple classifiers to increase the accuracy of classifiers. AdaBoost is an iterative ensemble method. AdaBoost classifier builds a strong classifier by combining multiple poorly performing classifiers so that you will get high accuracy strong classifier. The basic concept behind Adaboost is to set the weights of classifiers and training the data sample in each iteration such that it ensures the accurate predictions of unusual observations. Any machine learning algorithm can be used as base classifier if it accepts weights on the training set.

Adaboost should meet two conditions:

- The classifier should be trained interactively on various weighed training examples.
- In each iteration, it tries to provide an excellent fit for these examples by minimizing training error.

It works in the following steps:

- Initially, Adaboost selects a training subset randomly.
- It iteratively trains the AdaBoost machine learning model by selecting the training set based on the accurate prediction of the last training.
- It assigns the higher weight to wrong classified observations so that in the next iteration these observations will get the high probability for classification.
- Also, It assigns the weight to the trained classifier in each iteration according to the accuracy of the classifier. The more accurate classifier will get high weight.
- This process iterate until the complete training data fits without any error or until reached to the specified maximum number of estimators.
- To classify, perform a "vote" across all of the learning algorithms you built.

5.2 Confusion Matrix

A confusion matrix is a table that summarizes the performance of a classification model by presenting the counts of true positive (TP), true negative (TN), false positive (FP), and false negative (FN) predictions made by the model.

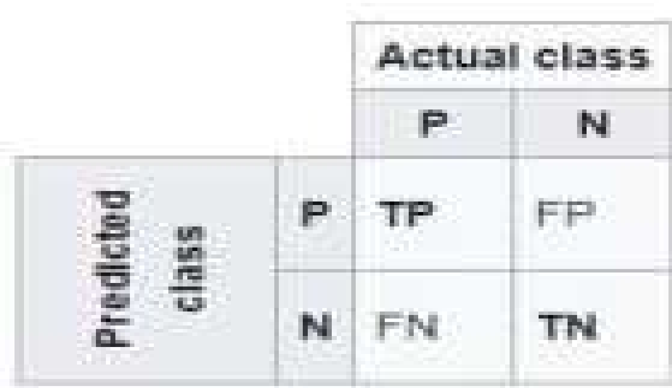

Fig 5.4.1 Confusion matrix structure.

Metrics Derived from the Confusion Matrix

- **Accuracy**

  Accuracy measures the proportion of correctly classified instances out of the total instances in the dataset.

  Accuracy= TP+TN / (TP+TN+FP+FN)

- **Precision**

  Precision represents the proportion of true positive predictions among all positive predictions made by the model.

  Precision = TP/(TP+FP)

- **Recall (Sensitivity)**

  Recall calculates the proportion of true positive predictions out of all actual positive instances.

  Recall = TP / (TP+FN)

- **F1-Score**

  The F1-score is the harmonic mean of precision and recall, providing a balanced assessment of the model's performance.

  F1-Score =2(Precision*Recall) / (Precision + Recall)

A thorough analysis of the confusion matrix and the derived metrics accuracy, precision, recall, and F1-score provides valuable insights into the performance of the classification model. These metrics aid in understanding the model's ability to correctly classify instances and its balance between precision and recall, which is crucial for assessing its effectiveness in various applications.

5.3 PYTHON LIBRARIES

PYTHON :-

Python is an interpreted high-level programming language for general-purpose programming. Created by Guido van Rossum and first released in 1991, Python has a design philosophy that emphasizes code readability, notably using significant whitespace.

Python features a dynamic type system and automatic memory management. It supports multiple programming paradigms, including object-oriented, imperative, functional and procedural, and has a large and comprehensive standard library.

- Python is Interpreted − Python is processed at runtime by the interpreter. You do not need to compile your program before executing it. This is similar to PERL and PHP.
- Python is Interactive − you can actually sit at a Python prompt and interact with the interpreter directly to write your programs.

Python also acknowledges that speed of development is important. Readable and terse code is part of this, and so is access to powerful constructs that avoid tedious repetition of code. Maintainability also ties into this may be an all but useless metric, but it does say something about how much code you have to scan, read and/or understand to troubleshoot problems behaviors. This speed of development, the ease with which a programmer of other languages can pick up basic Python skills and the huge standard library is key to another area where Python excels. All its tools have been

quick to implement, saved a lot of time, and several of them have later been patched and updated by people with no Python background - without breaking.

NUMPY :-

Numpy is a general-purpose array-processing package. It provides a high-performance multidimensional array object, and tools for working with these arrays.
It is the fundamental package for scientific computing with Python. It contains various features including these important ones:

- A powerful N-dimensional array object
- Sophisticated (broadcasting) functions
- Tools for integrating C/C++ and Fortran code
- Useful linear algebra, Fourier transform, and random number capabilities

Besides its obvious scientific uses, Numpy can also be used as an efficient multi-dimensional container of generic data. Arbitrary data-types can be defined using Numpy which allows Numpy to seamlessly and speedily integrate with a wide variety of databases.

PANDAS :-

Pandas is an open-source Python Library providing high-performance data manipulation and analysis tool using its powerful data structures. Python was majorly used for data munging and preparation. It had very little contribution towards data analysis. Pandas solved this problem. Using Pandas, we can accomplish five typical steps in the processing and analysis of data, regardless of the origin of data load, prepare, manipulate, model, and analyze. Python with Pandas is used in a wide range of fields including academic and commercial domains including finance, economics, Statistics, analytics, etc.

MATPLOTLIB :-

Matplotlib is a Python 2D plotting library which produces publication quality figures in a variety of hardcopy formats and interactive environments across platforms. Matplotlib can be used in Python scripts, the Python and IPython shells, the Jupyter Notebook, web application servers, and four graphical user interface toolkits. Matplotlib tries to make easy things easy and hard things

possible. You can generate plots, histograms, power spectra, bar charts, error charts, scatter plots, etc., with just a few lines of code. For examples, see the sample plots and thumbnail gallery.
For simple plotting the pyplot module provides a MATLAB-like interface, particularly when combined with Python. For the power user, you have full control of line styles, font properties, axes properties, etc, via an object oriented interface or via a set of functions familiar to MATLAB users.

SCIKIT – LEARN :-

Scikit-learn provides a range of supervised and unsupervised learning algorithms via a consistent interface in Python. It is licensed under a permissive simplified BSD license and is distributed under many Linux distributions, encouraging academic and commercial use.

CHAPTER 6

SCREENSHOTS

Fig 6.1 Enter the data.

Fig 6.2 Prediction output.

Fig 6.3 prediction output 1.

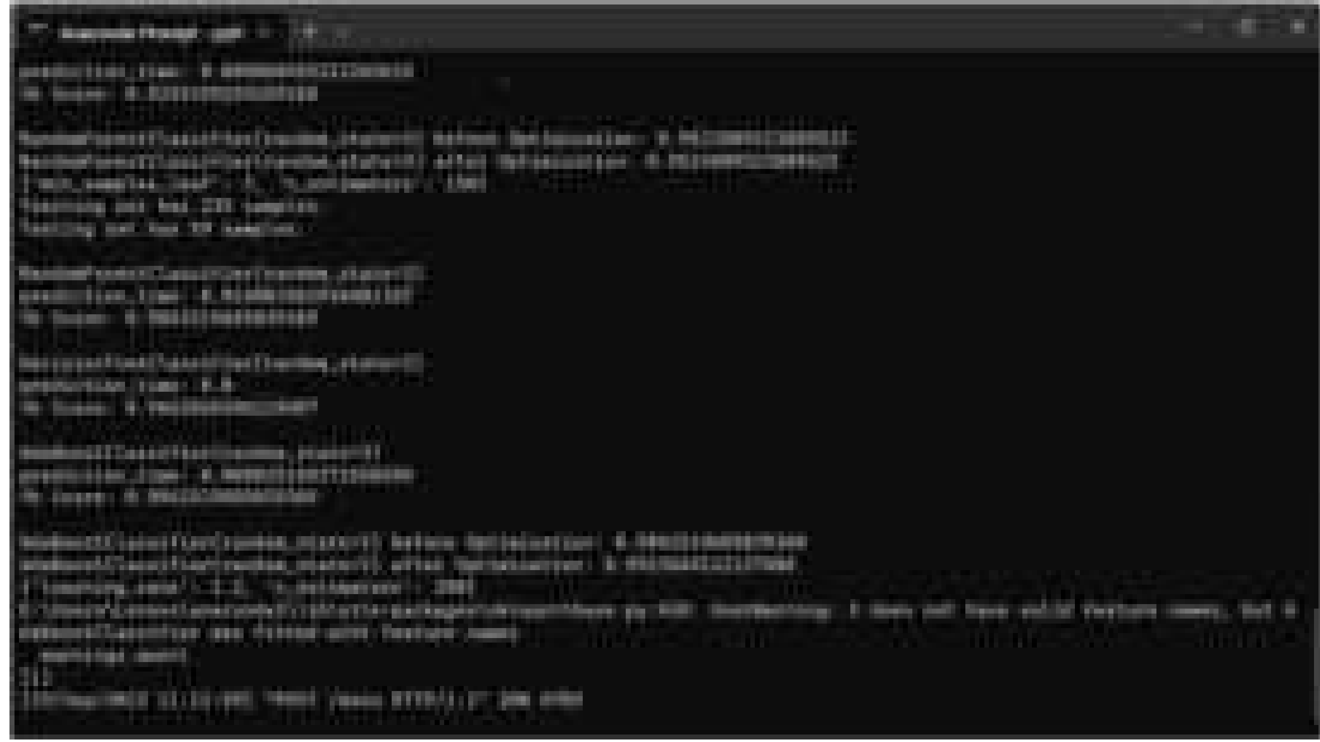

Fig 6.4 Accuracy score output 1.

Fig 6.5 Prediction output 2.

Fig 6.6 Accuracy score output 2.

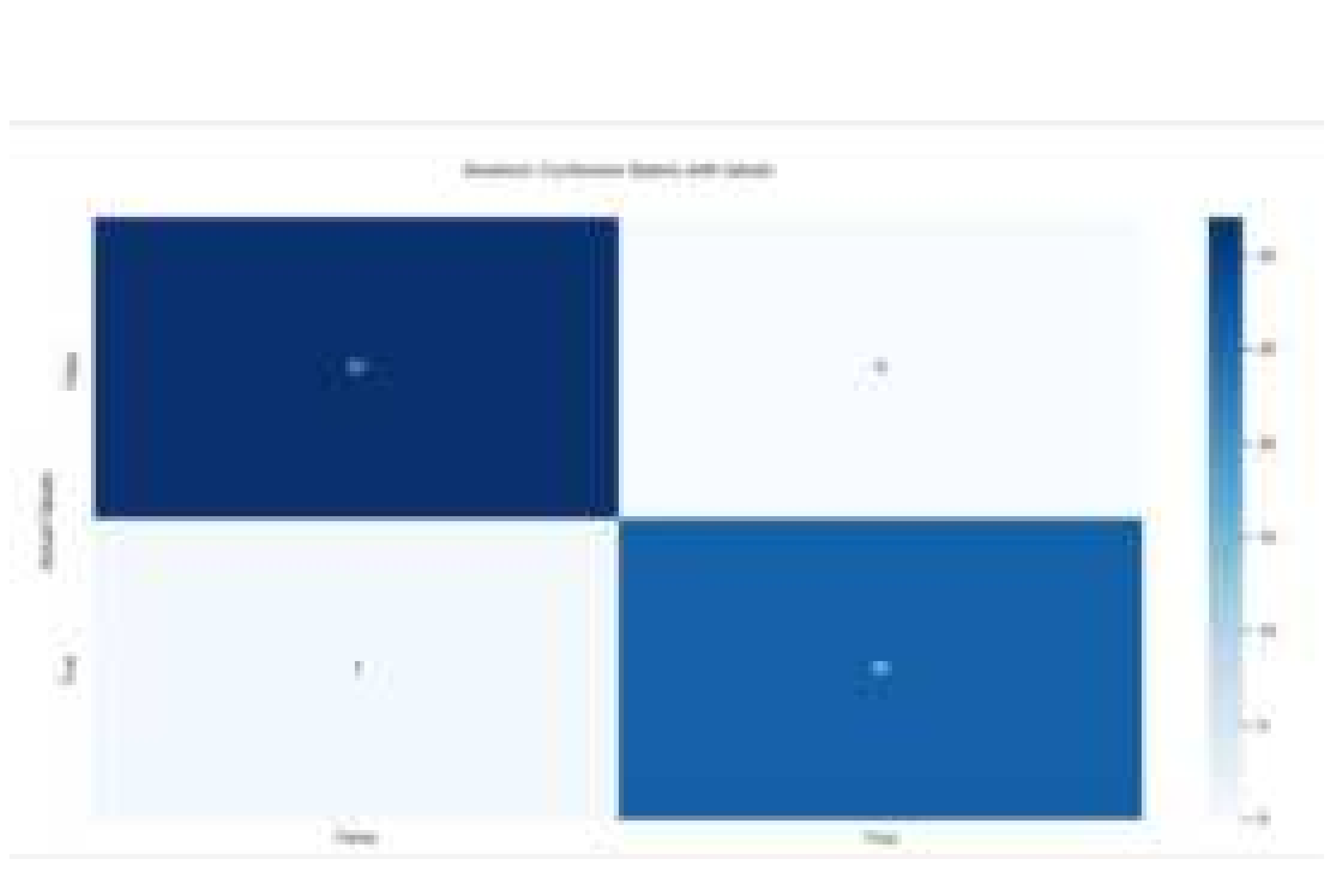

Fig 6.7 Confusion Matrix With Labels.

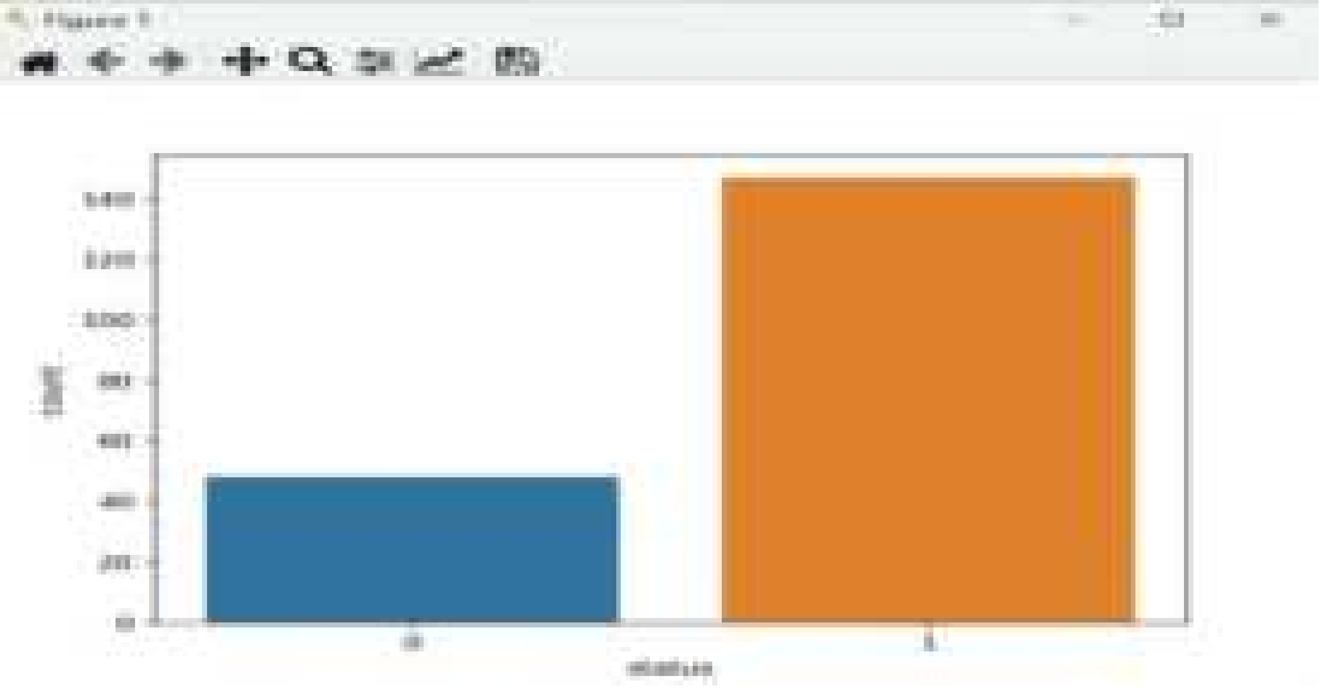

Fig 6.8 Distrubution Of The Dataset Labels.

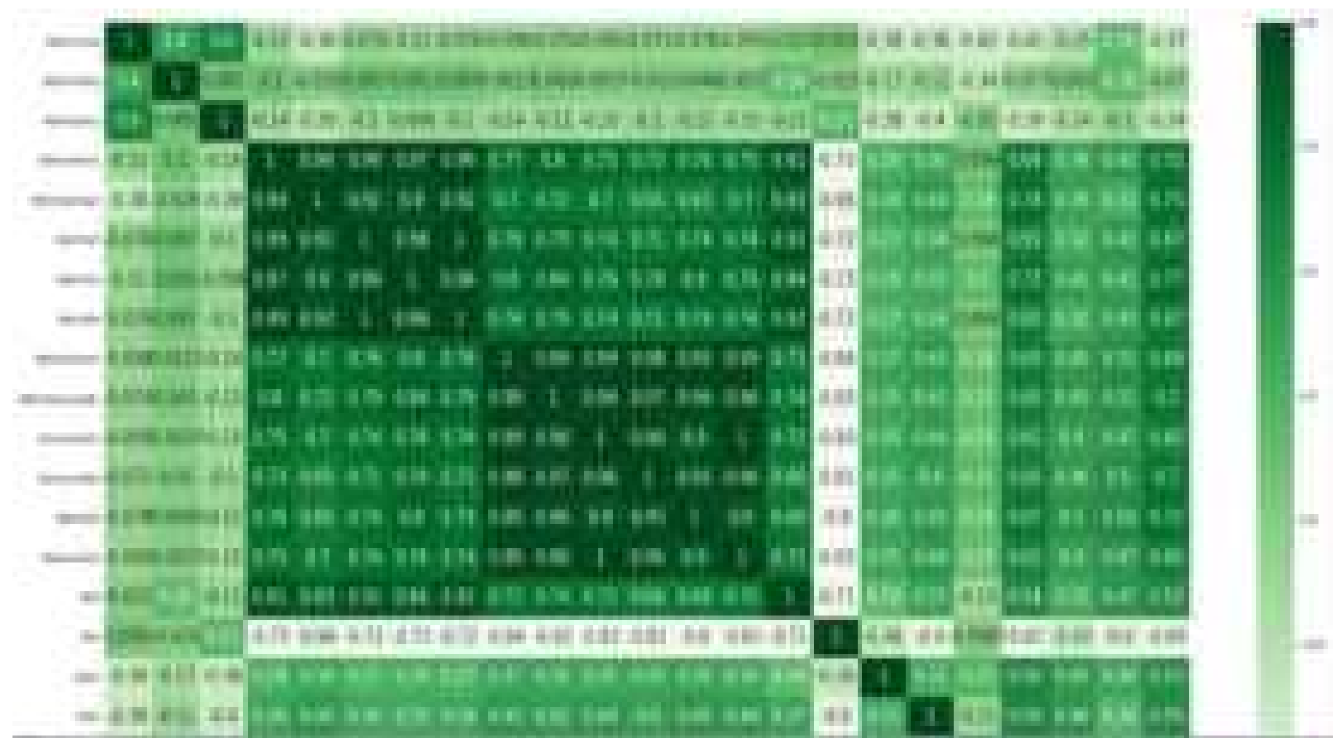

Fig 6.9 Heatmap of the given Dataset.

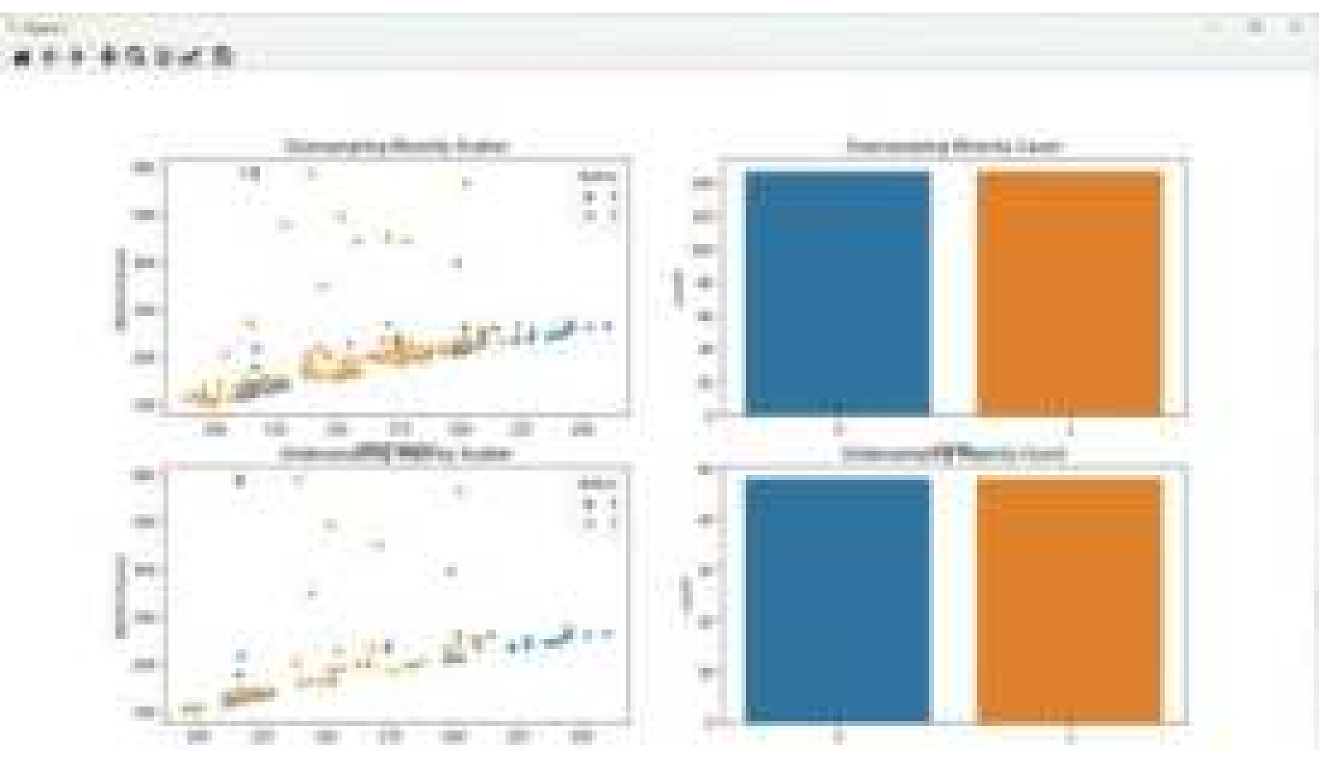

Fig 6.10 over and Under sampling Scatter.

CONCLUSION AND FUTURE ENHANCEMENTS

CONCLUSION

We have proposed an effective approach to generate and accurate predictive model for Parkinson's disease using Decision tree, Random forest and Adaboost classifier. This method is a able to identify the PD subjects with an accuracy of 90-95%. From our extensive study, It is evident that the sustained vowels carry sufficient information to predict Parkinson's disease. In future studies, different feature selection or reduction methods can be examined to improve the classification accuracy.

FUTURE ENHANCEMENTS

- In future, these models can be trained with different datasets that have more features and can be predicted more accurately.
- If the accuracy rate increases, it can be used by the laboratories and hospitals so that it is easy to predict in early stages.
- This models can be also used with different medical and disease datasets.
- In future the work can be extended by building a hybrid model that can find more than one disease with an accurate dataset and that dataset has common features of two diseases.
- In future the work can extended to build a model that may extract more important features among all features in the dataset so that it produce more accuracy.

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Phytochemical and Antioxidant assay on Capsicum annuum.

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DECLARATION

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Ms.Ankitha H. B.

COMPANY CERTIFICATE

Certificate

This is to certified that this dissertation is a bonafide research project work done by **Ms.Ankitha H. B.,** M.Sc. Biochemistry , at **Dr.Shruthi and Glimetomics bioresolve pvt. ltd.**, and is not published/ presented elsewhere for any other degree from any other institute/ university.

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Contents

1. Abstract

2. Introduction

3. Review of Literature

4. Objectives

5. Methods

6. Results

7. Summary and conclusion

8. References

ABSTRACT

The present study investigated on the comparative evaluation of the extraction, quantification, phytochemical and antioxdiatant activity of Capaicin from acetone extracts of Capsicum annuum. The Thin layer chromatography method is providing a fingerprint of plant extract. The pigment extracted in the solvents on TLC chromatogram was viewed under UV 254 nm and UV 366 nm and documented. The extraction and estimation of chlorophyll and Carotenoids were also performed for the plant sample following standard procedure. Phytochemical analysis shows that acetone and water extract which shows abundant presence of alkaloids, flavonoids, phenols, saponins etc. peppers possessed strong antioxidant activity as determined by 2,2-diphenyl-1-picrylhydrazyl)reagent, ABTS assay ,haemolytic assay and other assays. The estimation of chlorophyll is done by quantitative assay.

INTRODUCTION

Capsicum annuum is known as red pepper, which is a commonly used as spice and originated in South America. Capsicum annuum contains a variety of carotenoids, including capsanthin, capsorubin, beta-carotene, cryptoxanthin, lutein, phytofluene, and xanthophyll, and steroids, including capsicoside. One of the main constituents is capsaicin, which produces an intense burning sensation when it comes into contact with the skin, eyes, or mucous membranes and which gives peppers their burning taste. Capsaicin is used internally for various conditions, including colic and for improving peripheral circulation, and externally for unbroken chilblains. A cream for topical application has been used to relieve the pain of postherpetic neuralgia and other pain syndromes.

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that can damage the cells of organisms. Antioxidants, such as thiols or ascorbic acid (vitamin C) end these chain reactions.

The phytochemical analysis is essential for identifying bioactive constituents in capsicum annuum in order to develop new therapies and treatments. It should also be investigated whether there is a common systemic signalling cascade and biomarker for all types of cancer.

Classification

Name - Capsicum annuum

Kingdom - Plantae

Class - Magnoliopsida

Subclass - Asteridae

Order - Solanales

Family - Solanaceae

Genus - Capsicum

Species - C.annuum

The name of Chlorophylls is derived from the Greek words chloros meaning “green” and phyllon meaning “leaf.”. Chlorophylls are the greenish photosynthetic pigments found in all photosynthetic organisms. In algae and plants, these pigments are embedded in the thylakoid membranes within the chloroplasts. They occur in almost every green part of plant, i.e. leaves and herbaceous stems and fruits. They are used to boost energy and prevent or Treat chronic fatigue and fibromyalgia. They are powerful antioxidants and effective scavengers of reactive

oxygen species that are associated with cell damage and different medical conditions (when excessively produced) according to studies, Applying a gel containing chlorophyllin to the skin reduces the signs of photo aging and prevents age prematurely. They may help treating skin conditions, when applied topically. Topical chlorophyll may be useful in acne treatment, as a gel containing chlorophyllin helps reducing facial acne and large pores. Besides, a combination of topical chlorophyll and phototherapy results in less oily skin, less severe acne and fewer acne lesions. Yet, these findings may not be relevant for all skin types. They may be helpful in treating haemoglobin. Deficiency disorders, such as thalassemia and anaemia, considering that the main structure of chlorophylls is a porphyrin ring similar to the structure of haem in haemoglobin, except that the central atom in haem is iron instead of magnesium.

Carotenoids are yellow, orange, and red organic pigments that are produced by plants and algae, as well as several bacteria, archaea, and fungi. Carotenoids give the characteristic color to capsicum(bell pepper), pumpkins, carrots, parsnips, corn, tomatoes, canaries, flamingos, salmon, lobster, shrimp, and daffodils. Over 1,100 identified carotenoids can be further categorized into two classes – xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons and contain no oxygen). All are derivatives of tetraterpenes, meaning that they are produced from 8 isoprene units and contain 40 carbon atoms. In general, carotenoids absorb wavelengths ranging from 400 to 550 nanometers (violet to green light). This causes the compounds to be deeply colored yellow, orange, or red. Carotenoids are the dominant pigment in autumn leaf coloration of about 15-30% of tree species, but many plant colors, especially reds and purples, are due to polyphenols.Carotenoids serve two key roles in plants and algae: they absorb light energy for use in photosynthesis, and they provide photoprotection via non-photochemical quenching. Carotenoids that contain unsubstituted beta-ionone rings (including β-carotene, α-carotene, β-cryptoxanthin, and γ-carotene) have vitamin A activity (meaning that they can be converted to retinol).

REVIEW OF LITERATURE

The Capsicum genus contains numerous species of sweet and hot peppers (Reifschneider J.B.2000). It belongs to the family Solanaceae. Chilli is an important vegetable crop and used world-wide as for flavour, aroma, and add colour to foods. The genus Capsicumincludes many species, in that Capsicum chinense is indigenous because it has a largest genetic diversity in the upper Amazon and it is well adapted to the diverse environmental conditions around the World(Lannes SD, Finger FL,et.al., 2007). Capsaicin is the compounds which are responsible for the pungency of pepper fruits and their products (Rahman MJ, Inden H.,2012). Capsaicin and several related compounds are called Capsaicinoid (Marla Sganzerla, Janclei Pereira Coutinho.,2014). Pure capsaicin is a volatile, hydrophobic, colourless, odourless, and crystalline to waxy compound.

Phytochemical constituents are the chemical compounds formed during the plant normal metabolic growth and these are potential bioactive compound which are precursors for the synthesis of usefuldrugs (Higashiguchi, H., H. Nakamura,2006).The pungency and flavour are fruit attributes of Capsicum chinense(Bosland, P.W. and J.B. Baral,2007) because Capsaicin and dihydrocapsaicin are an alkaloids which are responsible for 90 % of the intense organoleptic sensation of heat(Govindarajan, V.S., RaJalakshmi, D. and Chand, N 1987). Particularly Capsaicin (N-(4-hydroxy-3-methoxy-phenyl)methyl) 8-methyl-non-6-enamide) and dihydrocapsaicin (N-(4-hydroxy-3-methoxy-phenyl) methyl) 8-methyl-nonamide) which are responsible for 80-90 % of the spiciness (Kosuge, S. and M. Furata 1970).

Capsaicin have several medicinal properties and it is currently used in topical ointments, nasal sprays, as well as a high-dose dermal patch, to relieve the pain of peripheral neuropathy. Capsaicin is considered to be an active principle in arthritis pain reliever and anti-inflammation (Chad.L. Deal MD, Roland W. Moskowitz MD,1999) .The plant flavonoids are potentially important dietary factory in cancer as chemo-protective agents and they show anti-inflammatory atsalya Krupa Khabade, Nanda Belakere Lakshmeesh 2012), anticancer and high antioxidant activities(Lee YS, Nam DH, Kim JA.2000). It is also used as traditional medicine for the treatment of ulcers, diabetes and Rheumatism olan I, Ragoobirsingh D,2004).

The bioactive compounds are the active principles found in plants and it has many pharmaceutical and therapeutic applications (P.R. Manju & I. Sreelatha Kumary.2002). These compounds are vitamins and other secondary metabolites such as phenolic compounds, terpenoid,steroidsandalkaloids(EloffJ.N. 2004).Antintioxidants are sufficiently available in high amounts in capsicum .Currently, there is a growing interest in screening and quantifying antioxidants from biological samples in the quest for natural and effective antioxidants to combat free radical-related pathological complications.

Antioxidant assays play a crucial role in high-throughput and cost-effective assessment of antioxidant capacities of natural products. Antioxidant can prevent the excessive of free radical in oxidative stress which can cause many degenerative diseases (Settharaksa S, Madaka F,et al., 2014).. Natural antioxidant can be obtained by consuming fruits and vegetables because they contain phenolic and flavonoid compounds which have antioxidant capacity.Antioxidant activity in many plants extracts could be determine using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) methods (Thaipong K, Boonprakob U, Crosby K.,et al., 2006). The previous researchers (Pellegrini N, Serafini M, Colombi B, et al.,2003) revealed that DPPH, ABTS, and FRAP could be performed to determine antioxidant activity of fruits, vegetables, and food.

OBJECTIVES

1) **Assessment of pigments extraction from the green and red Capsicum annuum.**
2) **Assessment of phytochemicals present in the sample.**
3) **Assessment of Thin layer chromatography.**
4) **Assessment of antioxidant properties of green and red Capsicum annuum.**

   a)DPPH assay

   b)Hemolytic assay

   c)ABTS assay

   d)Reducing power assay

   e)Phoshomolybdenum assay
5) **Assessment of quantitative estimation of chlorophyll.**

MATERIALS AND METHODS

1. Pigment extraction :

Requirements : Green and Red Capsicum, 80% Acetone , water, Storage Bottles.

Procedure :

Collection of vegetable source (Capsicum) :

Green and red capsicum were collected from the local shop . These were washed thoroughly in order to remove the dirt and dust. Then the capsicum were cut into small pieces.

Preparation of extract :

The experiment was done in two solvents i.e.acetone and water (aqueous).

Acetone extract : weigh 10g of green and red capsicums separately, add 50ml of 80% acetone stir the mixture well and allow it for 1 hour to extract the chlorophyll and carotenoids. Mixture it filtered using muslin cloth centifuged at 3000rpm for 10 minutes. Pellet is discarded and supernatant is stored in dark bottle in cold.

Water extract: Weigh 50gram of green and red capsicums separately into a beaker.Add 300ml of water to each beaker. Keep the mixture on magnetic stirrer for over night at particular temperature. Allow it to cool and filter the mixture using muslin cloth store the filtrate in cold.

2. Phytochemical analysis

The major phytochemicals present in capsicum are Tannins, Terpenoids, Phenols, Proteins, Saponin and Reducing sugar.

Procedure:

Test for Alkaloids:

1.Mayer's Test-

To 1ml of sample,4 to 5 drops of mayers reagent is added and development of white precipitate or yellow color indicates the presence of alkoloids.

2.Wanger's Test-

To 1 ml of sample was added to Wagner's reagent and the development of brown flocculent precipitate indicated the presence of alkaloids.

Test for Flavonoids:

To 1 ml of sample solution was taken in a test tube then 5 ml of dilutedammonia was added to the solution and add few drops of concentrated sulphuric acid in to the solution . At time yellow colour appears detect the presence of flavonoids.

Test for Saponins: .

To 2ml of sample was taken in test tube and 20ml of water was added and shaken vigorously for a stable. The foam formed indicated the presence of saponin.

Test for Tannins:

1.Ferric Chloride Test-

To 1ml of sample 1ml pottassium ferric cyanide was added after sometime 1ml of ferric chloride containing 0.1N HCl was added development of blackish blue indicate the presence of tennins

Test for Phenols:

To 1ml sample add 20 drops of 5% $FeCl_3$

Formation of blue or green colour indicated the presence of phenols.

Test for Quinones:

To 1ml of sample add 1ml dil.NaOH development of blue-green or re colour indicates the presence of quinones.

Test for Reducing Sugar:

1.Benedict's Test-

To 1ml of sample was treated with few drops of benedict's reagent. And place it in the boiling water bath for 3-5 minutes. Check for the formation of brick red colour.

Test for Proteins:

1. Ninhydrin Test-

To 1ml of sample add 0.25% of ninhydrin reagent and keep it in boiling water bath for 5-10 minutes. Development of blue colour indicates the presence of protein.

Test for terpenoids:

To 1ml sample add 0.4ml chloroform and 0.6ml conc.sulphuric acid. Development of reddish brown layer indicates the presence of terpinoids.

Test for anthocyanin:

To 1ml of sample add 1ml 2N HCl and 0.5ml of ammonia. Development of purplish blue color indicates the presence of anthocyanin.

Test for carbohydrates:

To 1ml sample add 2 drops of alcohlic α-napthal solution. Development of voilet ring at junction indicates the presence of carbohydrates.

Chemicals Required:

1. Alkaloids- Mayer's Reagent , Wagner's Reagent
2. Flavonoids- Ammonia Solution , Conc. Sulphuric Acid
3. Saponins – Water
4. Tanins – Potassium ferricyanide, Ferric chloride , Conc.HCl
5. Phenols – Ferric chloride
6. Quinons – Dil.NaOH
7. Reducing sugar – Benedicts reagent
8. Protein – Ninhydrin reagent
9. Terpenoids – Chloroform,Conc.sulphuric acid.
10. Anthocyanin- 2N Hcl,Ammonia.
11. Carbohydrates-Alcoholic α-napthal solution.

Reagent Preparation: For 5ml

1)1% ammonium Solution -

1ml - 100ml

Xml- 5ml

= 0.05ml of ammonia mixed in 5ml water

2)10% Ferric Chloride -

10g – 100ml

Xg – 5ml

= 0.5g of $FeCl_3$ dissolved in 5ml of water.

3) 0.5% Potassium ferricyanide-

2g – 100ml

Xg – 5ml

= 0.1g of Potassium ferricyanide in 5ml of water.

4) 0.25% Ninhydrin -

0.25g - 100ml

Xg – 5ml

= 0.01g of Ninhydrin in 5ml water.

5)2N Hcl –

166ml – 1000ml

Xml – 10ml

=1.66ml of Hcl in 10ml water.

3. Thin Layer Chromatography

Thin layer chromatography (TLC) is a technique used to separate mixtures into their individual components. A sample is applied to a thin layer of absorbent material, such as silica gel, and the mixture is then separated by how quickly each component moves. The faster a component moves, the higher its concentration.

Principle:

Thin layer chromatography (TLC) is a technique used in chemistry to separate mixtures of substances into their component parts. A thin layer of the mixture is placed on a flat surface, usually glass or a plastic sheet, and a solvent is then applied. The solvent travels up the thin layer by capillary action, and the different substances in the mixture are carried along with the solvent at different rates. This allows the different substances in the mixture to be separated into bands, which can then be analyzed to determine their composition.

Commercially available standard TLC plate was used with standard particle size range to improve reproducibility. The absorbent silica gel coated on an TLC plate of 7.3 cm length, 2.5 cm breadth and 0.3 cm thick plate. Small spot of the solution containing the sample was applied on the plate 1.0 cm from the bottom marked.

Procedure:

1. The Mobile Phase (Solvent) was prepared using chloroform and ethanol in ratio 7:3 for chlorophyll ,chloroform and methanol in ratio 8.85 : 1.14 for carotenoid.

2. The TLC chamber was wiped using ethanol and the tissue was adjusted inside the chamber and the extra length of tissue was cut. And mark was made on chamber to fill the mobile phase

3. Then the mobile phase was filled up to the mark and closed using the lid.

4. Then the TLC paper was taken by measuring the height of 7.5cm and width of 2.3cm and it was cut using the clean scissor.

5. The mark was made on the paper to load the sample and solvent front was marker and the paper was kept on heat surface for about 30seconds and the 10µl or 20 µl of sample was loaded using micropipette.

6. The after spotting the paper was carefully placed in TLC chamber and allowed to run.

7. Once the solvent reaches the solvent front and the bands are seen it is removed from the chamber and placed on the tissue paper.

8. The paper was kept in the UV chamber for visualization of spot.

9. Then kept in iodine chamber which was filled with the iodine for 10-15mins until the bands are properly visible.

$$\text{RF value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by the solvent}}$$

After Completion of TLC to measure the spectrum of spots :

- After running TLC totally 8 spots were separated.
- All the spots were marked and scraped off using the sterile blade and transferred into the separate Eppendorf tube.
- To the acetone extracted sample 400µl of acetone and for the aqueous extracted sample 400µl of water was added and centrifuged at 1000rpm for 5 minutes at 4°C .Then supernatant was collected in to other 8 Eppendorf tube.
- The Spectrum was measured from range 200 to 700nm.

Materials Required:

- TLC chamber, Tissue Paper , TLC Plates, Sample , Chloroform and ethanol (7:3), Chloroform and methanol(8.85 : 1.14)

Reagent Preparation:

To prepare 30ml 0f mobile Phase to run TLC.

Chloroform and ethanol (7:3)

21ml of chloroform and 9ml of ethanol.

Chloroform and methanol(8.85 : 1.15)

26.55ml of choroform and 3.45ml of methanol.

4. Antioxidant assays :

a)DPPH Assay

The 2,2-diphenylpicrylhydrazyl (DPPH) assay is widely used in plant biochemistry to evaluate the properties of plant constituents for scavenging free radicals. The method is based on the spectrophotometric measurement of the DPPH concentration change resulting from the reaction with an antioxidant.

Reagents Required:

• DPPH • Ascorbic Acid Preparation:

1. Ascorbic Acid-

Stock Solution – Concentration 15mg/ml

Dissolve 750mg of ascorbic acid in 50ml distilled water.

Working Solution –

Pipette 0.5ml of stock solution and make up to 50ml using distilled water.

2. 0.1mM DPPH –

Stock Solution -0.00197g of DPPH in 50 ml of absolute ethanol.

Working Solution- 1ml of Stock solution into 100 ml absolute ethanol.

Required Volume – 100ml of Working, 10ml of stock solution and 90ml ethanol.

Procedure:

- Prepare a stock solution in 7 clean test tubes by pipetting 100µl, 83.3µl, 66.6µl, 50µl, 33.3µl, 16.6µl, 0 µl of working ascorbic acid solution then make up the volume to 100µl using distilled water, vortex the tubes.
- Pipette above prepared 10µl each concentration of ascorbic acid making them into triplets into separate test tube then add 3ml of DPPH reagent to each tube.
- This gives the standard curve of DPPH assay, once standard curve is obtained again along with the standard both the sample was added i.e. water and ethanol extract was taken 50µl and 100µl each and were made into triples. And add 3ml of DPPH to all tubes

- Incubate all the tubes at room temperature in dark for 30 mins Then measure the absorbance at 517nm.

b) Haemolytic Assay

A.Isolation of RBC from whole blood.

Reagents Required:

- Lysis Buffer – 155mM Ammonium Chloride, 12mM Sodium Bicarbonate, 0.1mM EDTA, Sterile water – 100ml of water was autoclaved.
- PBS Buffer – 1.3M Sodium chloride, 70mM disodium hydrogen orthophosphate , 30mM Sodium dihydrogen orthophosphate.

Preparation of reagents – Weight = MW x M x V/1000

• 155mM Ammonium Chloride – Molecular weight 53.491g

$$\frac{53.491 \times 0.155 \times 50}{1000} = \frac{414.5}{1000} = 00414\text{g in 50ml}$$

• 12mM Sodium Bicarbonate – Molecular weight 84.007g

$$\frac{84.007 \times 0.012 \times 50}{1000} = \frac{50.40}{1000} = 0.504\text{g in 50ml water}$$

• 0.1mM EDTA - Molecular weight 372.24

$$\frac{372.24 \times 0.0001 \times 50}{1000} = \frac{1.861}{1000} = 0.00186\text{g in 50 ml water}$$

All these chemicals were weighed and dissolved one after the other into 50ml autoclaved sterile distilled water.

PBS Preparation –

• 1.3M Sodium chloride - Molecular weight 58.44g

$$\frac{58.44 \times 0.0013 \times 100}{1000} = \frac{7.597}{1000} = 0.00759\text{g in 100ml}$$

• 70mM disodium hydrogen orthophosphate - Molecular weight 141.96g

141.96 x 0.007 x 100   993.72
------------------------ =   --------- = 0.993g in 100ml water
1000 1000

• 30mM Sodium dihydrogen orthophosphate - Molecular weight 156.01g

156.01 x 0.003 x 100   468.03
-------------------------   =   -------- = 0.468g in 100ml water
1000   1000

This gives 10X buffer and then this diluted to 1x using distilled water by taking 1ml of PBS from 10X add 9ml of water gives 1x buffer.

Procedure:

• Draw the Blood into tube containing EDTA.

• Centrifuge at 1000rpm for 5minutes .

• Collect the pellets add PBS and wash 3times at 1000rpm for 5minutes.

• 1:9 dilute pellets in PBS.

B.Haemolytic Assay

Reagents Required: RBC, 1x PBS, tween 20, sample.

Procedure:

• To the clean plate reader samples were loaded in the triplets.

• For Blank - 100µl of PBS was taken.

• For Positive control - 50µl RBC and 50µl Tween 20 both were mixed well by sucking with the pipette.

• For Negative Control - 50µl PBS and 50µl RBC.

• Then for all the samples including the TLC samples 50µl RBC was added followed by 50µl sample i.e., Water extract, ethanol extract and TLC samples.

• After the sample is loaded the plate was incubated for one hour at 370 C.

• Then centrifuge the same at 1000rpm for 5minutes at 4oC.

• Collect the supernatant and measure the OD at 595.

• To calculate the percentage of RBC lysis by sample using formula,

Absorbance
-------------------- X 100
Positive Control

c)ABTS Assay

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation (ABTS is a stable free radical frequently used for estimating the□2,2'-Azino-bis(3-

ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation (ABTS total antioxidant capacity (TAC) of natural products.

Chemical preparation:

Stock ABTS:

Buffer preparation:0.4M sodium acetate buffer in 50ml de-ionized water pH of 5.8 is set using glacial acetic acid

$$\frac{0.4\times136.04\times50}{1000} = 2.721\text{g}/50\text{ml}$$

10ml of ABTS stock:

Add 7mM(0.007M) ABTS and 2.5mM potassium persulphate in 10ml sodium acetate buffer kept overnight in 4° C.

Working ABTS:

833µl stock ABTS in 50ml methanol.

Gallic acid:

Stock: 75mg(0.0075g) gallic acid in 50ml deionized water.

Working:Take 10ml stock and makeup to 100ml.

Procedure:

- Pipette 0, 10,20,30,40,50,60,70,80,90,100µl of working standard solution of gallic acid into clean test tube .
- Make up volume to 100µl using distilled water.
- Take 50µl of 4 samples in 4 different clean test tubes.
- Add 3ml of ABTS reagent to all the test tube including unknown sample.
- Measure OD at 737nm.

d) Reducing power assay:

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm.

Chemicals required:

PBS buffer(0.2M):

1.548g of disodium hydrogen phosphate and 0.58g of sodium dihydrogen phosphate is added to 50ml of deionised water pH 7 is set using Hcl or NaOH.

Potassium ferricyanide(1%):

0.1g of potassium ferricyanide in 10ml deionised water.

Trichloro acetic acid(10%):

1g of trichloro acetic acid in 10ml deionised water.

Ferric chloride(0.1%):

0.01g of ferric chloride in 10ml of deionised water.

Procedure:

- Take 4 centrifuge tubes and add 2.5ml of PBS buffer, 2.5ml of potassium ferricyanide and 1ml of 4 pigment extracted samples are added to 4 different tube.
- Incubate at 50°C for 20min
- Add 2.5ml of trichloro acetic acid
- Centrifuge at 3000rpm for 10min
- For the supernatant add 2.5ml of distilled water and 0.5ml of ferrichloride
- Absorbance is measured at 700nm

e)Phosphomolybdenum assay:

This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically.

Chemicals required :

Sodium phosphate :

Stock -0.436g of sodium phosphate in 1 ml deionised water

Working - 100µl of stock make up to 1ml using deionised water

Ammonium molybdate :

Stock -0.494g of ammonium molybdate in 10ml deionised water

Working -100µl of stock make up to 1ml using deionised water

Sulphuric acid(0.6M):

0.3ml of conc.sulphuric acid in 5ml deionised water.

Assay reagent:

0.06ml sulphuric acid,100µl sodium phosphate, 100µl ammonium molybdate and make up to 1ml using deionized water.

Procedure:

- Take 5 test tube, add 1ml of assay reagent to each test tube for 1 test tube 100µl of deionised water and used as blank, 1ml of 4 pigment extracted samples are added to 4 different tube.
- Keep the test tubes at 95° C for 90min in water bath.
- Cool the solution and measure the absorbance at 695nm

5) Quantitative estimation of chlorophyll:

The chlorophyll a concentration is determined spectrophotometrically by measuring the absorbance of the extract at various wavelengths. The resulting absorbance measurements are then applied to a standard equation

Chemicals required:

Fresh sample

Acetone:Ethanol(2:1)

Procedure :

- 0.5g of fresh extraction material was taken in pestle and mortor and crushed in less light
- Add 4ml of acetone:ethanol mixture,placed in 10ml tubes and mixed for 1min
- Incubate for 30min in freezer
- Centrifuge for 10min at 200rpm
- Supernatent were covered with aluminium foil and 5ml of acetone ethanol mixture was added and stirred for 1min
- Absorbance was read at chlorophyll a -642nm and 372nm

  Chlorophyll b- 626nm and 392nm

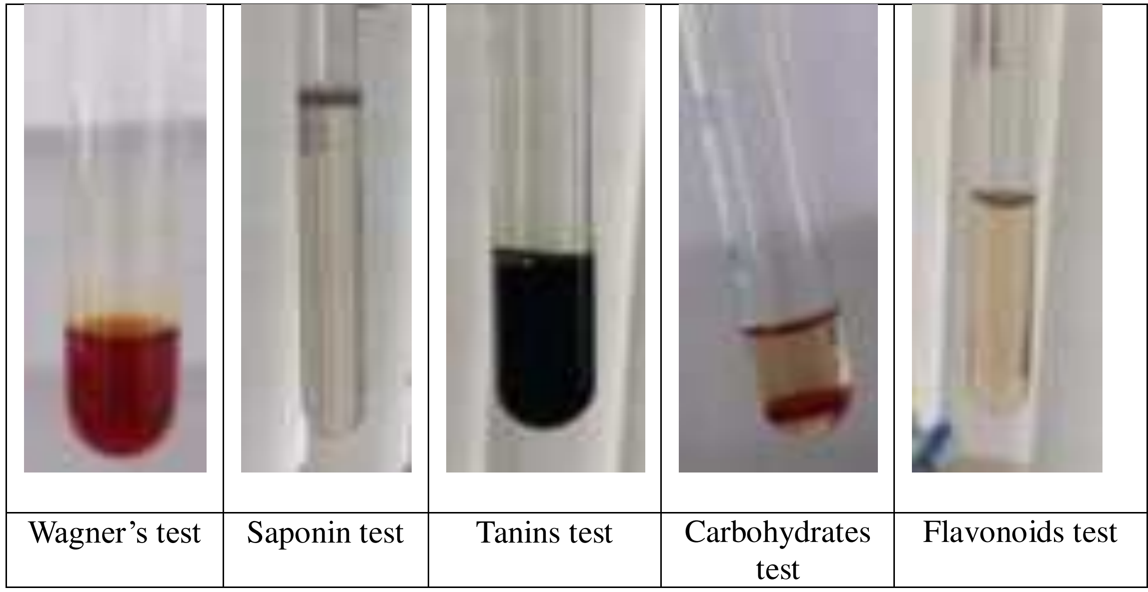
2.1 CHEMICALS

All standard chemicals and enzymes were purchased from Sisco Research Laboratories, Mumbai. All other analytical and laboratory grade chemicals were procured from Sisco Research Laboratories, Mumbai. The microbial culture media, agar agar were purchased from Himedia, standard compounds were purchased from Sisco Research Laboratories, Mumbai. All other analytical and laboratory grade chemicals were procured from Sisco Research Laboratories, Mumbai.

RESULT

1. Phytochemical test

Sl.No	Phytochemical	Chlorophyll solvent extract	Chlorophyll water extract	Carotenoids solvent extract	Carotenoids water extract
1	Alkaloids a)Mayer's test	-	-	-	-
	b)Wager's test	+	-	-	+
2	Saponins	+	+	+	+
3	Tannins	+	+	+	+
4	Phenols	+	-	+	-
5	Carbohydrates	+	-	-	-
6	Flavonoids	+	-	-	-
7	Anthocyanin	-	-	-	-
8	Protein	+	+	+	-
9	Terpenoids	+	-	+	+
10	Reducing sugar	+	+	+	-
11	Quinones	-	-	-	-



Wagner's test	Saponin test	Tanins test	Carbohydrates test	Flavonoids test

2. TLC

- Chloroform and ethanol in ratio 7:3 for chlorophyll
- Chloroform and methanol in ratio 8.85 : 1.14 for carotenoid.

Sample	Chlorophyll solvent extract				Chlorophyll water extract	Carotenoids solvent extract		Carotenoids water extract
Solvent distance(cm)	6.4				5.2	6.9		5.9
Solute distance (cm)	4	4.8	5.2	5.7	1	3.7	4.7	4
Rf value	0.62	0.75	0.81	0.89	0.19	0.53	0.68	0.67
Observation								

Spectrum analysis of TLC spots

	Absorbance	**nm**
Spot 1	1.11	209.27
Spot 2	0.96	209.27
Spot3	1.01	208.89
Spot4	0.73	209.66
Spot5	1.00	208.89
Spot6	1.06	208.89
Spot7	2.49	200.39
Spot 8	2.27	200

3.Antioxitant assays

A.Hemolytic assay

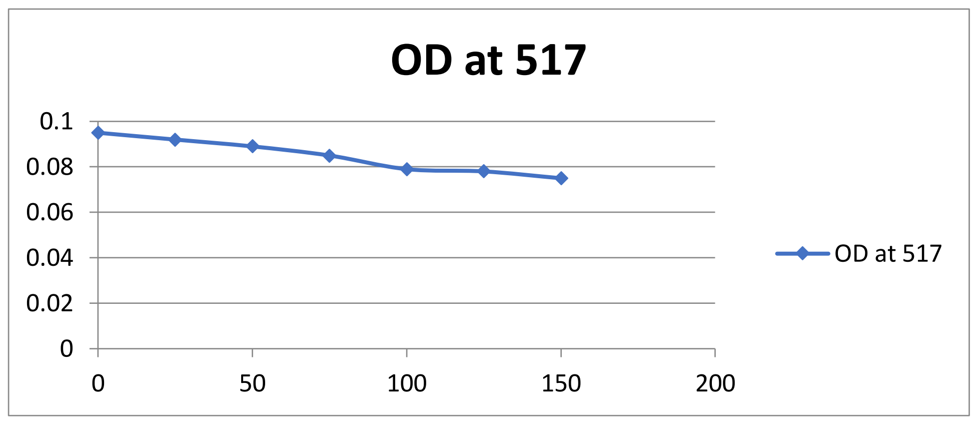
Sl no	Sample	OD at 595 nm
1	Blank	0.081
2	+ve control	0.947
3	- ve control	0.100
4	Chlorophyll solvent extract	0.175
5	Chlorophyll water extract	0.207
6	Carotenoids solvent extract	0.176
7	Carotenoids water extract	0.214

Sample	Absorbance	Percentage(%)
Chlorophyll solvent extract	0.175	18.47
Chlorophyll water extract	0.207	21.85
Carotenoids solvent extract	0.176	18.58
Carotenoids water extract	0.214	22.59

B.DPPH assay

Sl No	Volume of ascorbic acid(µg)	Conc of ascorbic acid(µg)	Volume of distilled water(µl)
1	100	150	0
2	83.3	125	16.6
3	66.6	100	33.3
4	50	75	50
5	33.3	50	66.6
6	16.6	25	83.3
7	0	0	100

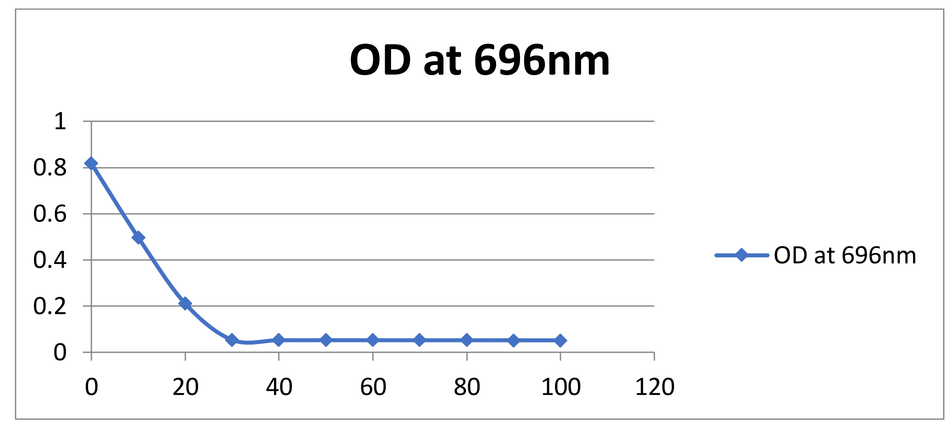
Sl No	Volume of ascorbic acid (µl)	Conc of ascorbic acid (µg)	Volume of DPPH(ml)		O.D at 517nm
1	10×3	0			0.095
2	10×3	25			0.092
3	10×3	50			0.089
4	10×3	75			0.085
5	10×3	100			0.079
6	10×3	125			0.078
7	10×3	150			0.075
8	Chlorophyll solvent extract a)10	-			0.094
9	b)20	-			0.092
10	c)30	-			0.093
11	d)40	-			0.087
12	e)50	-			0.085
13	Chlorophyll water extract a)10	-	3ml	Incubate for 30min for room temperture	0.094
14	b)20	-			0.093
15	c)30	-			0.091
16	d)40	-			0.087
17	e)50	-			0.087
18	Carotenoids solvent extract a)10	-			0.095
19	b)20	-			0.094
20	c)30	-			0.091
21	d)40	-			0.091
22	e)50	-			0.089
23	Carotenoids water extract a)10	-			0.094
24	b)20	-			0.095
25	c)30	-			0.092
26	d)40	-			0.090
27	e)50	-			0.088



Chlorophyll solvent extract		Chlorophyll water extract		Carotenoids solvent extract		Carotenoids water extract	
Vol(µl)	Conc(µg)	Vol(µl)	Conc(µg)	Vol(µl)	Conc(µg)	Vol(µl)	Conc(µg)
10	9.89	10	9.89	10	2.88	10	9.89
20	23.91	20	16.90	20	9.89	20	16.90
30	37.93	30	30.92	30	30.92	30	23.91
40	58.97	40	51.96	40	30.92	40	37.93
50	72.99	50	58.97	50	44.95	50	51.96

C.ABTS assay

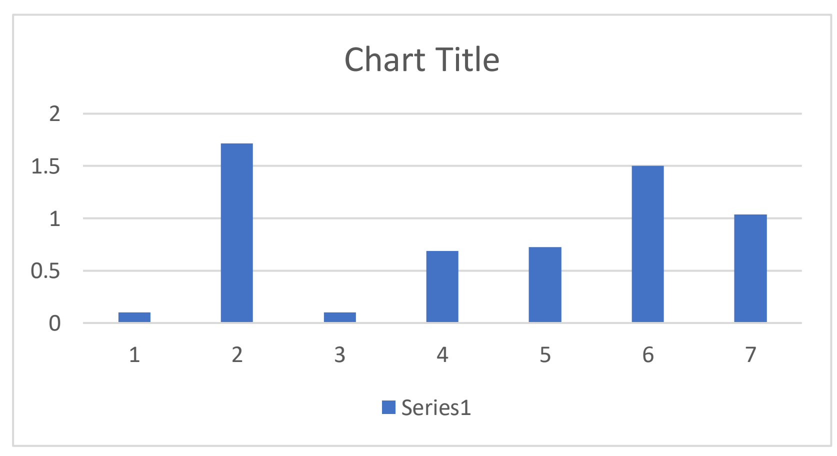
Sl. No	Volume of gallic acid(µl)		Conc of gallic acid (µg)	Volume of distilled water(µl)	Volume of ABTS(ml)	OD at 696nm
1	0		0	100		0.8169
2	10		10	90		0.4967
3	20		20	80		0.2106
4	30		30	70		0.0536
5	40		40	60		0.0523
6	50		50	50		0.0523
7	60		60	40		0.0522
8	70		70	30	3ml	0.0521
9	80		80	20		0.0520
10	90		90	10		0.0519
11	100		100	0		0.0517
12	Chlorophyll solvent extract	50	-	-		0.0583
13	Chlorophyll water extract	50	-	-		0.1255
14	Carotenoids solvent extract	50	-	-		0.0548
15	Carotenoids water extract	50	-	-		0.0620



sample	Concentration
Chlorophyll solvent extract	61.29µg
Chlorophyll water extract	54.87µg
Carotenoids solvent extract	61.62µg
Carotenoids water extract	60.93µg

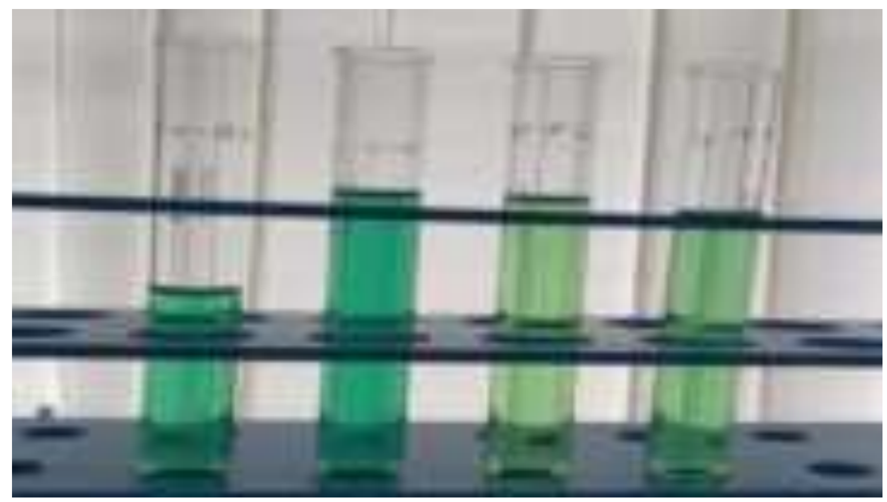
D.Reducing power assay

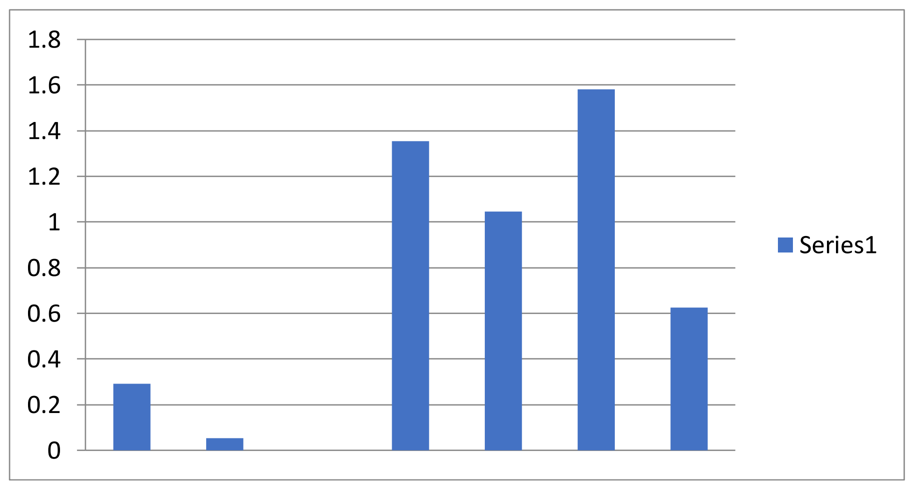
Sl no	**sample**	**OD at 700nm**
1	+ve control(100µl)	0.1030
2	+ve control(1ml)	1.7188
3	- ve control	0.0985
4	Chlorophyll solvent extract	0.6895
5	Chlorophyll water extract	0.7268
6	Carotenoids solvent extract	1.5046
7	Carotenoids water extract	1.0391



E.Phosphomolybdenum assay

Sl no	sample	OD at 700nm
1	+ve control(100μl)	0.2917
2	+ve control(1ml)	0.053
3	- ve control	0.0
4	Chlorophyll solvent extract	1.3532
5	Chlorophyll water extract	1.0459
6	Carotenoids solvent extract	1.5822
7	Carotenoids water extract	0.6262





4.Quantitative estimation of chlorophyll

absorbance	642nm	372nm
Chlorophyll a	0.1661	0.1720
absorbance	626nm	392nm
Chlorophyll b	0.0624	0.1712

Calculation: Chlorophyll a(mg/g) = (reading × A642) – (reading × A372)
= (0.1661) – (0.1720)
= 0.1037

Chlorophyll b(mg/g) = (reading × A392) – (reading × A626)
= (0.1712) – (0.0624)
= 0.0008

Total chlorophyll(mg/g) = 0.1037+0.0008
= 0.1045mg/g

RESULT: The total chlorophyll present in the sample = 0.1045 mg/g.

SUMMARY

Phytochemical and antioxidant assays are techniques used to analyze the chemical compounds present in plants (phytochemicals) and their ability to combat oxidative stress (antioxidant activity). These tests help identify and quantify various bioactive compounds in plants, such as flavonoids, phenols, alkaloids, and terpenoids. Common phytochemical assays include spectrophotometry, chromatography, and spectroscopy. Antioxidants are compounds that protect cells from damage caused by free radicals. Antioxidant assays assess the ability of plant extracts or compounds to neutralize free radicals. Common antioxidant assays include DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, FRAP (Ferric Reducing Antioxidant Power) assay, and ORAC (Oxygen Radical Absorbance Capacity) assay.These assays are crucial in understanding the potential health benefits of plant-based foods and medicines, as phytochemicals often contribute to their therapeutic properties. High antioxidant activity in plant extracts suggests their potential in reducing oxidative stress and preventing various diseases.

Phytochemical assays are laboratory tests used to analyze the chemical compounds present in plants. These assays help researchers identify and quantify bioactive substances with potential health benefits. Common phytochemical assays include measuring total phenolic content (TPC), total flavonoid content (TFC), total alkaloid content (TAC), total tannin content (TTC), and conducting antioxidant assays. Additionally, techniques like HPLC, GC-MS, TLC, and UV-Vis spectroscopy are employed to separate and analyze specific phytochemicals in plant extracts, providing valuable insights into their composition and potential uses.

Antioxidant assays are laboratory tests designed to evaluate the antioxidant activity of substances, typically in plant extracts or compounds. DPPH assay: In this assay, the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) is used to measure the ability of an antioxidant to scavenge free radicals. The reduction in DPPH absorbance indicates antioxidant activity.ABTS Assay: The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay assesses the capacity of antioxidants to reduce ABTS radicals, resulting in a decrease in absorbance.

CONCLUSION

Half page

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Phytochemical and Antioxidant assay on Brinjal

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COMPANY CERTIFICATE

Certificate

This is to certified that this dissertation is a bonafide research project work done by **Ms.Shubhashree H.Y.,** M.Sc. Biochemistry , at **Glimetomics bioresolve pvt. Ltd.** and is not published/ presented elsewhere for any other degree from any other institute/ university.

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Contents

1. Abstract
2. Introduction
3. Review of Literature
4. Objectives
5. Methods
6. Results
7. Summary and conclusion
8. References

ABSTRACT

The present study investigated on the comparative evaluation of the extraction, quantification, phytochemical and antioxidatant activity of chlorophyll and anthocyanin extracts of brinjal. The Thin layer chromatography method is providing a fingerprint of plant extract. The pigment extracted in the solvents on TLC chromatogram was viewed under UV 254 nm and UV 366 nm and documented. The extraction and estimation of chlorophyll and anthocyanin were also performed for the plant sample following standard procedure. Phytochemical analysis shows that acetone, methanol and water extract which shows abundant presence of alkaloids, flavonoids, phenols, saponins etc. Brinjal possessed strong antioxidant activity as determined by 2,2-diphenyl-1-picrylhydrazyl)reagent, ABTS assay ,haemolytic assay and other assays. The estimation of chlorophyll is done by quantitative assay.

INTRODUCTION

Brinjal or eggplant (Solanum melongena L.) is an important solanaceous crop of sub-tropics and tropics. The brinjal is of much importance in the warm areas of Far East, being grown extensively in India, Bangladesh, Pakistan, China and Philippines. It is also popular in Egypt, France, Italy and United States. In India, it is one of the most common, popular and principal vegetable crops grown throughout the country except higher altitudes.

Brinjal fruit (unripe) is primarily consumed as cooked vegetable in various ways. It is low in calories and fats, contains mostly water, some protein, fibre and carbohydrates. It is a good source of minerals and vitamins and is rich in total water soluble sugars, free reducing sugars, amide proteins among other nutrients. It contain a higher content of anthocyanin, phenols, glycoalkaloids (such as solasodine), and amide proteins. A high anthocyanin content and a low glycoalkaloid content are considered essential, regardless of how the fruit is to be used. Bitterness in eggplant is due to the presence of glycoalkaloids which are of wide occurrence in plants of Solanaceae family.

Brinjal is known to have ayurvedic medicinal properties and is good for diabetic patients. It has also been recommended as an excellent remedy for those suffering from liver complaints. Eggplant has rich natural variation in fruit color. There are two types of pigments that determine the color of eggplant fruit: anthocyanin and chlorophyll, whose content and proportion jointly determine the color of eggplant.

Classification

Name - Brinjal

Kingdom - Plantae

Class - Magnoliopsida

Subclass - Asteridae

Order - Solanales

Family - Solanaceae

Genus - Solanum

Species - Melongena

The name of Chlorophylls is derived from the Greek Words chloros meaning “green” and phyllon meaning “leaf.”. Chlorophylls are the greenish photosynthetic Pigments found in all photosynthetic organisms. In algae and plants, these pigments are embedded in the thylakoid membranes within the chloroplasts. They occur in almost every green part of plant, i.e. leaves and herbaceous stems and fruits. They are used to boost energy and prevent or Treat chronic fatigue and fibromyalgia. They are powerful antioxidants and effective scavengers of reactive oxygen species that are Associated with cell damage and different medical Conditions (when excessively Produced). According to studies, Applying a gel containing chlorophyllin to the skin. Reduces the signs of photo aging and prevents age Prematurely They may help treating skin conditions, when applied topically.Topical chlorophyll may be useful in acne treatment, as a gel containing Chlorophyllin helps reducing facial acne and large pores. Besides, a combination of topical chlorophyll and phototherapy results in less oily skin, less severe acne and fewer acne lesions. These findings may not be relevant for all skin types. They may be helpful in treating haemoglobin Deficiency disorders, such as thalassemia and anaemia, considering that the main structure of chlorophylls is a porphyrin ring similar to the structure of haem in haemoglobin, except that the central atom in haem is iron instead of magnesium.

Anthocyanin:

Anthocyanin’s (Greek anthos: flower and kyaneos: dark blue) represent a subclass of the phenolic compounds (Delgado-Vargas, 2000). They are water-soluble glycosides of anthocyanidins, which are largely responsible for the attractive pale yellow, orange, red, magenta, violet and blue colour of a wide range of plant tissues, principally flowers, leaves and fruits, besides storage organs, roots, tubers, stems and grains . They are ubiquitous in higher plants (occurring in more than 30 families), but normally are absent in liverworts, algae, and other lower plants, even though some anthocyanins have been identified in mosses and ferns. Anthocyanin as antioxidant- Living cells produce by products during metabolism in the form of reactive oxygen species (ROS) and free radicals, under normal and stressed conditions. ROS are a group of reactive molecules derived from molecular oxygen, such as superoxide, singlet oxygen , hydrogen peroxideand hydroxyl radical. They can induce cellular damage when excessively produced . Anthocyanins and anthocyanidins have a higher antioxidant properly.

REVIEW OF LITERATURE

The eggplant (Solanum melongena L.) is a herbaceous, vegetable crop with coarsely lobed leaves, white to purple flowers, fruit is berry and are grown around the world mainly for food representing one of the best dietary sources of biologically active polyphenolic compounds, vitamins, antioxidants and medicinal requirements.Brinjal is an economic flowering plant belonging to the family Solanaceae which contains 75 genera and over 2000 species (Biology of Brinjal, 2011) and are grown mainly for food and medicinal purposes (Igwe et al., 2003). Eggplant fruit popularly known as aubergine (UK), melanzana, garden egg, brinjal, Baingan (India) and is one of the most important vegetable crops grown on over 1.7 million worldwide.

Phytochemical studies have yielded flavonoids, alkaloids, tannins and steroids. (Kwon et al., 2007) It is widely distributed in India for its fruit. Anthocyanins, an important group of naturally occurring pigments of red and/or purple colored fruits, are the main phenolic compounds in eggplant peel (Mazza et al., 2004). Bitterness in eggplant is due to the presence of glycoalkaloids (Rai MK et al., 1997).

Brinjal contain arginine, aspartic acid, histidine, 5–HT, delphinidine −3 bioside (nasunin), oxalic acid, solasodine, ascorbic acid, tryptophan, etc. A bioflavonoid glycoside named solanoflavone is present in the brinjal (Shen G et al., 2005). The major anthocyanins were identified in extracts from the peels as delphinidin 3-(pcoumaroylrutinoside) -5-glucoside (nasunin), delphinidin 3-rutinoside , delphinidin 3-glucoside, and petunidin 3-(p-coumaroylrutinoside)-5-glucoside (petunidin 3RGc5G) (Keiko Azuma, 2008). Nasunin, a major component of anthocyanin pigment, was isolated from the eggplant peels, and its antioxidant activity was evaluated (Igarashi et al., 1993).

The antioxidant vitamins, including vitamin A, vitamin C and β-carotene were lower and some of the polyphenolic components, especially nasunin content, were higher in grilled eggplants, but they were unable to demonstrate better cardioprotective properties compared to the raw fruit(Noda et al., 2000). Various parts of the plant are useful in the treatment of inflammatory conditions, cardiac debility, neuralgias, and ulcer of nose, cholera, bronchitis and asthma. Besides, having many traditional uses, S. melongena is reported to exhibit many important pharmacological actions.

Eggplant is ranked as one of the top ten vegetables in terms of oxygen radical scavenging capacity due to the fruit's phenolic constituents (Cao et al., 1996). Phenolic compounds included flavonoid compounds which are commonly found in many plants have various effects such as antioxidant activity and antibacterial activity(Hodnick WF, Milosavljevic EB,et al.,1988). Antioxidant can prevent the excessive of free radical in oxidative stress which can cause many degenerative diseases. Natural antioxidant can be obtained by consuming fruits and vegetables because they contain phenolic and flavonoid compounds which have antioxidant capacity(Pellegrini N, Serafini M, et al.,2003)Previous researchers expressed that phenolic and flavonoid content could be correlated to their antioxidant activities (Pourmorad F, Hosseinimehr SJ,et al.,2002).

Eggplant (Solanum melongena) contained many flavonoid and tannin which can act as an antioxidant (Saleh GS.2015). Antioxidant activity in many plants extracts could be determine using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) methods (Thaipong K, Boonprakob U, Crosby K.,et al., 2006). The previous researchers (Pellegrini N, Serafini M, Colombi B, et al.,2003) revealed that DPPH, ABTS, and FRAP could be performed to determine antioxidant activity of fruits, vegetables, and food.

OBJECTIVES

1) **Assessment of pigments extraction from the green and purple Brinjal.**
2) **Assessment of phytochemicals present in the sample.**
3) **Assessment of thin layer chromatography.**
4) **Assessment of antioxidant properties of green and purple Brinjal.**

   a)DPPH assay

   b)Hemolytic assay

   c)ABTS assay

   d)Reducing power assay

   e)Phoshomolybdenum assay
5) **Assessment of quantitative estimation of chlorophyll.**

MATERIALS AND METHODS

1. Pigment Extraction

Requirements: Green and Purple Brinjal, 80% Acetone ,70% Methanol, water, Storage Bottles.

Procedure:

Collection of vegetable source (brinjal) :

Green and purple brinjal were collected from the local shop . These were washed thoroughly in order to remove the dirt and dust. Then the brinjal were cut into small pieces.

Preparation of extract :

The experiment was done in three solvents i.e.acetone , methanol and water (aqueous).

Acetone extract : weigh 10g of green brinjal and add 50ml of 80% acetone stir the mixture well and allow it for 1 hour to extract the chlorophyll. Filter using muslin cloth centifuge at 3000rpm for 10minutes.Pellet is discarded and supernatant is stored dark bottle in cold.

Water extract: Weigh 50gram of green and purple brinjal separately into a beaker.Add 300ml of water to each beaker.Keep the mixture on magnetic stirrer for over night at particular temperature.Allow it to cool and filter the mixture using muslin cloth store the filtrate in cold.

Methanol extract : weigh 10g of purple brinjal and add 50ml of 70% methanol stir the mixer well and allow it for 1 hour to extract the chlorophyll. Filter using muslin cloth centifuge at 3000rpm for 10minutes.Pellet is discarded and supernatant is stored dark bottle in cold.

2. Phytochemical analysis

The major phytochemicals present in sample **a**re Alkaloids, Flavonoids, Phytosterols , Tannins, Phenols, Proteins, Carbohydrates, Anthocyanin, Saponin.

Procedure:

1.Test for Alkaloids:

a.Mayer's Test-

To 1ml of sample,4 to 5 drops of mayers reagent is added and development of white precipitate or yellow color indicates the presence of alkoloids.

b.Wanger’s Test-

To 1 ml of sample was added to Wagner's reagent and the development of reddish brown colour indicated the presence of alkaloids.

3.Test for Flavonoids:

To 1 ml of sample solution was taken in a test tube then 5 ml of dilutedammonia was added to the solution and add few drops of concentrated sulphuric acid in to the solution . At time yellow colour appears detect the presence of flavonoids.

4.Test for Saponins: .

To 2ml of sample was taken in test tube and 20ml of water was added and shaken vigorously for a stable. The foam formed indicated the presence of saponin.

5.Test for Tannins:

1.Ferric Chloride Test-

To 1ml of sample 1ml pottassium ferric cyanide was added after sometime 1ml of ferric chloride containing 0.1N HCl was added.Development of blackish blue indicate the presence of tennins.

6.Test for Phenols:

To 1ml sample add 20 drops of 5% $FeCl_3$. Formation of blue or green colour indicated the presence of phenols.

7.Test for Phytosterols:

To 1ml of sample add 2ml chloroform and add few drps of H_2SO_4 .Development reddish colour layer indicates the presence of phytosterols.

8.Test for Anthocyanin:

To 1ml of sample add 1ml 2N HCl and 0.5ml of ammonia. Development of purplish blue color indicates the presence of anthocyanin.

9.Test for Carbohydrates:

To 1ml sample add 2 drops of alcohlic α-napthal solution. Development of voilet ring at junction indicates the presence of carbohydrates.

10.Test for Glycosides:

To 1ml sample add 1.5ml of chlroform and shaken well ,chloroform layer separated and 10% ammonia solution .Development of pink colour indicates the presence of glycosides.

11.Test for Proteins:

Ninhydrin Test-

To 1ml of sample add 0.25% of ninhydrin reagent and keep it in boiling water bath for 5-10minutes. Development of blue colour indicates the presence of protein.

Chemicals Required:

1. Alkaloids- Mayer's Reagent , Wagner's Reagent.
2. Flavonoids- Ammonia Solution , Conc. Sulphuric Acid.
3. Saponins – Water.
4. Tanins – Potassium ferricyanide, Ferric chloride , Conc.HCl.
5. Phenols – Ferric chloride.
6. Quinons – Dil.NaOH.
7. Reducing sugar – Benedicts reagent.
8. Protein – Ninhydrin reagent.

Reagent Preparation: For 5ml

1)1% ammonium Solution -

1ml - 100ml

Xml- 5ml

= 0.05ml of ammonia mixed in 5ml water.

2)10% Ferric Chloride -

10g – 100ml

Xg – 5ml

= 0.5g of $FeCl_3$ dissolved in 5ml of water.

3) 0.5% Potassium ferricyanide-

2g – 100ml

Xg – 5ml

= 0.1g of Potassium ferricyanide in 5ml of water.

4) 0.25% Ninhydrin -

0.25g - 100ml

Xg – 5ml

= 0.01g of Ninhydrin in 5ml water.

3. Thin Layer Chromatography

Thin layer chromatography (TLC) is a technique used to separate mixtures into their individual components. A sample is applied to a thin layer of absorbent material, such as silica gel, and the mixture is then separated by how quickly each component moves. The faster a component moves, the higher its concentration.

Principle:

Thin layer chromatography (TLC) is a technique used in chemistry to separate mixtures of substances into their component parts. A thin layer of the mixture is placed on a flat surface, usually glass or a plastic sheet, and a solvent is then applied. The solvent travels up the thin layer by capillary action, and the different substances in the mixture are carried along with the solvent at different rates. This allows the different substances in the mixture to be separated into bands, which can then be analyzed to determine their composition.

Commercially available standard TLC plate was used with standard particle size range to improve reproducibility. The absorbent silica gel coated on an TLC plate of 7.3 cm length, 2.5 cm breadth and 0.3 cm thick plate. Small spot of the solution containing the sample was applied on the plate 1.0 cm from the bottom marked.

Procedure:

1. The Mobile Phase (Solvent) was prepared using chloroform and ethanol in ratio (7:3) for chlorophyll ,n-butanol:acetic acid:water (4:1:5) for anthocyanin.

2. The TLC chamber was wiped using ethanol and the tissue was adjusted inside the chamber and the extra length of tissue was cut. And mark was made on chamber to fill the mobile phase.

3. Then the mobile phase was filled up to the mark and closed using the lid.

4. Then the TLC paper was taken by measuring the height of 7.5cm and width of 2.3cm and it was cut using the clean scissor.

5. The mark was made on the paper to load the sample and solvent front was marker and the paper was kept on heat surface for about 30seconds and the 10µl or 20 µl of sample was loaded using micropipette.

6. The after spotting the paper was carefully placed in TLC chamber and allowed to run.

7. Once the solvent reaches the solvent front and the bands are seen it is removed from the chamber and placed on the tissue paper.

8. The paper was kept in the UV chamber for visualization of spot.

9. Then kept in iodine chamber which was filled with the iodine for 10-15mins until the bands are properly visible.

$$\text{RF value} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by the solvent}}$$

After Completion of TLC to measure the spectrum of spots :

- After running TLC totally 7 spots were separated.
- All the spots were marked and scraped off using the sterile blade and transferred into the separate Eppendorf tube.
- To the acetone extracted sample 400µl of acetone, for methanol extracted sample 400µl of methanol and for the aqueous extracted sample 400µl of water was added and centrifuged at 1000rpm for 5 minutes at 4°C .Then supernatant was collected in to other 7 Eppendorf tube.
- The Spectrum was measured from range 400 to 700nm.
- Again from range 200 to 700nm.

Materials Required:

• TLC chamber • Tissue Paper • TLC Plates • Sample • Chloroform : Ethanol(7:3) and n-butanol: acetic acid: water(4:1:5) .

Reagent Preparation:

To prepare 30ml 0f mobile Phase to run TLC.

Chloroform and ethanol (7:3)

21ml of chloroform and 9ml of ethanol.

n-butanol , acetic acid and water(4:1:5)

12ml of n-butanol and 3ml of acetic acid and 15ml water.

4. Antioxidant assays :

A. DPPH Assay

The 2,2-diphenylpicrylhydrazyl (DPPH) assay is widely used in plant biochemistry to evaluate the properties of plant constituents for scavenging free radicals. The method is based on the spectrophotometric measurement of the DPPH concentration change resulting from the reaction with an antioxidant.

Reagents Required:

• DPPH • Ascorbic Acid Preparation:

1. Ascorbic Acid-

Stock Solution – Concentration 15mg/ml

Dissolve 750mg of ascorbic acid in 50ml distilled water.

Working Solution –

Pipette 0.5ml of stock solution and make up to 50ml using distilled water.

2. 0.1mM DPPH –

Stock Solution -0.00197g of DPPH in 50 ml of absolute ethanol.

Working Solution- 1ml of Stock solution into 100 ml absolute ethanol.

Required Volume – 100ml of Working, 10ml of stock solution and 90ml ethanol.

Procedure:

- Prepare a stock solution in 7 clean test tubes by pipetting 100µl, 83.3µl, 66.6µl, 50µl, 33.3µl, 16.6µl, 0 µl of working ascorbic acid solution then make up the volume to 100µl using distilled water, vortex the tubes.
- Pipette above prepared 10µl each concentration of ascorbic acid making them into triplets into separate test tube then add 3ml of DPPH reagent to each tube.
- This gives the standard curve of DPPH assay, once standard curve is obtained again along with the standard both the sample was added i.e. water and ethanol extract was taken 50µl and 100µ1 each and were made into triples. And add 3ml of DPPH to all tubes
- Incubate all the tubes at room temperature in dark for 30 mins Then measure the absorbance at 517nm.

B. Haemolytic Assay

A.Isolation of RBC from whole blood.

Reagents Required:

- Lysis Buffer – 155mM Ammonium Chloride, 12mM Sodium Bicarbonate, 0.1mM EDTA, Sterile water – 100ml of water was autoclaved.

- PBS Buffer – 1.3M Sodium chloride, 70mM disodium hydrogen orthophosphate , 30mM Sodium dihydrogen orthophosphate.

Preparation of reagents – Weight = MW x M x V/1000

• 155mM Ammonium Chloride – Molecular weight 53.491g.

$$\frac{53.491 \times 0.155 \times 50}{1000} = \frac{414.5}{1000} = 00414\text{g in 50ml.}$$

•12mM Sodium Bicarbonate – Molecular weight 84.007g.

$$\frac{84.007 \times 0.012 \times 50}{1000} = \frac{50.40}{1000} = 0.504\text{g in 50ml water.}$$

• 0.1mM EDTA - Molecular weight 372.24

$$\frac{372.24 \times 0.0001 \times 50}{1000} = \frac{1.861}{1000} = 0.00186\text{g in 50 ml water.}$$

All these chemicals were weighed and dissolved one after the other into 50ml autoclaved sterile distilled water.

PBS Preparation –

• 1.3M Sodium chloride - Molecular weight 58.44g.

$$\frac{58.44 \times 0.0013 \times 100}{1000} = \frac{7.597}{1000} = 0.00759\text{g in 100ml.}$$

• 70mM disodium hydrogen orthophosphate - Molecular weight 141.96g

$$\frac{141.96 \times 0.007 \times 100}{1000} = \frac{993.72}{1000} = 0.993\text{g in 100ml water.}$$

• 30mM Sodium dihydrogen orthophosphate - Molecular weight 156.01g

$$\frac{156.01 \times 0.003 \times 100}{1000} = \frac{468.03}{1000} = 0.468\text{g in 100ml water.}$$

This gives 10X buffer and then this diluted to 1x using distilled water by taking 1ml of PBS from 10X add 9ml of water gives 1x buffer.

Procedure:

• Draw the Blood into tube containing EDTA and centrifuge at 1000rpm for 5minutes .

• Collect the pellets add PBS and wash 3times at 1000rpm for 5minutes.

• 1:9 dilute pellets in PBS.

B.Haemolytic Assay

Reagents Required: RBC, 1x PBS, tween 20, sample.

Procedure:

- To the clean plate reader samples were loaded in the triplets.
- For Blank - 100µl of PBS was taken.
- For Positive control - 50µl RBC and 50µl Tween 20 both were mixed well by sucking with the pipette.
- For Negative Control - 50µl PBS and 50µl RBC.
- Then for all the samples including the TLC samples 50µl RBC was added followed by 50µl sample i.e., Water extract, ethanol extract and TLC samples.
- After the sample is loaded the plate was incubated for one hour at 370 C.
- Then centrifuge the same at 1000rpm for 5minutes at 4ºC.
- Collect the supernatant and measure the OD at 595.
- To calculate the percentage of RBC lysis by sample using formula,

$$\frac{\text{Absorbance}}{\text{Positive Control}} \times 100$$

C. ABTS Assay

ABTS is a stable free radical frequently used for estimating the 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation (ABTS) total antioxidant capacity (TAC) of natural products.

Chemical preparation:

Stock ABTS:

Buffer preparation:0.4M sodium acetate buffer in 50ml de-ionized water pH of 5.8 is set using glacial acetic acid.

$$\frac{0.4 \times 136.04 \times 50}{1000} = 2.721\text{g/50ml.}$$

10ml of ABTS stock:

Add 7mM(0.007M) ABTS and 2.5mM potassium persulphate in 10ml sodium acetate buffer kept overnight in 4º C.

Working ABTS:

833µl stock ABTS in 50ml methanol.

Gallic acid:

Stock: 75mg(0.0075g) gallic acid in 50ml deionized water.

Working:Take 10ml stock and makeup to 100ml.

Procedure:

- Pipette 0, 10,20,30,40,50,60,70,80,90,100µl of working standard solution of gallic acid into clean test tube .
- Make up volume to 100µl using distilled water.
- Take 50µl of 4 samples in 4 different clean test tubes.
- Add 3ml of ABTS reagent to all the test tube including unknown sample.
- Measure OD at 737nm.

D. Reducing power assay:

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm.

Chemicals required:

PBS buffer(0.2M):

1.548g of disodium hydrogen phosphate and 0.58g of sodium dihydrogen phosphate is added to 50ml of deionised water pH 7 is set using Hcl or NaOH.

Potassium ferricyanide(1%):

0.1g of potassium ferricyanide in 10ml deionised water.

Trichloro acetic acid(10%):

1g of trichloro acetic acid in 10ml deionised water.

Ferric chloride(0.1%):

0.01g of ferric chloride in 10ml of deionised water.

Procedure:

- Take 4 centrifuge tubes and add 2.5ml of PBS buffer, 2.5ml of potassium ferricyanide and 1ml of 4 pigment extracted samples are added to 4 different tube.
- Incubate at 50°C for 20min .Add 2.5ml of trichloro acetic acid.
- Centrifuge at 3000rpm for 10min For the supernatant add 2.5ml of distilled water and 0.5ml of ferrichloride.
- Absorbance is measured at 700nm

E.Phosphomolybdenum assay:

This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically.

Chemicals required :

Sodium phosphate :

Stock -0.436g of sodium phosphate in 1 ml deionised water.

Working - 100µl of stock make up to 1ml using deionised water.

Ammonium molybdate :

Stock -0.494g of ammonium molybdate in 10ml deionised water.

Working -100µl of stock make up to 1ml using deionised water.

Sulphuric acid(0.6M):

0.3ml of conc.sulphuric acid in 5ml deionised water.

Assay reagent:

0.06ml sulphuric acid,100µl sodium phosphate, 100µl ammonium molybdate and make up to 1ml using deionized water.

Procedure:

- Take 5 test tube, add 1ml of assay reagent to each test tube for 1 test tube 100µl of deionised water and used as blank, 1ml of 4 pigment extracted samples are added to 4 different tube.
- Keep the test tubes at 95° C for 90min in water bath.
- Cool the solution and measure the absorbance at 695nm.

5. Quantitative estimation of chlorophyll:

The chlorophyll a concentration is determined spectrophotometrically by measuring the absorbance of the extract at various wavelengths. The resulting absorbance measurements are then applied to a standard equation.

Chemicals required:

Fresh sample, Acetone:Ethanol(2:1).

Procedure :

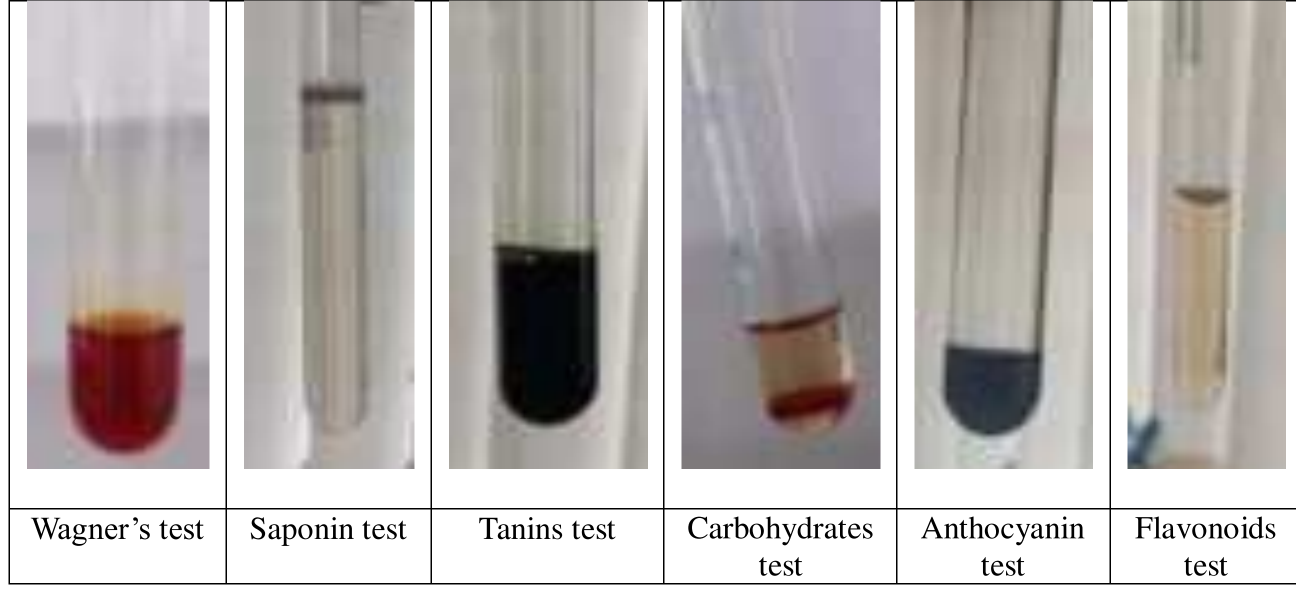
- 0.5g of fresh extraction material was taken in pestle and mortor and crushed in less light.
- Add 4ml of acetone:ethanol mixture,placed in 10ml tubes and mixed for 1min.
- Incubate for 30min in freezer.
- Centrifuge for 10min at 200rpm.
- Supernatent were covered with aluminium foil and 5ml of acetone ethanol mixture was added and stirred for 1min.
- Absorbance was read at chlorophyll a -642nm and 372nm.

Chlorophyll b- 626nm and 392nm.

RESULT

1. Phytochemical analysis

Sl.No	Phytochemical	Chlorophyll solvent extract	Chlorophyll water extract	Anthocyanin solvent extract	Anthocyanin water extract
1	Alkaloids a)Mayer's test	-	-	+	-
	b)Wager's test	+	+	+	+
2	Saponins	+	+	+	+
3	Phytosterols	+	-	+	-
4	Phenols	+	+	+	-
5	Tannins	+	+	+	+
6	Flavonoids	+	+	+	-
7	Anthocyanin	-	-	+	+
8	Protein	-	-	-	-
9	Carbohydrates	+	-	+	-
10	glycosides	-	-	-	-



Wagner's test	Saponin test	Tanins test	Carbohydrates test	Anthocyanin test	Flavonoids test

2. Thin layer chromatography

- Chlorophyll solvent and water extract - Chloroform : Ethanol(7:3).
- Anthocyanin solvent and water extract - n-butanol:acetic acid:water(4:1:5)

Sample	Chlorophyll solvent extract			Chlorophyll water extract	Anthocyanin solvent extract	Anthocyanin water extract	
Solvent distance(cm)	6.4			5.4	4.5	5.6	
Solute distance (cm)	Spot 1 5.9	Spot 2 5.3	Spot 3 4.8	Spot 4 1.6	Spot 5 2.1	Spot 6 1.2	Spot 7 2.3
Rf value	0.921	0.828	0.75	0.296	0.466	0.214	0.410
Observation							

Spectrum analysis of TLC spots

	Absorbance	**nm**
Spot 1	0.94	206.92
Spot 2	1.01	208.89
Spot3	0.96	209.27
Spot4	2.49	200.39
Spot5	1.68	201.16
Spot6	2.27	200
Spot7	1.55	200.39

Antioxidant assays

A.Hemolytic assay

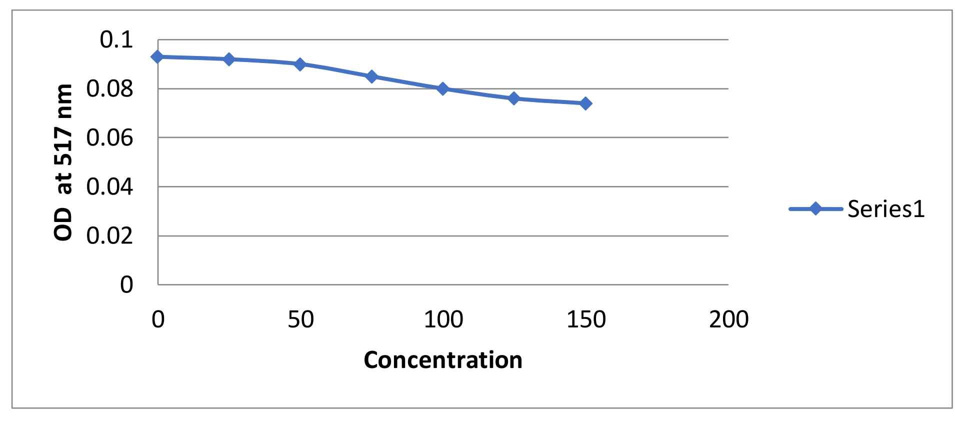
Sl no	Sample	OD at 595 nm
1	Blank	0.083
2	+ve control	1.108
3	- ve control	0.080
4	Chlorophyll solvent extract	0.191
5	Chlorophyll water extract	0.232
6	Anthocyanin solvent extract	0.205
7	Anthocyanin water extract	0.292

sample	Absorbance	Percentage(%)
Chlorophyll solvent extract	0.191	17.23
Chlorophyll water extract	0.232	25.70
Anthocyanin solvent extract	0.205	18.50
Anthocyanin water extract	0.292	26.35

B.DPPH assay

Sl No	Volume of ascorbic acid(µg)	Conc of ascorbic acid(µg)	Volume of distilled water(µl)
1	100	150	0
2	83.3	125	16.6
3	66.6	100	33.3
4	50	75	50
5	33.3	50	66.6
6	16.6	25	83.3
7	0	0	100

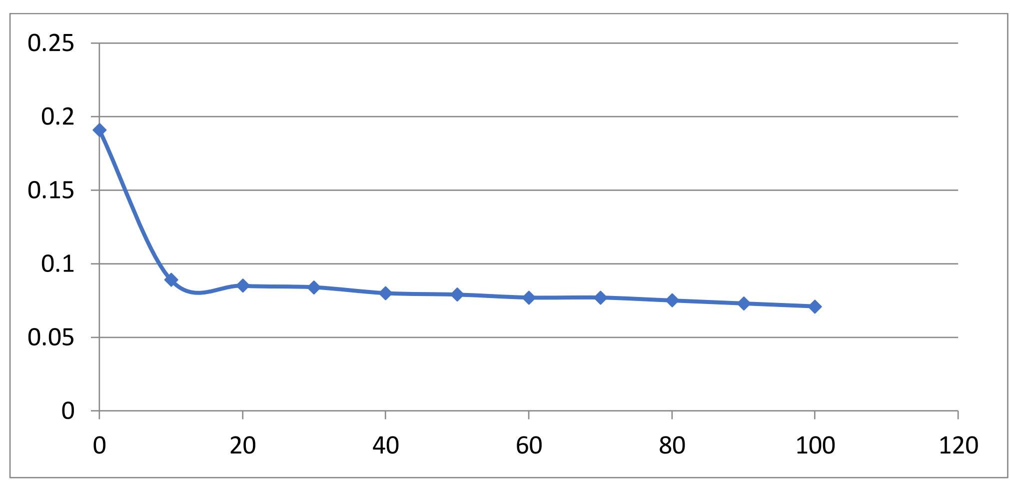
Sl No	Volume of ascorbic acid (µl)	Conc of ascorbic acid (µg)	Volume of DPPH(ml)		O.D at 517nm
1	10×3	0			0.093
2	10×3	25			0.092
3	10×3	50			0.090
4	10×3	75			0.085
5	10×3	100			0.080
6	10×3	125			0.076
7	10×3	150			0.074
8	Chlorophyll solvent extract a)10	-			0.079
9	b)20	-			0.075
10	c)30	-			0.074
11	d)40	-			0.074
12	e)50	-			0.073
13	Chlorophyll water extract a)10	-	3ml	Incubate for 30min for room temperture	0.090
14	b)20	-			0.082
15	c)30	-			0.082
16	d)40	-			0.084
17	e)50	-			0.080
18	Anthocyanin solvent extract a)10	-			0.079
19	b)20	-			0.073
20	c)30	-			0.079
21	d)40	-			0.077
22	e)50	-			0.076
23	Anthocyanin water extract a)10	-			0.093
24	b)20	-			0.092
25	c)30	-			0.085
26	d)40	-			0.083
27	e)50	-			0.082



Chlorophyll solvent extract		Chlorophyll water extract		Anthocyanin solvent extract		Anthocyanin water extract	
Vol(µl)	Conc(µg)	Vol(µl)	Conc(µg)	Vol(µl)	Conc(µg)	Vol(µl)	Conc(µg)
10	111.19	10	35.86	10	111.19	10	15.32
20	138.58	20	90.65	20	152.28	20	25.17
30	145.43	30	90.65	30	111.19	30	70.10
40	145.43	40	96.95	40	124.89	40	83.80
50	152.28	50	104.34	50	131.73	50	90.65

C.ABTS assay

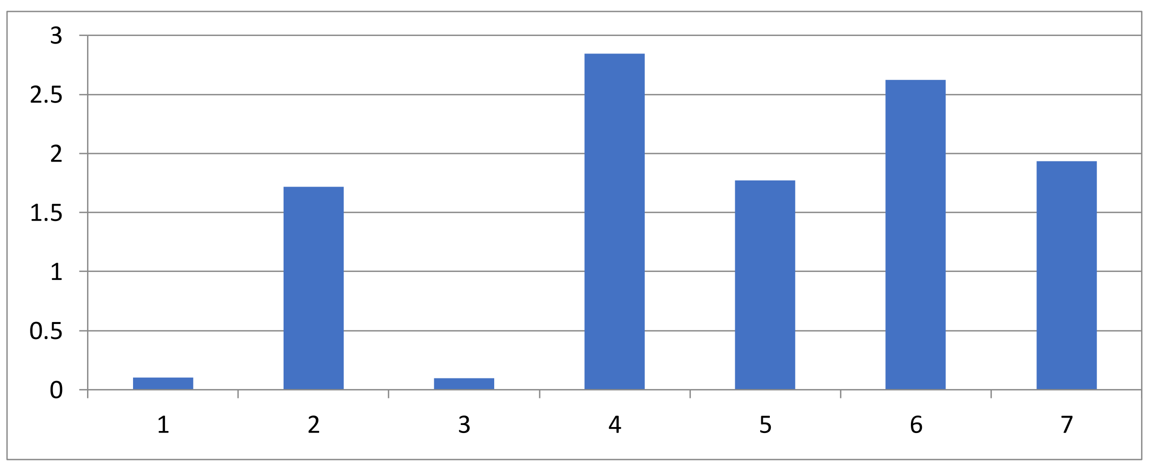
Sl. No	Volume of gallic acid(µl)		Conc of gallic acid (µg)	Volume of distilled water(µl)	Volume of ABTS(ml)	OD at 696nm
1	0		0	100	3ml	0.191
2	10		10	90		0.089
3	20		20	80		0.085
4	30		30	70		0.084
5	40		40	60		0.080
6	50		50	50		0.079
7	60		60	40		0.077
8	70		70	30		0.077
9	80		80	20		0.075
10	90		90	10		0.073
11	100		100	0		0.071
12	Chlorophyll solvent extract	50	-	-		0.078
13	Chlorophyll water extract	50	-	-		0.080
14	Anthocyanin solvent extract	50	-	-		0.071
15	Anthocyanin water extract	50	-	-		0.083



sample	concentration
Chlorophyll solvent extract	56.80µg
Chlorophyll water extract	55.58µg
Anthocyanin solvent extract	61.05µg
Anthocyanin water extract	53.75µg

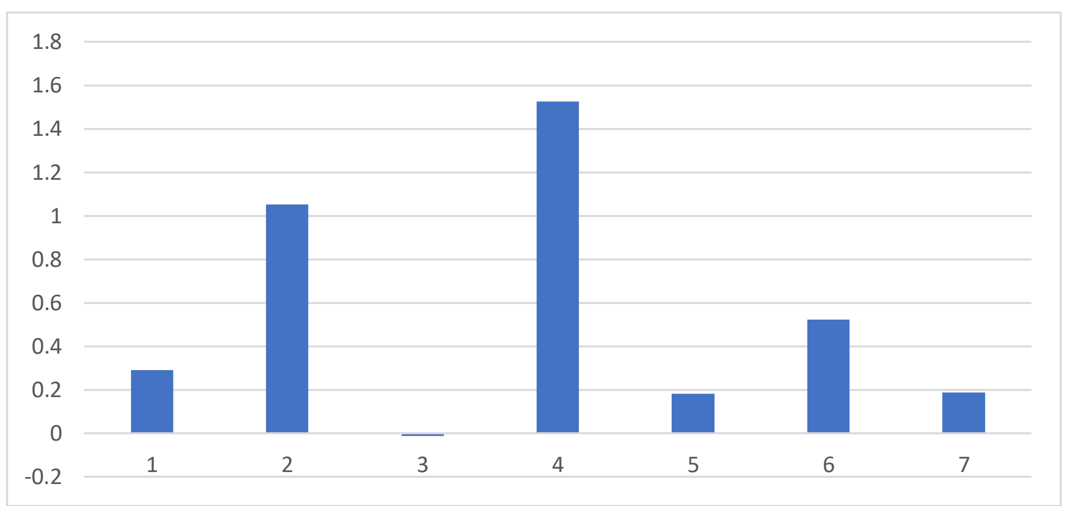
D.Reducing power assay

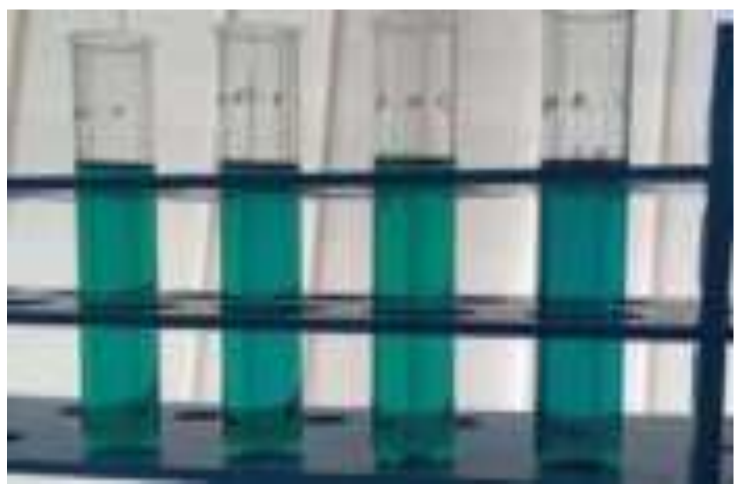
Sl no	sample	OD at 700nm
1	+ve control(100µl)	0.1030
2	+ve control(1ml)	1.7188
3	- ve control	0.0985
4	Chlorophyll solvent extract	2.8427
5	Chlorophyll water extract	1.7705
6	Anthocyanin solvent extract	2.6209
7	Anthocyanin water extract	1.9340



E.Phosphomolybdenum assay

Sl no	sample	OD at 700nm
1	+ve control(100µl)	0.2917
2	+ve control(1ml)	0.053
3	- ve control	-0.0126
4	Chlorophyll solvent extract	1.5262
5	Chlorophyll water extract	0.182
6	Anthocyanin solvent extract	0.5229
7	Anthocyanin water extract	0.1884





5.Quantitative estimation of chlorophyll

Absorbance	642nm	372nm
Chlorophyll a	0.0968	0.2968
Absorbance	626nm	392nm
Chlorophyll b	0.2688	0.0643

Calculation: Chlorophyll a(mg/g) = (reading × A642) – (reading × A372)
= (0.0968) – (0.2968)
Chlorophyll b(mg/g) = (reading × A392) – (reading × A626)
= (0.2688) – (0.0643)
Total chlorophyll(mg/g) = Chlorophyll a + Chlorophyll b
= 0.1611 + 0.5656
= 0.7267mg/g

RESULT: The total chlorophyll present in the sample = 0.7267 mg/g.

SUMMARY

Phytochemical and antioxidant assays are techniques used to analyze the chemical compounds present in plants (phytochemicals) and their ability to combat oxidative stress (antioxidant activity). These tests help identify and quantify various bioactive compounds in plants, such as flavonoids, phenols, alkaloids, and terpenoids. Common phytochemical assays include spectrophotometry, chromatography, and spectroscopy. Antioxidants are compounds that protect cells from damage caused by free radicals. Antioxidant assays assess the ability of plant extracts or compounds to neutralize free radicals. Common antioxidant assays include DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, FRAP (Ferric Reducing Antioxidant Power) assay, and ORAC (Oxygen Radical Absorbance Capacity) assay.These assays are crucial in understanding the potential health benefits of plant-based foods and medicines, as phytochemicals often contribute to their therapeutic properties. High antioxidant activity in plant extracts suggests their potential in reducing oxidative stress and preventing various diseases.

Phytochemical assays are laboratory tests used to analyze the chemical compounds present in plants. These assays help researchers identify and quantify bioactive substances with potential health benefits. Common phytochemical assays include measuring total phenolic content (TPC), total flavonoid content (TFC), total alkaloid content (TAC), total tannin content (TTC), and conducting antioxidant assays. Additionally, techniques like HPLC, GC-MS, TLC, and UV-Vis spectroscopy are employed to separate and analyze specific phytochemicals in plant extracts, providing valuable insights into their composition and potential uses.

Antioxidant assays are laboratory tests designed to evaluate the antioxidant activity of substances, typically in plant extracts or compounds. DPPH assay: In this assay, the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) is used to measure the ability of an antioxidant to scavenge free radicals. The reduction in DPPH absorbance indicates antioxidant activity.ABTS Assay: The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay assesses the capacity of antioxidants to reduce ABTS radicals, resulting in a decrease in absorbance.

CONCLUSION

Half page

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PROJECT

REPORTON

“Elucidationofantitumoractivityof*PureNE–Placebo*oncancercells”

SUBMITTEDTO

DEPARTMENTOFSTUDIESINBIOTECHNOLOGY

Pooja Bhagavat Memorial Mahajana Education

CentrePost Graduate wingof

SBRRMahajanaFirstGradeCollege(Autonomous)

Metagalli,K.R.Sroad,Mysore-570016,Karnataka

In Partial Fulfilment for the Requirement of Award of

The**DEGREE IN MASTER OF SCIENCE IN**

BIOTECHNOLOGYSUBMITTEDBY

MS. VAISHNAVI

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Vaishnavik

DECLARATION

I, Ms. VAISHNAVI K, hereby declare that dissertation entitled **"Elucidation of antitumoractivityof*PureNE-Placebo*oncancercells"**submittedinpartialfulfilmentoftherequirements for the award of the degree of **Master of science in Biotechnology,** is a recordof original research work carried out by me under the external guidance of **Dr. Bharathi PSalimath,Director,SanorvaBiotech.Pvt.Ltd.,Mysore,Karnataka,**andInternalguidanceof**Dr. Jyoti Bala Chauhan, professor, Department of studies in Biotechnology, PoojaBhajavat Memorial Education Centre Post Graduate Wing Of SBRR Mahajana FirstGradeCollege(Autonomous),MetagalliKRS Road Mysore.**

Thisprojectreporthasnotformedthebasisfortheawardofanyotherdegree/Diploma/Associatechip/ Fellowshiporanothersimilartitleofanycandidateofanyuniversity.

Date:

Place:Mysore

Vaishnavik

Departmentofstudies in Biotechnology

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Post-Graduationwing,

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Metagalli,KRSroadMysore

INDEX

SL.no	Contents	Pageno
1	Introduction	1-3
2	Reviewof Literature	4-6
3	Objectives	7
4	MaterialsandMethods 3.1. Instrumentation 3.2. Preparationofbuffersandreagents 3.3. *IN VITRO* Assay 3.4. *IN VIVO* Assay	8-23
5	Resultsanddiscussion	24-39
6	MajorfindingsandConclusion	40
7	References	41-42

LISTOFFIGURES/GRAPHS

FIGURESANDGRAPHS	Pageno
Figure1.1:Tumorangiogenesis	2
Figure1.2:Tumormetastasis	3
Figure1.3.Failedappoptosis	3
Figure3.1.Mediafiltrationunit	14
Figure3.2.Vialscontainingfreezingmixturewithcells	15
Figure3.4.Formazanformationin MTTAssay	18
Figure4.1.1.Hemocytometerchambercontaininglive cellsunderaninvertedmicroscope	24
Figure4.1.2.A.MicroscopeimageofMDA-MB-231 cells	25
Figure4.1.2.B.Microscopicimageofformationof formazancrystals	25
Graph4.1.2.A. MTTassayabsorbancegraph	26
Graph4.1.2.B.Percentof cellviabilityMDAMB-231 cellsaftertreatmentwiththecompound	27
Figure4.1.2.C.Colourcomplex formed afterMTTassay indicatingcellviability	27
Figure4.1.3.A.TreatmentofMDAMB-231 cellswith thecompound	28
Graph4.1.3.A.ELISAabsorbancegraph	28
Graph4.1.3.B.ELISAConcentrationgraph	29

Figure4.1.3.B.ResultsofELISAshowing coloured complexindicatingthepositive effectofthecompound	29
Graph4.1.4.Numberofcells treatedwithcompound	30
Figure4.2.1.ImageofEATbearing Swissalbino mice beforeandaftertreatingwiththecompound	31
Graph4.2.1.Representationofbodyweightofmice	32
Figure4.2.2.Imageofbloodveins fromtheperitoneal regionofthemice	33
Graph4.2.2.Representationofnumberofbloodvessels inperitonealregionofmice	33
Figure4.2.3.Imageofcollectedascitesalongwith packedcellsaftercentrifugation	34
Graph4.2.3.Ascitesfluid andpackedcellsvolumeof controlandtreatedmice	35
Figure4.2.4,Cellsinahemocytometer(a)Control(b) Treatedmice	35
Graph4.2.4.Representationofestimatedcellcount	36
Figure4.2.5.Determinationofproteinconcentration	38
Graph4.2.5.Representationofproteinestimationagainst BSAstandard	38
Figure4.2.6.Proteinbands resolvedongelafterSDS-PAGEand CBBstaining	39

ABBREVATION

Abbreviation	Explanation
DMSO	DimethylSulfoxide
PBS	Phosphatebuffersolution
CBB	Coomassiebrilliantblue
FBS	Fetalbovine serum
PNPP	Phenylphosphate
APS	Alkalinepersulphate
SDS	Sodiumdodecylsulphate
ELISA	Enzymelinkedimmunosorbentassay
EAT	Ehrilichascitestumor

LIST OF TABLES

SL no	Title	Page no
4.1.1	Numberofcellsin4cornersquareof the hemocytometer	24
4.1.2	ResultofviabilityofMDA-MB231 cellsas observed fromMTT assay	26
4.1.3	ELISAreadingobtained fromabsorbanceat405nm	28
4.1.4	Totalnumberofcellsaftertreatingwithcompound PureNE-Placebo	30
4.2.1	Detailsofbodyweightofcontrol and treated mice	32
4.2.2	Numberofbloodvesselsinperitonealofmice	33
4.2.3	Estimatedofpackedcellsandascitesfluidvolume	34
4.2.4	Estimationofcellcountofmice	36

ISOLATION AND CHARACTERIZATION OF PATHOGENIC BACTERIA FROM STREET FOOD AND ITS ANTIBIOTIC RESISTANCE ASSAY

A Project Report

Submitted to

Department of Studies in Biotechnology
Pooja Bhagavat Memorial Mahajana
PG Wing of SBRR Mahajana First Grade College (Autonomous)
Metagalli, KRS Road, Mysuru - 570016

In Partial Fulfillment of the Requirements
for the Award of Degree

MASTER OF SCIENCE
IN
BIOTECHNOLOGY

Submitted by

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CERTIFICATE

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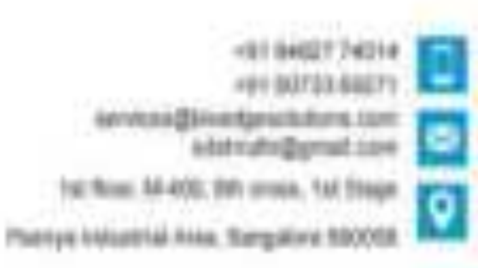

No: BES/CER/283/23-24 Sep 28th, 2023

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This is to certify that **Ms. Harshitha G. D. [Reg No. P01BH21S0004]** M.Sc. Biotechnology from Pooja Bhagavath Memorial Mahajana Education Centre PG Wing of SBRR Mahajana First Grade College (autonomous) affiliated to University of Mysuru, has successfully completed project entitled "**ISOLATION AND CHARACTERIZATION OF PATHOGENIC BACTERIA FROM STREET FOOD AND ITS ANTIBIOTIC RESISTANCE ASSAY**" at BioEdge Solutions, Bangalore under the guidance of Dr. SHRUTHI SD during 22/05/2023- 8/8/2023. She has successfully learnt and handled Microbiology and Molecular Biology techniques. I am pleased to state that she has completed the term with punctuality, hard work and as an inquisitive student.

Yours sincerely,

Place: Bangalore

Date: 28-09-2023

For BioEdge Solutions

Proprietress

DECLARATION

I, Ms. HARSHITHA G D with **Reg no: P01BH21S0004** hereby declare that the project work entitled "**ISOLATION AND CHARACTERIZATION OF PATHOGENIC BACTERIA FROM STREET FOOD AND ITS ANTIBIOTIC RESISTANCE ASSAY"** Submitted in partial fulfillment of the degree of master of science in biotechnology is a record of original research work carried out by under guidanceof **Dr. S D Shruthi, BioEdge Solution. Bangalore ,** and **Ms, Smitha Grace SR Assistant Professor, Department of Biotechnology,Pooja Bhagavat Memorial Institute Post Graduate Wing of SBRR Mahajana's College Mysuru,Karnataka.**

This project work has not formed the basics for award of any other degree\diploma associates or any other title of any candidate of any university.

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It is truly and for surely a great pleasure for me to present my major project work, a utmost loved, dedicated work of mine and it is my sheer luck that I got to do my project work under a noble, amicable, generous, jovial guide who stood by my side not only as a teacher, but as a mentor, friend, well-wisher and a strict, well-disciplined external guide **Dr**. **S D SHRUTHI** and internal guide **Ms. SMITHA GRACE S.R**.

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CONTENTS

Serial Number	Particulars	Page Number
1	INTRODUCTION	1-2
2	AIM AND OBJECTIVE	3
3	REVIEW OF LITERATURE	4-17
4	MATERIALAND METHOD	18-23
5	RESULTAND DISUSSION	24-32
6	CONCLUSION	33
7	REFERENCE	34-37

ABSTRACT

Food safety today is a fundamental need of food quality. For this investigation, we purchased Pani-puri samples from cart sellers near the campus. The samples were brought to the lab in an ice-cold state. Additionally, they underwent sub-culture and pour plate bacteriological analysis. Pour plate techniques involved serial dilution of the sample, followed by the selection of one tube from a higher dilution and one tube from a lower dilution, and the pipetting of 200 l of each sample onto Nutrient agar plates. The plates were then incubated for 20 hours at 370C, during which time they were checked for signs of growth. We performed a subculture using bacteriological agar. Bacterial growth was found in varied degrees in all of the tested food samples. 90 percent of the sampled foods had bacterial counts above the acceptable limits (10^4cfu/ml) and 10% of the samples had bacterial counts less than (<10^4cfu/ml). The foodstuffs analyzed contained six different bacterial species. Various methods were used to identify Staphylococcus aureus, Bacillus cereus, Vibrio spp., Salmonella spp., Escherichia coli, and Shigella spp. From samples of street food, many harmful microorganisms were discovered. The research showed that street food is a potential source of food borne illnesses, necessitating the development of workable safety measures for street food.

ISOLATION AND CHARACTERIZATION OF PATHOGENIC BACTERIA FROM STREET FOOD AND ITS ANTIBIOTIC RESISTANCE ASSAY

INTRODUCTION

Street food is a popular and convenient dining option enjoyed by people worldwide. However, it often involves a range of potential food safety risks, as it is typically prepared and served in open-air environments where hygiene standards may be less stringent than in formal restaurants or kitchens. One significant risk associated with street food is the presence of pathogenic bacteria, which can have detrimental effects on health when ingested. In this discussion, we will explore some common pathogenic bacteria found in street food and the adverse health effects they can cause.Street food is a popular and accessible culinary choice worldwide. However, due to its open preparation environment and sometimes limited hygiene standards, it can become a breeding ground for harmful microorganisms. Identifying pathogenic bacteria is essential to ensure that the food consumed on the streets is safe and free from contamination.

The identification of pathogenic bacteria in street food through antibiotic resistance assays is a vital aspect of food safety research. By subjecting these microorganisms to antibiotic susceptibility testing, we gain insights into their resistance patterns, which can be indicative of their potential to cause infections and the risks they pose to consumers. This study aims to elucidate the prevalence and characteristics of antibiotic-resistant pathogenic bacteria in street food, highlighting the need for improved food handling practices and antibiotic stewardship to safeguard public health.

The identification of pathogenic bacteria in street food through antibiotic resistance assays is a vital aspect of food safety research. By subjecting these microorganisms to antibiotic susceptibility testing, we gain insights into their resistance patterns, which can be indicative of their potential to cause infections and the risks they pose to consumers. This study aims to elucidate the prevalence and characteristics of antibiotic-resistant pathogenic bacteria in street food, highlighting the need for improved food handling practices and antibiotic stewardship to safeguard public health.

The quality of the raw food and the sanitary circumstances under which the food is processed may therefore be determined by microbiological investigation of foods and food contact surfaces (Michael et al., 2004). Microorganisms can readily migrate throughout the kitchen by attaching themselves to humans, food, and equipment. They reside there and can do so effortlessly. If the equipment is not thoroughly cleaned and sterilized before being used to produce another item, bacteria may transfer from the equipment to the food. It is crucial to check the quality of the food you eat as well as its wholesomeness, which means that any food you intend for human consumption should be pure and free of contamination, especially from pathogenic and rotting bacteria. Failure to ensure the safety and wholesomeness of the food consumed by the public might lead to some illness. To reduce contamination by microorganisms to a minimum level, and obtain good keeping quality of the products, the raw materials should regularly be monitored and examined. Food contacts surfaces are a major concern for food service facilities in controlling the spread of food-borne pathogens, surfaces such as bench tops, table, etc. may have bacterial on them from contact with people, raw foods, dirty equipment or other things such as cartons that have been stored on the floor. If the bench tops are not properly cleaned, any food on them will be contaminated by the bacterial.

Ultimately, the overarching goal of this study is to safeguard public health. By comprehensively understanding the presence and characteristics of antibiotic-resistant pathogenic bacteria in street food, policymakers, health authorities, and street food vendors can work together to implement strategies that reduce the risk of food borne illnesses and protect consumers.

Hence the following aims and objective have been conducted. To identify pathogenic bacteria present in the street food.

Aims and objectives

- Collection of samples from street side pani puri stall near college.
- To isolate pure colonies and identify using both Morphology and Microscopic technique.
- To identify obtained bacteria using biochemical tests.
- To characterize the organism using 16s rRNA sequencing method.
- To check the antibiotic resistance of the obtained pathogenic organism.

REVIEW OF LITERATURE

Isolation of pure colonies techniques

For isolation of pure colonies in drinking water first we do serial dilution, Membrane filtration, Streak plate method, pour plate method and spread plate method. Serial dilution is a series of sequential dilutions that are performed to convert a dense solution into a more useable concentration. In biology, serial dilution is often associated with reducing the concentration of cells in a culture to simplify the operation. Serial dilution is frequently used to avoid having to pipette very small volumes (1-10 micro liter) to make a dilution of solution (Avishai et al., 2014). In serial dilution, the density of cells is reduced in each step to make it easier to calculate the concentration of the cells in the original solution by calculating the total dilution over the entire series. In the late 1950s, membrane filters were developed as an alternative to the most probable number (MPN) method for microbial detection of water samples. In contrast to cellulose esters or other comparable polymeric materials, membrane filters are thin porous sheet structures. In order to catch microorganisms, membrane filters have a known uniform porosity of specified size (often 0.45mm).

On selective agar media, samples were cultivated. The spread plate method was used to calculate CFUs after serial dilution. MALDITOF MS was used to do the molecular characterisation and identification of the bacterial isolates, and the Kirby-Bauer disk diffusion assay and VITEK system were used to assess the antibiotic susceptibility of all the positive cultures (Kumar et al., 2021). Colonies were purified using the streaking plate method of twice subculturing. All isolates were determined to be Gram negative Bacilli using young cultures and gram staining. Primary and secondary biochemical identification tests were performed on each and every isolate that was Gram negative. In order to make sample cultures and for PCR purposes, the bacteria that were chosen to make antibiograms were streaked on to nutrient agar slant. Streaking is a technique used to isolate a pure strain from a single species of microorganisms, often bacteria. Streaking is a rapid and ideally a simple process of isolation dilution. This technique is done by diluting a comparatively large concentration of bacteria to a smaller concentration

Identification of pure colonies

Morphology: The configuration, margin elevation, shape, size and color of the isolates colonies should be observed under light microscope

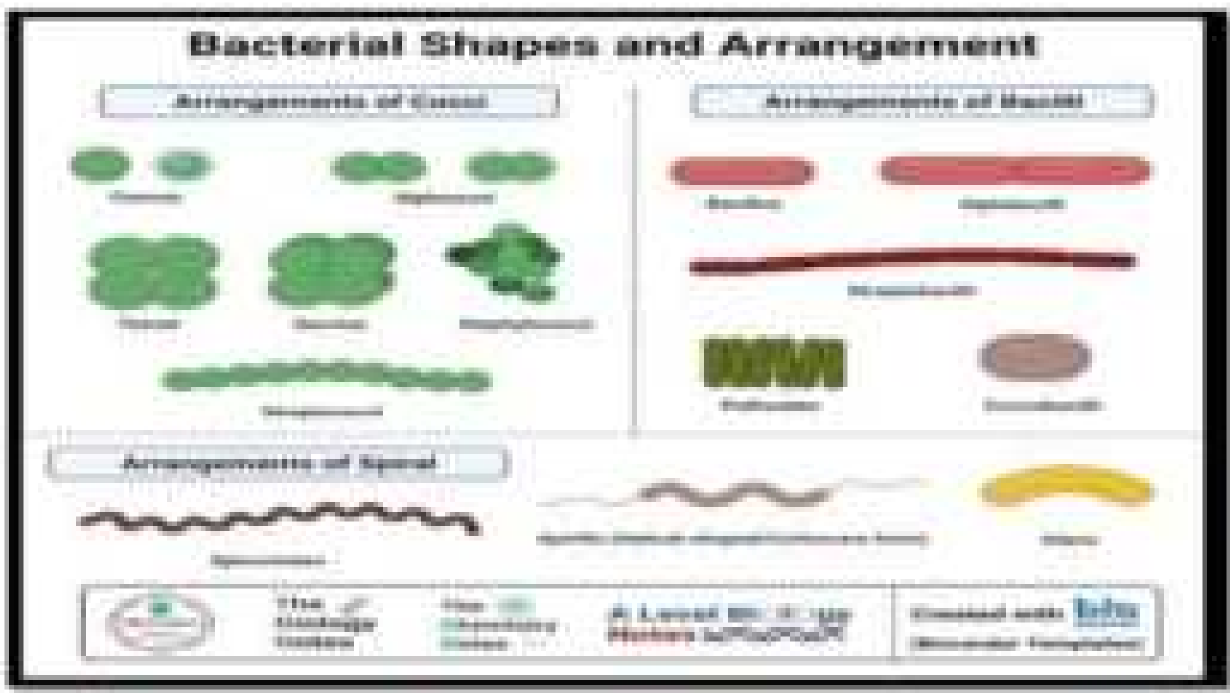


Fig.1 Bacterial shapes and arrangements

Microscopic test

Gram's stain: The gram staining is one of the most crucial staining techniques in microbiology. It gets its name from the Danish bacteriologist Hans Christian Gram first introduced it in 1882, mainly to identify organisms. When bacteria is stained with primary stain crystal violet and fixed by the mordant, some of the bacteria are able to retain the primary stain and some are decolourise by alcohol. The cell walls of positive bacteria have a thick layer of proteins-sugar complexes called peptidoglycan and lipid content is low. Decolorizing the cells causes this thick cell wall to dehydrate and shrink, which closes pores in the cell wall and prevents the stain from exiting the cell. So, the ethanol cannot remove the crystal violet-iodine complex that is bound to the thick layer of peptidoglycan of Gram positive bacteria and appears blue or purple color. Gram positive bacteria are Actinomyces, Bacillus, Clostridium, Corynebacterium, Gardne8rella, Lactobacillus, Staphylococcus, Streptococcus, Streptomyces exetra. Gram negative bacteria are E. coli, Salmonella, Shigella, and other Enterobacteriaceae, Pseudomonas, Moraxella, Helicobacter, Stenotrophomonas, Bdellovibrio, Acetic acid bacteria, Legionella exetra.

Biochemical tests: These tests include Oxidase, Catalase, Indole, Citrate utilization test. From mast diagnostics isolated pure colony can be placed on a filter paper using a sterile wire loop.

Oxidase testing: Organisms that manufacture cytochromes produce an intracellular oxidase enzyme. The oxidation of cytochrome c is catalyzed by this oxidase enzyme. Oxidase-positive organisms colour the reagent blue or purple because they contain cytochrome C as a component of the respiratory chain. Oxidase-negative organisms do not oxidize the reagent, leaving it colorless within the test's parameters. These organisms lack cytochrome c as a component of their respiratory chain.

Test for indole: Bacteria that express the tryptophanase enzyme can deaminate and hydrolyze the amino acid tryptophan. By using the intermediary molecule indole pyruvic acid, tryptophan is transformed into indole through reductive deamination. The tryptophan molecule's amine (-NH2) group is removed during the deamination reaction, which is catalyzed by tryptophanase. The enzyme's end products are indole, pyruvic acid, ammonium, and energy. As an enzyme, pyridoxal phosphate is needed.

Citrate utilization: Citrate agar is used to test the organism ability to utilize the citrate as a source of energy. The medium contains citrate as the soul carbon source and inorganic ammonium salts ($NH_4H_2PO_4$) as the soul source of nitrogen bacteria that can grow on this medium produce an enzyme, citrate permease, capable of converting citrate to pyruvate. Pyruvate can then enter the organism metabolic cycle for the production of energy. Growth is indicative of utilization of citrate, and intermediate metabolite in the Kreb's cycle. When the bacteria metabolize citrate, the ammonium salts are broken down to ammonia, which increases alkalinity. The shift in pH turns the bromothymol blue indicator in the medium green to blue above pH 7.6.

Christensen developed an alternative citrate test medium that does not require the organism to use citrate as a soul carbon source. Christensen medium contains both peptone and cystine. The citrate negative bacteria can also grow on this medium. A positive reaction shows that the organism can use citrate but not necessarily as sole carbon source.

Sanger sequencing of 16sRNA

The typical 16S rRNA sequencing method used in diagnostic labs is still based on the first generation sequencing method, which involves PCR amplification, qualitative product detection and separation by gel electrophoresis, and amplicon purification with ethanol. Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli are among the most prevalent infections that may be detected and genotyped using traditional Sanger sequencing methods.

Despite being widely used for PCR, only a small number of studies have examined its usefulness for pathogen sequencing and typing, which would validate this claim. The standard Sanger sequencing method, also known as "first generation sequencing," is still the basis for the widespread 16S rRNA sequencing technique used in diagnostic labs today. This method involves PCR amplification, product qualitative detection and separation by gel electrophoresis, purification of the amplicon through ethanol precipitation, sequencing by an amplification reaction, and final capillary electrophoresis.

Due to time-consuming, laborious, high operation skills requirement and potential hazard of ethidium bromide in agarose gel electrophoresis, the first generation sequencing technique has not been commonly used in most diagnostic laboratories. To save time and reduce workload,

they make improvement and propose a new combined protocol involving direct sequencing of the product generated by diagnostic SYBR Green real-time PCR. The PCR product is diagnosed via the amplifying curve, and specificity of the product is determined by analysis of the melting curve, avoiding the step of agarose gel electrophoresis. In addition, we optimized all hands-on instrument steps by using modern reagents, by means of sequencing 16S rRNA gene of reference and clinical pathogenic strains, we validated the applicability and also found the shortcomings of 16S rRNA gene sequencing method for identification .

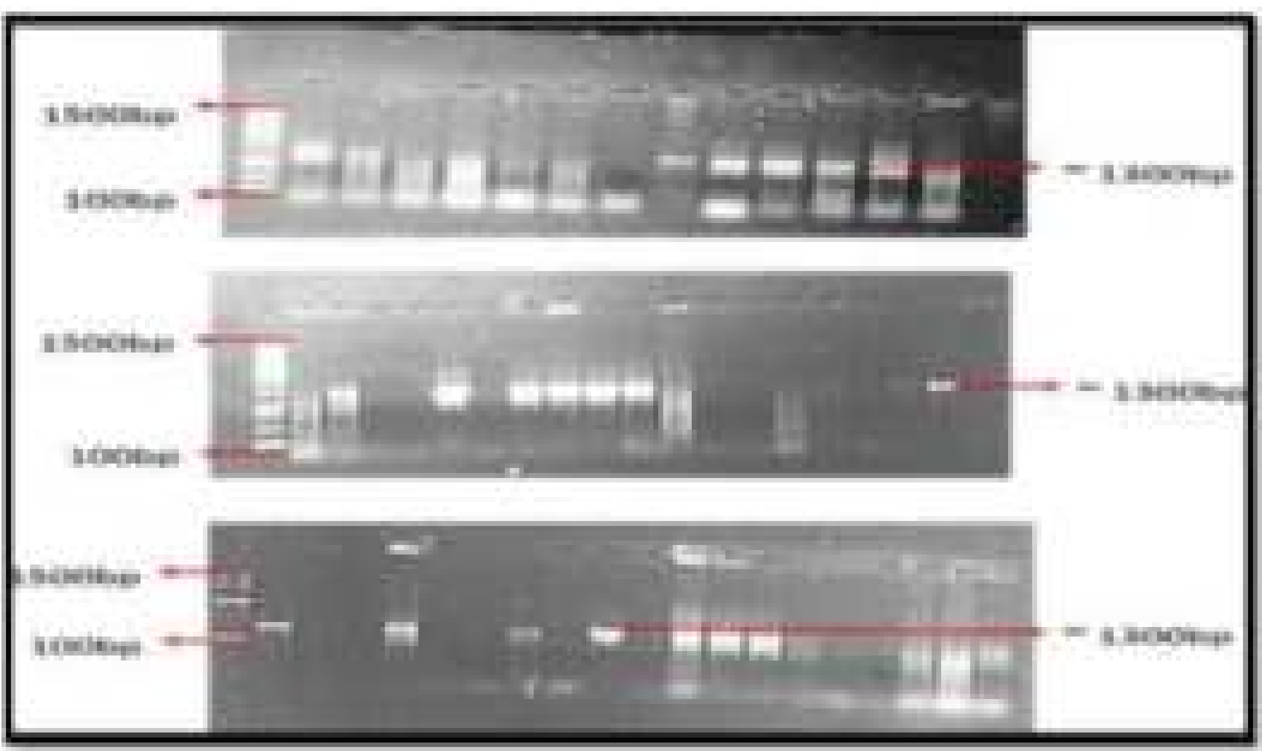


Fig 2: Ethidium bromide stained agarose gel resolving the PCR amplification fragment of 16s rRNA gene 1.3Kb from bacteria. (~1300Kbp) 16s rRNA gene as PCR product. Lane M : DNA marker (1000bp).

Antimicrobial susceptibility test:

Antimicrobial susceptibility testing aims to identify potential treatment resistance in common pathogens as well as susceptibility to preferred medications for specific diseases. Broth microdilution and quick automated instrument methods that use goods that are sold in stores are the two most popular testing techniques. The gradient diffusion method and disk diffusion are manual techniques that offer flexibility and potential cost savings. Each method has a strength and a weakness, and some methods produce quantitative results (such as Minimum Inhibitory Concentration), while others are intermediate or resistive.

Susceptibility testing of individual isolates is important with species that may possess acquired resistant mechanisms (Eg: Members of the Enterobacteriaceae, Pseudomonas species, Staphylococcus species, Enterococcus and *Streptococcus Pneumoniae*).

Most commonly used susceptibility testing method

Broth dilution test: one of the earliest antimicrobial susceptibility testing methods was the macro broth or tube -dilution method (Ericsson et al., 1971). The advantage of this technique was the generation of a quantitative result (i.e, the MIC). The principle and disadvantages of the macro dilution method were the tedious, manual task of preparing the antibiotic solutions for each test.

Antimicrobial gradient technique: The antimicrobial gradient diffusion technique works on the premise that an antibiotic concentration gradient can be established in an agar medium to assess susceptibility. Because it can test the medications the lab selects, the gradient diffusion method offers inherent versatility. When testing a fastidious organism that needs enriched media or a particular incubation environment, this method works well when only a MIC for one or two medicines is required (for example, when testing Penicillin and Ceftriaxone with Pneumococci).

Disk diffusion test: The disk diffusion susceptibility method is simple and practical and has been well-standardized.it is used to determine the susceptibility of clinical isolates of bacteria to different antibiotics. The advantages of the disk method are the test simplicity that does not require any special equipment. Antibiotic susceptibility test was performed using Kirby-Bauer disc diffusion method (Kirby et al., 1966). The following antibiotic discs (mast diagnostics UK) at the final concentrations that are indicated were used; grams, Streptomycin (S) 10 Micro grams, erythromycin (E) 15 Micro grams, Chloramphenicol(C) 30 Micro grams, Neomycin (NE) 30

Micro grams, amoxicillin (A) 10 Micro grams, Ciprofloxacin (CIP) 5 Micrograms, Trimethoprim (OT) 30 Micro grams these antibiotics were chosen because they are either used in both medicine and animal veterinary practice or because previous studies have reported microbial resistance to them.

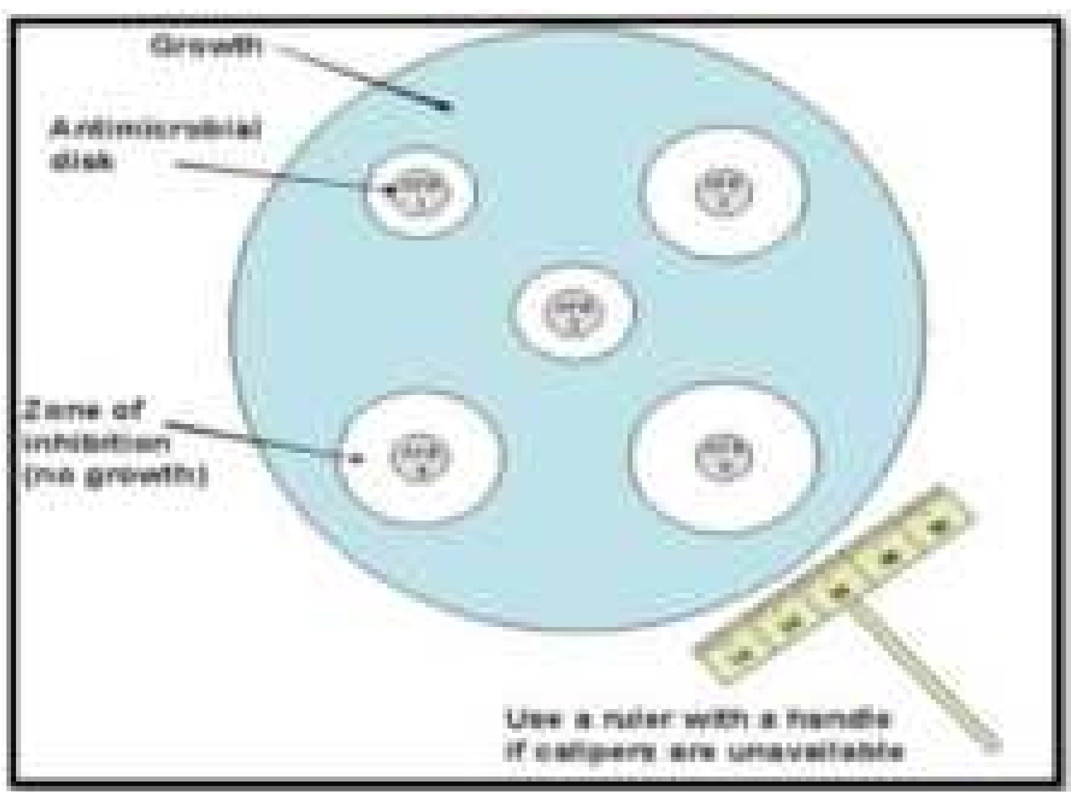


Fig 3. Antimicrobial susceptibility testing (Centers for disease control and prevention).

Isolation and identification of coliform bacteria from water sources of Hazara division, Pakistan

Coliforms were specifically determined in this investigation. Using a 100ml sample of drinking water from common sources, E. coli, P. aeruginosa, Salmonella, and H. pylori were isolated and identified. The WHO advises against the presence of Coliforms in any 100 ml sample of water that is intended for consumption. A total of 90 samples were gathered for this study from the Hazara division's three separate cities of Mansehra, Abbottabad, and Haripur. Following the use of staining to identify bacterial species, a culture technique was employed to identify harmful bacteria. In Mansehra, 15 samples (16.66%), 18 samples (20%), and 16 samples (17.77%) from Abbottabad and Haripur, respectively, were found to be pathogenic. Salmonella, P. aeruginosa, H. pylori, and E. coli, four distinct bacterial species, were discovered. coli was mostly isolated species that was identified in 24 samples (26.66%) followed by P. aeruginosa 11 samples (12.22%), H. pylori 8 samples (8.88%) and Salmonella 6 samples (6.66%). This study concludes that disinfection of water should be implemented to reduce water borne diseases, water supplying departments have to follow WHO standards for better public health and to control disease outbreak by coliforms water used for drinking is highly contaminated in Hazara division (Mansehra, Abbottabad and Haripura cities). As summer progressed number of pathogenic bacteria increased isolation of 4 different bacterial species indicate high number of water borne diseases in Hazara division. So water authorities should have to take steps to control coliforms in drinking water in order to prevent population from water borne diseases.

Isolation and 16s rRNA Identification of microorganisms from drinking water

Bottled water is an important source of drinking water in the KSA, which as limited resources of fresh water. Continuous monitoring of water quality and effectiveness of the treatment processes, and obeying regulations, are required to ensure that the water quality meets set standards and to meet the increasing demand for good quality tap or bottled water. The study revealed that most of the chemical parameters were within the world health organization limits (WHO) regardless of the brand name. Number of E. coli was detected throughout their study period. Water bottling plants and municipal water systems need to be thoroughly inspected and monitored so that only

businesses that consistently produce water with acceptable bacteriological purity are permitted to produce water for consumption by the general public. To improve the quality and shelf life of bottled water, it is advised that the Saudi Food and Drug Authority (SFDA) promulgate the industry's standardized process. Additionally, the findings of their investigation pointed to the necessity of enhancing hazard analysis critical control point (HACCP) systems in order to continuously monitor the source of the water samples at supermarkets and bottling facilities.

Isolation and characterization of bacteria from drinking Water fountains at a school canteen in Davao city

This study examined the microorganisms in drinking water fountains and hand-washing faucets at a Davao City school cafeteria. Tests on the isolates' morphology and biochemistry were done to characterize them. The majority, if not all, of the children were utilizing water fountains when Pseudomonas aeruginosa and Coagulase Negative Staphylococcus (CoNS) were discovered there. The study's main objective was to locate any potential bacteria in the school canteen water fountain, which is one of the campus's water fountains. Additionally, the isolates of bacteria utilized in the study were identified using biochemical techniques: The research in this study suggests that the use of molecular tests like PCR is highly suggested to further authenticate the discovered isolates for Pseudomonas aeruginosa, which is a frequent cause of pneumonia and urinary tract infections.

Isolation and identification of high fluoride resistant bacteria from street food samples of Dindigul district, Tamilnadu, South India

Fluoride pollution is one of the major issues in India and world wide. Water sample were collected analyze the physicochemical characteristics, from the results, acceptable limits pH ranges, low and high electrical conductivity values, high level of TDS (Total dissolved solids) and chloride(cl-)values, less than desirable and higher than permissible F-concentrations was found at first, ninety three colonies were screened using rapid hicoliform agar plates. After that 66 F- resistance colonies were picked up from 50Mm Naf (sodium fluoride containing LB agar

plates. F- resistance isolated showed their salt tolerance ranged from 4% to 7% NaCl resistance. F- resistant isolates were identified as Enterobacter cloacae strain, Enterobacter species, E. coli strain all strains submitted in the NCBI database with accession numbers (MW131637, MW131639, MW131650655). F-resistant gene 'crcB' was successfully amplified from the resistant isolates using gene specific prioners. The results have demonstrated that fluoride resistant bacteria would be useful for fluoride determination.

Isolation of environmental bacteria from surface and drinking water Mafikeng, South Africa and characterization using their antibiotic resistance profiles

In this work, environmental bacteria from different raw water sources and the drinking water distribution system in Mafikerg, South Africa, were isolated, identified, and their antibiotic resistance profiles were determined. Fecal indicator bacteria, as well as Acromonal and Pseudomonas species, were examined in water samples from five different sites. All examined organisms were resistant to erythromycin and amoxicillin, the two antibiotics with the highest prevalence of resistance. All isolates were susceptible to ciprofloxacin, neomycin, and streptomycin for pseudomonas species, coliforms, and face infections. The findings show that a variety of bacterial species, as well as occasionally fecal and total coliforms, are present in the drinking water from Mafikeng. that different microorganisms are resistant to specific antibiotic groups.

Isolation and characterization of stream water bacteria from ESA-OKE metropolis

Water is necessary for life, but many people lack access to clean, safe drinking water, and many suffer fatal diseases from bacteria that are spread through the water. In order to identify the bacteria isolated from the stream, the study accessed the bacteria mean counts of the three reliable streams in the ESA-OKE metropolis. Water samples 100.1 from dilutions of 10 and 10 were used to calculate the viable bacterial count. Bacteria plate counting and subculturing were done using the pour plate and streak plate techniques, respectively. For the characterization of bacteria, selective and differential media were also employed. According to the results of the plate count, Afon OKE had a greater count than the others. It also showed that this plate included more Staphylococcus aureus than the others, while the Arubes plate contained more pseudomonas aeruginosa than the others. The study concluded that the stream should be monitor

from the source since they are very close to residential areas and outbreak of water borne disease in imminent as well as the water inspection from the source should be considered very important.

Isolation of pathogenic bacteria from drinking water of school wells and tanks in Jalalabad of Nangarhar province, Afghanistan.

Water borne illness are prevalent and have caused significant losses to the habitants of Nangarhar province. This was due to lack of knowledge about water borne pathogens and accessibility to safe drinking water. This study was conducted to isolate and identify Shigella, Salmonella and E. coli from drinking water of Jalalabad government and private schools. Collectively, 300 drinking water samples was collected and subjected to microbiological test of *E. coli, Shigella* and *Salmonella* isolation in collected culture medium followed by gram staining and biochemical test. The results revealed that the prevalence of *Shigella, Salmonella* and *E. coli* was 60%, 38% and 20.7% in wells and 63.3%, 34% and 30.7% in water tanks respectively. In conclusion, the drinking water of schools in Jalalabad city is not safe from microbiological point of view and the prevalence of the common water borne pathogens is significantly associated with studied fetal.

Isolation and characterization of pathogenic bacteria from Kundu river water of Nandyal, Kurnool, Andhra Pradesh, India

The author's major goal is to investigate and characterize the pathogenic strains that are present in the water of the Kundu River and to determine how they would react to the human population using this water for a variety of home uses. They take a sample of water from the Kundu River, and using microbiological techniques, they isolate the contaminated bacteria. Analyses of the morphology and biochemistry were performed. Gram staining was used to distinguish between gram positive and gram negative after being seen morphologically under a microscope. Biochemical tests have been conducted to determine the genus of the bacteria. In order to determine how damaging antibiotics might be if they infect nearby populations, antibiotic susceptibility tests are also carried out. Based on a morphological and biochemical analyses, authors were found that the kundru river water is highly contaminated with pathogenic bacteria. Most of the bacteria isolated and identified in this study belong to E. coli, Klebsiella pneumoniae,

Pseudomonas aeruginosa, Staphylococcus aureus and Shigella dysenterial. Main of these were showing resistance towards the antibiotics in the antibiotic susceptibility test. This type of research can be useful in bringing the awareness both in the government and public regarding the safety precautions and measures to be taken to prevent contamination of water resources.

Isolation and identification of free living Amoeba from tap water in Sivas, Turkey.

The author's major goal is to investigate and characterize the pathogenic strains that are present in the water of the Kundu River and to determine how they would react to the human population using this water for a variety of home uses. They take a sample of water from the Kundu River, and using microbiological techniques, they isolate the contaminated bacteria. Analyses of the morphology and biochemistry were performed. Gram staining was used to distinguish between gram positive and gram negative after being seen morphologically under a microscope. Biochemical tests have been conducted to determine the genus of the bacteria. In order to determine how damaging antibiotics might be if they infect nearby populations, antibiotic susceptibility tests are also carried out. The free-living amoeba (FLA) that infects people both opportunistically and non-opportunistically.From 6 areas in the province of Sivas, the authors gathered a total of 150 samples of tap water. The samples were filtered before being seeded onto non-nutritional agar with E. coli spread. Out of 150 samples, 33 (or 22%) tested positive for FLA. Both morphological and PCR using the 18s r DNA gene were used to identify FLA. Acanthamoeba Castellani, Acanthamoeba Polyohaga, and Hartmannella Vermiforims were identified as three distinct species as a result of morphological investigations and the incomplete sequencing of the 18s r DNA gene. The majority of the pathogenic Acanthamoeba strains, genotype T4, were discovered to be present in all types of Acanthamoeba Polyohaga and Acanthamoeba castellanii. Finally, the authors noted the data that showed the presence and dispersion of FLA species in tap water in various regions of Sivas, Turkey. Additionally, health hazards associated with the region's tap water must be considered due to the existence of the temperature-tolerant Acanthamoeba genotype T4. Clinical diagnosis of the amebic illnesses is challenging, and a patient may experience therapeutic delays.

Molecular identification of biological contamination in different drinking water resources of the Jazan region, Saudi Arabia.

Drinking water quality plays a remarkable role in human infections and diseases. In this study authors used polymerase chain reaction (PCR) techniques to detect bacterial pathogens. In addition, they have done physiochemical analyses on drinking water samples from several sources, they were collected total 123 drinking water samples from different areas in the Jazan region in Saudi Arabia: ground water (40 samples), bottled water (15 samples), tap water (52 samples), and water purifications shops (16 samples). To isolate the Bacterial Pathogens were spread water samples on nutrient and MacConkey agar a16s ribosomal RNA technique. In 87(70.7%) of the 123 drinking water samples, there was no pathogen growth on the 2 cultures medium. However, 36(29.3%) of the samples were found to be contaminated with bacteria. The physiochemical analyses indicated that the water samples were within the Saudi drinking water standards, the bacteria were resistance to Cefotoxime, cefotaxime/clavulanic acid, erythromycin, penicillin G, Rifampin and Sulfamethoxazole-Primethoprim, respectively. Finally, they findings and suggested that bottled drinking water is the safest source of drinking water in Jazon; Therefore, regular monitoring studies are highly recommended.

MATERIALS AND METHODS

Sample collection:

In the month of May 2023, pani puri street sample was collected near lab aseptically in a sterile bottle with cap. The bottle was labelled as SSP and stored in clean place.

Serial dilution:

Five vials have been taken and marked with the numbers 10-1, 10-2, 10-3, 10-4, and 10-5. Using a sterile micropipette, 1 ml of the obtained material was taken and added to the 10-1 vial. To the 10-2 to 10-5 tube was put 900 l of distilled water. Currently, 1 cc of the mixture is transferred into the second tube from the 10-2 dilution. The overall dilution factor in the second tube is now 10-2. The remaining tube is then subjected to the identical process, with the exception that 100 l from the preceding tube is added to the subsequent 900 microliters of diluents.

Preparation of Luria Bertani Agar:

Suspend 40 gram of the powder in 1 liter of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute. To completely dissolve the powder, Autoclave at 121°C for 15 minutes. Test samples of the finished product for performance using stable, typically control cultures. Final pH 7.0 ± 0.2.

Pour plate method:

Consider two petri dishes with the labels 10-2 and 10-4. Pour the LB agar media into the petri plates to harden the media. Then, using a 1 ml micropipette, add 200 l of the solution for 10-2 and 10-4 tubes of samples to the petri plate containing the LB agar. Then, distribute the plates with a cotton swab. The plates are then incubated for 24 hours at 37 °C. Following incubation, the morphology of the plates was evaluated before doing pure culture.

Isolation of pure colonies:

Using mixed bacterial culture 5 colonies from each plate were collected and streaked on LB agar plates the plates were labelled as 10^{-2} (SSP1, SSP2, SSP3 and SSP4) and then plates are incubated for 24hrs at 37 ^{0}c, and gram staining was done.

Morphology and microscopic study:

Colonies that absorb nutrients were used to study morphological traits such shape, size, margin, elevation, texture, and colour. Gram reaction, form, and color were examined under a microscope. By looking at the colony under a microscope, it was possible to detect the color of the bacteria.

Gram staining of bacteria:

To differentiate the bacteria between gram positive and gram negative, gram staining was done. made a thin smear of culture on glass slides, dried the smear and heat, covered the smear one by one with crystal violet(60sec), grams iodine(60sec),95% ethanol(20sec), and safranin(40sec). Air dried the slides after wash with distill water and observe under microscope.

Biochemical tests:

Biochemical analysis was done in order to know which type of bacteria are present in the current water sample of the study. The biochemical tests include Catalase test, Oxidase test, Methyl red test and Voges Proskauer test. This test was performed according to standard methods.

Catalase test:

Five slides have been taken and labeled as KW3, KW4, BW1, BW3, and BW5. Add a little amount of bacterial culture to these slides, followed by hydrogen peroxide (H2O2), and let the slides for 10 minutes before checking for effervescence.

Oxidase test:

Five filter papers have been extracted and are marked as KW3, KW4, BW1, BW3, and BW5. Small amounts of bacterial culture and one drop of the Oxidase reagent (Tetramethyl-p-phenylenediamine) should be added to the filter paper. After 10 minutes, check to see if the color of the filter paper has changed.

Methyl red test:

Five test tubes have been taken and are marked KW3, KW4, BW1, BW3, and BW5. Add 4 ml of MRVP broth to this test tube, add a bacterial culture, and incubate for 48 hours at 37 °C. Add a drop of methyl red indicator after incubation, shake well, and watch for a color shift.

Voges Proskauer test:

Five test tubes have been taken and are marked KW3, KW4, BW1, BW3, and BW5. Add 4 ml of MRVP broth to this test tube, add a bacterial culture, and incubate for 48 hours at 37 °C. After incubation, add two drops of the Alpha Naphthol indicator and the potassium hydroxide, and shake well. After a few minutes, check for color change.

Sanger sequencing of 16sRNA

Extraction of genomic DNA:

DNA isolation is a process of purification of DNA from sample using a combination of physical and chemical methods. The first isolation of DNA was done in 1869 by Friedrich Miescher. A technique to lyse the cells gently and solubilize the DNA. Enzymatic or chemical methods to remove containing proteins, RNA, or macromolecules.

There are 3 basic steps involved in DNA extraction, that is, lysis, precipitation and purification. In lysis, the nucleus and the cell are broken open, thus releasing DNA. This process involves mechanical disruption and uses enzymes and detergents like chloroform to dissolve the cellular proteins and free DNA. The other step, which is known as precipitation the free DNA from the cellular debris. After separation of DNA from aqueous solution, it is then rinsed with alcohol, a process known as purification. Purification removes all the remaining cellular debris and unwanted material.

Method: Out of the ten pure bacterial cultures from the two water samples are chosen for DNA isolation. Add 1ml of CTAB Buffer and 2ml of each of the five vials. Each tube's appropriate bacterial sample should be added. The tubes should be incubated for 30 minutes at 60°C and then left at room temperature for 10 minutes. Add 1 ml of a chloroform and isopropanol mixture, and then centrifuge the tubes for 10 minutes at 10,000 rpm. Transfer the fluid from the upper layer, which contains DNA, to another set of tubes after centrifuging. To precipitate the DNA, add 1 ml of ice-cold isopropanol, mix well, and then place the tubes in the refrigerator at 20 C overnight. After incubation transfer DNA solution to DNA columns collection tubes and centrifuge at 10K rpm for 1 minute and discard the flow through solution. Add 700 µl of 2X wash buffer to wash the DNA and centrifuge at 10K rpm for 1 minute and discard the flow through solution. Add 600 µl of 2X wash buffer to wash the DNA and centrifuge at 10K rpm for 1 minute and discard the flow through solution. The DNA is bind in collection tube that tube is put inside the 1.7ml DNA collection tube and add 20 µl of Elution buffer and leave it for 5 minute. Add 1 ml of RNase mix well and leave it for 30 Minute at room temperature.

Agarose gel electrophoresis:

The most efficient method for isolating DNA fragments with sizes ranging from 100 bp to 25K bp is agarose gel electrophoresis. Agarose is made up of repeating agarobiose (L- and D-galactose) subunits and is extracted from the seaweed genera Gelidium and Gracilaria. Agarose polymerase forms a network of bundles during gelation whose pore widths control the gel's molecular sieving capabilities. DNA separation was revolutionized by the application of agarose gel electrophoresis. DNA was typically separated using sucrose density gradient centrifugation before agarose gels were widely used, although this method only offered an approximation of size.

Methods: weigh 1g of agarose and mix agarose powder with 100ml 0f 1X TAE buffer. Microwave for 1-3 minutes until the agarose is completely dissolved. And let agarose solution cool down till you can comfortably keep your hand on the flask. Pour the agarose into a gel tray with the well comb in place. Wait till the gel will completely solidified. Remove well comb from the gel. Place the agarose gel into the gel box. Fill gel box with 1_xTAE until the gel is covered. Mix the 2µl of DNA with 2µl of dye (Bromophenol blue). Carefully load a molecular weight ladder into the first lane of the gel. Carefully load the sample into the additional wells of the gel. Run the get from negative to positive side at 90 volts 250mA for 30 Minute. Visualize the DNA fragments in UV light transilluminator. Fragments of DNA are separated in the gel depending on their length (smaller molecules travel faster than larger molecules in gel).

Sanger sequencing of 16s rRNA

Five PCR strips was taken to those strips add 1µl of ITS2 FORWARD primer and ITS2 reverse primer. Then add 5µl of respective DNA sample to each tube and add 12.5µl of master mix then the strips are run in PCR machine for 1hr 28min. After PCR the samples were electrophoresed here weigh 1g of agarose and mix agarose powder with 100ml 0f 1X TAE buffer microwave for 1-3 minutes until the agarose is completely dissolved. And let agarose solution cool down till you can comfortably keep your hand on the flask. Pour the agarose into a gel tray with the well comb in place wait till the gel will completely solidified. Remove well comb from the gel and place the agarose gel into the gel box. Fill gel box with 10ml of TAE buffer and 480ml of water

until the gel is covered. Mix the 2µl of DNA with 2µl of dye (Bromophenol blue).Load the sample into the additional wells of the gel after carefully loading a molecular weight ladder into the gel's first lane. Run the gel for 30 minutes at 90 volts and 250 mA from the negative to the positive side. Use a UV light transilluminator to see the DNA fragments. DNA fragments are sorted in the gel based on their length (smaller molecules move through the gel more quickly than bigger molecules). Pieces of the bands are divided up and added to the S tubes. Add 400 l of gel solution (Buffer QG solubilization buffer) to these tubes, and keep them in a dry bath for 20 minutes at 55 °C. After dry bath transfer DNA solution to column collection tube and spin for 1min at 10K rpm. Then add 700µl of 1x wash buffer and spin for 1min at 10K rpm and dry spin for 2min at 10K rpm. Transfer column tube to DNA collection tube and add 20µl of elution buffer and leave it for 3min in room temperature and spin at 10K rpm for 1min. PCR strips are taken and add 1 of 27F primer and 2µl of DNA sample. Add 4.5 of water and 2µl of 5x buffer. Then add 0.5µl of master mix (Big dye terminator) and spin for 1min at 3K rpm just to mix the solution and kept the PCR strips in Eppendorf for 1hr 28min. Then PCR product were linked to 3130 XL genetic analyzer. The obtained dot ab1 files were observed through Finch TV.

Antimicrobial susceptibility test:

Add 1ml of LB media and 1ml of Enterococcus faecalis bacterial culture to a 1.5 ml tube, then vortex for 1 minute to mix the cultures. Pour the LB medium into the designated plate 1 and plate 2 of two petri plates, then wait for the plates to harden. After each plate has solidified, add 500 l of culture, spread it with a cotton swab, and then let it continue to solidify. In the first plate, add Amoxicillin, Azithromycin, Doxycycline, Norfloxacin, and Cephalexin antibiotics after the solidification process is complete. In the second plate, add Ciprofloxacin, Cefpodoxime, Cefixime, Amoxicillin clavulanate, and Metronidazole. Incubate the plates for 18 to 24 hours. Watch for the appearance of the zone after incubation.

Results

The aim of the study was to isolate and identify the bacteria exist in the street side pani puri. Further the isolated bacteria were tested for biochemical test, gram staining, molecular identification of 16s RNA and antibiotic susceptibility test.

Serial dilution method was followed in order to reduce the number of colonies and also to isolate the bacteria. The serially diluted water samples is spread on LB agar plates and incubated for bacterial growth and also to isolate pure colonies.

Sample	Size	Shape	Margin	Elevation	Texture	Pigmentation
SSP1	Large	Irregular	Undulate	Raised	Rough	Creamish
SSP2	Small	Circular	Round	Flat	Smooth	Creamish
SSP3	Medium	Irregular	Umbonate	Flat	Smooth	Creamish
SSP4	Large	Irregular	Crateriform	Raised	Rough	White
SSP5	Medium	Irregular	entire	flat	smooth	White

Table 1. Morphological characteristic of isolates from Pani puri sample.

Sub-culturing to obtain pure colonies

Five colonies in total were isolated from each sample, and they were kept alive on LB agar plates that were regularly kept at 4 C. The five isolates were given the SSP1, SSP2, SSP3, SSP4, and SSP5 designations. All five isolates' pure cultures were saved for later use.

Morphological characterization of bacteria isolated from Sample

The morphological characteristics such as color, size, shape and nature of colonies of all isolates were determined by growing them on LB agar plates. In addition, all the isolates were tested by gram staining to check whether they belong to gram positive or gram negative. The details of the morphological characteristics of isolates were shown in table 1 and ground water isolates were shown in table 1.

Microscopy : Gram Staining

Five distinct isolates were chosen from the total number of colonies collected. They are all Gram positive bacteria, according to staining, which revealed that two of the strains were gram positive rods, two were gram positive cocci in a cluster, and one was gram positive rod.

Biochemical characterization of bacteria isolated from sample:

The material was used to isolate the five colonies. Biochemical characterization was done on all of these isolates. The biochemical characterization of water samples revealed variation in how they responded to several biochemical tests.

Catalase test:

In this test SSP3 and SSP4 shows positive that means Staphylococcus and pseudomonas bacteria are present in sample. SSP1, SSP2 and SSP5 shown negative it means Bacillus present in bore water.

Oxidase Test:

In this test SSP3, SSP4 and SSP5 shows positive that means *Staphylococcus Aureus* bacteria is present in both water sample. SSP1 and SSP2 shows negative that means *Bacillus cereus* is present in pani puri water sample.

Methyl red test:

In this test all are showing positive it means *E. coli* is present in pani puri water sample.

Voges Proskauer test:

In this test SSP3 and SSP5 shows negative that means *E. coli* bacteria is present in both water sample. SSP1 and SSP3 shows positive it means *Enterococcus* is present in bore water. SSP4 shows partially positive.

Sanger sequencing of 16sRNA

Extraction of genomic DNA:

There are 3 basic steps involved in DNA extraction, that is, lysis, precipitation and purification. DNA bands are obtained by the method called Agarose Gel Electrophoresis.

Fasta sequences

>SSP1_27F_F01.ab1

ACGTAGCTGACGGATTCTCGTAAGCGCACGCAGGCGGTCTGTCAAGTC
GGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGCTGGCAGG
CTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATG
CGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGA
AGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGAT
ACCCTGGTAGTCCACGCCGTAAACACGTTCTACTTGGAGGCTGTGCCCT
TGAAGCGTGGCTTCCGGAGCTAACGCGTTAAGTAGACCGCCTGGGGAG
TACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAA
GCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCT
ACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGA
ACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATG
TTGGGTTAAGCTTTGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCG
GTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAA
GGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACAC
ACGTGCTACAATGGCGCATACAAAGAACGTCGAGAGCAAGCGGACCTC
ATAAAGTGCGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGA
AGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGT
TCCGGGACGTCACCATGGGAGTGGGTTGCAAAAGAAGTAGGTAGCTT
AACCTTCGGGAGGGCGCTTACC

Hit: *Enterobacter sp*. 24B 16S ribosomal RNA gene, partial sequence

Percentage identity: 99.99%

Query coverage: 98%

E value: 0.0

Accession no: ON669291.1

>SSP2_16S

TCGGTTCGGAGAACGGGGGAGGGGGTAACGGGATAGTGTGTCAAGGTA
AAACTATACCGTTACCGTGTTCCCGCTGACCCGCGACTACTACCGATTC
CGGCTTCTGCAGGTGAGTTGCAGCCTGCAATCCGAACTGAGAGAAGCT
TTAAGAGATTTGCATGACCTCGCGGTCTAGCGACTCGTTGTACTTCCCA
TTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGT
CATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCGCTAGAGTGCCC
AACTAAATGATGGCAACTAACAATAAGGGTTGCGCTCGTTGCGGGACT
TAACCCAACATCTCGGGAAAGCTCTATCTCTAGAGTGGTCAAAGGATG
TCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCT
CCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCG
GTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTTGCTGCAGCACTGAAG
GGCGGAAACCCTCCAACACTTCGCCACTGGTGTTCCTCCATATATCTAC
GCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTGCACTCAAG
TCTCCCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACAT
CAGACTTAAGAAACCGCCTGCGCTCGCTTTACGCCCAATAATCCGGAC
GACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGGTAGCGT
GCTTTCTGGTTAAATGACAGTCAAGGGACGTTCAGTACTAACGATACAT
AGTCCTTCTCTACATCGAAGATATACGATGGTTCACTCGCGAGTAGGCG
AGCACCGCTCTCAGATCCCCTAGTCGTTGGAACTGGAGCTACGTGCCCA
GTGCATATACACCGGGATTCATCTGCGGGCGACACCAAGCA

Hit : *Enterococcus faecalis partial* 16S rRNA gene, isolate strain #125 (MBG-DUTH)/170518_10 partial sequence

Percentage identity: 99.99%

Query coverage: 79%

E value: 1e-134

Accession no: OU548755.1

>SSP3_27F_D12.ab1

GCTCAGGACGAACGCTGGACGGACGTAGCGGCGTGCCTAATACATGCA
AGTCGAGCGGACAGATGGGAGCTACGTAGCTAGCAGCTAGTTGGTGAG
GTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGAT
CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGC
AGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGC
GTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGA
ACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAA
AGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCA
AGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCAGGCGGTTTCTTA
AGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAAC
TGGGGAACTTGAGTGCAGAAGAGGAGAGTGACGTAGCTGAATTCCACG
TGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAG
GCGACTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAG
CGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGC
TAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCAAACGCATTAAG
CACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTG
ACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAAC
GCGAAGAAGCAGCTAACGCATTAAGTGCCCCGCCTTGGGGAGTACGGC
CGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGC
GGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGGCT
TGACATACACCGGAAAACCCTGGAGACAGGGTCCCCCTTGTGGTCGGT
GTACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGT
TAAGTCCCGCAACGAGACGTGCAACCCTTGTCCCGTGTTGCCAGCAGG
CCCTTGTGGTGCTG

Hit: *Bacillus licheniformis* strain MBK1 16S ribosomal RNA gene, partial sequence

Percentage identity: 99.99%

Query coverage: 84%

E value: 0.0

Accession no: ON254199.1

>SSP4_27F_B06.ab1

ACGATGCTAGCTAGGACGACGTAGCGAGCTTGCTCCCTGATGTTAGCG
GCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGATA
ACTCCGGGAAACCGGGGCTAATACCGGATGCTTGTTTGAACCGCATGG
TTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCG
GCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCG
TAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCC
CAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAA
AGTCTGACGGAGCAACGAAGCGTGAGTGATGAAGGTTTTCGGATCGTA
AAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTAC
CTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGACGTTTATTG
GGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCC
CGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGA
AGAGGAGAGTGGAATTCCACGTGTAGCCCTGAAATGCGTAGAGATGTG
GAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTG
AGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCC
ACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGT
GCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGGAGTACGGTCGCAA
GACTGAAACTCAAAGGAATTGACGGCGAGCCCATTTATACCCATATAA
ATCAGCATCCATGGTGGGATTTATCGCGGCCTAAGGCAGACGGACGAT
GCTAGCT

Hit: *Bacillus subtilis* strain WHZ-20 16S ribosomal RNA gene, partial sequence

Percentage identity: 99.99%

Query coverage: 89%

E value: 0.0

Accession no: ON907586.1

>SSP5_27F_A05.ab1

TAGTTGGTGAGGTAACGGCTCACCCGACGATGCGTAGCCGACTGAGAG
GGTGATCGGCCACACTGGGGAGCAACGCCGCGTGAGTGATGAAGGTTT
TCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGGTTCAAATAGGG
CGGCACCTTGACGGTACCTACCAGAAAGCCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGG
CGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCG
GCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGA
GGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAG
GAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCAC
GCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGC
TGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGAC
TGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTGTTGACATCCTC
TGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGT
GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTAAGTCCCGCAAC
GAGCGCAACCCTTGATCTTAGTTGCACCGATACCAGGATCTTGCCATCC
TATGGAACTGCCTCGGTGAGTTTCTCCTTCATTACAGAAACGGCTTTTT
CAAAAATATGGTATTGATAATCTTGAATATGAATAAATTGCAGTTTCAT
TTGATGCTCC

Hit: Bacillus velezensis strain MBK3 16S ribosomal RNA gene, partial sequence

Percentage identity: 99.99%

Query coverage: 85%

E value: 0.0

Accession no: ON259692.1

Sample	Code	Hit	Percentage identity	Query coverage	E value	Accession number
SSP1	KW3_27F_F01.ab1	*Enterobacter Sp.*	99.99%	98%	0.0	ON669291.1
SSP2	KW4_16S	*Enterococcus faecalis*	99.99%	79%	1e-134	OU548755.1
SSP3	Bw1_27F_D12.ab1	*Bacillus licheniformis*	99.99%	84%	0.0	ON254199.1
SSP4	BW3_27F_B06.ab1	*Bacillus subtilis*	99.99%	89%	0.0	ON907586.1
SSP5	BW5_27F_A05.ab1	*Bacillus velezensis*	99.99%	85%	0.0	ON259692.1

Table 2. Sanger sequencing of 16S rRNA

Sl.no	Antibiotics	Zone of inhibition in mm
1	Amoxicillin	1.5
2	Azithromycin	3.8
3	Doxycycline	3.7
4	Norfloxacin	3.5
5	Cephalexin	1.3
6	Ciprofloxacin	4
7	Cefpodoxime	1.6
8	Cefixime	1.8
9	Amoxicillin	1.8
10	Metronidazole	2.2

Table 3. Antibiotic susceptibility test

Discussion

The Pani puri water was found to be tainted with numerous bacteria in the current study. Bacillus subtilis, Bacillus species, Bacillus licheniformis, Bacillus velezensis, and Enterobacter species were tentatively identified as the bacteria isolated from the sample based on the sanger sequencing of 16s rRNA. Some of the bacteria isolated in this study are members of the genus of pathogenic bacteria.

Most of these bacteria like *Enterococcus faecalis* cause many serious human infections such as Gut commensal, Urinary tract infection, Endocarditis, Bacteremia and Wound infections. *Bacillus licheniformis* cause sepsis in an immunocompetent patient. *Bacillus velezensis* is non-pathogenic. Enterobacter species causes many nosocomial infections, and less commonly community acquired infections including urinary tract infection, respiratory tract infection, soft tissue infections, osteomyelitis, and endocarditis. Every year millions of people die in developing countries mainly due to the disease caused by water contamination. Water contamination with pathogenic bacteria is serious concern not only in developing countries but also developed countries.

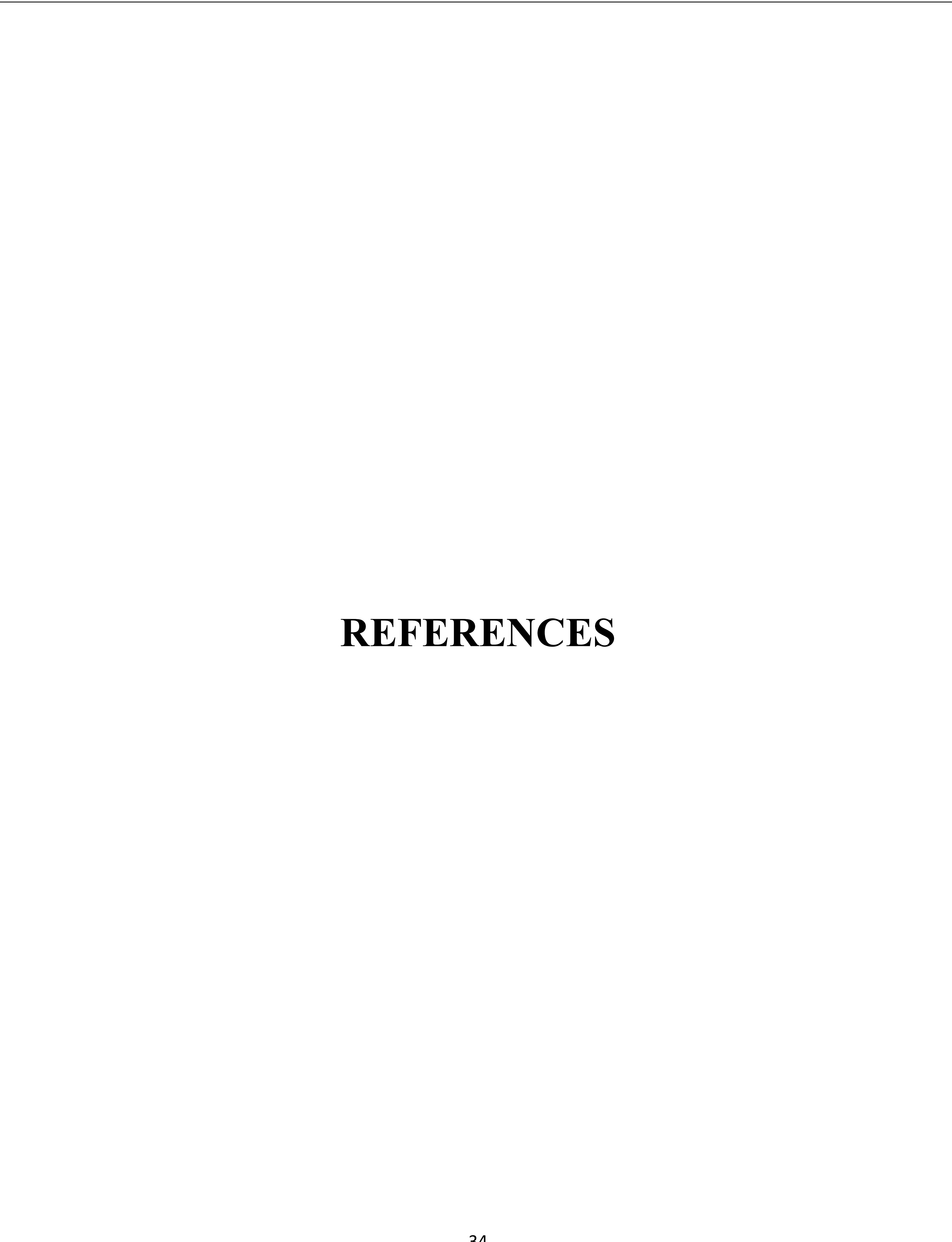
Numerous sources of street food, both in developed and developing nations, are vulnerable to contamination by various harmful bacteria. Salmonella, E. coli, Shigella, and other germs may also be present, depending on the local microorganism population. Many of these bacteria have developed resistance to a number of commercially available antibiotics. It is preferable to take less antibiotics less frequently since else these pathogenic microorganisms are difficult to get rid of because they steadily acquire resistance.

In this study highlights the presence of pathogenic bacteria in pani puri water. Therefore, care should be taken to free from the infections caused by these pathogenic bacteria especially at the time of rainy season. It is better to recommend to use good quality water for preparation of foods.

We have got 5 organisms in water sample *Bacillus subtilis, Enterococcus faecalis, Bacillus licheniformis, Bacillus velezensis and Enterobacter specie.* These organisms contradicting in street foods, soil and fruits. *Enterococcus faecalis* is corelating with rain water and Bacillus subtilis is corelating with pond water.

Conclusion

We carried out this investigation to alert scientists about the presence of dangerous bacteria in pani puri water. Additionally, it raises public awareness of the extent of contamination and its detrimental effects on the inhabitants of the Mysore area, where our college is located. The goal of this experiment was to identify the harmful bacteria found in street food. Sanger sequencing of the 16S rRNA and an examination of the bacteria's susceptibility to antibiotics are the two techniques used to identify pathogenic bacteria. Bacillus Subtilis, Enterococcus faecalis, Enterobacter species, Bacillus licheniformis, and Bacillus velezensis are the harmful bacteria found in food. The benefits of this endeavor for students and society include the fact that one of the biggest factors affecting human health is drinking water quality. However, street food quality in many countries, especially in developing countries is not desirable and poor food preparation quality has induced many water borne diseases. This special issue of exposure and health was edited to gain a better understanding of impacts of drinking water quality on public health so that proper action can be taken to improve the food quality condition in many countries. We can continue this work further by measuring physiochemical parameters, and major metal analysis, trace metal analysis, anion analysis, fluoride and chloride test and we can also find out the inorganic, organic, biological and radio logical contaminants.



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PHYTOCHEMICAL PROFILING AND ANTIMICROBIAL EVALUTION OF LEAF EXTRACT FROM *LANTANA CAMARA*

A Project Report

Submitted to

Department of Studies in Microbiology
Pooja Bhagavat Memorial Mahajana
PG Wing of SBRR Mahajana First Grade College (Autonomous)
Metagalli, KRS Road, Mysuru - 570016

In Partial Fulfilment of the Requirements
for the Award of Degree

MASTER OF SCIENCE
IN
MICROBIOLOGY

Submitted by

ABHISKEK V R
Reg. No. P01BH21S0245

Department of Studies in Microbiology
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Under the Guidance of

Ms. Sahanashree K S
Project Coordinator
Dextrose Technologies Pvt. Ltd.
Bengaluru

SEPTEMBER – 2023

Date:

CERTIFICATE

We certify that this project entitled **"Phytochemical Profiling and Antimicrobial Evaluation of Leaf Extract from *Lantana camara*"** submitted in partial fulfilment of the requirement for the degree of Master of Science in Microbiology to the department of Studies in Microbiology, Pooja Bhagavat Memorial Mahajana PG wing of SBRR Mahajana First Grade College, Metagalli, KRS Road, Mysuru, affiliated to University of Mysore. It is an original research work carried out by **Mr. Abhishek V R** at **Dextrose Technologies Pvt., Ltd., Bengaluru.**

Dr. Harish R	**Dr. Ramesh S.R**	**Prof. C.K Renukarya**
Co-Ordinator	**Chief Scientist and Head**	**Director**
Department of Microbiology	School of Life Science	Mahajana PG Wing

Date: 22/07/2023

CERTIFICATE

This is to certify that **Mr. Abhishek V R** with **Reg No: [illegible]** M.Sc. Microbiology student from **Pooja Bhagavat Memorial Mahajana PG Wing of SBRR Mahajana First Grade College (Autonomous), Mysuru,** has successfully completed his final year dissertation project entitled **"Phytochemical Profiling and Antimicrobial Evaluation of Leaf Extract from Lantana camara"** at Dextrose Technologies Pvt. Ltd., Bangalore from **22nd May 2023 to 22nd July 2023.**

We wish him all success in his future endeavours.

[illegible] K S
Project Coordinator
Dextrose Technologies Pvt. Ltd.
Bangalore- 560068

Aravind [illegible]
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Dextrose Technologies Pvt. Ltd
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CERTIFICATE

This is to certify that the project entitled **"Phytochemical profiling and antimicrobial evaluation of leaf extract from *Lantana camara*"** being submitted by **Abhishek V R** with register number **P01BH21S0245** in partial fulfillment for the award of the degree of **Master of Science in Microbiology**, is a bonafide work carried out by him from 22nd May 2023 to 22nd July 2023.

The results embodied in this report have not been submitted to any other university or institute for the award of any degree/diploma.

Date:-
Place:- Bengaluru

Sahanashree K S
Project Coordinator
Dextrose Technologies
Pvt., Ltd. Bengaluru

Aravind Ganessin
Managing Director
Dextrose Technologies
Pvt., Ltd. Bengaluru

Signature of
Internal guide
Dr. Harish R

DECLARATION

I, hereby declare that the project work entitled "**Phytochemical Profiling and Antimicrobial Evaluation of Leaf Extract from *Lantana camara*"** Is bonafide work and has been prepared under the supervision of **Ms. Sahanashree K S,** Project Coordinator, Dextrose Technologies Pvt. Ltd., Bangaluru. It gives us proud privilege and honour to thank and express our sincere regards to those who have been instrumental in the completion of this project.

Date:
Place: Bangalore **Mr. Abhishek VR**

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Mr. Abhishek VR

INDEX

SL. NO	CONTENT	PAGE NO
01	Introduction	1
02	Review of Literature	11
03	Aim and Objectives	19
04	Materials and Methods	21
05	Results and Discussions	32
06	Conclusion	42
07	References	44

LIST OF TABLES

SL. NO	TITLE	PAGE NO
1	Taxonomy of plant sample	02
2	Microorganisms used for the antimicrobial assay	06-10
3	Phytochemical analysis tests	22
4	Solvents for thin-layer chromatography	23
5	Instruments and glassware's	23
6	Media compositions of Lennox LB Agar and Brain Heart Infusion agar	23-24
7	Qualitative test for phytochemicals	27-29
8	Phytochemical test results	33-34
9	Thin layer chromatography results	35-36
10	Antimicrobial analysis results	37
11	GC-MS results	40-41

LIST OF FIGURES

SL. NO	TITLE	PAGE NO
1	Soxhlet extraction	25
2	Rotatory evaporator	26
3	*Lantana camara* leaf powder and crude extraction	33
4	Tubes showing results of phytochemical tests	34
5	TLC sheets showing a different bands under UV raditions	37
6	Plates showing zone of inhibitions	38-39

ABSTRACT

Lantana camara leaf. is widely used in traditional medicine around the world and is recognized as both a notorious weed and a popular decorative garden plant. Small ruminants are poisonous to several taxa of the highly varied *L. camara* complex, and this effect has been linked to the kinds and proportions of particular triterpenes ester metabolites. *L.camara* also generates a variety of metabolites in good quantities, some of which have been demonstrated to have advantageous biological properties. In order to assess the potential for utilizing the substantial biomass of lantana that is now available, all these factors are taken into account in this research. Included is the photochemistry of additional Lantana species. The phytochemical profiling and antimicrobial evaluation of leaf extract from *Lantana camara* are discussed in the present study. In order to evaluate the extract's potential antimicrobial efficacy against a variety of diseases, the research intends to identify and quantify the phytochemical components that are present in the extract. This study advances our knowledge of Lantana camara's therapeutic potential as well as its prospective uses in developing natural medicines and antibacterial drugs.

KEYWORDS: - Phytochemicals, Antimicrobial, *Lantana camara*

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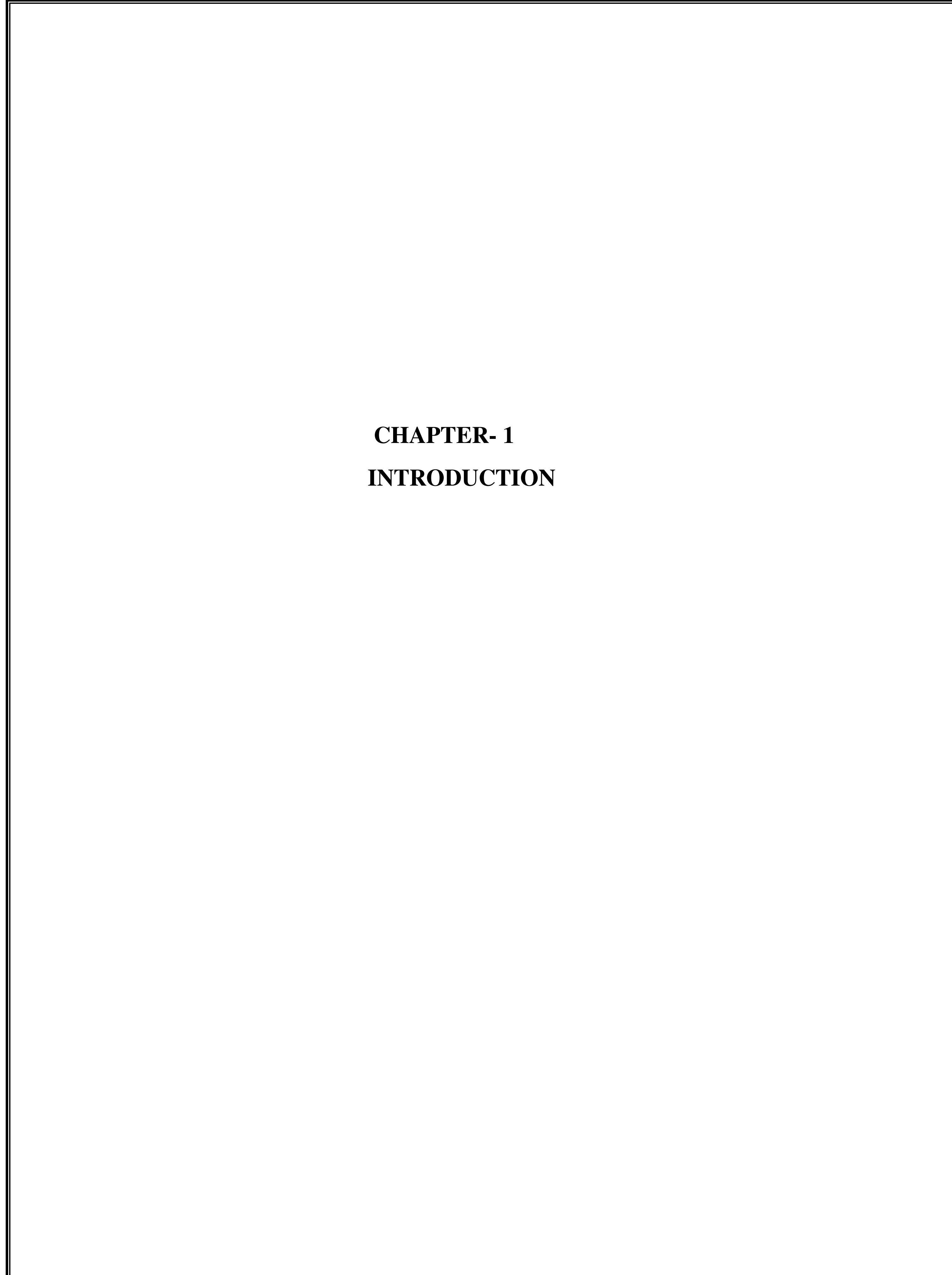


CHAPTER- 1

INTRODUCTION

Introduction

Lantana camara is a low erect, rugged hairy, evergreen shrub (Verbenaceae) native to tropical America. Known by several common names viz., blacksage, cuasquito, angel lip, flowered sage, shrub verbena, white sage and wild sage all over the world, it is a significant weed of which there are some 650 varieties in over 60 countries or island groups. L. camara has several uses, mainly as a herbal medicine and in some areas as firewood and mulch. It is also used for the treatment of cancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumours, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atoxy of abdominal viscera. In some countries, it is planted as a hedge to contain or keep out livestock. Extracts from the lantana leaves exhibit antimicrobial, insecticidal and nematicidal activity and also contain verbascoside, which possesses antimicrobial, immunosuppressive and antitumor activities. Lantanoside, linaroside and camarinic acid have been isolated and are being investigated as potential nematocides. Lantana oil is sometimes used for the treatment of skin itches, antiseptic for wounds, leprosy and scabies (Saraf et al. 2011)

1.1 Taxonomy of plant sample: -

Family: - Verbenaceae

Species:- *Lantana camara L.*

Details: -

TABLE:-1

Family	Verbenaceae
Species	*Lantana camara L.*
Meaning of the Name	Lantana - after an Italian name for the similar Viburnum. Camara - vaulted chamber, possibly referring to fruit.
Global description	L. camara is a thorny shrub upright, half climbing or sometimes more or less hanging, reaching 2-3 m in height. The stems and branches are angular, bearing curved spines, arranged along the edges. The leaves are simple, opposite, decussate with rough lamina, oval, regularly dentate with acute apex. The inflorescence is a hemispherical head, axillary or terminal, yellow, pink or orange colored, made up of many small tubular flowers. The fruits are small drupes fleshy, about 3 mm in diameter, varying in color from blue to black.
Cotyledons	The cotyledons are carried by a stalk from 5 to 7 mm long, covered with long hair. The lamina is oval, 5 mm long and 6 mm wide, apex emarginated, light green.

First, leaves	Simple, opposite, decussate, attached by a short hairy petiole, 2 to 3 mm long. The blade is small, oval, 10 to 12 mm long and 6-8 mm wide, hispid, with margin toothed.
Habit	Scrambling, woody shrub, up to 4m high.
Underground system	Taproot.
Stem	Four-angled, covered with short stiff hairs and re-curved prickles.
Leaves	The leaves are simple, opposite, decussate carried by a petiole, 1.5 to 2 cm long. The blade is leathery, oval to broadly oval shaped, 5 to 8 cm long and 3-4 cm wide, truncated to subcordate at the base, acute or acuminate at the top. Rough and hispid on the upper surface, pubescent on the lower surface. The leaf margin is regularly toothed.
Inflorescence	Flowers are in large umbel round shape, 2 to 6 cm in diameter. Compact, flat-topped inflorescences supported by a peduncle 3 to 7 cm long, pubescent and glandular.
Flowers	Flowers surrounded by an involucre of bracts narrowly ovate, long from 5 to 7 mm, green. Floral pedicel 6 to 12 mm long. Corolla tube curved along 10 to 12 mm, with ascending hairs inside, opening in the top four rounded lobes spread 6 to 8 mm in diameter. The first flower is often white, turning yellow, orange or pink with age.
Fruits	Small, fleshy, purplish black berries.
Seeds	Seeds sub-spherical, about 3 mm in diameter.
Biology	Evergreen, perennial plant. Multiplies by seeds. Flowers and fruits throughout the year. Seeds dispersed by birds, monkeys, and washed downstream by rivers.
Ecology	Occurs in bushel, forest, forest-edge, riparian or riverine, staff villages, roadsides, and other disturbed areas.
Origin	Native to South and Central America.
Use	Flowers visited by bees and butterflies. Fruit eaten by birds, monkeys, and insects. Young stems browsed by bushbuck. Cultivated for ornament and hedging.

(Anon n.d.)

1.2. Phytochemicals: -

A significant source of chemicals used as medications, agrochemicals, tastes, perfumes, colours, bio pesticides, and food additives, plants often create a large number of secondary metabolites that are biosynthetically derived from primary metabolites. Recent studies have shown that the medicinal plants have a variety of pharmacological effects, including those that are antimicrobial, reproductive, anti-inflammatory, antipyretic and analgesic, cardiovascular, central nervous, gastrointestinal, antioxidant, ant diabetic, dermatological, anticancer, hepato- and Reno protective, immunological, and many others.

Traditional uses of Lantana camara include treating gastropathy, tetanus, epilepsy, dysentery, and carminative illnesses as well as respiratory infections and disorders (cough, cold, asthma, and bronchitis). The plant was found to include alkaloids, glycosides, steroids, saponins, flavonoids, coumarin, tannins, carbohydrates, and hydroxycitric acid, according to a phytochemical examination of Lantana camara. Proteins, phytosteroids, fixed oils, lipids, triterpenoids, hydroxyl Anthraquinones, and Anthraquinones glycosides. Previous pharmacological studies showed that L. camara had a wide range of therapeutic effects, including those that were antimicrobial, ant parasitic, gastrointestinal, anxiolytic, hypoglycaemic, cardiovascular, antioxidant, anticancer, anti-inflammatory, analgesic, wound healing, antiurolithiatic, hepatoprotective, reproductive, antihemorrhoidal, and thrombin inhibition(Chaitali, Rao, and Vikhe 2022).

1.3 Phytochemical constituents of *Lantana camara* leaf extract: -

Phytosterols, glycosides, carbohydrates, phenolic compounds, saponins, alkaloids, flavonoids, and tannins were found in L. camera leaf extracts, which contrasts with earlier reports that triterpenoids, steroids, carbohydrates, lactones, proteins, flavonoids, resins, tannins, and fixed oils were present in various organic extracts of L. camera leaves. These phytochemicals are the leading contenders for the plant's therapeutic properties (Kalita et al. n.d.).

1.2. PHARMACOLOGICAL ACTIVITIES:

1.2.1 Antibacterial Activities of *Lantana Camara:*

Extracts of leaves and flowers obtained with ethyl acetate were studied for antibacterial activities. Leaf and flower ethyl acetate extracts exhibited considerable antibacterial activities against the bacteria used where the value of zone of inhibition ranged from 10-21 and 9-15 mm, respectively. For flower and leaf ethyl acetate extracts, the zone of inhibition (mm) measured ranged from 10 -21 and 9-15 mm, respectively. Thus, the study of the biochemical parameters viz., lipids, carbohydrates and proteins has revealed similarity among the four different *L camara*. However, antibacterial activities vary from one *L. camara* to the other and also according to the type of tissue used

1.2.2. Wound healing activity of *Lantana camara L.*

Lantana camara is used in herbal medicine for the treatment of skin itches, as an antiseptic for wounds, and externally for leprosy and scabies. The objective of our study is to investigate burn wound healing activity of the leaf extract of *L.camara* in rats. The animals were divided into two groups of 6 each. The test group animals were treated with the ethanol extracts of *L. camara* (100 mg kg-1 day-1*)* topically and the control group animals were left untreated. Healing was assessed by the rate of wound contraction, period of epithelialization. Antimicrobial activities of the extract against the specific microorganisms were assessed. The extract showed antimicrobial activity against *Staphylococcus aureus*, *Klebsiella Pneumoniae* and *E. coli.* Extract treated wounds were healed in about 21 days which is not distinct from the controls. Our data suggest that *L.camara* has antimicrobial activity but not wound healing promoting activity on burn wound.

1.2.3. **Anti-Mycobacterium Activity of *Lantana Camara:***

Chloroform and methanol extracts of *L. camara* collected from South-western Uganda were screened against three strains of *Mycobacterium tuberculosis* using the agar-well diffusion method. H37Rv, the rifampicin-resistant TMC-331 and a non-resistant wild strain (28-25271). The MIC and MBC were determined using the Agar dilution method on Middle brook 7H11. The methanol extract showed the highest activity against all the three strains used, with zones of inhibition of 18.0 -22.5 mm and MIC values of 20 µg/ml for H37Rv and 15 µg/ml for both TMC-331 and wild stain. The values for rifampicin were 1.0 µg/ml for both H37Rv and wild strain but rifampicin hardly showed any activity on TMC-331. The MBC value for the methanol extract of *L. camara* was 30µg/ml for the H37Rv, and 20µg/ml for both the TMC-331 and wild strains of *M. tuberculosis*. The MBC for rifampicin was 2.0µg/ml for both H37Rv and the wild strain.*it is* conclude that *L. camara* contains principles active against *M. tuberculosis*, which merit further research. (Chaitali et al. 2022).

1.3. GC-MS

Gas chromatography mass spectrometry (GC-MS) consists of two very different analytical techniques: gas chromatography (GC) which is hyphenated (hence uses a hyphen not a forward slash) to mass spectrometry (MS). Usually, the analytical instrument consists of a gas chromatograph that is hyphenated via a heated transfer line to the mass spectrometer, and the two techniques take place in series. However, some specialist and usually miniature or portable instruments contain the whole GC-MS within a single box. GC is a separation science technique that is used to separate the chemical components of a sample mixture and then detect them to determine their presence or absence and/or how much is present. GC detectors are limited in the information that they give; this is usually two-

dimensional giving the retention time on the analytical column and the detector response. Identification is based on comparison of the retention time of the peaks in a sample to those from standards of known compounds, analysed using the same method. However, GC alone cannot be used for the identification of unknowns, which is where hyphenation to an MS works very well. MS can be used as a sole detector, or the column effluent can be split between the MS and GC detector(s)MS is an analytical technique that measures the mass-to-charge ratio (m/z) of charged particles and therefore can be used to determine the molecular weight and elemental composition, as well as elucidating the chemical structures of molecules. Data from a GC-MS is three-dimensional, providing mass spectra that can be used for identity confirmation or to identify unknown compounds plus the chromatogram that can be used for qualitative and quantitative analysis (Narciso, Luz, and Bettencourt Da Silva 2019).

1.4. Microorganisms used for the antimicrobial assay:-

Antimicrobial susceptibility tests can guide the physician in drug choice and dosage for difficult-to-treat infections. Results are commonly reported as the minimal inhibitory concentration (MIC), which is the lowest concentration of drug that inhibits the growth of the organism.

TABLE :-2

1.4.1 Bacillus cereus:-

Domain:	Bacteria
Clade:	Terrabacteria
Phylum:	Bacillota
Class:	Bacilli
Order:	Bacillales
Family:	Bacillaceae
Genus:	*Bacillus*

B. cereus is a rod-shaped bacterium with a Gram-positive cell envelope. Depending on the strain, it may be anaerobic or facultative anaerobic. Most strains are mesophilic, having an optimal temperature between 25 °C and 37 °C, and neutralophilic, preferring neutral pH, but some have been found to grow in environments with much more extreme conditions.

These bacteria are both spore-forming and biofilm-forming, presenting a large challenge to the food industry due to their contamination capability. Biofilms of *B. cereus* most commonly form on air-liquid interfaces or on hard surfaces such as glass. *B. cereus* displays flagella motility, which has been shown to aid in biofilm formation via an increased ability to reach surfaces suitable for biofilm formation, to spread the biofilm over a larger surface area, and to recruit planktonic, or single, free-living bacteria. Biofilm formation may also occur while in spore form due to the varying adhesion ability of spores.Their flagella are located all around the cell body that can bundle together at a single location on

the cell to propel it. This flagella property also allows the cell to change directions of movement depending on where on the cell the flagellum filaments come together to generate movement. (Enosi Tuipulotu et al. 2021)

TABLE:-3

***1.4.2. Pseudomonas aeruginosa*:-**

Domain:	*Bacteria*
Phylum:	*Pseudomonadota*
Class:	*Gammaproteobacteria*
Order:	*Pseudomonadales*
Family:	*Pseudomonadaceae*
Genus:	*Pseudomonas*

Pseudomonas aeruginosa is a common encapsulated, gram-negative, aerobic–facultative, rod-shaped bacterium that can cause disease in plants and animals, including humans. A species of considerable medical importance, *P. aeruginosa* is a multidrug-resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms, and its association with serious illnesses – hospital-acquired infections such as ventilator-associated pneumonia and various sepsis syndromes.

The organism is considered opportunistic insofar as serious infection often occurs during existing diseases or conditions – most notably cystic fibrosis and traumatic burns. It generally affects the immunocompromised but can also infect the immunocompetent as in hot tub folliculitis. Treatment of *P. aeruginosa* infections can be difficult due to its natural resistance to antibiotics. When more advanced antibiotic drug regimens are needed adverse effects may result.(Diggle and Whiteley 2020)

TABLE:-4

1.4.3. Staphylococcus haemolyticus

Domain:	Bacteria
Clade:	*Terrabacteria*
Phylum:	*Bacillota*
Class:	*Bacilli*
Order:	*Bacillales*
Family:	*Staphylococcaceae*
Genus:	*Staphylococcus*

Staphylococcus haemolyticus is a member of the coagulase-negative staphylococci (CoNS). It is part of the skin flora of humans, and its largest populations are usually found at the axillae, perineum, and inguinal areas.*S. haemolyticus* also colonizes primates and domestic animals. It is a well

known opportunistic pathogen, and is the second-most frequently isolated CoNS (*S. epidermis's* is the first). Infections can be localized or systemic, and are often associated with the insertion of medical devices. The highly antibiotic-resistant phenotype and ability to form biofilms make *S. haemolyticus* a difficult pathogen to treat. Its most closely-related species is *Staphylococcus Borealis*. (Falcone et al. 2006)

TABLE:-5

***1.4.4 Staphylococcus aureus*:-**

Domain:	*Bacteria*
Clade:	*Terrabacteria*
Phylum:	*Bacillota*
Class:	*Bacilli*
Order:	*Bacillales*
Family:	*Staphylococcaceae*
Genus:	*Staphylococcus*

Staphylococcus aureus is a Gram-positive spherically shaped bacterium, a member of the Bacillus, and is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. It is often positive for catalase and nitrate reduction and is a facultative anaerobe that can grow without the need for oxygen. Although *S.* aureus usually acts as a commensal of the human microbiota, it can also become an opportunistic pathogen, being a common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins, and the expression of a cell-surface protein that binds and inactivates antibodies. *S. aureus* is one of the leading pathogens for deaths associated with antimicrobial resistance and the emergence of antibiotic-resistant strains, such as methicillin-resistant *S. aureus* (MRSA), is a worldwide problem in clinical medicine. Despite much research and development, no vaccine for *S. aureus* has been approved.

An estimated 20% to 30% of the human population are long-term carriers of *S. aureus*, which can be found as part of the normal skin microbiota, in the nostrils, and as a normal inhabitant of the lower reproductive tract of females. *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia, and sepsis. It is still one of the five most common causes of hospital-acquired infections and is often the cause of wound infections following surgery. Each year, around 500,000 hospital patients in the United States

contract a staphylococcal infection, chiefly by *S. aureus*. Up to 50,000 deaths each year in the U.S. are linked to *S.aureus* infections (Fitzgerald 2014).

TABLE:-6

1.4.5. Escherichia coli:-

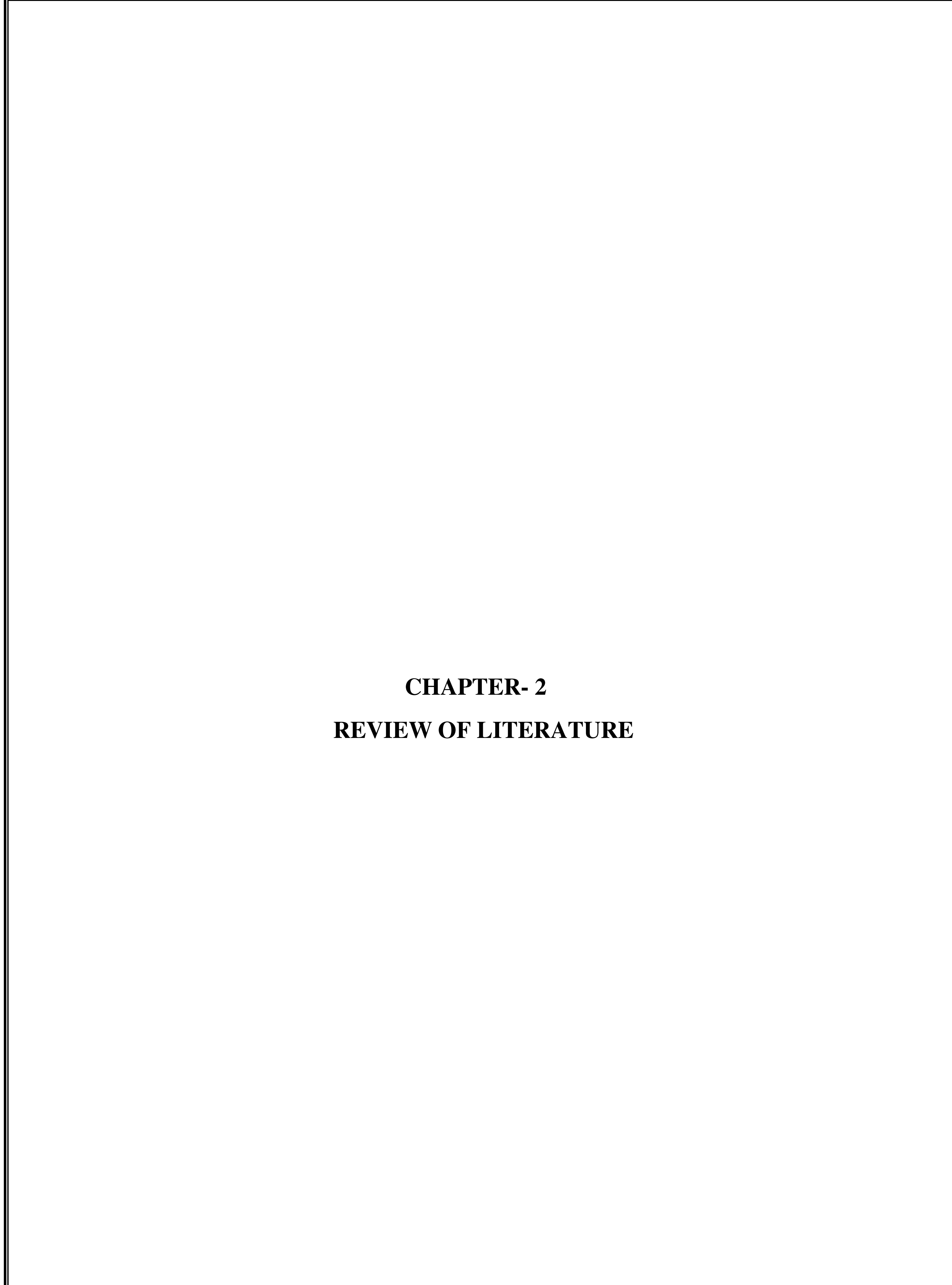
Domain:	*Bacteria*
Phylum:	*Pseudomonadota*
Class:	*Gammaproteobacteria*
Order:	*Enterobacterales*
Family:	*Enterobacteriaceae*
Genus:	*Escherichi*

Escherichia coli is a Gram-negative, facultative, rod-shaped, coliform bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes such as EPEC, and ETEC are pathogenic and can cause serious food poisoning in their hosts, and are occasionally responsible for food contamination incidents that prompt product recalls. Most strains are part of the normal microbiota of the gut and are harmless or even beneficial to humans (although these strains tend to be less studied than the pathogenic ones). For example, some strains of *E. coli* benefit their hosts by producing vitamin K2 or by preventing the colonization of the intestine by pathogenic bacteria. These mutually beneficial relationships between *E. coli* and humans are a type of mutualistic biological relationship — where both the humans and the *E. coli* are benefitting each other. *E. coli* is expelled into the environment within faecal matter. The bacterium grows massively in fresh faecal matter under aerobic conditions for three days, but its numbers decline slowly afterward.

E. coli and other facultative anaerobes constitute about 0.1% of gut microbiota, and faecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells can survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for faecal contamination A growing body of research, though, has examined environmentally persistent *E. coli* which can survive for many days and grow outside a host.

The bacterium can be grown and cultured easily and inexpensively in a laboratory setting and has been intensively investigated for over 60 years. *E. coli* is a chemo heterotroph whose chemically defined medium must include a source of carbon and energy. *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields

of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. Under favourable conditions, it takes as little as 20 minutes to reproduce.(Tenaillon et al. 2010)



CHAPTER- 2

REVIEW OF LITERATURE

Review of Literature

Lantana camara is a potential medicinal resource for treating various medical issues, with essential oil and plant extracts possessing various bioactivities, including antibacterial properties. The paper reviews the antimicrobial activities of L. camara, evaluating methanolic extracts from different parts of the plant. The extract showed the highest activity against Alternaria alternate and M. phaseolina, the lowest activity against R. solani, and no activity against A. niger. The leaf and seed extracts of L. camara showed potential to inhibit seed-borne fungi and enhance seed germination. The ethanol extract of L. camara showed minimal inhibition of mycelia growth of A. flavus but effective inhibition of aflatoxin B1 production. Medicinal plants contain phytochemicals or secondary metabolites that can act individually, additively, or in synergy to improve human health. The active principle of the extracts disrupts the permeability barrier of cell membrane structures, inhibiting bacterial growth. EOs may interact with and affect the plasma membrane, interfering with respiratory chain activity and energy production. The paper emphasizes the need to document and preserve traditional methods of treatment using medicinal plants for future reference. Lantana camara L. is an evergreen plant found throughout India and has been traditionally used in treating various ailments, supported by scientific data. However, most pharmacological studies were preliminary and required intensive validation of its therapeutic potential, side effects, and interactions for safe modern medicine usage (Girish K 2017).

Lantana camara, an invasive plant species, was studied for its potential antimicrobial and antioxidant properties. Extracts from the leaves and flowers of Lantana camara showed high antioxidant, indicating their potential as natural antioxidants. Chemical analysis of the extracts revealed the presence of various metabolites, and GC-MS analysis identified 66 bioactive compounds with potential functions such as anti-inflammatory, ant androgenic, antitumor, and antimicrobial activities. In vitro studies confirmed the antimicrobial effect of the extracts against phytopathogens, suggesting their potential use in plant disease management. Molecular analysis further revealed that the phytochemicals in Lantana camara could inhibit the growth of phytopathogens by targeting specific enzymes. Overall, the study highlights the potential of Lantana camara extracts as a natural source of antimicrobial and antioxidant compounds for medicinal and agricultural application (Mansoori et al. 2020).

The study found that methanolic extracts of *Lantana camara* and Lippia chevalieri exhibited antioxidant and antibacterial activities. *L. chevalieri* extracts showed the highest antioxidant activity, while *L. camara* displayed the broadest antimicrobial spectrum, particularly against *Shigella flexneri* and *Pantoea spp*.The polyphenolic composition of the extracts was characterized using HPLC-DAD and *L. chevalieri* extracts was found to be rich in phenolic compounds. L. camara extracts contained phenol acid and flavone compounds, which may explain its antimicrobial activity. The study also determined the Minimum Inhibitory

Concentrations (MIC) and minimum bactericidal concentrations (MBC) of the extracts against various pathogenic bacteria. The extracts showed promising antimicrobial effects, with low MIC and MBC values. *Lippia chevalieri* was identified as a valuable source of antioxidant compounds, with a complex phenol composition. The reducing power of *L. chevalieri* extracts was almost twice that of *Lantana camara* extracts, suggesting a correlation between reducing capability and phenol composition complexity (Mindiédiba Jean et al. 2012).

The study concludes that the ethanolic extracts of Lantana camara and Lantana montevidensis have antibacterial activity against both gram-positive and gram-negative bacteria, including multi-resistant strains. The extracts showed promising results in inhibiting the growth of the tested bacteria, indicating their potential as natural antibacterial agents.

Another study concludes that the juices of Lantana camara and Ocimum gratissimum have larvicidal efficacy against the larvae of malaria vectors, including Aedes aegypti, Anopheles subpictus, and Culex quinquefasciatus. This suggests that these weeds could be explored as potential control measures for malaria vectors. This indicate that the ethanolic extracts of Lantana camara and Lantana montevidensis showed promising results in inhibiting the growth of the tested bacteria, suggesting their potential as natural antibacterial agents. Specifically, the L. montevidensis fresh leaves extract demonstrated the best results against Pseudomonas aeruginosa and multi-resistant Escherichia coli.(Barreto et al. 2010)The study examined the phytochemical make-up and antibacterial properties of Lantana camara leaf extracts in ethanol and methanol against clinical infections. Powdered leaves were soaked in each solvent to create the extracts, which were then concentrated and filtered. The agar well diffusion method was used to calculate the extracts' minimum inhibitory concentration (MIC). The findings demonstrated that E. coli was the most resistant to the plant extracts, whereas *V. cholerae* was the most sensitive. The phytochemicals included in the extracts displayed antibacterial activity, indicating their potential as replacements for human infections with resistance. Due to improved organic component solubility in ethanol, the ethanol extract demonstrated higher inhibitory action than the methanol extract. The study suggested employing Lantana camara leaf extract as a natural antibiotic (Ezebo et al. 2021).

The analysis of the essential oil extracted from Nigerian Lantana camara L. leaves revealed the presence of 32 different compounds, the primary ones being 1,8 -cineol, sabinene, and caryophyllene. A total of six strains were used to assess the essential oil's antibacterial efficacy; it shown moderate action against *Candida albican, Bacillus subtilis*, *Staphylococcus typhi, Pseudomonas aeruginosa*, and *Bacillus aureus*. With an LC50 value of 0.01, which indicates significant toxicity, the essential oil also displayed cytotoxicity. The essential oil from Nigerian Lantana camara leaves has never been examined for its

cytotoxicity and antibacterial activities. The essential oil's chemical makeup is consistent with earlier reports from Nigeria and Iran, although it differs from species found in North Brazil. Several novel substances not previously mentioned in the Nigerian species were detected in the essential oil (Sonibare and Effiong 2008).

In the study, Lantana camara plants with yellow, lavender, red, and white blooms were examined for their biochemical makeups and antibacterial properties. The compositions of the plants' carbohydrates and lipids were similar, with larger concentrations of carbohydrates in the flowers and higher concentrations of lipids in the leaves. The numerous L. camara varieties shared commonalities, as shown by the electrophoresis of leaf and flower proteins. Leaf and floral ethyl acetate extracts shown significant antibacterial activity against the examined microorganisms. Although the various L. camara varieties shared comparable biochemical properties, the study found that their antibacterial activity differed depending on the tissue type and between them. Secondary metabolites found in L. camara plants include steroids, alkaloids, phenolics, terpenoids, phytosterols, saponins, tannins, and phytosterols. The chemical composition of L. camara plants is influenced by genetic factors rather than environmental and seasonal factors (Sam and Hayat Khan 2009).

The essential oils of Lantana camara leaves and flowers were analysed using GC-FID and GC-MS, and a total of fifty-nine compounds were identified, comprising the majority of the volatile constituents in both oils. The main components of the leaves oil were α-zingiberene, γ-curcumene, β-caryophyllene, ar-curcumene, 1,10-di-epi-cubenol, β-curcumene, and caryophyllene oxide, while the flowers oil was dominated by β-caryophyllene, ar-curcumene, γ-gurjunene, γ-curcumene, and β-curcumene. The antimicrobial activity of the oils was evaluated using the tube dilution method, and both oils showed activity against Gram-positive bacteria and fungi. The leaf oil was particularly effective against *Micrococcus flavus* and *Proteus mirabilis*, while the flower oil was effective against *Aspergillus niger* and *Penicillium chrysogenum*. The essential oils of Lantana camara have been reported to have various activities, including insecticidal, adulticidal, antibacterial, and cytotoxic activities. The chemical composition of Lantana camara essential oil can vary depending on the region, and this study found some constituents in higher quantities compared to previous reports. Gram-positive bacteria and fungi were generally more sensitive to the essential oils than Gram-negative bacteria (Joshi 2012).

The study investigated the chemical composition and biological activities of the essential oil of Lantana camara leaves from Burkina Faso. The essential oil showed good radical scavenging power and moderate reducing power compared to other antioxidants. The main components of the essential oil were caryophyllene oxide, spathulenol, humulene-1, 2-epoxide, β-caryophyllene, E-nerolidol, and α-humulene. The essential oil exhibited antibacterial activity, particularly against Escherichia coli, and inhibitory action on all fungal strains tested. The results suggest that the essential oil of Lantana camara could be used as an

antifungal agent, antioxidant, and potential antibacterial agent against Escherichia coli. The extraction yield of the essential oil from dried leaves of L. camara was higher than previous studies in Nigeria, Egypt, and India, but lower than a study in Benin. The DPPH radical scavenging power of the essential oil of L. camara depends on its concentration. The study supports the traditional uses of Lantana camara and highlights its potential as a natural source of bioactive compounds with antioxidant and antimicrobial properties (Zénabou et al. 2018).

The study focused on the phytochemical analysis and antifungal potential of different parts of Lantana camara L., including fruits, leaves, and stem. The phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids in all three parts of L. camara. In vitro antifungal activity testing was conducted using different concentrations of methanolic extracts from the selected parts of L. camara against Colletotrichum gloeosporioides Pens, a fungal pathogen.

The results showed that the metabolic fruit extract had the highest antifungal activity, reducing the biomass of C. gloeosporioides by up to 66%. The effective n-hexane fraction of the fruit extract was identified as containing major antifungal compounds, including cyclopropane, carboxylic acid, 5 -heptonic acid, 2,2-dimethyl1-4-pentenoate, and 2-Propyloctahydro-1-benzothiophene. The presence of these compounds in L. camara fruit suggests its potential as a natural alternative for synthetic fungicides in controlling C. gloeosporioides. The phytochemical analysis of L. camara extracts also showed the presence of other secondary metabolites, such as phenols, carbohydrates, proteins, steroids, and triterpenes, which may contribute to its antifungal activity (Bashir et al. 2019).

The study analysed the chemical composition of the essential oil of Lantana camara Linn. from Uttarakhand, India, and found that monoterpenes and sesquiterpenes hydrocarbons, followed by oxygenated monoterpenoids, were the predominant components of the essential oil. The essential oil and extracts of Lantana camara Linn. Exhibited antibacterial activity against all tested microbial strains. Among the tested bacterial strains, M. luteus showed the highest zone of inhibition for both the aqueous extract and essential oil. A varying degree of efficacy was observed in the zone of inhibition, suggesting different levels of antibacterial activity. The antibacterial activity of the essential oil and leaf extracts may be attributed to the presence of various active constituents in the leaves. The study suggests that the most active extract, particularly central (E & Z), can be further isolated and evaluated for its therapeutic antimicrobial properties (Seth et al. 2012).

The essential oils of Lantana camara flowers (FLCO) and leaves (LLCO) exhibited antimicrobial activity against Bacillus cereus and Bacillus subtilis, suggesting their potential use in medical purposes, food, and perfumery industries. The oils also showed moderate antioxidant activity, which increased with increasing

concentrations. Transmission electron microscopy (TEM) micrographs confirmed the effects of FLCO and LLCO on morphological and ultra-structural alterations in B. cereus spores. The chemical composition of the oils was analysed using gas chromatography-mass spectrometry, and several compounds, including humulene, davanone, and caryophyllene, were identified. The antimicrobial activity of the oils was tested using a TLC bio autographic assay, and the minimum inhibitory concentration (MIC) was determined. B. subtilis and B. cereus were susceptible to the tested oils, while other bacteria, fungi, and Staphylococcus aureus were resistant (El Baroty et al. 2014).

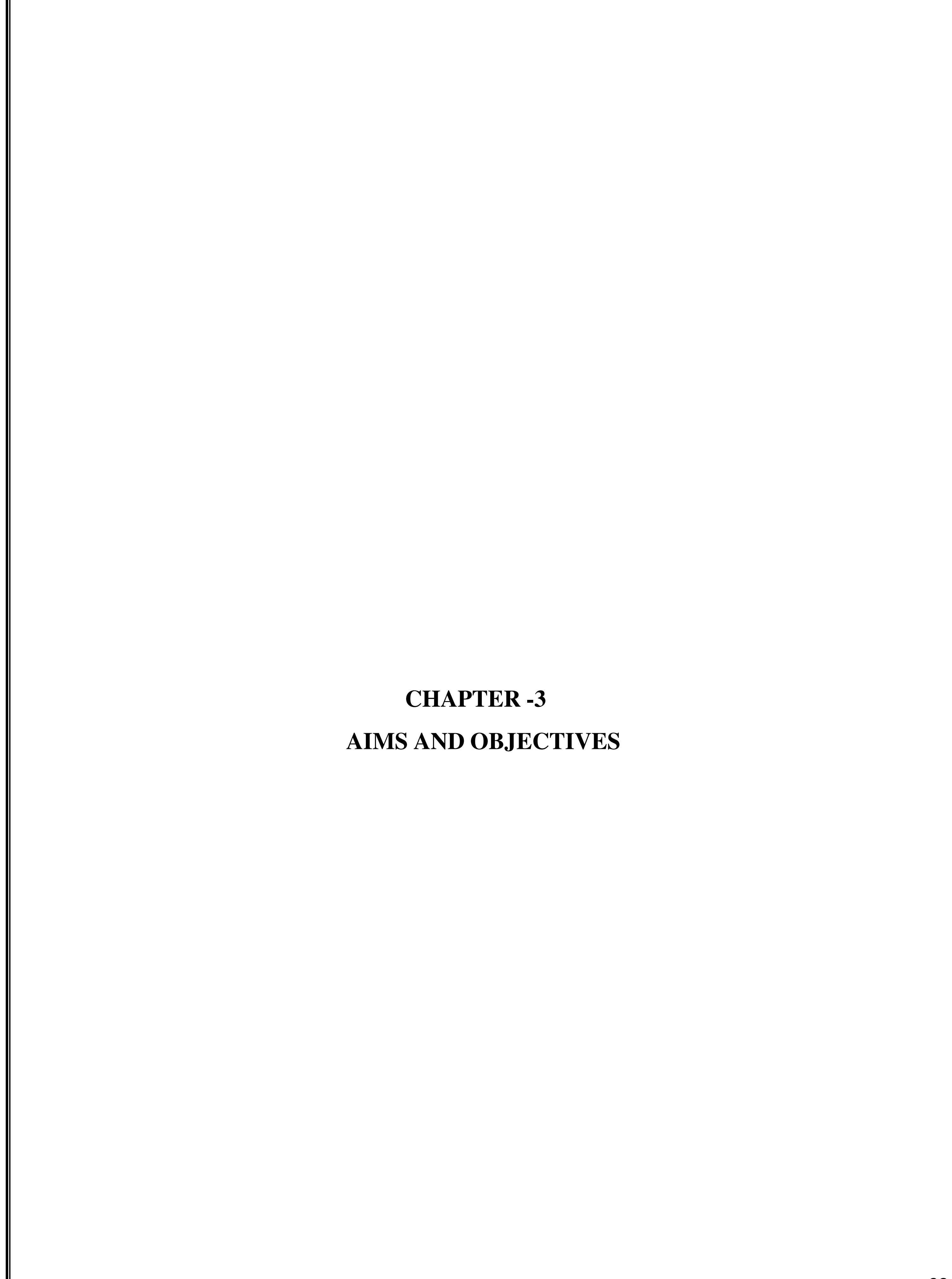
This paper reports the biochemical composition and antibacterial activities of the leaves hydrosol of Lantana camara. The hydrosol of leaves obtained with waste of essential oil was studied for antibacterial activities against Gram-negative and Gram-positive bacteria. The antibacterial activities were evaluated using zone of inhibition and MIC by Dilution method. The leaf hydrosol exhibited considerable antibacterial activities against Gram-positive bacteria, with the zone of inhibition values ranging from 10-11 mm. The micro dilution assay gave MIC values ranging from 200 to 400 mg/ml for Gram-positive bacteria. The hydrosol showed no antibacterial activity against Gram-negative bacteria. Qualitative tests detected the presence of terpenoids, saponins, and tannins in the Lantana camara leaf hydrosol. Alkaloids and flavonoids could not be detected in the leaf hydrosol. Gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa were found to be resistant, while Gram-positive bacteria Bacillus cereus, Staphylococcus aureus, and Micrococcus luteus were sensitive to the leaf hydrosol (Dubey et al. 2011).

In this paper they have shown that Lantana camara contains phytoconstituents such as triterpenes, flavones, coumarin, steroids, and iridous glycosides. The plant exhibits antimicrobial, fungicidal, insecticidal, and nematicidal properties, and possesses antimicrobial, immunosuppressive, and anti-tumour activity. Lantana oil is sometimes used for the treatment of skin itches, as an antiseptic for wounds, and externally for leprosy and scabies. However, most of the pharmacological studies conducted so far have been preliminary and carried out in animals, and further intensive preclinical and clinical studies are required to evaluate the efficacy and toxicity of these plant products (Reddy 2013).

In this paper, they show promising anti-mycobacterial activity against strains of Mycobacterium tuberculosis, including drug-resistant strains. The methanol extract of L. camara exhibited the highest activity, indicating its potential as a lead compound for the development of a tuberculosis drug. The plant is widely used in East Africa for various purposes, including herbal medicine, as a source of firewood, and for its microbicide, fungicide, and insecticide properties. In an acute toxicity study, the methanol extract of L. camara was found to be non-toxic to mice. Further research is needed to verify the activity of L. camara against multidrug-resistant tuberculosis (MDR-TB) and to identify the specific compounds responsible for

its antimycobacterial activity. The discovery of Lantana camara's antimycobacterial activity, particularly against drug-resistant strains, is considered a significant finding in the search for new drugs to combat tuberculosis (Kirimuhuzya et al. 2009).

In this paper, they used different solvents (ethyl acetate, methanol, acetone, and chloroform) that have varying effects on the extraction of phytoconstituents from Lantana camara leaves, with methanol solvent recovering the highest amount of extractable compounds and having the highest phenolic and flavonoid content. Methanol extract of L. camara leaves showed the highest antioxidant activity, as indicated by lower IC50 values in DPPH and hydroxyl scavenging assays. All solvent extracts of L. camara showed antimicrobial activity, with methanol extract having the highest inhibition activity against both Gram-negative and Gram-positive bacteria. Methanol leaf extract of L. camara containing many bioactive compounds may be utilized as a therapeutically source for developing beneficial drugs to manage various human diseases and disorders (Swamy, Sinniah, and Akhtar 2015).



CHAPTER -3

AIMS AND OBJECTIVES

AIM AND OBJECTIVES

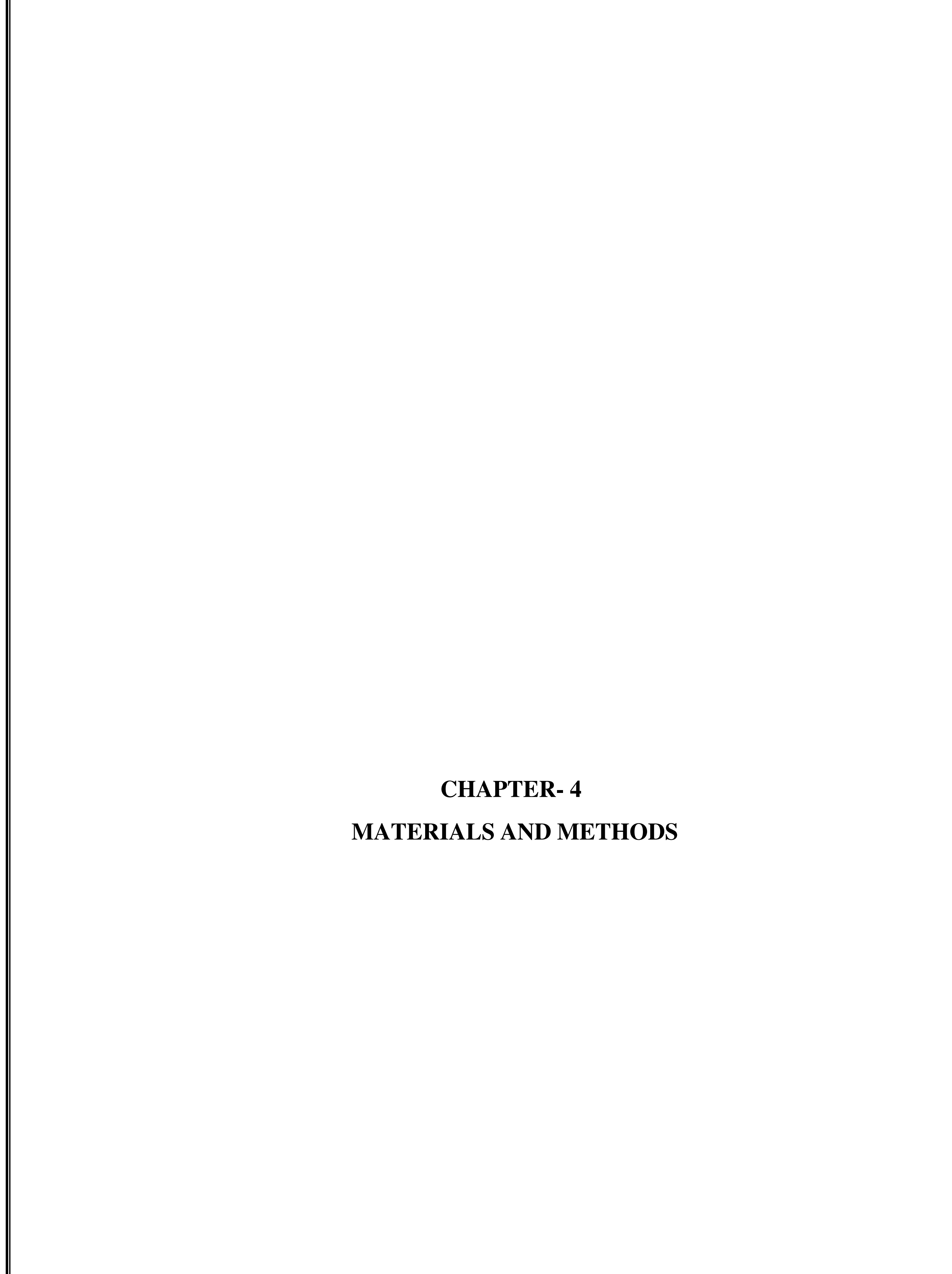
AIM OF THIS STUDY

The aim of the present study is to **isolate the leaf extract of *Lantana camara* and to evaluate the antimicrobial activity of the leaf extract**.

OBJECTIVES OF THIS STUDY

The study "**Phytochemical Profiling and Antimicrobial Evaluation of Leaf Extract from *Lantana camara*"** was under taken with the following objectives.

1. Extraction of metabolites from leaf samples for analysis.
2. Conduct a qualitative phytochemical analysis on the extracted sample.
3. Evaluate the antimicrobial activity of the extract.
4. Perform GCMS analysis on the sample and conduct molecular docking studies.



CHAPTER- 4

MATERIALS AND METHODS

MATERIALS AND METHODS

4.1) MATERIALS:-

TABLE:-7

Chemicals	Quantity	Purpose/test
80% methanol	200 ml	Solvent for Soxhlet extraction
PHYTOCHEMICAL ANALYSIS:		
Iodine solution		Alkaloids test
Sodium hydroxide		Flavonoids test, tannins test,
Ammonia solution		Flavonoids test
Conc. Sulphuric acid		Flavonoids test, glycosides test, terpenoids test, sterols test
Ferric chloride		Tannins test, glycosides test, phenols test
Sodium chloride		Tannins test
Glacial acetic acid		Glycosides test
Chloroform		Glycosides test, Terpenoids test, Sterols test
Potassium hydroxide(KOH)		Quinones test

TABLE 8 - SOLVENTS FOR THIN-LAYER CHROMATOGRAPHY:-

Chloroform	Ammonia solution
Glacial acetic acid	Ethanol
Methanol	Formic acid
Ethyl acetate	
n-hexane	
Diethyl ether	
Dichloromethane	

TABLE 9 - INSTRUMENTS AND GLASSWARES

INSTRUMENTS:	PURPOSE:
Mortar and pestle	Grinding of sample
Round bottom flask	Soxhlet extraction
Soxhlet apparatus	Soxhlet extraction
Rotary vapour	Soxhlet extraction
Test tubes	Phytochemical analysis
TLC sheet	TLC
Screw cap bottles	Media preparation
Petriplates	Antimicrobial assay
Autoclave	Sterilization
Laminar air flow	
Eppendrof tube	Sample storage
Micropipettes, sterile tips, and other requirements	

TABLE 10 -MEDIA COMPOSITION:-

Lennox LB Agar composition

Ingredients	gram/liter
Peptone	10g
Yeast extract	5g
Sodium Chloride	5g

TABLE 11 - Brain Heart Infusion (BHI) Agar

BHI	6 g
Peptic digest of animal tissue	6g
Nacl	5 g
Dextrose	3 g
Pancreatic digest of Gelatin	14.5 g
Na_2HPO_4	2.5 g
Distilled water	1liter
Agar-agar	15 g

4.2) METHODS:

4.2.1. a) Collection of the plant material:

The plant for the sample Lantana camera was selected from the garden near the home. The clean leaf of the Lantana camara plant was selected and taken for the experiment.

4.2.2. b) <u>Preparation of the sample:</u>

The leaf that was collected from the garden was dried in the sunlight and ground up to a small powder-like. The powder is stored for further use.

4.2.3. c) <u>Extracting the crude sample:</u>

4.2.4. i) <u>Soxhlet extraction:</u> The sample powder is then subjected to a hot extraction procedure employing the Soxhlet extraction to extract the crude sample. In order to test the sample, 80% methanol is employed as the solvent.

We used 10 g of powdered Lantana camara leaf in a Whatsmann filter paper for the Soxhlet extraction. The Soxhlet apparatus is set up by attaching it to the water supply that will deliver the cold water. The 80% methanol is poured into the round-bottom bottle and linked to the Soxhlet apparatus once the sample in the filter paper has been placed inside. The experiment is conducted at 64.70 C, which is methanol's boiling point. The solvent in the round bottom flask that is attached at the bottom will evaporate in this procedure and flow toward the cold-water supply. After the vapours cool, they will condense in the Soxhlet flask that contains the sample through the condensation process. This solvent reacts with the sample, changing its color to match the sample color, and the mixture of the sample and the solvent, which is 80% methanol and leaf powder, will be collected in a round-bottom flask that also contains the solvent. This process is carried out for a number of cycles. After 33 cycles of the experiment, the solvent started to become a mild shade of green color. It indicates complete extraction of the sample. The round-bottom flask is filled with the sample and solvent. The flask is then kept to cool for the next process.

Fig: 1 Soxhlet extraction

4.3. ii) Concentrating the plant extract by evaporating the solvent

The rotary evaporator is a device that separates the solvent from a sample in order to obtain a crude sample that will be beneficial for our experiment. The round bottom flask in which the sample with the solvent is collected is linked to the rotary vapour apparatus, which is then turned on using a specific temperature and speed. The rotary vapour apparatus is also connected to a cold-water source.

This method utilized the condensation principle just like the soxhlet container extraction did. The flask that is attached at the other end will be used to condense the evaporating 80% methanol that is used as the solvent. The solution-containing round bottom flask rotates at a specific rate and comes into touch with the hot water held in a with constant heat supply for the water. Rotation of the round bottom flask with the mixture of solvent and extract, the hot water causes the solvent to evaporate. The flask is removed from the rotary vapour when the solvent has entirely evaporated. A sample is taken, then it is stored to cool.

The sample is poured in a petriplates and left for drying to obtain the crude sample by evaporating the remaining moisture content of the solvent in room temperature. After evaporation, the crude sample is separated from the plated it is collected.

The crude extract we got is 1gram. The sample is then diluted using the alcohol in the dilution of 1 microliter of alcohol per 1 microgram of the crude sample. The diluted crude extract is used for the further tests.

Fig: 2 Rotatory evaporator

4.4 Qualitative test for phytochemicals

Tests for alkaloids:

TABLE:-12

TESTS	INFERENCE
1. Hager's test: 100 microliter of extract + 1-2 ml of hager's reagent Hager's reagent: 1 g of picric acid in 100 ml of water (1% or 2%)	Yellow crystalline precipitate indicates the presence of alkaloids.
2. Iodine test: 3ml of extract solution + few drops of iodine solution	A blue colouration forms, which disappears on boiling and reappears on cooling.

Tests for flavonoids:

TESTS	INFERENCE
1. Alkaline reagent test: 1ml extract + 2ml of 2% NaOH solution + few drops of dil. HCl	An intense yellow colour becomes colourless with addition of dil. Acid indicates the presence of flavonoids.
2. Ferric chloride test: 0.5 or 1ml of extract aqueous solution + few drops of 10% ferric chloride solution	A green precipitate forms indicating the presence of flavonoids.
3. Ammonia test: 3g of extract + 5ml dil. Ammonia solution + conc. Sulphuric acid	Yellow colour develops indicating presence of flavonoids.
4. Conc. Sulphuric acid test: 1g of plant extract + conc. Sulphuric acid	An orange colour develops indicating the presence of flavonoids.

Tests for tannins:

Tests	Inference
1. Ferric chloride tests: Baymer's test: 1ml of extract + 10ml distilled water + 2-3 drops of 10% or 0.1% ferric chloride.	brownish-green / blue-black colour indicates the presence of tannins
2. 10 % Na OH test: 0.4 ml of plant extract + 4ml of 10% NaOH + shake well	the formulation of emulsion indicates the presence of tannins.
3. Gelatin test: 1ml of plant extract is dissolve in 5ml distilled water + 1% gelatin solution +10% Nacl	White- precipitate formation indicates the presence of tannins

Tests for glycosides:

1. Keller-Killani test: 1ml extract + 1-1.5 ml of glacial acetic acid + 1-2 drops of 5% ferric chloride + conc. Sulphuric acid (along the side of the test tube)	The reddish brown colour indicates the presence of glycoside. Brown ring formation at the junction and bluish green upper layer.
2. Borntrager's test: to 2ml of extract, 3ml of chloroform is added and	Pink colour develops which indicates the

shaken, chloroform layer is separated and 10% ammonia solution added to it.	presence of glycosides.
3. Salkowski's test: add 2 ml sulphuric acid concentrated to the whole aqueous plant crude extract.	A reddish brown colour form indicated the presence of the steroidal aglycone part of glycoside.

Tests for saponins:

Tests	Inference
1. Froth test: 0.5 gm of extract + 5-10 ml distilled water and shake well	Froth formation persists on warming in a water bath for 5 min and the addition of 3 drops of olive oil emulsion formation

Tests for terpenoids:

1. Salkowski test: 100 mg crude extract + 2 ml chloroform + 2 ml conc. sulphuric acid (along the side of test tube)	A reddish-brown colouration of the inter face indicates the presence of terpenoides.
2. 2 ml chloroform + 5 ml plant extract + boil with 3 ml conc. sulphuric acid	Grey colour formation indicates presence of terpenoides

Test for sterols :

1. Hesse's response: 2 ml of plant extract + 2 ml chloroform + 2 ml conc. Sulphuric acid + shake well	The chloroform layer appeared red or pink and the acid layer appears greenish yellow fluorescent.
2. Salkowski's test: extract + few drops of conc. Sulphuric acid + shake well and allow to stand	Red colour appears in the lower layer indicating the presence of sterols.

Tests for phenols:

Tests	Inference
1. Extract + 5 ml distilled water (dissolve) + few drops of 5% $FeCl_3$ solution.	Dark green colour indicates the presence of phenol compounds.

4.5. Thin layer chromatography:-

The method of thin layer chromatography is used to separate non-volatile mixtures. It is carried out on glass, plastic, or aluminium foil that has been lightly covered with an adsorbent substance. It uses cellulose, silica gel, or aluminium oxide. The separation concept is how it operates. When a component splits, a spot appears. There is a retention factor (Rf) for each place.

RF = (sample travel distance / solvent travel distance)

Prepare the solvents first, as they will be used to separate the samples. Different phytoconstituents that are to be separated by the samples require different solvents.

4.5.1 The following measurements of chemicals are used to prepare those solvents:

1. For alkaloids: For 10 ml of solution, we have to add 5.5 ml of chloroform, 2.7 ml of glacial acetic acid, 1ml of methanol and 680 µl distilled water.

   16:8:3:2 is the ratio.
2. For flavonoids: For 10 ml of solution, we have to add 7.2 ml ethyl acetate, 400 µl distilled water, 1.3 ml methanol and 900 µl n-hexane.

   16:1:3:2 is the ratio.
3. For terpenoids: For 10 ml of solution we have to add 7.9 ml of n-hexane, 1.9 ml of diethyl ether, and 0.09 ml of glacial acetic acid in a 10 ml solution.

   80:20:1 is the ratio.

   For glycosides: For 10 ml of solution we have to add 6.6 ml of chloroform, 1.9 ml of methanol, and 0.9 ml of ammonia solution.

   7:2:1 is the ratio.
4. For tannins: For 10 ml of solution we have to add 5.9 ml chloroform, 3.18 ml methanol, and 0.9 ml distilled water.

   65:35:10 is the ratio.
5. For saponins: For 10 ml of solution we have to add add 6.0 ml dichloromethane, 3.1 ml methanol, and 0.9 ml of distilled water.

   65:35:10 is the ratio.
6. For steroids: For 10 ml of solution we have to add 9.3 ml of chloroform, 0.5 ml of ethanol, and 0.04 ml of distilled water.
7. For phenols: For 10 ml of solution, add 0.7 ml of formic acid, 6.7 ml of ethyl acetate, 0.7 ml of acetic acid, and 1.7 ml of distilled water to make a phenol solution.

   7:67:7:7:17 is the ratio.

The prepared solvents are put into the screw-top bottle.

The TLC developing silica gel or aluminium foil sheets are applied to the papers, which are then cut into thin strips of 2 cm wide and 10 cm long.

The sample is stored for separation into the solvent-containing bottles after being spotted on the sheet at a location 1 cm above the margin. Separate additions of the sample are made into the solvents that are being prepared for the specific phytoconstituents.

The sheets are taken and the solvent run distance is measured when the solvent has covered the entire sheet or up to 80% of it.

Then the TLC sheets have been exposed to UV light, the spots are located and the sample's distances are measured.

Calculation is done for the retention factor.

4.6 Antimicrobial assay:-

The antimicrobial assay involves stopping the growth of the organisms by the sample. You must use the agar well diffusion method for this.

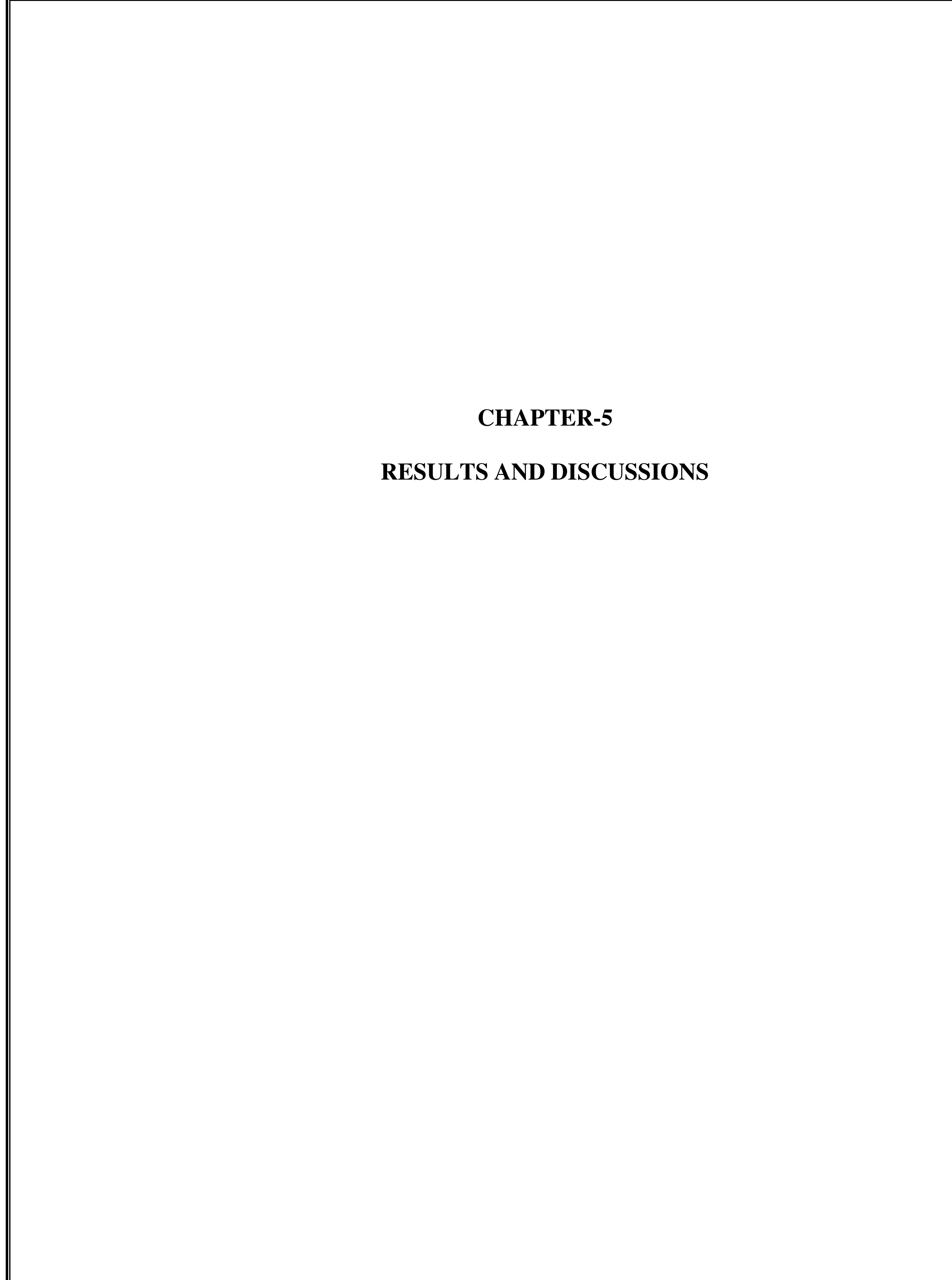
Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, and *Staphylococcus haemolyticus* are the selected organisms. By adding 100 µl of the organism culture to 10 ml of distilled water, the organism culture is diluted.

The media for the chosen microorganisms' growth has been prepared. The media that have been chosen are BHI and LB agar media. The glassware and the media are ready, and they are all stored together for autoclave sterilization. Additionally, the surface of the laminar air flow is cleaned with alcohol and exposed to UV radiation to sterilize it and kill any microorganisms that may be present. All of the equipment is kept inside a laminar air flow after sterilization. For identification, the petri dishes are labelled. The media is then moved to the petriplates using the pour plate method. In order to solidify the media plates were left inside the laminar airflow.

After the solidification of media, the wells are prepared on the agar bed. The diluted microorganism culture is inoculated by using the spread plate method by using the L shaped glass rod. Each microorganism is inoculated to separate petri plate. The wells that are created after solidification of media for that wells, the samples for the identification of antimicrobial activity are added. We added

100 microliter of Lantana camara leaf extract for one well and 50 microlitre of the sample for the another well in the agar media and those plates are kept for the incubation at 36°C for 24 hours.

After 24 hours, the plates were checked for microbial growth inhibition in various amounts of sample loaded into the wells. The inhibitory zone was observed, measured, and recorded. These zones of inhibition demonstrated the sample's antibacterial effectiveness against various pathogens.



CHAPTER-5

RESULTS AND DISCUSSIONS

5.1 Results and Discussions

5.1.1 Soxhlet extraction

10g of *Lantana camara leaf* powder was packed and made them bags like T-bags and then that was used in soxhlet extraction, after soxhlet extraction the round bottom flask was transferred for Rotatory evaporator after rotatory evaporator, we got the extraction about 0.8g and 1.4g of crude sample and transferred for Eppendrof tubes.

Fig:-3
***Lantana camara* leaf powder**

Fig:-4
Extraction of crude sample

Fig:-5
Crude extraction

5.1.2 PHYTOCHEMICALS ANALYSIS

TABLE: 13

Phytochemical test results

The phytochemical test which was conducted in lab, showed the results as mentioned above. As the researchers conducted the tests before on this topic, they concluded tests results in a different ways, so that in several projects they found a different result.

In my experiment, I found that the only substances missing from the Hager's and iodine tests were the alkaloids. The remaining compounds, including flavonoids, tannins, saponins, terpenoids, sterols, phenols, and glycosides, had successful outcomes.

1. Alkaloids test:		
a) Hager’s test b) Iodine test	a) No precipitate formed b) No blue colour	Alkaloids are absent
2. Flavonoids test:		
a) alkaline reagent test	a) De-colourization of yellow colour	

b) Ferric chloride test c) Ammonia test d) Cons. Sulphuric acidtest	b) Green precipitate formed c) No yellow colourformation d) No orange colourformation	Flavonoids are present
3. Tannins test:		
• Ferric chloride test(Braymer's test) • 10%NaOH test • Gelatin Test	a) No brownish greencolour formed b) No emulsion c) No white precipitatio n	Tannins are absent
4. Saponins test:		
• Froth test	Froth formed	Saponins are present
5. Glycosides test:		
• Keller-Killani test • Borntager's test • Salkowski's test	Brown ring formed No brown colourformed Reddish brown colourformed	Glycosides are present
6. Phenols test:	Dark green colour	Phenols are present
7. Terpenoids test:		
• Salkowski's test	Reddish brown coloration	Terpenoids are present
8. Sterols test:		
• Salkowski's test	Red colour appeared	Sterols are present

Fig: 6 Tubes showing results of phytochemical tests

5.2 THIN LAYER CHROMATOGRAPHY:

The following table shows the results of thin-layer chromatography calculated accordingly using the formula.

RETENTION FACTOR $= \frac{distance\ travelled\ by\ solute\ from\ baseline}{distance\ travelled\ by\ solvent}$

TABLE: 14

RF values of the sample for different solvents:

Sl.no	Solvents	Solvent run	Solute run	Rf value
1	Alkaloids	6.5cm	Solute 1= 6.3cm	$Rf = \frac{6.3}{6.5} = 0.96$
2	Flavonoids	6.7 cm	Solute 1= 6.3cm	$Rf = \frac{6.3}{6.7} = 0.94$
3	Terpenoids	6 cm	Solute1= 5.4cm	$Rf = \frac{5.4}{6} = 0.9$
4	Glycosides	6.1cm	Solute1 = 3.8	$Rf = \frac{3.8}{6.1} = 0.62$
5	Tannins	6.4cm	Solute1 = 6.cm	$Rf = \frac{6}{6.4} = 0.93$
6	Saponins	7.2cm	Solute1 =1.2cm Solute2 = 2.1cm Solute3 = 2.8cm Solute 4=	$Rf = \frac{1.2}{7.2} = 0.16$ $Rf = \frac{2.1}{7.2} = 0.29$ $Rf = \frac{2.8}{7.2} = 0.38$ $Rf = \frac{4.2}{7.2} = 0.58$

			4.2 cm Solute 5= 5.2cm Solute 6 = 6.4cm	$Rf = \frac{5.2}{7.2} = 0.81$ $Rf = \frac{6.4}{7.2} = 0.88$
7	Steroids	6.7cm	Solute1 = 1.5cm Solute2 = 2.2cm Solute3 = 4.3cm Solute = 5.6cm Solute5= 6.1cm Solute6= 6.5cm	$Rf = \frac{1.5}{6.7} = 0.22$ $Rf = \frac{2.2}{6.7} = 0.32$ $Rf = \frac{4.3}{6.7} = 0.64$ $Rf = \frac{5.6}{6.7} = 0.83$ $Rf = \frac{6.1}{6.7} = 0.91$ $Rf = \frac{6.5}{6.7} = 0.97$
8	Phenols	6.1cm	Solute1 = 3.2cm Solute2 = 5.9cm	$Rf = \frac{3.2}{6.1} = 0.52$ $Rf = \frac{5.9}{6.1} = 0.967$

Thin-layer chromatography was used to extract the combinations from the sample I had selected for the experiment. Different liquids that are utilized as solvents for the various phytochemicals are demonstrated to have various actions.

The **Lantana camara** extracted sample utilized in the test clearly had the phytochemicals, according to the solute's results.

Fig:7 TLC sheets showing a different bands under UV raditions

5.3 .ANTIMICROBIAL ANALYSIS

The area where the growth of the isolated organisms is inhibited can be used to identify the antimicrobial activity of a sample. The size of the zone varied among the various bacteria. These are counted and measured.

TABLE: 15

Zone of inhibition by the organisms isolated in BHI agar media

Organisms isolated	100µl sample	50µl sample
Bacillus cereus	2.275mm	1.6mm
Pseudomonas aeruginoas	2.1mm	1.95
Staphylococcus haemolyticus	1.975mm	1.725mm
Staphylococcus aureus	1.85mm	1.6mm
E.coli	Shows no zone of inhibition	No zone of inhibition.

For different concentrations of the sample that had been used, the zone of inhibition of the development of the bacteria that were inoculated for the antimicrobial tests was different.

According to the results seen, the zone of inhibition shown by the different microorganisms is ***Bacillus cereus*** showed 2.27mm, ***Pseudomonas aeruginoas*** showed 2.1mm, ***Staphylococcus haemolyticus showed*** 1.975mm***, Staphylococcus aureus*** showed 1.85mm, E.coli showed no zone of inhibitions in 100µl of the sample.

In 50µl of the sample, zone of inhibition shown by ***Bacillus cereus*** showed 1.66mm, ***Pseudomonas aeruginoas*** showed 1.95mm, ***Staphylococcus haemolyticus*** showed 1.725mm, ***Staphylococcus aureus*** showed 1.6mm and ***E.coli*** showed no zone of inhibitions.

5.3.1. Plates showing the zones of inhibition in 100 µl and 50 µl

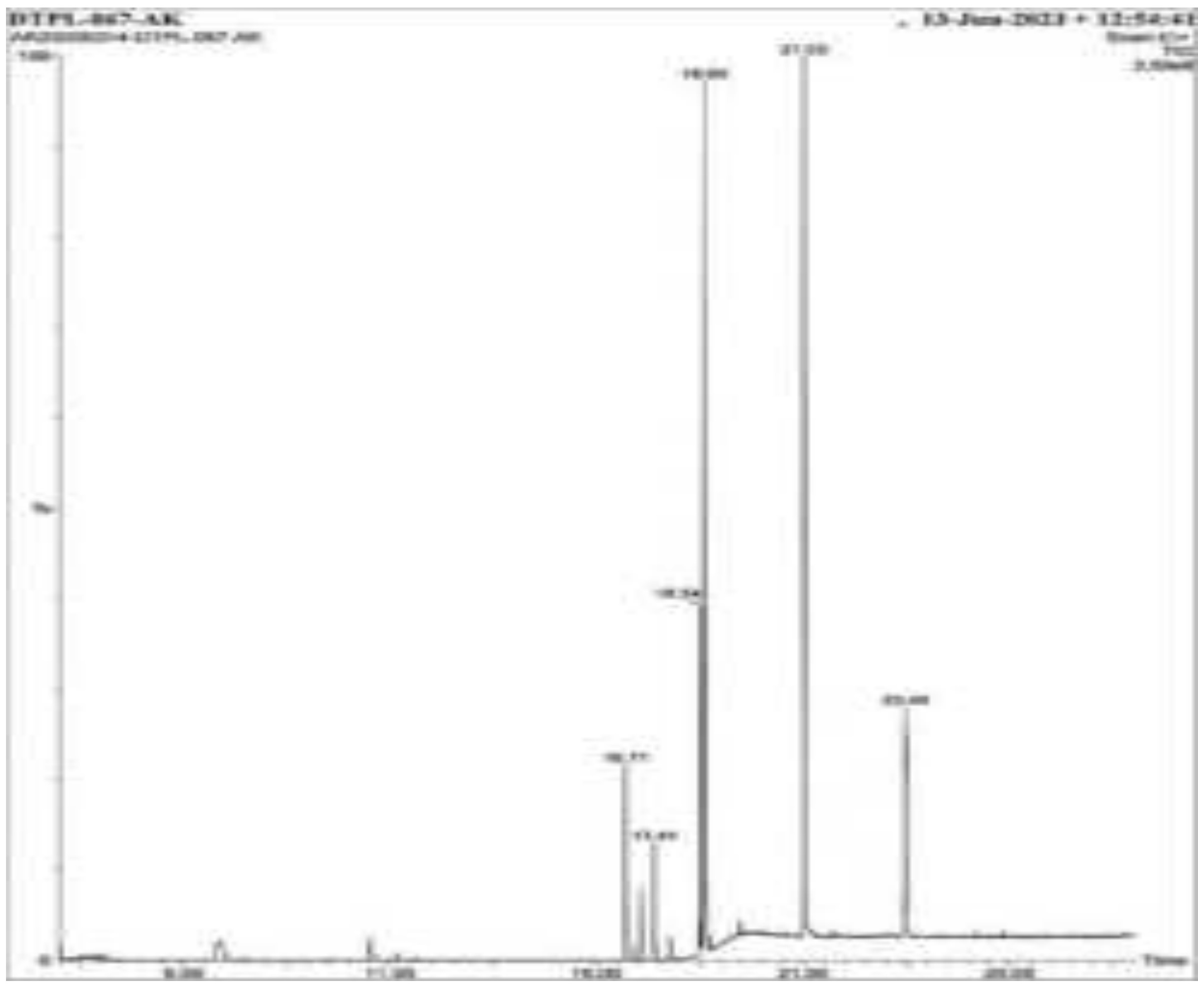
Fig:-8 *Bacillus cereus* ***Fig:-9*** *Pseudomonas aeruginoas*

Fig:10 *Staphylococcus haemolyticus* ***Fig:-11*** *Staphylococcus aureus*

Fig:-12 *E.coli* shows no zone of inhibitions

GC-MS Result

Bioactive compounds identified from the crude extract of lantana camara leaf:-



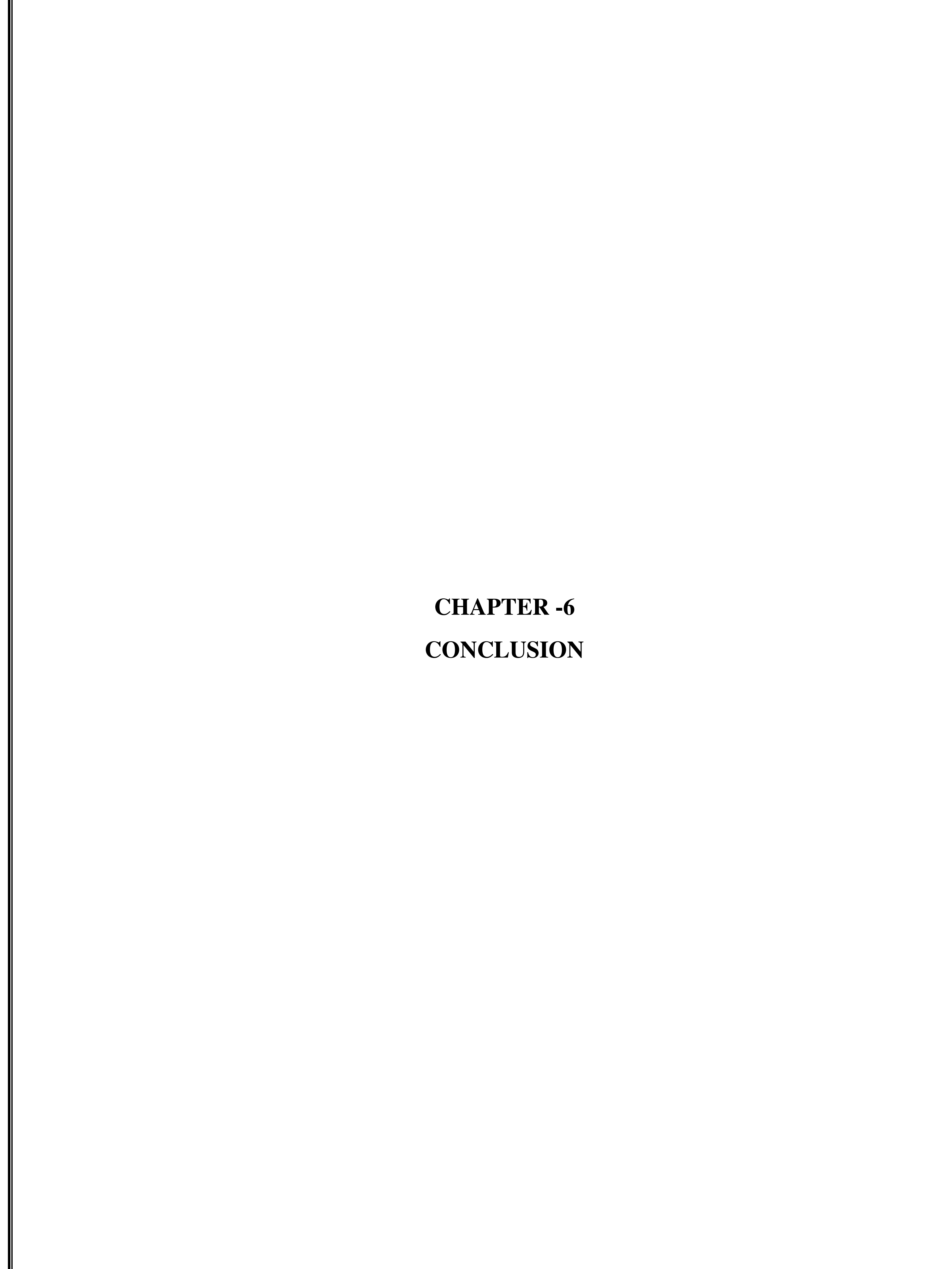
TIC of Lantana camara leaf extract by GC-MS

Biological activity of few compounds that are showed in above graph

TABLE:-16

Retention time (in min)	Hit	Name of the compound	Formula	Molecular weight (in KDa)	Biological activity
Rt 16.71	Hit 1	Neophytadiene	$C_{20}H_{38}$	278	Anti-inflammatory agent, antimicrobial agent,
	Hit 2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	Antibacterial, Antioxidant property
	Hit 3	1,14-Tetradecanediol	$C_{14}H_{30}O_2$	230	Antimicrobial activity against Gram-positive bacteria, Anti-inflammatory activity
	Hit 4	3-Hexadecyne	$C_{16}H_{30}$	222	Antioxidant activity
	Hit 5	1-Octadecyne	$C_{18}H_{34}$	250	Antibacterial, Antifungal, Antioxidant, Decrease blood cholesterol
	Hit 6	10-Undecen-1-ol	$C_{11}H_{22}O$	170	Flavoring agent
	Hit 7	7-Octen-1-ol, 3,7-dimethyl	$C_{10}H_{20}O$	156	Antimicrobial, Antifungal, Anthelmintic, and Antioxidant activities
	Hit 8	Pentadecanal	$C_{15}H_{30}O$	226	Antimicrobial agent
	Hit 9	Oxirane, tetradecyl	$C_{16}H_{32}O$	240	Antimicrobial agent
	Hit 10	1-Pentadecyne	$C_{15}H_{28}$	208	Induce autophagy in mammalian cells
Rt 17.4	Hit 1	1,3-Dioxane, 4,6-bis(2-methylpropyl), trans	$C_{12}H_{24}O_2$	200	Anti-tumor, Anti-oxidation, Anti-inflammatory, immune regulation
	Hit 2	4-Isothiazolecarboxylic acid, 3-methyl	$C_5H_5NO_2S$	143	Antioxidant, Antimicrobial including Antibacterial, Antifungal, Anticancer, Antiallergic, Antihypertensive, Anti-inflammatory,
	Hit 3	3-Buten-2-one, 4-(dimethyl amino)-4-methoxy-	$C_7H_{13}NO_2$	143	Antitumor activity
	Hit 4	2-Propenal, 3-(dimethyl amino)-3-ethoxy-	$C_7H_{13}NO_2$	143	Antitumor activity
	Hit 5	Isobutyramide, N-isobutyl	$C_8H_{17}NO$	143	Antibacterial, Antifungal, Anti-inflammatory, Anticancer, and Antiviral properties.
	Hit 1	Cyclohexane ethanol	$C_8H_{16}O$	128	Anticancer, Antioxidant, cytotoxic, Anti-inflammatory and Antimicrobial activities.

Rt 17.0	Hit 3	3-Nonen-1-ol,	$C_9H_{18}O$	142	Antioxidant, Antimicrobial
	Hit 4	1-Undecyne	$C_{11}H_{20}$	152	Antimalarial and Antitrypanosomally agent
	Hit 6	8-Dodecenol	$C_{12}H_{24}O$	184	Anticancer, Antimicrobial, Antioxidant
	Hit 7	1-Tridecyne	$C_{13}H_{24}$	180	Herbicidal
Rt 18.6	Hit 1	Phytol	$C_{20}H_{40}O$	296	Anxiolytic, Cytotoxic, Autophagy and Apoptosis inducing, immune modulating, Antioxidant, Antimicrobial, and Anti-inflammatory activities
	Hit 5	Citronellol	$C_{10}H_{20}O$	156	Antibacterial, Antifungal and Cardiovascular, antidiabetic
	Hit 6	Cyclohexanol, 5-methyl-2-(1-methylethyl)	$C_{10}H_{20}O$	156	Antimicrobial, Antibacterial, Antifungal, Anticancer, Antiviral, Anti-inflammatory
	Hit 7	Isophytol	$C_{20}H_{40}O$	296	Anticancer, Antioxidant activities
	Hit 8	Oxirane, tetradecyl	$C_{16}H_{32}O$	240	Anti-inflammatory, Immunosuppressive, and antitumor activities
Rt 23.4	Hit 1	Squalene	$C_{30}H_{50}$	410	Oxygen-scavenging agent.
	Hit 2	Supraene	$C_{30}H_{50}$	410	Anticancer, Antioxidant, and Detoxicant bioactivities
	Hit 5	4,8,12-Tetradecatrien-1-ol, 5,9,13-trimethyl	$C_{17}H_{30}O$	250	Antibacterial activity
	Hit 6	3,7,11,Trimethyl-8,10-dodecedienylacetate	$C_{17}H_{30}O_2$	266	Antimicrobial activity
	Hit 7	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	$C_{14}H_{24}O$	208	Anti-tumour, Anticancer



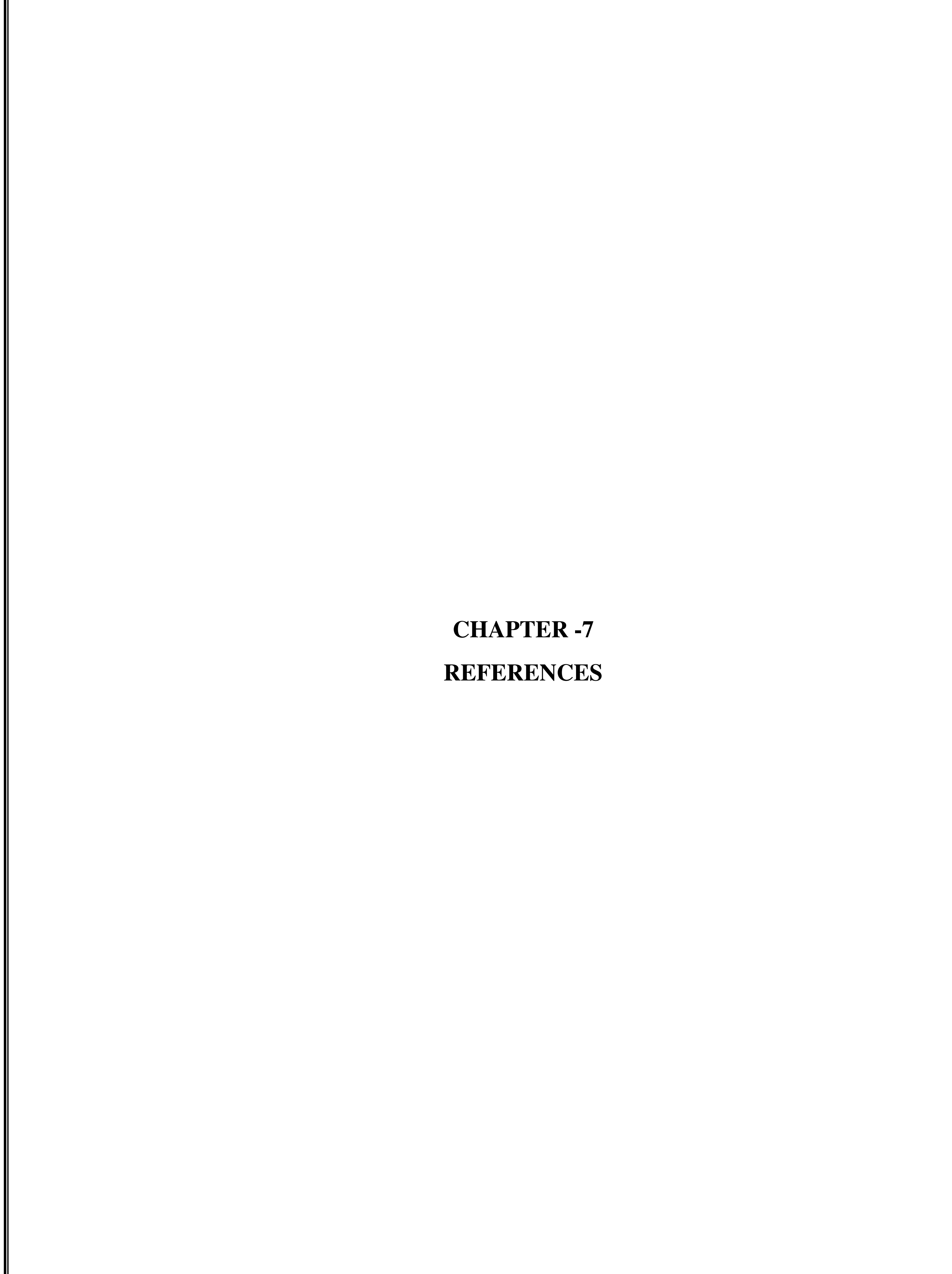
CHAPTER -6

CONCLUSION

CONCLUSION

The phytochemical profiling and antimicrobial evaluation of leaf extract from Lantana camara reveal promising potential for its application in both the pharmaceutical and agricultural sectors. The presence of various bioactive compounds, such as alkaloids, flavonoids, and tannins, suggests its potential for medicinal use. Furthermore, the demonstrated antimicrobial activity against a range of pathogens underscores its role in combating infectious diseases. Further research is warranted to isolate and identify specific compounds responsible for these effects and to explore their mechanisms of action. Overall, this study paves the way for the development of novel natural products derived from Lantana camara for medicinal and agricultural purposes.

The antimicrobial assessment and phytochemical profiling of Lantana camara leaf extract show considerable potential for its use in the pharmaceutical and agricultural industries. Alkaloids, flavonoids, and tannins are a few examples of bioactive substances that imply this plant may have medical use. Furthermore, its importance in preventing infectious disorders is highlighted by the antibacterial activity that has been shown to be effective against a variety of pathogens. To isolate and pinpoint the precise substances that are in charge of these effects and to investigate their methods of action, more investigation is required. Overall, this research opens the door to the creation of innovative natural compounds for agricultural and medical uses derived from Lantana camara.



CHAPTER -7

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ISOLATION AND ANTIMICROBIAL ASSESSMENT OF PIGMENT PRODUCING BACTERIA FROM SOIL SAMPLE

A Project Report

Submitted to

Department of Studies in Microbiology
Pooja Bhagavat Memorial Mahajana
PG Wing of SBRR Mahajana First Grade College (Autonomous)
Metagalli, KRS Road, Mysuru - 570016

In Partial Fulfilment of the Requirements
for the Award of Degree

MASTER OF SCIENCE
IN
MICROBIOLOGY

Submitted by

ADARSH K G
Reg. No. P01BH21S0183

Department of Studies in Microbiology
Pooja Bhagavat Memorial Mahajana
PG Wing of SBRR Mahajana First Grade College (Autonomous)
Metagalli, KRS Road, Mysuru - 570016

Under the Guidance of

Ms. Sahanashree K S
Project Coordinator
Dextrose Technologies Pvt. Ltd.
Bengaluru

SEPTEMBER – 2023

Date:

CERTIFICATE

We certify that this project entitled **"Isolation and Antimicrobial Assessment of Pigment Producing Bacteria from Soil Sample"** submitted in partial fulfillment of the requirement for the degree of Master of Science in Microbiology to the department of Studies in Microbiology, Pooja Bhagavat Memorial Mahajana PG wing of SBRR Mahajana First Grade College, Metagalli, KRS Road, Mysuru, affiliated to University of Mysore. It is an original research work carried out by **Mr. Adarsh K G** at **Dextrose Technologies Pvt., Ltd., Bengaluru.**

Dr. Harish R	**Dr. Ramesh S.R**	**Prof. C.K Renukarya**
Co-Ordinator	**Chief Scientist and Head**	**Director**
Department of Microbiology	School of Life Science	Mahajana PG Wing

DECLARATION

I, **Mr. Adarsh K G** with **Reg No. P01BH21S0183** hereby declare that this project work entitled **"Isolation and Antimicrobial Assessment of Pigment Producing Bacteria from Soil Sample"** submitted in partial fulfilment of the requirements for the award of the degree of Master of Science in Microbiology, is a record of original project work carried out by me under the guidance of **Ms. Sahanashree K S**, Project Coordinator, Dextrose Technologies Pvt. Ltd., Bengaluru.

This project work has not formed the basics for the award of any other Degree/ Diploma or any other similar title of any candidates of any university.

Date:

Place: Mysuru

ADARSH K G

P01BH21S0183

Department of studies in Microbiology

Pooja Bhagavat Memorial Mahajana

PG Wing of SBRR Mahajana First Grade College

Mysuru – 570016

Date: [illegible]

CERTIFICATE

This is to certify that **Mr. Adarsh K C** with Reg No.: **[illegible]** MSc. Microbiology student from **Pooja Bhagavat Memorial Mahajana PG Wing of SBRR Mahajana First Grade College (Autonomous), Mysuru** has successfully completed his final year dissertation project entitled **"Isolation And Antimicrobial Assessment Of Pigment Producing Bacteria From Soil Samples"** at Dextrose Technologies Pvt. Ltd., Bangalore from **22nd May 2023 to 22nd July 2023**.

We wish him all success in his future endeavours.

[illegible] K S	**[illegible]**
Project Coordinator	Managing Director
Dextrose Technologies Pvt. Ltd.	Dextrose Technologies Pvt. Ltd
Bangalore- [illegible]	Bangalore- [illegible]

Signature of Internal Guide
(Dr. [illegible])

Dextrose Technologies Pvt. Ltd.,
#124, 2nd floor, Kengeri Satellite Town, Bengaluru-560060

CERTIFICATE

This is to certify that the project entitled **“Isolation and Antimicrobial Assessment of Pigment Producing Bacteria from Soil Sample”** being submitted by **Adarsh K G** with register number **P01BH21S0183** in partial fulfillment for the award of the degree of **Master of Science in Microbiology**, is a bonafide work carried out by him from 22nd May 2023 to 22nd July 2023.

The results embodied in this report have not been submitted to any other university or institute for the award of any degree/diploma.

Date:-
Place:- Bengaluru

Sahanashree K S	**Aravind Ganessin**	**Signature of Internal guide**
Project Coordinator Dextrose Technologies Pvt., Ltd. Bengaluru	Managing Director Dextrose Technologies Pvt., Ltd. Bengaluru	(Dr. Harish R)

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- ADARSH K G

LIST OF UNITS AND ABBREVATIONS

ABBREVATIONS	EXPANSION
%	Percentage
℃	Degree Celsius
Min	Minutes
ml	Milli liter
nm	Nano meter
gm	Grams
µg	Microgram
pH	Power of hydrogen
M	Molar
g/l	Grams per Liter
rpm	Rotation per minute
OD	Optical density
VP	Voges- proskaurs
MR	Methyl red
UV	Ultra- violet
NaCl	Sodium chloride
TIC	Total Ion Chromatogram
Rt	Retention time
LB	Luria-Bertani
TSB	Tryptone Soya Broth
KOH	Potassium hydroxide
GC-MS	Gas chromatography-Mass spectrometry

ABSTRACT

Pigment-producing bacteria are of significant interest due to their potential applications in various fields, including food, pharmaceuticals, and biotechnology. This study aimed to isolate and characterize pigment-producing bacteria from agricultural soil, which could serve as a valuable source for novel pigments. Agricultural soil samples were collected from different locations, representing diverse agricultural practices. The samples were processed through serial dilution and spread plate techniques to obtain pure bacterial isolates. The isolated colonies were screened for pigment production by visual observation of coloration on agar plates. The pigment-producing isolates were further characterized through morphological, biochemical, and microscopic analysis. Preliminary results indicated the successful isolation of several pigment-producing bacterial strains from the agricultural soil samples. The isolated strains exhibited two types of colours, including, orange and yellow. Morphological observations revealed variations in colony morphology, such as size, shape, and texture. Biochemical tests provided insights into metabolic characteristics, The identified pigment-producing bacteria demonstrated potential for pigment production, offering possibilities for novel natural pigments with commercial applications. Further characterization, including pigment extraction and quantification. This study contributes to the exploration of microbial diversity in agricultural soil and highlights the potential of these microorganisms as sources of pigments. The findings hold promise for the development of sustainable and eco-friendly pigments, reducing reliance on synthetic dyes in various industries.

Key words: Soil microorganism, Pigment producing bacteria, Bacterial pigment, antimicrobial activity.

TABLE OF CONTENTS

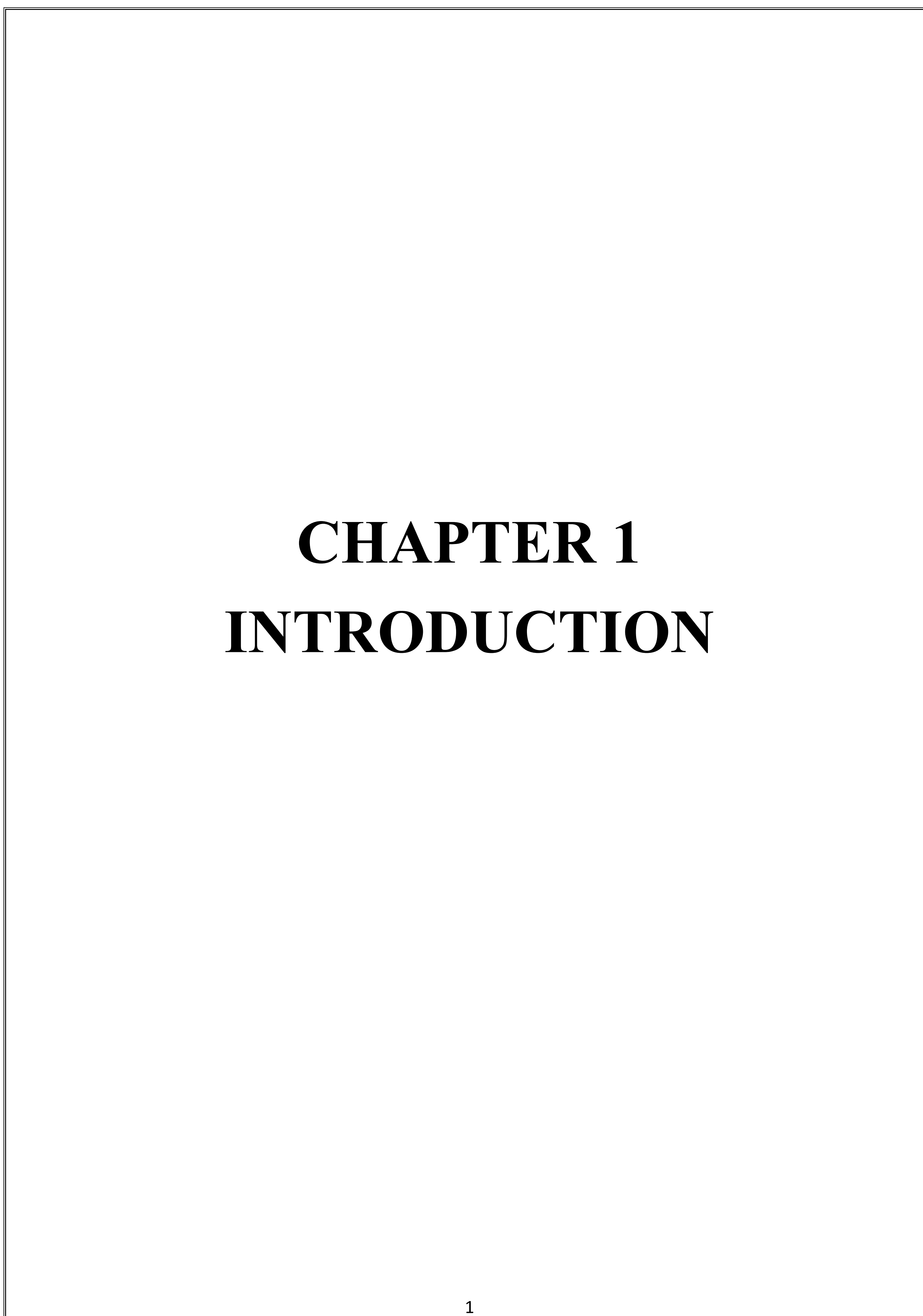
TITLE	CONTENTS	PAGE
Chapter 1	**INTRODUCTION**	1
	1.1 Soil microorganisms	2
	1.2 Bacterial secondary metabolites	3
	1.3 Bacterial pigments	4
	1.4 Pigment producing bacteria	5
	1.5 Factors affecting the production of pigment in pigment producing bacteria	7
	1.6 Advantages of pigment producing bacteria	7
	1.7 Applications of natural Pigments Produced by Bacteria	9
Chapter 2	**REVIEW OF LITERATURE**	11
Chapter 3	**AIM AND OBJECTIVES**	20
Chapter 4	**MATERIALS AND METHODS**	22
	4.1 Collection of soil sample from agriculture field	23
	4.2 Isolation of pigment producing bacteria from agriculture soil	23
	4.3 Purification of pigment producing bacteria	24
	4.4 Pigment production from orange and yellow pigment producing bacteria	24
	4.5 Extraction of pigment from isolated pigment producing bacteria	25
	4.6 Optical Density	25
	4.7 Antimicrobial activity of extracted pigments against the human pathogens	26
	4.8 Biochemical and morphological characterization of isolated yellow pigment producing bacteria	27
	4.9 Identification of bioactive compounds by GC-MS	30
Chapter 5	**RESULTS AND DISCUSION**	31
	5.1 Isolation of pigment producing bacteria	32
	5.2 Pigment production from orange and yellow pigment producing bacteria	32
	5.3 Extraction of pigment from pigment producing bacteria	33
	5.4 Maximum absorption of the pigments	33
	5.5 Antimicrobial activity of extracted pigments against human pathogens	34
	5.6 Biochemical and morphological characterization of isolated yellow pigment producing bacteria	35
	5.7 Identification of bioactive compounds by GC-MS	38
Chapter 6	**CONCLUSION**	41
Chapter 7	**REFERENCES**	43

LIST OF FIGURES

FIGURE	CONTENT	PAGE
Figure 1	Pure culture of (A) orange and (B) yellow pigment producing bacteria	31
Figure 2	Production of pigments in their respective screwcap bottles	32
Figure 3	(A) Orange and (B) Yellow pigments extracted respective pigment producing bacteria	33
Figure 4	Maximum absorption of pigments	33
Figure 5	Antimicrobial activity of isolated pigment producing bacteria	34
Figure 6	Gram’s staining	35
Figure 7	Catalase test	35
Figure 8	Indole test	36
Figure 9	Methyl red test	36
Figure 10	Voges Proskauer test	37
Figure 11	Citrate utilization test	37
Figure 12	Carbohydrate fermentation (dextrose)	38
Figure 13	Total Ion Chromatogram of bacterial crude extract	39

LIST OF TABLES

TABLE	CONTENT	PAGE
Table 1	Composition of Nutrient broth	22
Table 2	Composition of Luria-Bertani agar	22
Table 3	Composition of Tryptone Soya agar	22
Table 4	Composition of BHI agar	26
Table 5	Composition of 1% Tryptone broth	28
Table 6	Composition of MR broth	28
Table 7	Composition of VP broth	29
Table 8	Composition of Simmon's Citrate agar	29
Table 9	Composition of carbohydrate fermentation broth	30
Table 10	Antimicrobial activity of Pigments (0.05 ml concentration)	34
Table 11	Bioactive compounds identified from the crude extract of yellow pigment producing bacteria	40



CHAPTER 1
INTRODUCTION

1. INTRODUCTION

1.1 Soil microorganisms

Soil is indeed a rich source of microbial growth. It is estimated that a single gram of soil can contain billions of microorganisms, including bacteria, fungi, protozoa, viruses, and archaea. These microorganisms play crucial roles in soil fertility, nutrient cycling, organic matter decomposition, and overall ecosystem functioning.

Soil serves as a thriving habitat for microbial growth due to the following key factors:

Microbial Diversity: Soil harbours an immense diversity of microorganisms. Different types of soil, such as forest soil, grassland soil, or agricultural soil, can have distinct microbial communities due to variations in environmental factors like pH, moisture content, organic matter content, and plant cover.

Nutrient Availability: Soil provides microorganisms with essential nutrients required for their growth, such as carbon, nitrogen, phosphorus, and trace elements. Organic matter, including decaying plant and animal material, serves as a food source for many soil microbes.

Microbial Interactions: Soil microorganisms interact with each other in complex ways. They can form symbiotic relationships, such as mycorrhizal associations between fungi and plant roots, where both partners benefit. Microbes can also compete for resources or produce antimicrobial compounds to inhibit the growth of other organisms.

Decomposition and Nutrient Cycling: Soil microorganisms play a vital role in breaking down complex organic compounds, such as dead plant and animal material, into simpler forms. This process, known as decomposition, releases nutrients back into the soil, making them available for plants and other organisms.

Disease Suppression: Some soil microorganisms have the ability to suppress plant pathogens, protecting plants from diseases. For example, certain bacteria and fungi present in the soil can produce antibiotics or enzymes that inhibit the growth of harmful pathogens enhancing soil structure, promoting water infiltration and retention, and mitigating the impacts of environmental stressors like pollution or drought. They can also break down certain pollutants and toxins, detoxifying the soil. (McCauley, A., et al. 2005)

1.2 Bacterial secondary metabolites

Bacterial secondary metabolites are organic compounds produced by bacteria that are not essential for their primary growth and survival but play important roles in ecological interactions and adaptation. These metabolites are diverse in structure and function, and they contribute to the ecological success and versatility of bacteria. Here are some examples of bacterial secondary metabolites and their functions:

Antibiotics: Perhaps the most well-known group of bacterial secondary metabolites, antibiotics are compounds produced by bacteria that inhibit the growth of other microorganisms. They serve as a defence mechanism for bacteria against competing microorganisms in their environment. Examples include penicillin, streptomycin, and tetracycline.

Pigments: Bacteria can produce pigments of various colours, such as red, yellow, green, and blue. These pigments serve multiple purposes, including protection against harmful ultraviolet (UV) radiation, detoxification of harmful compounds, and signalling between bacterial cells.

Iron Chelators**:** Bacteria produce compounds called siderophores that bind to iron, an essential nutrient, and scavenge it from the environment. Siderophores enable bacteria to acquire iron even in iron-limited conditions and enhance their survival and competitiveness.

Antifungals: Some bacteria produce secondary metabolites that are effective against fungi. These antifungal compounds help bacteria compete with fungal pathogens or protect plants from fungal infections. Examples include griseofulvin and fusaric acid.

Quorum Sensing Molecules: Bacteria utilize quorum sensing to communicate and coordinate behaviours within their populations. Quorum sensing molecules are small organic compounds that bacteria produce and release into the environment. These molecules allow bacteria to detect population density and regulate various processes, such as biofilm formation, virulence factor production, and antibiotic resistance.

Toxins: Some bacteria produce toxins as secondary metabolites, which can harm other organisms or provide a competitive advantage. For example, certain strains of Escherichia coli produce toxins that cause food poisoning symptoms in humans.

Plant Growth-Promoting Compounds: Certain bacteria produce secondary metabolites that promote plant growth and development. These compounds can enhance nutrient uptake, stimulate root growth, and protect plants against pathogens. Examples include auxins, cytokinins, and siderophores.

Understanding the microbial life in soil is important not only for agriculture and environmental management but also for various scientific fields, including microbiology, ecology, and biotechnology. Researchers continue to explore the diversity and functions of soil microorganisms to unlock their potential for sustainable agriculture, bioremediation, and the development of novel bioactive compounds. (Tyc,Osong., et al. 2014)

1.3 Bacterial pigments

Bacterial pigments are organic compounds produced by bacteria that contribute to their characteristic colors. These pigments are synthesized by bacteria as secondary metabolites, meaning they are not essential for basic cellular functions but serve important roles in adaptation, survival, and ecological interactions.

Diversity and Colors: Bacterial pigments exhibit a wide range of colors, including red, orange, yellow, green, blue, brown, and purple. The coloration arises from the chemical structure and composition of the pigments. Different bacterial species produce specific pigments, resulting in their characteristic colors.

Types of Bacterial Pigments: Bacterial pigments can be classified into various groups based on their chemical nature. Some common types include carotenoids, melanins, flavins, prodigiosins, quinones, phenazines, and pyocyanin. Each type has unique properties and function

Bacterial pigments serve multiple functions in bacterial survival and ecological interactions which is listed below.

Protection: Pigments can protect bacteria from harmful environmental factors such as UV radiation, oxidative stress, and toxic compounds. They absorb or scatter damaging light and act as antioxidants, neutralizing reactive oxygen species.

Antimicrobial Activity: Many bacterial pigments exhibit antimicrobial properties. They can inhibit the growth of other bacteria or microorganisms, serving as a competitive advantage in the microbial community.

There exists a diverse range of bacterial pigments, encompassing various types, which include:

Carotenoids, Melanin, Violacein, pyocyanin, prodigiosin, indigiodine, fluorescent pigments. These bacterial pigments exhibit beneficial effects on the growth and survival of the bacteria.

Carotenoids: Carotenoids are a class of pigments that include yellow, orange, and red colors. They are widely produced by bacteria and serve various functions, including photoprotection, antioxidant

activity, and light harvesting during photosynthesis. Examples of bacterial carotenoids include lycopene, astaxanthin, zeaxanthin, and β-carotene.

Melanins: Melanins are dark pigments produced by bacteria, similar to those found in human skin and hair. They provide protection against UV radiation, oxidative stress, and other environmental factors. Melanins also have metal-chelating properties, helping bacteria tolerate high metal concentrations.

Violacein: Violacein is a purple pigment produced by *Chromobacterium violaceum* and some other bacteria. It has been found to possess anti-inflammatory properties by inhibiting the production of inflammatory cytokines and enzymes. Violacein has shown promise in preclinical studies as a potential treatment for inflammatory diseases, such as colitis and rheumatoid arthritis.

Pyocyanin: Pyocyanin is a blue-green pigment produced by the bacterium *Pseudomonas aeruginosa*. It contributes to the virulence of *P. aeruginosa* and plays a role in damaging host tissues and interfering with the immune response. Pyocyanin also has antimicrobial properties, aiding *P. aeruginosa* in competing with other bacteria.

Fluorescent pigments: produced by soil bacteria are known as bacterial pigments or bacterial fluorescent pigments. These pigments are synthesized by certain types of bacteria and exhibit fluorescence, meaning they emit visible light when exposed to ultraviolet (UV) light. (Sinha., et al. 2017)

1.4 Pigment producing bacteria

There are several bacterial species known for their ability to produce pigments. Here are some examples of pigment-producing bacteria and the colors associated with them:

Chromobacterium violaceum: This bacterium produces a purple pigment called violacein. It is commonly found in soil and water environments and is known for its antimicrobial properties.

Pseudomonas aeruginosa: Pseudomonas aeruginosa can produce several pigments, including pyocyanin (blue-green), pyoverdine (fluorescent green), and pyorubin (reddish-brown). These pigments contribute to the bacterium's virulence and competitiveness.

Streptomyces spp: Many species of the genus Streptomyces produce a variety of pigments, including red, orange, yellow, and purple. These pigments are also often associated with *Bacillus* spp. Some Bacillus species produce pigments such as anthracene (green), the formation of spores by these

filamentous bacteria prodigiosi (red), and pyrrolnitrin (yellow-green). These pigments are involved in various physiological functions, including defence mechanisms and communication

Arthrobacter spp: can yellow pigment called carotenoid. These bacteria are commonly found in soil and have the ability to survive harsh conditions.

*Actinobacteria: Actinobacter*ia, a phylum that includes many antibiotic-producing bacteria, often produce pigments such as various shades of yellow, orange, red, and brown. These pigments may have roles in protection against environmental stressors.

Myxococcus xanthus: Myxococcus xanthus is a social bacterium that forms multicellular structures called fruiting bodies. It produces a yellow pigment called xanthusindole, which is involved in cell-cell communication and coordination during fruiting body development.

Vibrio species: Some species of *Vibrio* bacteria, such as *Vibrio cholerae* and *Vibrio fischeri*, produce pigments. Vibrio cholerae produces a dark brown pigment called melanin, while Vibrio fischeri produces a luminescent greenish-yellow pigment known as luciferase.

Streptococcus mutans: Streptococcus mutans is a bacterium associated with dental cavities and tooth decay. It produces a pigment called mutanobactin, which contributes to the formation of dental plaque.

Roseobacter species: *Roseobacter* bacteria are known for their production of a pink pigment called roseobacticides. These pigments have been found to have antimicrobial properties and are thought to be involved in competitive interactions with other microorganisms.

Arthrobacter species: Arthrobacter bacteria produce a variety of pigments, including yellow, orange, and red pigments. These pigments are typically carotenoids, which help protect the bacteria from oxidative stress and UV radiation.

These are just a few examples, and there are many more pigment-producing bacteria with different colors and functions. The production of pigments by bacteria can have various ecological roles, such as protection against stressors, communication, and antimicrobial activities. (Suman vikas bhat., et al.2013)

1.5 Factors affecting the production of pigment in pigment producing bacteria

The production of pigments in bacteria can be influenced by various factors. Here are some key factors that can affect pigment production:

Nutrient availability: The availability and composition of nutrients in the growth medium can significantly affect pigment production. Bacteria require specific nutrients to synthesize pigments,

and deficiencies or excesses of certain nutrients can impact pigment synthesis. For example, iron availability can influence the production of pigments such as pyoverdine.

Environmental conditions: Environmental factors such as temperature, pH, light intensity, and oxygen levels can influence pigment production. Different bacteria have specific environmental preferences for optimal pigment production. For instance, some bacteria produce more pigments under specific light conditions, while others may require anaerobic (oxygen-free) environments.

Quorum sensing: Quorum sensing is a cell-to-cell communication mechanism used by bacteria to regulate gene expression in response to population density. Pigment production in some bacteria is regulated by quorum sensing systems, where the presence of signaling molecules triggers pigment synthesis. The density of bacterial cells in the environment can, therefore, affect the production of pigments.

Stress and environmental stimuli: Bacteria can produce pigments as a response to stress or specific environmental stimuli. Factors such as exposure to UV radiation, heavy metals, toxins, antibiotics, or other challenging conditions can induce pigment production as a protective or adaptive response.

It's important to note that the factors influencing pigment production can vary significantly depending on the specific bacterium and the type of pigment being produced. Understanding these factors and their interactions can help researchers optimize pigment production or manipulate it for various applications. (Chidambaram kulandiasamy venil., et al. 2014)

1.6 Advantages of pigment producing bacteria

The usage of bacterial pigments offers several advantages:

Natural sourcing: Bacterial pigments are derived from microorganisms, making them a natural and sustainable alternative to synthetic dyes or pigments produced from chemical processes.

Biodegradability: Bacterial pigments are often biodegradable, meaning they can be broken down by natural processes, minimizing their environmental impact compared to non-biodegradable synthetic pigments.

Diversity of colors: Bacterial pigments exhibit a wide range of colors, including red, yellow, orange, green, and purple, providing versatility for various applications.

Stability and lightfastness: Many bacterial pigments show good stability under different environmental conditions, including lightfastness, meaning they retain their color intensity even when exposed to light.

Non-toxicity: Bacterial pigments are generally considered non-toxic or have low toxicity levels, making them safer for use in various applications, such as food, cosmetics, and textiles. Antioxidant and antimicrobial properties: Some bacterial pigments possess antioxidant and antimicrobial properties, which can provide additional benefits in applications such as food preservation or skincare products.

Novelty and uniqueness: Bacterial pigments offer a unique and distinct color palette that cannot be easily replicated by synthetic dyes, providing an opportunity for innovative and eye-catching product designs.

Wide color range: Bacterial pigments exhibit a diverse array of colors, ranging from red, orange, yellow, green, blue, to purple. This extensive color palette provides flexibility and opens up opportunities for a broad range of applications, including dyeing, coloring agents, and artistic purposes.

Economic viability: The production of bacterial pigments can be cost-effective, especially when using microbial fermentation methods. Microorganisms can be cultured on a large scale, providing a sustainable and economically viable source of pigments, which may offer an advantage over synthetic dyes or pigments derived from non-renewable resources

Overall, the usage of bacterial pigments presents a promising and eco-friendly approach in various industries, offering a range of advantages including natural sourcing, biodegradability, diverse colors, stability, non-toxicity, potential health benefits, and uniqueness. (Sinha., et al. 2017)

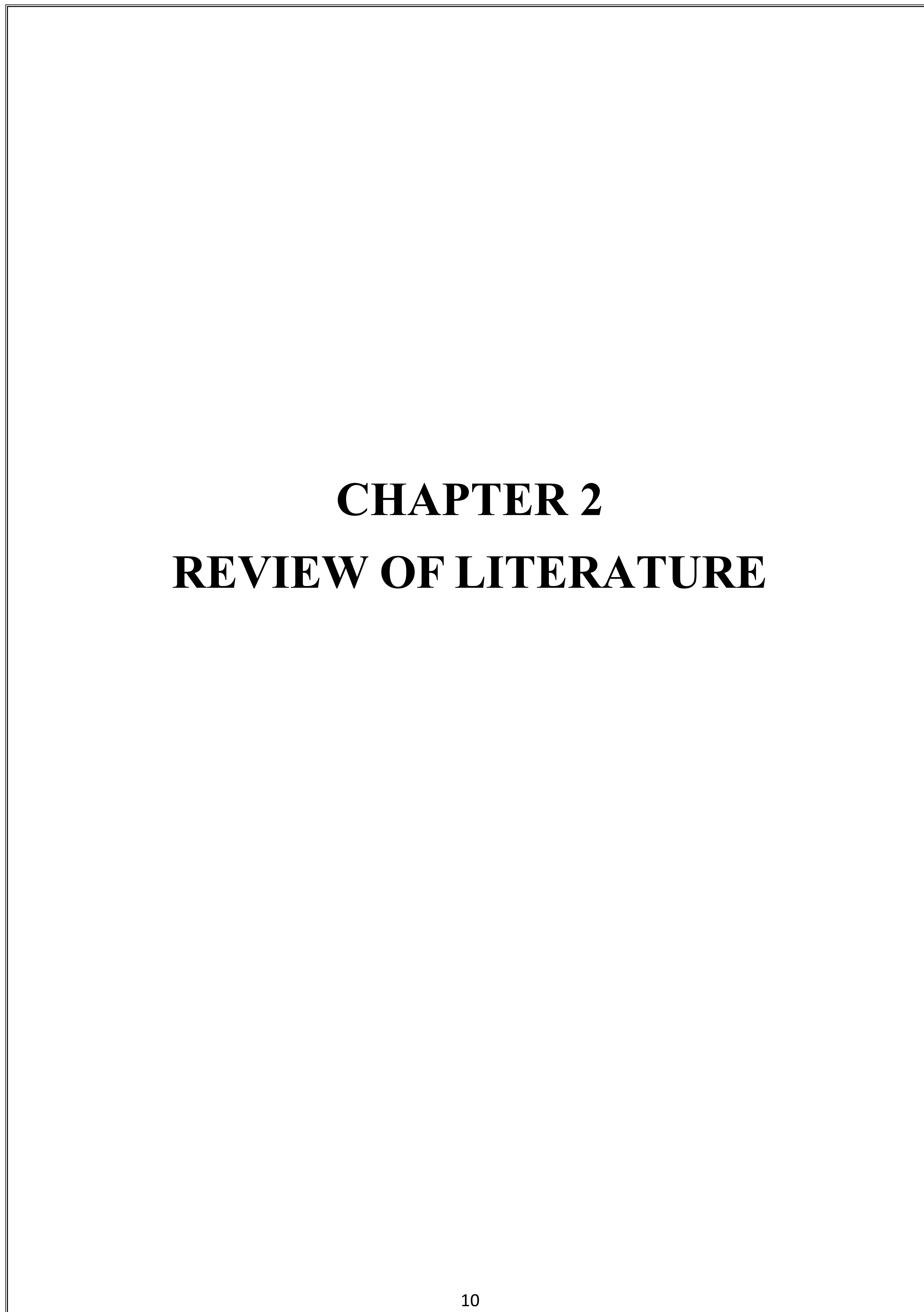
1.7 Applications of natural Pigments Produced by Bacteria

Food Industry: Natural food colors are gaining popularity in the food industry due to concerns about health issues associated with synthetic color additives. Using natural colors is essential for consumer health. Research into natural sources of food colors is vital to produce safer alternatives. Bacterial pigments offer benefits, being naturally occurring and environmentally friendly. They can be genetically modified to produce desired colors, providing healthy and safe natural alternatives. These pigments also help preserve biodiversity and reduce environmental harm compared to synthetic dyes.

Pharmaceutical Industry: Bacterial pigments like prodigiosin have shown potential in treating various diseases, including cancer, due to their antimicrobial, anticancer, and immune-suppressive properties. Pigments like melanin have protective properties and are used in sunscreens. These pigments have shown promise in developing new therapeutic agents, including anticancer drugs with fewer side effects. Flexirubin, carotenoids, and pyocyanin have also exhibited antimicrobial properties.

Dyeing Industry: Bacterial pigments are being explored as natural dyes for textiles. They offer an environmentally friendly alternative to synthetic dyes, which can be harmful to workers and the environment. Prodigiosin and violacein have been successfully used as natural dyes in fabrics like cotton, silk, and polyester. Other pigments from bacteria, such as anthraquinone and pink pigments, show potential in dyeing various fibers.

Limitations and Future Perspective: The use of bacterial pigments holds promise across various industries, but challenges remain. Efforts should focus on efficient pigment harvesting and consistent performance parameters. Continued research into genetically engineering bacteria for pigment production and exploring different applications is crucial. As a health science frontier, bacterial pigments offer exciting potential for safe and sustainable solutions in multiple sectors. (Priti S. Durgade., et al. 2022)



CHAPTER 2
REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The study conducted by P, Sasidharan., et. al. (2013) says that thirteen yellow pigment-producing bacterial strains were isolated from air and soil samples, with RS7, RSS3, RS13, and RS14 showing significant pigmentation abilities. Among them, RS7 exhibited the highest free radical scavenging activity (72%) according to the DPPH method. HPLC analysis confirmed that the isolated strains are natural producers of Astaxanthin. Through biochemical characterization and 16S rDNA gene sequencing analysis, RS7 and RS13 were identified as *Exiguobacterium aurantiacum*, while RSS3 and RS14 were identified as *Exiguobacterium profundum*. These findings suggest that *Exiguobacterium* species have potential applications in producing carotenoids, particularly Astaxanthin, known for its antioxidant properties. Further exploration of these strains could lead to the development of novel bioactive compounds and applications in various industries, such as pharmaceuticals and nutraceuticals.

The review of Minal M. Trivedi., et. al (2022) provides an extensive overview of pigments produced by bacteria, highlighting their diverse range of colors, textures, and gradients. The non-toxic nature of these pigments makes them environmentally friendly and suitable for various applications, including dyes, food products, pharmaceuticals, textiles, cosmetics, and other industrial uses. Natural pigments derived from microorganisms hold significant medicinal and industrial importance, offering antioxidant, antimicrobial, additive, color intensifier, and anticancer properties. Additionally, their use presents economic benefits. This study emphasizes the potential of bacterial pigments and their applications across different fields, underscoring the need for further research and exploration to harness their full potential for a sustainable and innovative future.

The study of M, Waghela., et. al. (2018) focused on microbial pigments produced by bacteria, which can serve as characteristic features for identification. Bacterial pigments hold great promise for diverse applications due to their biodegradability and environmental compatibility. The research aimed to isolate microorganisms from various food samples capable of producing pigments with antimicrobial properties. Three pigmented colonies were successfully isolated, and the antibacterial activity of the extracted pigments was evaluated against both Gram-positive and Gram-negative bacteria. The results demonstrated that the pigments exhibited inhibitory effects on both types of bacteria, indicating their antibacterial nature. This study highlights the potential of bacterial pigments as natural antimicrobial agents, which could have significant implications for various industries and applications in the future.

The study of M, U, Hizbullahi., et. al (2017) pigments play a crucial role in various industries, offering a wide array of colours, including water-soluble variants. The nontoxic nature of pigments produced by numerous microorganisms makes them environmentally friendly and suitable for applications in dyes, food products, pharmaceuticals, cosmetics, and other industrial uses. Additionally, natural pigments from biological sources possess medicinal importance, serving as antioxidants, antimicrobials, additives, colour intensifiers, and even anticancer agents, while also being economically viable. Several bacteria, such as *Agrobacterium aurantiacum*, *Staphylococcus aureus*, *Chromobacterium violaceum,* Serratia marcescens, *Bacillus* species, and *Flavobacterium* sp, exhibit the ability to produce pigments with varying colours, including pink-red, golden yellow, purple, red, creamy, and yellow, respectively. Industrial production of natural food colorants through microbial fermentation offers numerous advantages, such as cost-effectiveness, easier extraction, higher yields via strain improvement, consistent availability of raw materials, and no seasonal limitations. This review emphasizes the significance of bacterial pigments, their wide-ranging applications, and their potential for enhancing various industries in an economically and environmentally sustainable manner.

The study of Pooja, Ratnakaran., et. al. (2020) highlights the worldwide significance of pigments obtained from bacteria due to their natural colour, ease of production, and non-toxic nature, making them an appealing alternative to chemical counterparts. The research involved the isolation of pigment-producing bacteria from various sources such as rotten vegetables, soil, and air flora, resulting in ten bacterial isolates (PR 1 to 10). Through morphological and Gram's nature analysis, these isolates were identified using VITEK 2 at Nucleus Diagnostic Laboratory, Kalyan. The study also investigated the effects of different physical (temperature, pH, incubation period) and chemical (NaCl concentration, tryptone) parameters on pigment production. The pigments were successfully extracted using ethanol, methanol, chloroform, and acetone as solvents. Characterization of the isolated pigments using UV-Vis Spectrophotometer and TLC revealed their nature as carotenoids. This research contributes valuable insights into the potential applications of bacterial-derived carotenoids in various industries, given their natural origin and advantageous properties.

Bacteria produce pigments for various ecological purposes, and their role in nature is significant. These pigmented bacteria can be isolated, cultured, and purified from diverse environmental sources like water bodies, soil, and plants. Ordinary media, such as Nutrient agar, can be utilized for their isolation. In this investigation, bacteria were isolated from 12 different environmental samples, resulting in the identification of 22 different pigment-producing organisms. To extract pigments from

these bacteria, a common method involves treating the cultures with ethyl acetate followed by centrifugation. This process facilitates the separation and collection of the pigments from the bacterial cells. The study of pigment-producing bacteria and the extraction of their pigments hold potential for various applications in industries such as food, cosmetics, and pharmaceuticals, and further research in this area may lead to valuable discoveries and innovations (Asifa, Kelkar., et.al. 2018).

The study conducted by V, K, Suman., et. al. (2013) various food samples obtained from local markets in Kashmir were utilized to isolate pigment-producing bacteria. Two bacteria were successfully isolated, one exhibiting orange pigmentation and the other yellow pigmentation. These isolated bacteria were then cultivated into pure axenic cultures. Morphological observations revealed that both isolates were Gram-positive cocci and non-motile. Taxonomic characterization was conducted, and the isolates were identified as *Micrococcus nishinomiyaensis* and *Micrococcus luteus*.

The study also investigated the conditions that favour pigment production by these bacteria. The maximum pigment production was observed at a temperature of 35°C, pH 9, and a NaCl concentration of 4% (W/V). These findings are valuable for understanding the growth and pigment production behaviour of *Micrococcus nishinomiyaensis* and *Micrococcus luteus*, and they contribute to our knowledge of microbial pigmentation in food samples. Further research in this area may lead to potential applications in various industries, such as food colouring or natural pigment production.

The study of S. R. Sahoo., et. al. (2020) says isolated thirty halophilic bacteria from the soil of Karwar mangrove regions, Karnataka, and examined their pigment production, antibiotic, and proteolytic activities. Among the isolates, KA16SPiv produced a fluorescent green pigment, while KA16SK2HS produced a pink pigment. The optimal conditions for pigment production were observed at pH 8 and 37°C. The pigments could be extracted using chloroform and methanol, and their absorption spectra suggested a phenazine skeleton for the green pigment and a prodigiosin skeleton for the pink pigment. Furthermore, a polyphasic taxonomic analysis revealed that KA16SPiv belonged to the species *Pseudomonas aeruginosa*, with accession no: MF377544, and KA16SK2HS was identified as *Salinicoccus roseus*, with accession no: MF377542. This research provides valuable insights into the pigment production capabilities of halophilic bacteria and their taxonomic identification. Understanding the pigment production and other activities of these bacteria contributes to our knowledge of microbial diversity and potential applications in various fields, such as biotechnology, pharmaceuticals, and environmental management.

The research conducted by Nafisa Anzum., et. al. (2022) focused on isolating and identifying pigment-producing bacteria from the unique Ratargul Swamp Forest soil, the only freshwater swamp forest in Bangladesh. The soil samples showed strongly acidic pH values ranging from 4.71 to 5.48. The bacterial load in the samples varied significantly across different media, with the highest and lowest counts observed on nutrient agar. A total of 71 bacterial isolates were obtained, and 11 potential pigment-producing isolates were selected for further investigation. Among these, nine were Gram-positive, and two were Gram-negative, all displaying rod-shaped morphology. Further molecular marker analysis confirmed the identity of seven isolates up to the generic level, while *Erwinia stewartii* was identified as Aeromonas sobria. Eight of the isolates exhibited the ability to produce three different types of pigments, including red, yellow, and dark orange. These pigment-producing bacteria hold potential for various biotechnological applications. This research contributes to the understanding of microbial diversity and the biotechnological significance of pigment-producing microorganisms, opening avenues for future applications in various industries and fields.

The study of J. U. Vora., et. al. (2015) focused on orange and yellow pigmented bacteria isolated from the soil samples of Kharaghoda area, Gujarat. These halophilic, Gram-positive bacteria were identified as *Kocuria* spp. through 16s rRNA gene sequencing, exhibiting salt tolerance up to 12% NaCl. The gene sequences were submitted to NCBI and assigned the names *Kocuria* KM243757 & JO1 KM216829. The highest pigment extractions were obtained using a 2:1 mixture of 85% methanol: acetone. The extracted pigments showed Rf values of 0.32-0.97 in TLC method, and their maximum absorption spectrum was observed at 466 nm by UV-Visible Spectroscopy. The pigments were identified as carotenoids with various potential applications.

Colour plays a crucial role in enhancing the attractiveness and acceptance of various items. While synthetic colours are commonly used due to their reliability and cost-effectiveness, health concerns have led to the prohibition of some of these dyes. As an alternative, innocuous pigments derived from microbes are widely employed in industries like dye, food, pharmaceutical, and cosmetics. This study focused on isolating pigment-producing bacteria from soil and investigating the parameters influencing pigment synthesis. The bio colorant obtained from isolates A1 and A2 was applied to cotton fabric using the solvent method. Through biochemical and molecular analysis, both isolates were identified as *Pseudomonas aeruginosa* and *Salinococcus roseus*, respectively. The research revealed that pigment production was optimal under neutral pH and shaking conditions. Furthermore, different incubation times were observed for pigment synthesis between the two isolates. Notably, only *Pseudomonas aeruginosa* exhibited antibacterial activity against *E. coli* and *Staphylococcus*

aureus, suggesting potential applications in antimicrobial textiles or other relevant industries (Ashitha.K.Sanuj., et. al. 2021).

Bacterial pigments, also known as chromo bacteria, play a vital role in various applications in the current scenario. In this study, the aim was to isolate bacterial pigments from diverse soil samples, including garden areas, lakes, and agricultural regions, as well as from different flower samples such as Hibiscus, Gokarna, Rose, and Marigold, and vegetable samples like Spinach and Lufa from kitchen waste. Six bacterial isolates were selected for pigment production and identified based on morphological and biochemical tests. The majority of the isolated pigment producers were found to be Gram-negative and cocci in shape. This research provides valuable insights into the potential of these pigmented bacteria for various industrial and environmental applications (Trupti Kulkarni, et. al. 2022).

The study of Ajay, S Kumar, A., et. al. (2017) aimed to isolate microorganisms from various geographical locations capable of producing pigments with antimicrobial activity. Soil and water samples were collected from different areas with varying climatic conditions. Five pigmented colonies were isolated, and the isolated organisms were characterized using the Bergey's Manual of Determinative Bacteriology. Pigments were extracted from the isolates, and their antimicrobial activity was evaluated. The extracted pigments exhibited inhibitory effects on both Gram-positive and Gram-negative bacteria, as well as on fungi. The findings suggest that the soil and water samples harbor diverse organisms with antibacterial and antifungal properties. This research highlights the potential of these pigmented microorganisms as sources of natural antimicrobial agents, which could have significant implications in various fields, including medicine, agriculture, and environmental protection.

The production of potential pigments using bacterial sources holds great promise for diverse industrial applications. Soil samples from various terrestrial, rhizosphere, and forest habitats in Karnataka were collected to recover pigment-producing actinomycetes. From the investigation, 25 strains were isolated on starch casein agar medium, and further studies based on phenotypic, biochemical, morphological, and 16S+ rRNA gene sequencing indicated that strain BJZ10 belonged to the Streptomyces species, an actinomycete. Enhanced pigment production was achieved using starch casein broth medium, and actinobacterial pigments were extracted exclusively using methanol solvent. UV-visible spectroscopy characterized the pigment with absorption spectra ranging from 220 to 250 nm. FTIR analysis revealed functional groups such as alkane, alkyls, alkynes, alcohols, esters, and sulphates. Antibacterial activity testing demonstrated significant results against *Bacillus cereus*

and *Escherichia coli*. The study highlights *Streptomyces* sp. strain BJZ10's brown pigment for its potential antimicrobial activity (Zareenkousar Kazi., et. al. 2022).

Pigments obtained from natural sources have garnered worldwide interest as synthetic pigments pose significant environmental hazards and toxicity. Bacterial pigments play a crucial role in various industries, including food, pharmaceutical, and textiles. In the current study, pigmented bacteria were isolated from the soil of different regions with varying geographical and climatic conditions. The main objective was to isolate and identify these pigmented bacteria and investigate their antibacterial activity. Four pigmented bacterial isolates were obtained and characterized using the Bergey's Manual of Determinative Bacteriology. Pigment extraction was done using chloroform as the solvent, and UV spectrometer analysis determined the maximum absorption of each sample. Paper chromatography was used for further pigment analysis. Antibacterial activity testing demonstrated inhibitory action against pathogens such as *E. coli, Pseudomonas,* and *Staphylococcus* species. This study highlights the potential for large-scale production, purification, and application of these pigments in various industries, especially those sourced from soil with diverse organisms possessing antibacterial and antifungal activities (Sadia, Qayyum et al 2020)

Bacteria play a crucial role in producing various pigments with diverse functions, such as photosynthesis. To harness these pigments efficiently and economically, there is a need to develop low-cost production processes. The use of agro-industrial residues offers a promising solution to reduce substrate costs. Isolation of pigment-producing bacterial strains from environmental sources can be achieved using different growth mediums. After extraction, the pigments can be purified and characterized using advanced analytical techniques like TLC, UV–vis Spectroscopy, FTIR, ESI–MS, NMR, HPLC, and Gel Permeation Chromatography. The application of these methodologies not only aids in understanding the physical and chemical properties of the pigments but also facilitates their potential industrial applications in various fields such as food, pharmaceuticals, and cosmetics (W. A. Ahmad et al. 2012)

The study of Samina Bashir et al (2022) highlights the significant threat posed by disease and pests in agriculture and the environment, necessitating alternative approaches to chemical control agents. The research focused on bioactive pigment-producing bacteria from saline agricultural fields in Gujrat, Pakistan, as potential antagonists against plant pathogenic fungi and bacteria. *Streptomyces chromofuscus* exhibited the highest activity against most tested fungal and bacterial phytopathogens, followed by *Nonomurae salmonae*, while *Actinocorallia libanotica* showed limited activity. This study sheds light on the antimicrobial potential of rare actinomycetes like *Nonomurae salmonae* and *Actinocorallia libanotica* against plant pathogenic bacteria, particularly *P. syringae* and *X.*

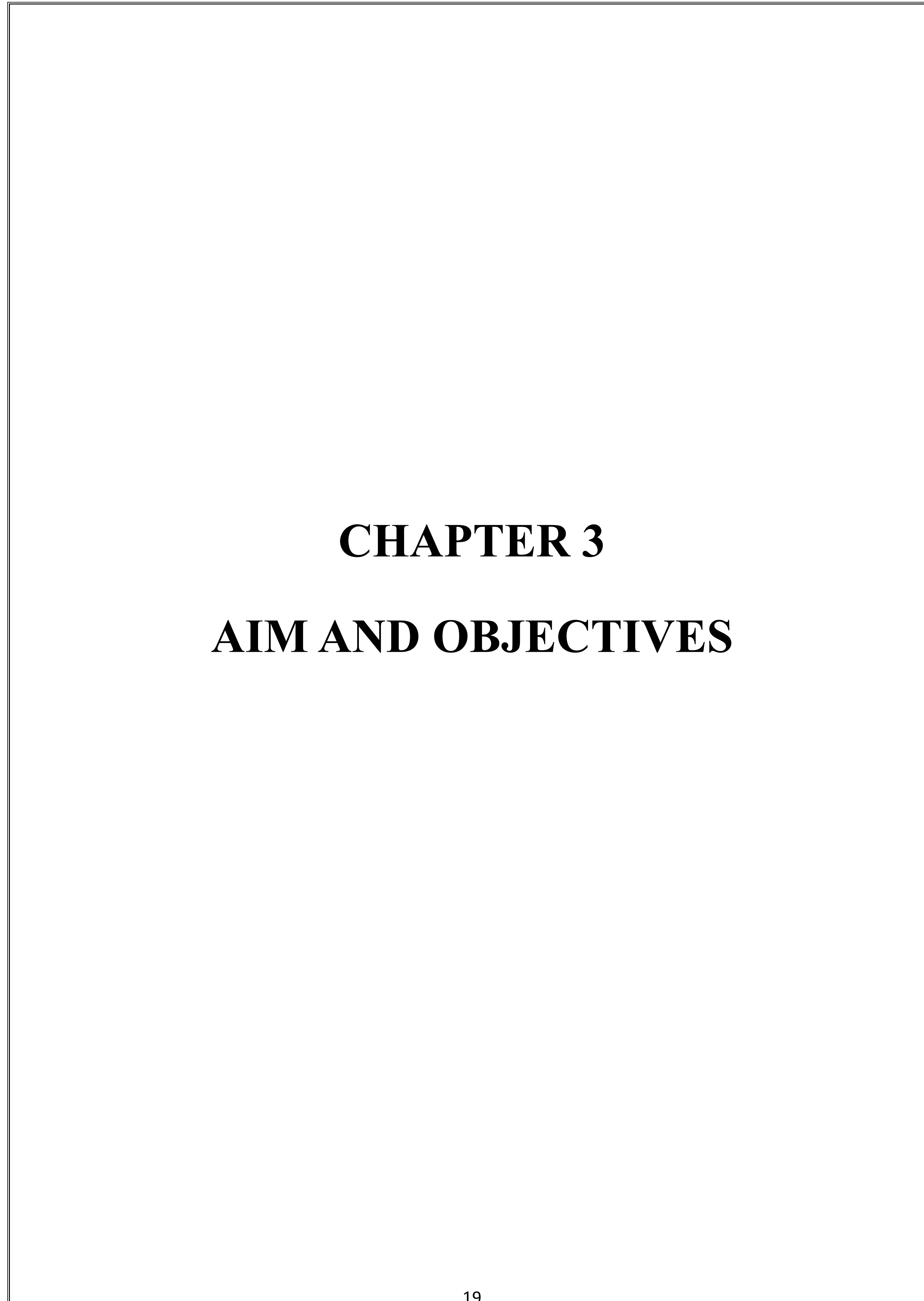
axonopodis. The findings emphasize the importance of advanced isolation techniques to discover promising bioactive compounds from rare microbial species. Further research should explore the mechanisms, optimal conditions, and active components involved in the antagonistic effects of these bioactive pigment-producing bacteria.

An extracellular pigment-producing Penicillium filamentous fungi from soil were studied for optimal culture conditions, leading to the highest pigment production at 2% soluble starch (670 units), 880 units of peptone, pH 9.0 (900 units), 30°C temperature (950 units), 200 rpm agitation (920 units), and 4-day-old inoculums (850 units). The pigments' properties were evaluated under various physico-chemical conditions, including sunlight, fluorescent light, UV light, high temperature, and exposure to preservatives (sodium bisulfate, ascorbic acid, and citric acid). Detailed investigations were conducted to assess the pigment's stability and residual content under these conditions. Further research may explore the potential applications of these pigments (Gunasekaran, S. et al. 2008)

In the study of Ulf Thane, et al (2006), exogenous pigments from Penicillium, Epicoccum, and Monascus fungi were extracted and characterized using quantitative colorimetry. Comparison with water-soluble natural colorants revealed a diverse colour range in the red-orange-yellow region of the CIELAB colour space. Fungal extracts exhibited unique hues in the red and yellow spectra, similar to or brighter than reference natural colorants. Principal component analysis effectively distinguished different colours based on a* and b* values, grouping fungal colour extracts according to their similarity or difference from existing colorants. Interestingly, colour diversity was observed not only between different fungal genera or species but also within the same species when media changed. These findings suggest that pigment-producing ascomycetous fungi, aside from Monascus, contribute novel colour shades, enriching the natural colorant palette. Utilizing multivariate approaches enhances colorant comparison and classification.

The evaluation of pigment content in microalgae is crucial for assessing cell growth and trophic levels in water. Spectrophotometric analyses are commonly used due to their practicality and reliability for chlorophyll evaluation. To compare different pigment quantification methods, simplicity, pigment identification range, and analysis time were considered. Factors affecting pigment extraction yield were tested for maximizing efficiency, including the choice of solvent, cell wall disruption technique, extraction time, and empirical correlations. *Nannochloropsis gaditana*, rich in chlorophyll, served as the biological material for the study. Methanol was identified as the most suitable solvent for chlorophyll extraction, using the Lichtenthaler correlation after 24 hours. Cell wall disruption, especially with freezing/unfreezing using liquid N2, improved extraction efficiency. Hydrophilic

solvents, like methanol, were effective in extracting polar pigments, such as carotenoids, regardless of the disruption method. These findings aid in optimizing pigment extraction techniques for microalgae analysis (M. Henriques., et al 2007).



CHAPTER 3

AIM AND OBJECTIVES

3. AIM AND OBJECTIVES

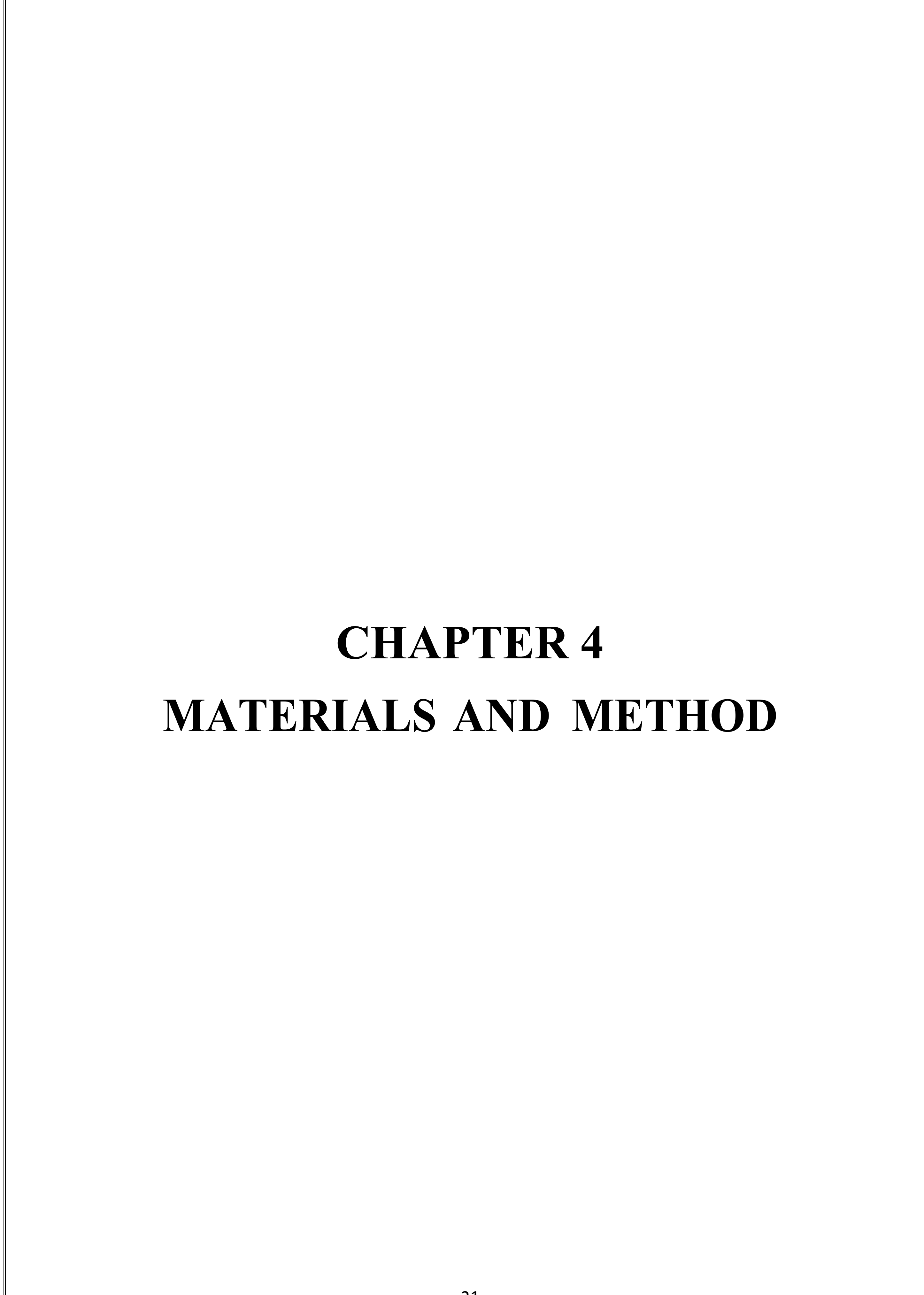
AIM OF THIS STUDY

The aim of the present study is to **isolate pigment producing bacteria from soil sample and assessment of its antimicrobial activity**.

OBJECTIVES OF THIS STUDY

The study **"Isolation and Antimicrobial Assessment of Pigment Producing Bacteria from Soil Sample"** was undertaken with the following objectives:

- **Isolation of pigment producing bacteria**
- **Production of pigments by the bacterial isolate**
- **Antimicrobial activity of the bacterial isolate**
- **Biochemical characterization of the antimicrobial positive organism.**



CHAPTER 4
MATERIALS AND METHOD

4. MATERIALS AND METHOD

MATERIALS

Media

Nutrient agar

Purpose - Cultivation of pigment producing bacteria present in the agriculture soil.

Table 1. Composition of Nutrient agar

Composition	Amount (g/l)
Peptone	5.0
Beef extract	1.5
Agar	15.0
Sodium chloride	5.0
Distilled water	1 litre

Luria-Bertani (LB) agar

Purpose - Cultivation of pigment producing bacteria present in the agriculture soil.

Table 2. Composition of Luria-Bertani agar

Composition	Amount (g/l)
Tryptone	10.0
Yeast extract	5.0
Sodium chloride (NaCl)	10
Agar	15
Distilled water	1 litre

Soya bean casein digestive agar also known as Tryptone Soya agar (TSA)

Purpose - Culturing of pigment-producing bacteria and provide a favourable growth medium that supports the development of visible pigments.

Table 3. Composition of Tryptone Soya agar

Composition	Amount (g/l)
Tryptone	15.0
Soya peptone	5.0
Sodium chloride (NaCl)	5.0
Agar	15.0

Distilled water	1 litre

Tryptone Soya Broth (TSB) - It has the same composition as mentioned above for Tryptone Soya Agar, but without the addition of agar.
Purpose - Cultivation of pigment-producing strains and provide a favourable growth medium that supports the development of visible pigments.

Equipment - Inoculation loop, Autoclave, Incubator, Centrifuge, Microscope, pH meter, Refrigerator, UV- spectrophotometer, Magnetic stirrer, Micropipette, Weighing balance.

Glass wares - L-shape glass rod, Screw cap bottles, Petri dishes, Test tubes, Cuvette, glass rod, pipette, Durham tube, measuring cylinder, glass slide and cover slips.

Chemicals - 70% ethanol, sodium chloride, ethyl acetate, hydrogen peroxide,95% ethanol, disodium phosphate, dipotassium phosphate, iodine, potassium diphosphate, potassium hydroxide, sodium nitrate, sodium citrate,

Dyes - Crystalline violet, safranin, methyl red, phenol red, bromomethyl blue.

4. Methods

4.1 Collection of soil sample from agriculture field

Soil was taken from a field of post-harvested turmeric in the Kallahalli village of Gundlupet Taluk of Chamarajanagar District. The soil was dug up for 10 cm deep using a sterilized spatula, and 100 gram of the sample was then transferred to a sterilized, leak-proof polyethene bag, labelled appropriately and which was then brought into the lab and kept at room temperature.

4.2 Isolation of pigment producing bacteria from agriculture soil

4.2.1 Serial dilution

The mixed solution from the test tube labelled 10^{-1} was obtained using the micropipette. 1 ml of the mixed solution from the test tube labelled 10^{-1} was transferred to the second test tube labelled 10^{-2}, resulting in a 1:10 dilution of the mixed solution. 1 ml of the mixed solution from the test tube labelled 10^{-2} was transferred to the third test tube labelled 10^{-3}, creating a 1:10 dilution of the previously diluted solution. The above dilution process was repeated for the remaining test tubes labelled 10^{-4}, 10^{-5}, and 10^{-6}, where 1 ml from the previous tube was transferred to the next tube, resulting in a 1:10 dilution each time. After each transfer, the contents of each test tube were mixed thoroughly to ensure proper dispersion of the solution. (Winthroap- Youngh, G. 2013)

4.2.2 Spread plate method

A test tube labelled as $10^{-4,}$ containing the diluted soil sample, was obtained. The spreading tool, an L-shaped glass rod, was sterilized by flaming until it turned red hot. Once sterilized, it was allowed to cool. Using a sterilized micropipette, 0.1 ml of the diluted soil sample was aseptically transferred onto the centre of both the LA (Luria Agar) (Table. 2) and NA (Nutrient Agar) (Table.1) plates. The inoculum was quickly spread evenly over the entire surface of the LA and NA plates using the sterilized spreading tool. After spreading the sample, the agar plates were left to dry with their lids partially open for a few minutes to allow any excess moisture to evaporate. Once dry, the plates were inverted. The inverted plates were then incubated at 37°C for a period of 24 to 48 hours. This incubation period allowed the bacteria present in the soil sample to grow and form visible colonies on the agar plates. (Winthroap- Youngh, G. 2013)

4.3 Purification of pigment producing bacteria

4.3.1 Streak plate technique

After incubation, the culture plates displayed a range of colonies, including both pigment-producing and non-pigment-producing bacteria. Among these colonies, we were able to identify distinct yellow and orange pigment-producing bacterial colonies. To specifically isolate the yellow and orange pigment-producing bacteria, a pure culture technique called streak plate technique is employed. First, a loopful of the yellow pigment-producing bacteria is taken using inoculation loop and inoculated onto a freshly prepared LB agar plate using the continuous streak plate technique. Similarly, another loopful of the orange pigment-producing bacteria is inoculated onto a separate LB agar plate. The streak plate technique involves streaking the bacteria across the agar surface in a continuous zig zag pattern to obtain isolated colonies. The plates are then incubated at 37°C for 24-48 hours in an inverted position to ensure optimal growth. (Winthroap- Youngh, G. 2013)

4.4 Pigment production from orange and yellow pigment producing bacteria

100 ml of Soya bean casein digestive medium also known as Tryptone Soya Broth (TSB) was prepared (Table. 3) and sterilized. The sterilized TSB was then transferred into two 250 ml screw-cap bottles, which were labelled as "yellow" and "orange" respectively, with 50 ml allocated for each

bottle. Using aseptic techniques, 5 loopfuls of yellow pigment-producing bacteria were inoculated into the broth culture in the "yellow" labelled bottle. Similarly, 5 loopfuls of orange pigment-producing bacteria were inoculated into the broth culture in the "orange" labelled bottle. The caps of the bottles were tightly screwed to ensure proper sealing. The screw-cap bottles were placed on a

magnetic striker to keep them in a stationary position. The bottles were incubated at room temperature for a period of 3 days. Throughout the incubation period, the development of pigment in each bottle was observed. After 3 days of incubation, the bottles were examined to assess the presence and intensity of pigment production in both the "yellow" and "orange" labelled bottles. The results were recorded and analysed for further evaluation.

4.5 Extraction of pigment from isolated pigment producing bacteria

To extract pigments from pigment-producing bacteria, the incubated bacterial culture was carefully transferred into separate Falcon tubes, each labelled with yellow and orange colours. The tubes were subjected to centrifugation at 4000 rpm for 25 minutes, facilitating the separation of the bacterial cells from the supernatant. The supernatant, containing the bacterial culture, was then gently removed, while the pellet containing the bacterial cells was retained. For pigment extraction, ethyl acetate was added to each Falcon tube, ensuring complete submergence of the bacterial pellet. The tubes were centrifuged once again at 4000 rpm for 10 minutes, causing the ethyl acetate to separate into an upper layer, distinct from the pellet containing the pigment-producing cells. After centrifugation, the ethyl acetate layer was left to undergo air drying, allowing for the evaporation of the solvent. This process resulted in the concentration of the pigment present in the solution. The dried pigment was subsequently collected, yielding a concentrated pigment extract suitable for further analysis and application. (Sinha, S., et al. 2017)

4.6 Maximum absorption of the pigments

The optical density measurement involved collecting the supernatant from pigment extracts of both yellow and orange samples. Subsequently, the supernatant was transferred to cuvettes, and optical density readings were taken. A blank solution with distilled water was employed to establish the zero-reference point. The investigation included scanning the wavelength range from 400nm to 700nm in

10nm increments. This process facilitated the identification of the maximum absorption points for both extracted pigments in the samples. (Zareenkousar kazi., et al.2022)

4.7 Antimicrobial activity of extracted pigments against human pathogens

The well diffusion assay was performed to assess the antimicrobial activity of the extracted yellow and orange pigments against human pathogens, including

- *Escherichia coli*
- *Enterococcus faecalis*
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Staphylococcus haemolyticus*

0.2 ml of both the yellow and orange pigments were individually weighed and mixed with 0.25 ml of ethyl acetate solvent. The mixture was thoroughly vortexed to ensure proper mixing of the pigments.

Luria-Bertani (LB) agar, (Table. 2) Brain Heart Infusion (BHI) agar (Table.4) media are prepared and sterilized. Along with agar plates, were prepared. Two wells were created on each agar plate using a microtip. To eliminate any potential contamination, the plates were exposed to UV light for 15-20 minutes. The bacterial strains, including *E. coli, S. aureus, P. aeruginosa,* and *S. haemolyticus,* were inoculated onto LB agar plates using the spread plate method. *E. faecalis* was inoculated onto BHI agar plate. Followed by 50µL of the yellow pigment sample was added to one well, and 50µL of the orange pigment sample was added to another well on each plate respectively, after allowing the plates to settle for 30 minutes, they were incubated at 37°C for a period of 24-48 hours. The plates were carefully observed, and the results were recorded. The antimicrobial activity was evaluated by examining the inhibition zones around the wells. The clear zones indicated the presence of antimicrobial activity against the respective bacterial strains. The plates were carefully observed, and the results were recorded. The findings of the antimicrobial activity were interpreted and discussed in relation to the potential applications of the extracted yellow and orange pigments as natural antimicrobial agents against the tested human pathogens. (Sinha, S., et al. 2017)

Table 4. Composition of BHI agar

Ingredient	Amount (g/l)
Brain Heart Infusion	37.0

Proteose Peptone	10.0
Dextrose (Glucose)	2.0
Sodium Chloride (NaCl)	5.0
Disodium Phosphate	2.5
Di-Potassium Phosphate	2.5
Agar	15.0
Distilled water	1 litre
pH	7.4

4.8 Biochemical and morphological characterization of isolated yellow pigment producing bacteria

4.8.1 Gram's staining

The process begins by placing a drop of water onto a clean microscope slide. Next, a loopful of bacterial culture is added to the slide, and a smear is created by spreading the culture evenly. The smear is then heat-fixed to immobilize the bacterial cells and promote adhesion to the slide. Once the smear is prepared, crystal violet stain is applied and left on for approximately 30 seconds. Afterward, the slide is washed with distilled water to remove excess stain. The smear is then flooded with Gram's iodine solution, allowing it to react with the crystal violet. Following the iodine treatment, the slide is washed again to remove the excess iodine solution. The decolorization step is carried out by exposing the slide to 95% ethanol. This step removes the crystal violet-iodine complex from certain types of bacteria. After decolorization, the slide is rinsed with water to eliminate any residual decolorizing agent. To provide contrast, the counterstain, typically safranin, is added to the smear for around 1 minute. Finally, the slide is observed under a microscope using a 100X objective lens. Gram-positive bacteria appears purple or violet, while Gram-negative bacteria appears pink or red, due to the differences in their cell wall composition. (K R Aneja Experiments in Microbiology, fourth edition., 2003)

4.8.2 Catalase test

The Catalase Test is a biochemical test used to determine the presence of the enzyme catalase in a bacterial culture. The test is performed as follows: Place a small amount of the bacterial culture on a clean slide, add a few drops of hydrogen peroxide directly to the bacterial culture on the slide, Observe the reaction closely. If catalase is present in the bacterial culture, it will catalyse the breakdown of hydrogen peroxide into water and oxygen gas. This will result in the release of bubbles

of oxygen gas, which can be seen as effervescence or bubbling on the slide. (K R Aneja Experiments in Microbiology, fourth edition., 2003)

4.8.3 IMViC Tests

4.8.3.1 Indole Test

The indole test is used to test an organism's ability to utilize tryptophan and produce indole. Take a sterilized tube containing tryptophan broth, inoculate the yellow pigment producing bacterial growth culture to the tube and incubate the tube at 37°C for 24 hrs then add 1ml of Kovac's reagent to the test tube then observe the tube for the presence or absence of cherry red colour ring in the test tube.(K R Aneja Experiments in Microbiology, fourth edition., 2003)

Table 5. Composition of 1% Tryptone broth

Chemicals	Quantity (g/l)
Peptone	10.0
Distilled water	1000 ml

4.8.3.2 Methyl Red Test

Preparation of MR broth, pour the broth into the test tube and sterilize it for 20min then inoculate the yellow pigment producing bacterial culture to the MR tubes, keep it for incubation at 37°C for 24 hrs and add 5drops of methyl red indicator to the culture test tube, observe the positive test appearance red colour and the negative test remain yellow colour. (K R Aneja Experiments in Microbiology, fourth edition., 2003)

Table 6. Composition of MR broth

Chemicals	Quantity (g/l)
Peptone	7.0
Glucose	5.0
Potassium phosphate	5.0
Distilled water	1000 ml
pH	6.9

4.8.3.3 Voges - Proskauer Test

For the preparation of VP broth, pour broth into the test tube and sterilize it for 20min then inoculate the yellow pigment bacterial culture to the VP broth tube keep it for incubation at 37°C for 24 hrs and add 6 drops of VP l reagent [alpha naphthol] and 2 drops of VP 2 reagent [40%KOH] to observe the colour change in the broth medium. (K R Aneja Experiments in Microbiology, fourth edition., 2003)

Table 7. Composition of VP broth

Chemicals	Quantity (g/l)
Peptone	7.0
Glucose	5.0
Potassium phosphate	5.0
Distilled water	1000 ml
pH	6.9

4.8.3.4 Citrate Utilization Test

Preparation of Simmon's citrate agar slants pour the agar into the sterilized test tube allow it to solidify and then inoculate [streak] the citrate slant from the cultured Petri plate and keep it for incubation at 37°C for 24-48 hrs then observe the slant culture for the growth and colouration of the medium. positive test turns green to blue and the negative test remains the same green in colour. (K R Aneja Experiments in Microbiology, fourth edition., 2003)

Table 8. Composition of Simmon's Citrate agar

Chemicals	Quantity (g/l)
Sodium nitrate	1.0
Dipotassium phosphate	1.0
Sodium chloride	5.0
Sodium citrate	2.0
Agar	15.0
Bromomethyl blue	0.8
Distilled water	1000 ml
pH	6.9

4.8.4 Carbohydrate Fermentation Test.

Preparation of respective carbohydrate fermentation broth like dextrose fill the broth to the test tube add Durham's tube to it without an air bubble, inoculate the yellow pigment producing bacterial culture to the tubes and keep it for incubation at 37°C for 24-48 hrs. Observes the change in red colour and appearance of air bubbles [due to the production of acid and gas]. (K R Aneja Experiments in Microbiology, fourth edition., 2003)

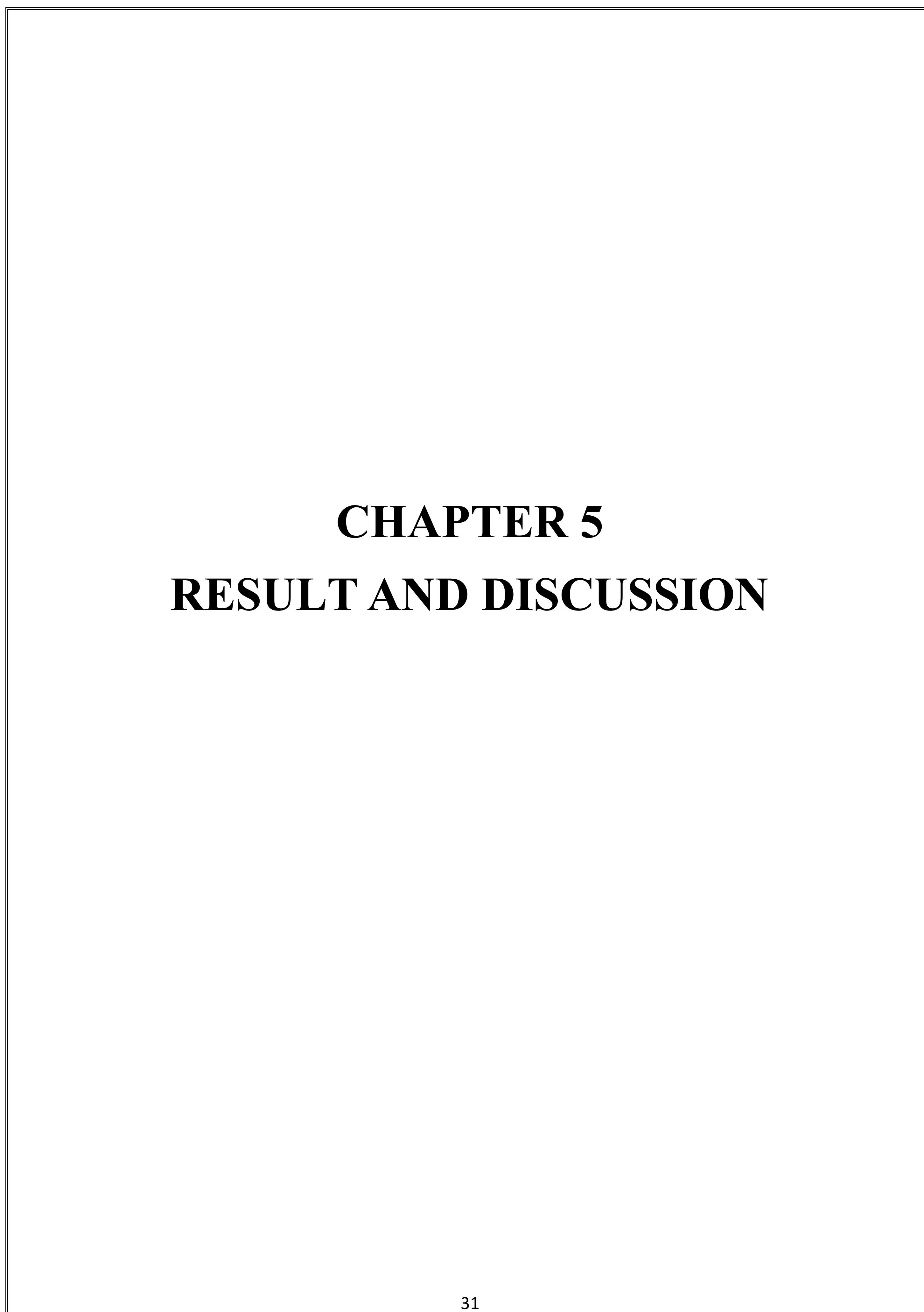
Table 9. Composition of carbohydrate fermentation broth

Chemicals	Quantity (g/l)
Peptone	10.0
NaCl	15.0
Dextrose	10.0
Phenol red	0.018
Distilled water	1000 ml
pH	7.3

4.9 Identification of bioactive compounds by GC-MS

Gas chromatography–mass spectrometry (GC–MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. GC-MS can be used to study liquid, gaseous or solid samples. Analysis begins with the gas chromatograph, where the sample is effectively vaporized into the gas phase and separated into its

various components using a capillary column coated with a stationary (liquid or solid) phase. The compounds are propelled by an inert carrier gas such as helium, hydrogen or nitrogen. As components of the mixture are separated, each compound elutes from the column at a different time based on its boiling point and polarity. The time of elution is referred to as a compound's retention time. GC has the capacity to resolve complex mixtures or sample extracts containing hundreds of compounds. Bacterial crude extract was prepared and outsourced for the analysis. The bioactive compounds present in the sample was identified by the retention time (Rt) and the molecular weight of the compounds compared with the standards of GC-MS.

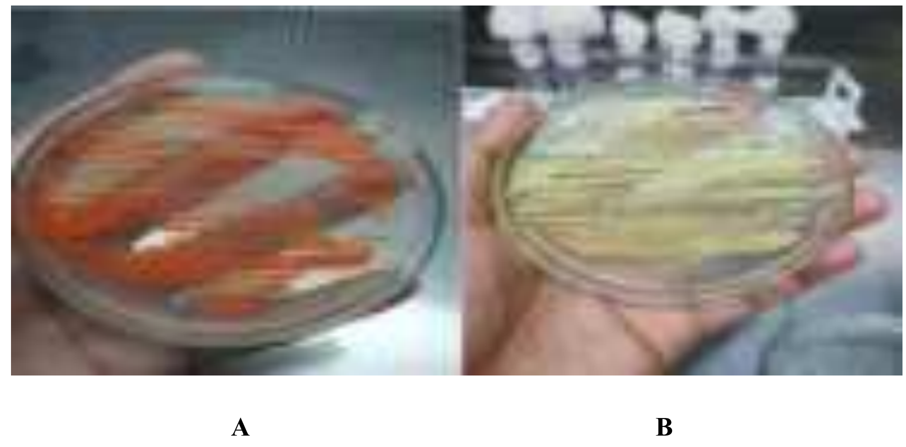


CHAPTER 5
RESULT AND DISCUSSION

5. RESULTS AND DISCUSSION

5.1 Isolation of pigment producing bacteria

In mixed culture plates, I successfully isolated bacteria capable of producing yellow and orange pigments. Both the selected yellow and orange pigment-producing bacteria were then cultivated in pure cultures using the streak plate method on freshly prepared LB agar media.



A **B**

Figure 1. Pure culture of (A) orange and (B) yellow pigment producing bacteria

5.2 Pigment production from orange and yellow pigment producing bacteria

Following a 3-day incubation period on a magnetic stirrer, the screw cap bottles containing TSB media inoculated with the respective yellow and orange pigment-producing bacteria displayed noticeable colour changes. This observation confirms the successful production of pigments in their respective screw cap bottles.

Figure 2. Production of pigments in their respective screwcap bottles

5.3 Extraction of pigment from pigment producing bacteria

Various methods are employed for the extraction of pigments. One commonly used approach involves centrifugation, where the addition of ethyl acetate aids in cell lysis, facilitating the extraction of intracellular pigments. The extracted pigments, which appeared in shades of yellow and orange, were subjected to measurement of their optical density. Furthermore, an assessment of their antimicrobial activity was conducted.

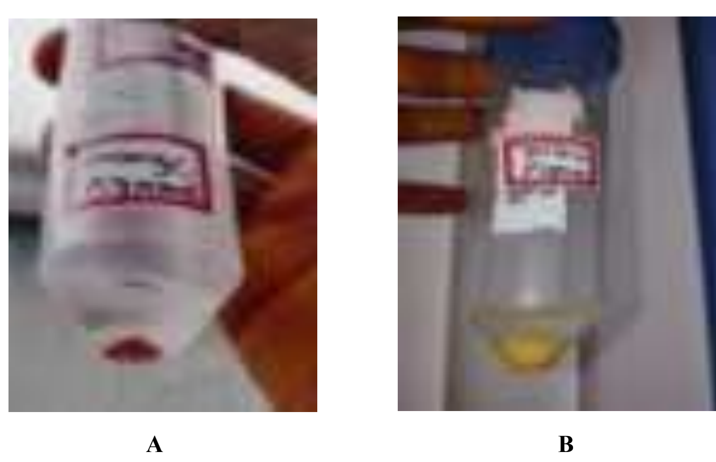


Figure 3. (A) Orange and (B) Yellow pigments extracted respective pigment producing bacteria

5.4 Maximum absorption of the pigments

The investigation included scanning the wavelength range from 400nm to 700nm in 10nm increments (Figure 4). This process facilitated the identification of the maximum absorption points for both extracted pigments in the samples. Yellow pigment has the maximum absorption of 0.98nm at 570nm and orange pigment has the maximum absorption of 0.91at 580nm.

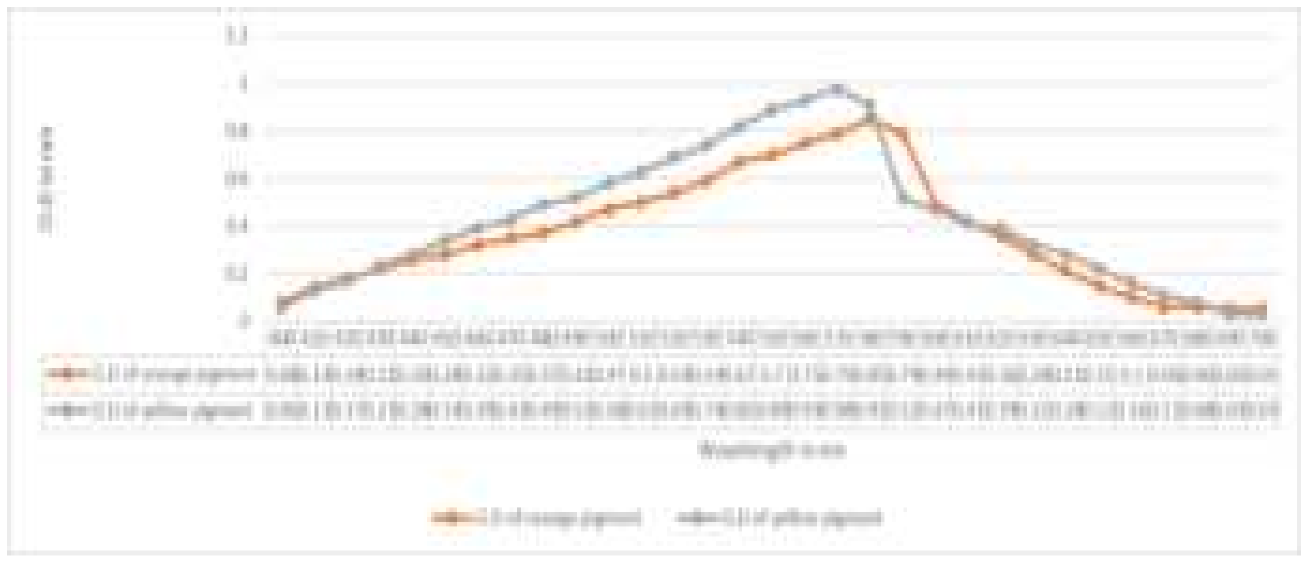


Figure 4. Maximum absorption of pigments

5.5 Antimicrobial activity of extracted pigments against human pathogens

The antimicrobial activity of extracted pigments against human pathogens was investigated after a 24-hour incubation period, and the results were evaluated based on the zones of inhibition observed surrounding the wells on agar plates. The yellow pigments displayed a positive response against inoculated human pathogens, specifically *E. coli, E. faecalis, S. aureus,* and *S. haemolyticus*. The presence of clear zones of inhibition around the wells containing the yellow pigments indicated that these pigments effectively inhibited the growth and survival of these particular human pathogens at a concentration of 50µL. These findings suggest that the yellow pigments possess antimicrobial properties with the potential to combat these specific pathogens. In contrast, the yellow pigments showed a negative response against *P. aeruginosa*. The absence of significant zones of inhibition around the well containing the yellow pigments suggested that they did not exert notable inhibitory effects on the growth of *P. aeruginosa*. Consequently, the yellow pigments may not be as effective in inhibiting the growth of this specific human pathogen. Similarly, the orange pigments demonstrated a negative response against all the tested human pathogens, including *E. coli, E. faecalis, S. aureus, S. haemolyticus, and* *P. aeruginosa* at a concentration of 50 µL. The lack of zones of inhibition around the wells containing the orange pigments indicated a limited or absent antimicrobial activity against these human pathogens.

Figure 5. Antimicrobial activity of isolated pigment producing bacteria

Table 10. Antimicrobial activity of Pigments (0.05 ml concentration)

Microorganisms	**Pigments**	
	Yellow	**Orange**
E. coli,	15mm	Nil
E. faecalis	14mm	Nil
S. aureus	14mm	Nil
S. haemolyticus	13mm	Nil
P. aeruginosa	Nil	Nil

5.6 Biochemical and morphological characterization of isolated yellow pigment producing bacteria

5.6.1 Gram staining

Gram staining procedure was conducted on the sample, resulting in a negative outcome. Upon microscopic examination using oil immersion at 100X magnification, the bacteria appeared as red cocci-shaped organism.

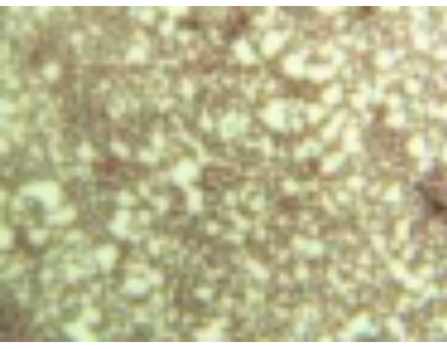


Figure 6. Gram's staining

5.6.2 Catalase test

The culture of bacteria that produces a yellow pigment has exhibited a positive result in the catalase test. Upon treating the bacterial culture with hydrogen peroxide, it showed the formation of effervescence, indicating the presence of catalase enzyme.

Figure 7. Catalase test

5.6.3 IMViC tests

5.6.3.1 Indole test

The isolated yellow pigment-producing bacteria in the samples exhibited a negative result in the indole test. The sample did not change the culture medium by forming a red ring at the top of the culture in the test tube after the addition of Kovac's reagent.

Figure 8. Indole test

5.6.3.2 Methyl red Test [MR]

In the Methyl Red Test [MR], the isolated bacteria producing a yellow pigment showed a positive result. Upon the addition of the methyl red reagent to the cultured broth, the colour changed to red, indicating a positive reaction.

Figure 9. Methyl red test

5.6.3.3 Voges Proskauer Test [VP]

The isolated yellow pigment producing bacteria from the sample, yielded negative result in the Voges Proskauer Test [VP]. Specifically, there were no observed colour changes to red in the culture medium after the addition of the two VP reagents, KOH and alpha-naphthol.

Figure 10. Voges Proskauer test

5.6.3.4 Citrate Utilization Test

The bacterial culture isolated from the samples, which produce a yellow pigment, yielded negative results in the Citrate utilization Test. Following the incubation period, there were no observable colour changes in the cultured medium, which remained green and did not turn blue. In this study, obtaining positive results would indicate a change of colour in the cultured medium from green to blue in the tube after incubation.

Figure 11. Citrate utilization test

5.6.4 Carbohydrate Fermentation Test

In this experiment, dextrose sugar is used in the test tube, and the broth is inoculated with an isolated yellow pigment producing bacterial culture that produces a yellow pigment. After the incubation period, the culture tube changes colour from red to yellow, and with no air bubbles are observed in the Durham's tube. According to the study by Bayada A. Hassan, when acid is produced in the inoculated tube, the broth's colour changes to yellow, and gas production is indicated by the appearance of air bubbles in the inverted Durham's tube. In the absence of sugar fermentation, there are no observable changes in the cultured broth, and it remains red.

Figure 12. Carbohydrate fermentation (dextrose)

5.7 Identification of bioactive compounds by GC-MS

The chromatogram (Figure 13) predicted the presence of numerous compounds, which were identified according to their retention time, peak area, molecular weight and molecular formula are shown in Table 10. The compounds present in my bacterial crude extract were determined by comparing the mass/ charge and molecular weight of the compound with the library or standards provided by the technical experts. 7- Octynoic acid, 8- Nonyoic acid, Hexanoic acid, Octanoic acid, Nonanoic acid are the few bioactive compounds identified. 7- Octynoic acid has the antimicrobial activity.

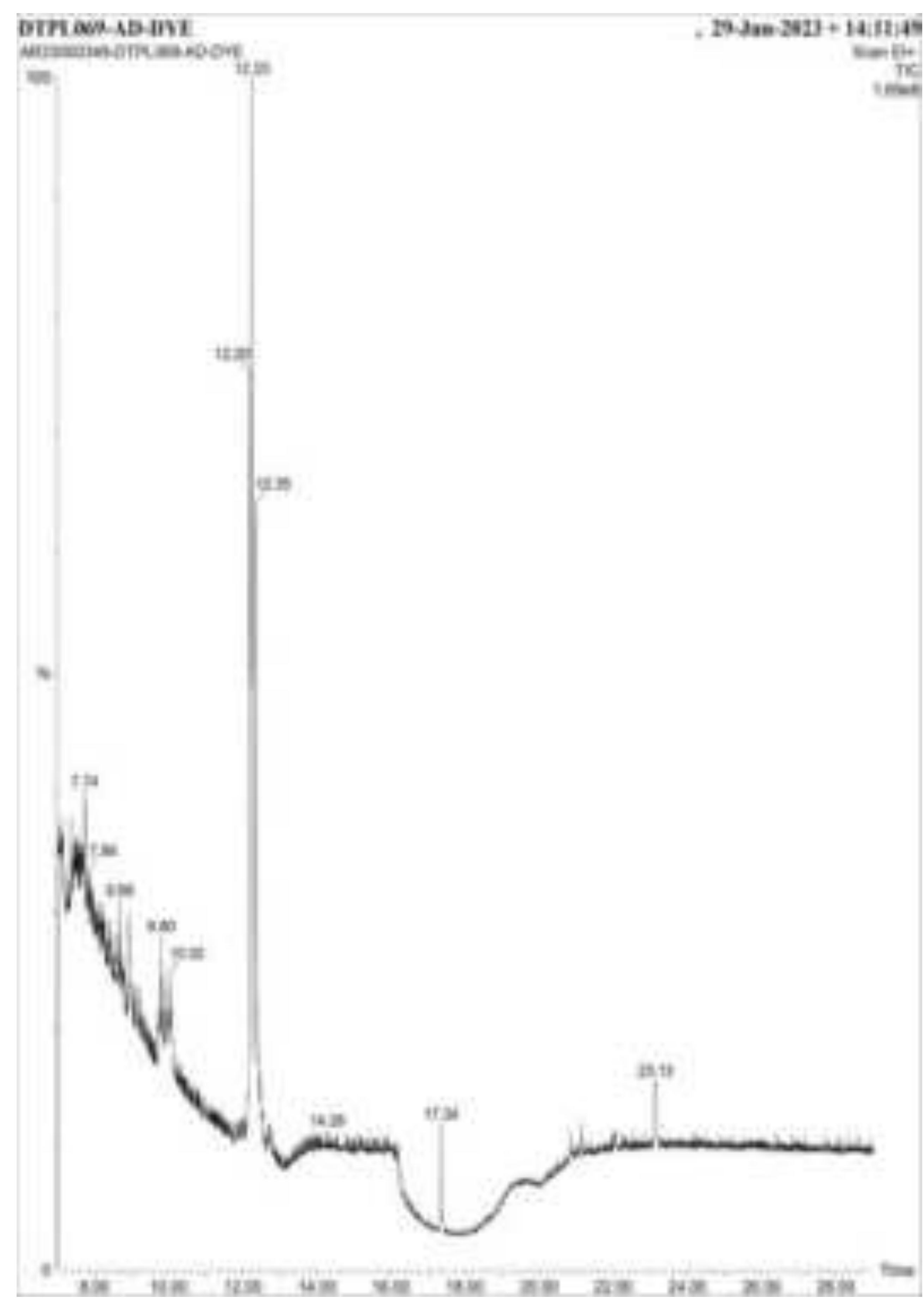
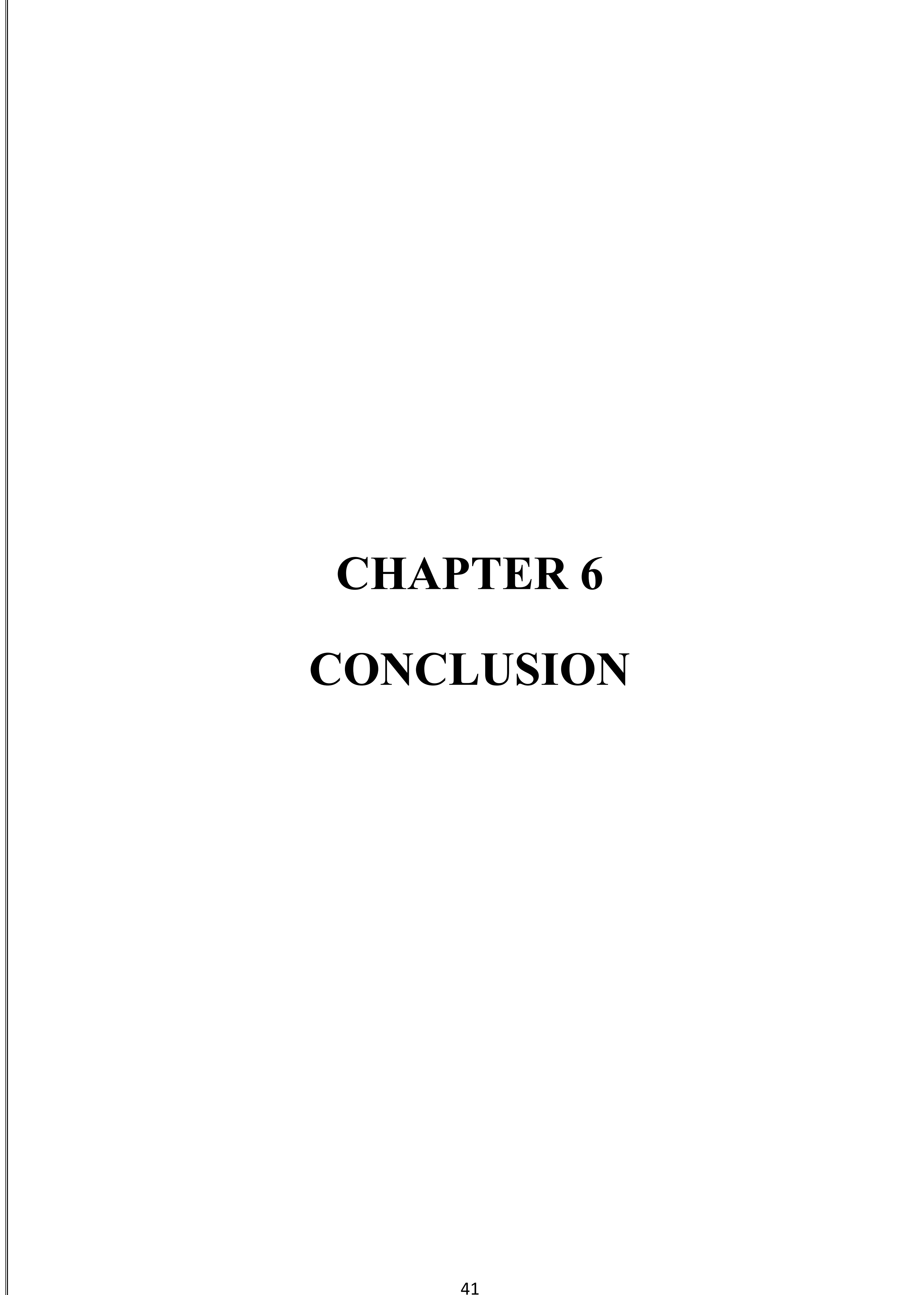


Figure 13. Total Ion Chromatogram of bacterial crude extract

Table 11. Bioactive compounds identified from the crude extract of yellow pigment producing bacteria

Retention time (in min)	Hit	Name of the compound	Formula	Molecular weight (in KDa)	Biological activity
Rt 12.25	Hit 1	7- Octynoic acid	$C_9H_{14}O_2$	154	Antimicrobial
	Hit 2	Methylcyclohexylacetate	$C_9H_{16}O_2$	156	Antibacterial
	Hit 3	8- Nonyoic acid	$C_{10}H_{16}O_2$	168	---------------
	Hit 4	Hexanoic acid	$C_8H_{16}O_2$	144	---------------
	Hit 5	10- Undecynoic acid	$C_{12}H_{20}O_2$	196	Anticancer, antioxidant
	Hit 6	Octanoic acid	$C_9H_{18}O_2$	156	Antifungal
	Hit 7	Pentanoic acid	$C_6H_{12}O_2$	116	Anticancer
	Hit 9	7- Nanenoic acid	$C_{10}H_{18}O_2$	170	Antibacterial
Rt 12.35	Hit 4	Hydrazine, trimethyl	$C_3H_{10}N_2$	74	Antifungal, antibacterial
	Hit 8	Acetamide, Oxime	$C_2H_6N_2O$	74	--------------
Rt 17.3	Hit 1	2 Methyl heptanoic acid	$C_8H_{16}O_2$	144	--------------
	Hit 2	Undecanoic acid	$C_{12}H_{24}O_2$	200	Antifungal
	Hit 3	Nonanoic acid	$C_{10}H_{20}O_2$	172	Antioxidant, antibacterial
	Hit 6	Methyl 2- butoxy acetate	$C_7H_{14}O_3$	146	---------------
	Hit 7	Dodecanoic acid	$C_{13}H_{16}O_2$	214	Antibacterial
	Hit 9	Decanoic acid	$C_{11}H_{22}0_2$	186	Antibacterial, anti-inflammatory



CHAPTER 6

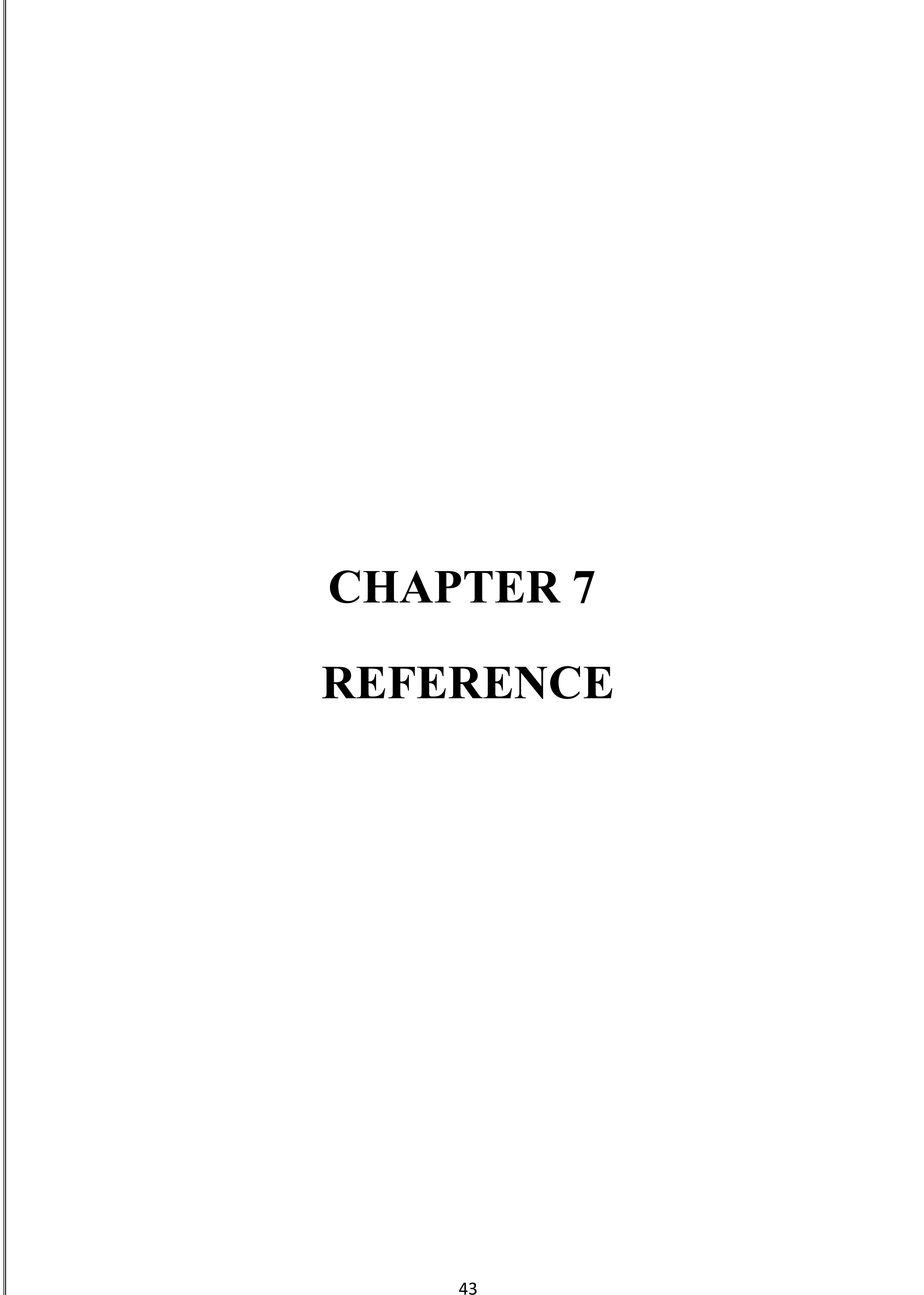
CONCLUSION

6. CONCLUSION

The soil microbial community stands as a complex and vital collection of organisms within the biosphere, The study focused on isolating and extracting pigments from soil-dwelling organisms, aiming to explore their suitability as alternatives to antibiotics for antimicrobial purposes.

The investigation centered on microbial pigments with potential antimicrobial properties, presenting an alternative to conventional antibiotics. Notably, a local microbial isolate demonstrated the capacity to produce pigments displaying antibacterial effects. The yellow pigment derived from a bacterial strain exhibited noteworthy antibacterial activity against key human pathogens such as *E. coli, E. faecalis, S. aureus, and S. haemolyticus* in in-vitro.

As a result, this study has highlighted the diverse range of pigments produced by various organisms within soil samples, many of which possess antibacterial capabilities. These findings indicate a potential avenue for leveraging these pigments in pharmaceutical and cosmetic industries. Further exploration into the antimicrobial potential of these pigments is warranted, potentially offering significant benefits for mankind's well-being.



CHAPTER 7

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UNIVERSITY OF MYSORE

A STUDY ON ANALYSIS OF IMPORTS AND EXPORTS OF INDIA WITH REFERENCE TO BALANCE OF PAYMENT CONDITION

Submitted by
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Reg.No: P01BH21C0001

Under the Guidance of
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Project Report Submitted to the University of Mysore in Partial Fulfilment of Requirements of IV Semester M. Com Degree Examination: September-2023

SBRR MAHAJANA FIRST GRADE COLLEGE PG-WING
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SEPTEMBER-2023

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Roopesh Kumar N
Assistant Professor & HOD
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DECLARATION

I hereby declare that the report entitled **A STUDY ON ANALYSIS OF IMPORTS AND EXPORTS OF INDIA WITH REFERENCE TO BALANCE OF PAYMENT CONDITION** is a record of independent research carried out by me under the guidance of **ROOPESH KUMAR N,** Assistant professor, Department of Studies in commerce, Pooja Bhagavat Memorial Mahajana Education Centre K R S Road, Metagalli, and Mysuru. I further declare that the findings in the project report are independent study done by me. I also declare that I have not submitted this project report in any other University/Institution for the award of any Fellowship/Diploma/Degree.

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I extend my sincere thanks to **Dr. Srinivas K R**, Assistant Professor, Department of studies in commerce, Pooja Bhagavath Memorial Mahajana Education Centre.

I extend my sincere thanks to **Teaching faculties and Library staff** of Pooja Bhagavath Memorial Mahajana Education Centre for rendering required support

Finally, I would like to thank **My Parents and Friends** and those who have directly or indirectly contributed for this work.

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CONTENTS

CHAPTER	DESCRIPTION	PAGE NO
1	INTRODUCTION	1-7
2	LITERATURE REVIEW	8-12
3	BALANCE OF PAYMENT IN INDIA	13-24
4	ANALYSIS OF PERFORMANCE OF BALANCE OF PAYMENTS WITH RESPECT TO IMPORTS AND EXPORTS.	25-51
5	FINDINGS, SUGGESTIONS AND CONCLUSION	51-55
	BIBLIOGRAPHY WEBSITES	

LIST OF TABLES

Table Number	Particulars	Page Number
4.1	Table 4.1: Trade during March 2023	42
4.2	Table 4.2: Trade during FY 2022-23 (April-March)	44
4.3	Table 4.3: Trade excluding Petroleum and Gems &Jewellery during March 2023	46
4.4	Table 4.4: Trade excluding Petroleum and Gems &Jewellery during FY 2022-23 (April-March)	47

LIST OF GRAPHS

Table Number	Particulars	Page Number
4.1	Overall Trade during March 2023	43
4.2	Overall Trade during FY 2022-23 (April-March)	44
4.3	Merchandise Trade during March 2023	45
4.4	Trade excluding Petroleum and Gems & Jewellery during March 2023	46
4.5	Trade excluding Petroleum and Gems &Jewellery during FY 2022-23 (April-March)	47
4.6	Services Trade during March 2023	48
4.7	Services Trade During FY 2022-23 (April-March)	49

“ANALYSIS OF PERFORMANCE OF SELECT INITIAL PUBLIC OFFEREINGS ”

Project Report Submitted to the University of Mysore in Partial Fulfilment of Requirements of IV Semester M. Com Degree Examination: September-2023

Submitted by

Apoorva M V

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Under the Guidance of

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DECLARATION

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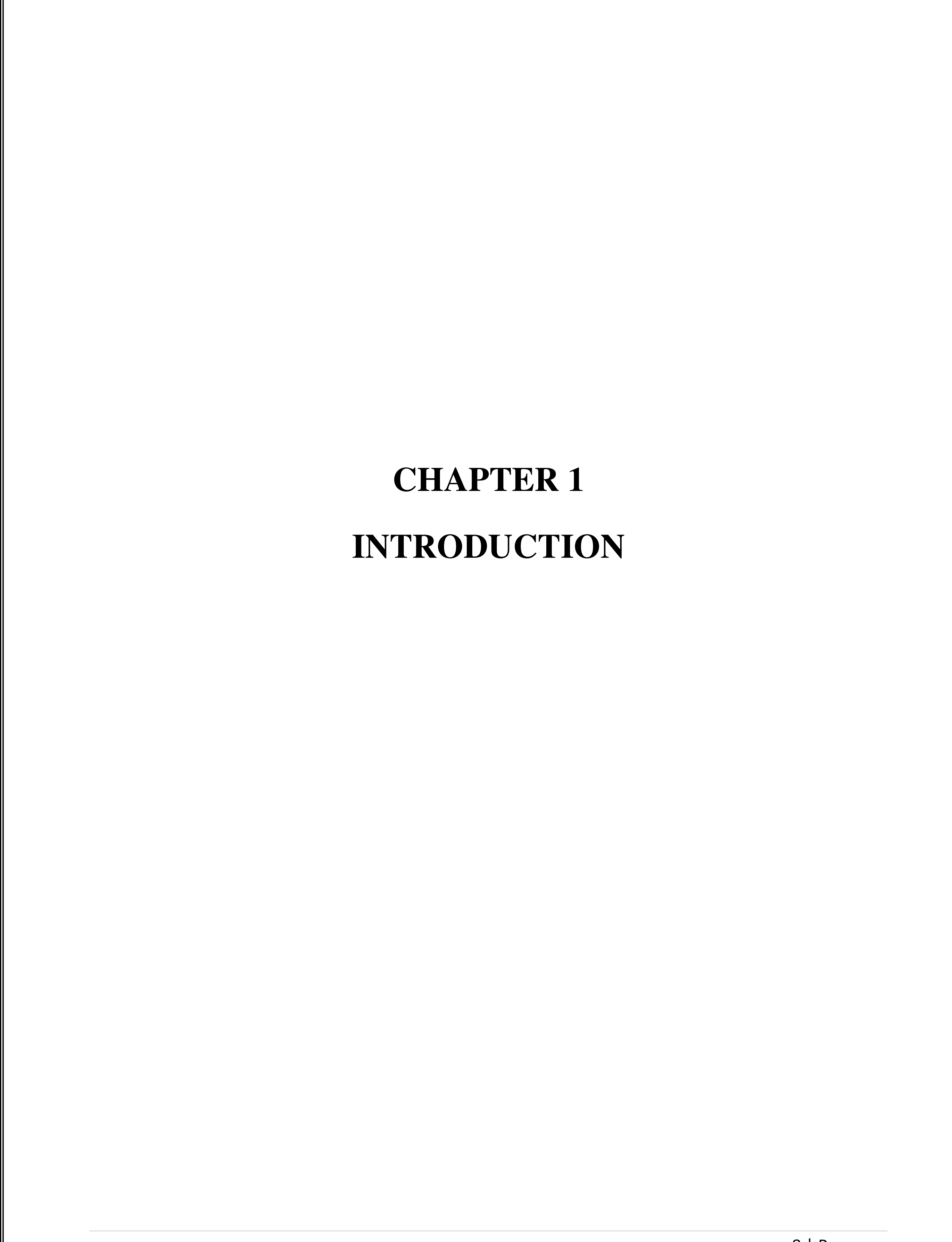
INDEX

CHAPTER NO.	DECRIPTION	PAGE NO
1.	INTRODUCTION	8-14
2.	REVIEW OF LITERATURE	15-19
3.	INITIAL PUBLIC OFFER IN INDIA- AN OVERVIEW	20-26
4.	DATA ANALYSIS AND INTERPRETATION	26-35
5.	FINDINGS, SUGGESTIONS AND CONCLUSION	36-37
	BIBLIOGRAPHY	38-39

	LIST OF TABLE	
TABLE NO.	**TABLE NAME**	**PAGE NUMBER**
1	Table showing **Number of IPO listed on NSE (2010-2014)**	13
2	Table showing **Sample selection**	29
3	Table showing **Descriptive statistics of Issue price, Issue size, Issue oversubscription andMarket return of IPOs Listed in NSE during year 2010 to 2014**	30-31
4	Table showing **Average returns of IPO companies during 2010 to 2014.**	33
5	Table showing **reression results**	34



CHAPTER 1

INTRODUCTION

1. Introduction:

The present paper seeks to compare the performance of a set of IPOs launched during 2016 to 2019. India in 2018 after three years of their listing using market based and fundamental financial indicators. The Indian Capital Market is witnessing a massive surge in IPOs (Initial Public Offering) for the past few years. This has not only made IPO a lucrative avenue for investment but has also made as a significant topic for corporate as well as academic research. As defined in the literature by Securities & Exchange Board of India (SEBIReport), an IPO is defined as "When an unlisted company makes either a fresh issue of shares or convertible securities or offers its existing shares or convertible securities for sale or both for the first time to the public, it is called an IPO" (SEBIReport). Putting into simple terms, IPO refers to the process by which a privately held company offers its share for the first time to the general public.

Public issue or Initial Public Offerings (IPOs) has become a very popular way of raising finance in India. The performance of these IPOs fluctuates over a period and investors, in the past, have incurred huge losses by investing in them. While IPOs offer appreciable returns on the listing day, their long-term performance is generally poor. In this study, the long-term price performance of 117 IPOs released over the period of 2009-2013 by way of market abnormal excess returns (MAER) was done to identify the factors which govern their performance. The research concludes that the listing day price and times subscribed are always positively correlated irrespective of the industry or sector. IPOs released in any sector, age of the firm, and times subscribed show positive correlation for the entire sample.

- In the main markets (i.e., BSE and NSE), there were 18 IPOs (including one InvIT) in Q4 2022 versus 24 IPOs in Q4 2021 and four IPOs in Q3 2022. This represents a decrease of 25% compared to Q4 2021 and an increase of 350% compared to Q3 2022.
- Indian stock exchanges (BSE and NSE including SMEs) ranked third in the world in terms of the number of IPOs in 2022. There were no cross-border deals.

Raising fund is a difficult problem faced by any organisation. Entrepreneurs need capital to fund projects, to pay employees and other miscellaneous purposes. Fund can be raised from

different sources like banks, institutional/angel investors, finance companies, and debentures etc. All these ways have an element of risk associated with them. The rates of interest to be paid take a huge toll on a company's finances. Initial Public Offering (IPOs) has proved to be a much safer alternative to raise fund.The government institutions and corporate companies raise finance through debt and/ or equity. The unlisted companies can issue shares through Initial Public Offering (IPO) from the primary market. It is an opportunity for these companies which are planning to expand, diversify, and grow with better future business prospects. For an investor holding shares issued through IPO can consider it as a mere speculative opportunity in short term or an opportunity to earn high dividends with capital appreciation in the long run. The study attempts to evaluate the value of share premium and pricing on listing day, assess progressive growth of IPO return. Further the study compares short-term performance with the long-term performance of IPO returns using Wilcoxon Signed Rank Test. The sample includes twenty-six companies issued IPOs that were successfully listed in the year 2020. The study considered a period of three years from the date of issue for analysis, i.e., 2020 to 2023. The study found that return on IPO fluctuated during the study period. Among the select sample, twenty IPOs have provided returns on the listing day. The IPOs traded on the stock exchange are found to be promising in long term when compared to short term period. It is also found that the companies that have overpriced issue price have failed to grow during the study period. This study acknowledges the fact that holding investment for a longer period provides an opportunity to earn higher returns. The study suggests the investors to hold investment for more than one year for better returns. Further the investors can sell the shares that are overpriced by the end of the listing day to minimize the losses.A financial system is a system that allows the exchange of funds between lenders, investors and borrowers. It promotes savings and investment in the economy and enlarges the resources flowing into the financial assets which are more productive than the physical assets. A financial market is a market in which people trade financial securities, commodities and other fungible items of value at low transaction costs and at prices that reflective supply and demand. Financial market has significant role to play in this context because it is a part of the financial system. It provides the financial resources needed for the long term and sustainable development of different sectors of the economy. Investors have access to a large number of financial markets and

exchanges representing a vast array of financial products. Some of these markets have always been open to private investors; others remind the exclusive domain of major

international banks and financial professional until the very end of the twentieth century. Financial market is divided into money market and capital market. Primary market facilitate securities to the investors and assist the corporate sector in arranging funds in the form of public issue, offer for sale, private placement and right issue. Public issue can be further classified into initial public offer (IPOs) and further public offer (FPO). An initial public offering (IPO) is a company's first offering of equity to the public. IPO is a major source of capital for firms. IPO's are important milestone in any company's growth as it progresses from being a start- up/private limited company to public limited. Successful IPO can generate tremendous amount of wealth for company promoters as well as pre IPO investors.

HISTORY OF IPO:

Back in 1994, Morgan Stanley Mutual Fund came out with an IPO of its first closed-ended Morgan Stanley Growth Fund (MSGF). That was long before investors really understood that a mutual fund is not a stock and it is just a summation of all its investments. Even as the issue opened, there were long queues outside the banks and application counters leading to the issue getting heavily oversubscribed. In fact, the grey markets quoted the MSGF IPO at a premium of 500 % and many gullible investors even ended up buying the same. When the MSGF finally listed, investors were shocked that it had listed at a huge discount to its NAV of Rs.10. Investors who had bought in the grey market at hefty premiums were virtually wiped out. But why did this happen? Firstly, investors failed to realize that a mutual fund does not have a stock price but an NAV. The NAV was a sum of the value of its holdings. Secondly, the MSGF came in 1994 when the markets were close to the peak. So MSGF ended up buying most stocks at peak prices with little upside left. Thirdly, investors did not realize that closed-ended funds, by default, trade at a discount to their NAV and in the Indian context, the discount was much higher than global benchmarks. Lastly, back then there was no transparency for closed ended funds and investors had no clue about what was the investment portfolio of the fund. But then, MSGF goes down in the history of Indian capital markets as one of the classic examples of an IPO mania stretching itself too far. Morgan Stanley AMC eventually sold out to HDFC MF in 2013 and exited the Indian MF business altogether.

Table 1: Number of IPO listed on NSE (2010-2014)

Year	No. of IPOs listed in NSE
2010	63
2011	30
2012	12
2013	3
2014	5
Total	113

NEED FOR STUDY:

The IPOs have been offered at one price and the price of the IPOs are found to be different on the day of listing in the market. Many IPOs have listed over and above the offer price and many IPOs have listed below the offer price. Hence, this study is conducted to know the performance of IPOs after their listing. This study is conducted to analyse how the select IPOs have performed .

OBJECTIVES OF THE STUDY:

*To understand about the concept of IPO.

*To provide the investors a clear picture about the IPOs launched during 2010 to 2014.

* To identify the factors affecting IPOs' listing day performance.

*To measure the IPOs' listing day performance.

*To analyze the impact of various factors; issue price, issue size, over subscription.

HYPOTHESIS:

H01: the variable issue price donot influence the initial performance of ipos

Ha1: the variable issue price will influence the inital performance of ipos

H02: the variable issue size donot influence the inital performance of ipos

Ha2: the variable issue size will influence the inital performance of ipos

H03: the variable oversubscription donot influence the inital performance of ipos

Ha3: the variable oversubscription will influence the inital performance of ipos

H04: the variable market index donot influence initial performance of ipo

Ha4: the variable market index will influence initial performance of ipo

RESEARCH METHODOLOGY:

Time duration of Study:

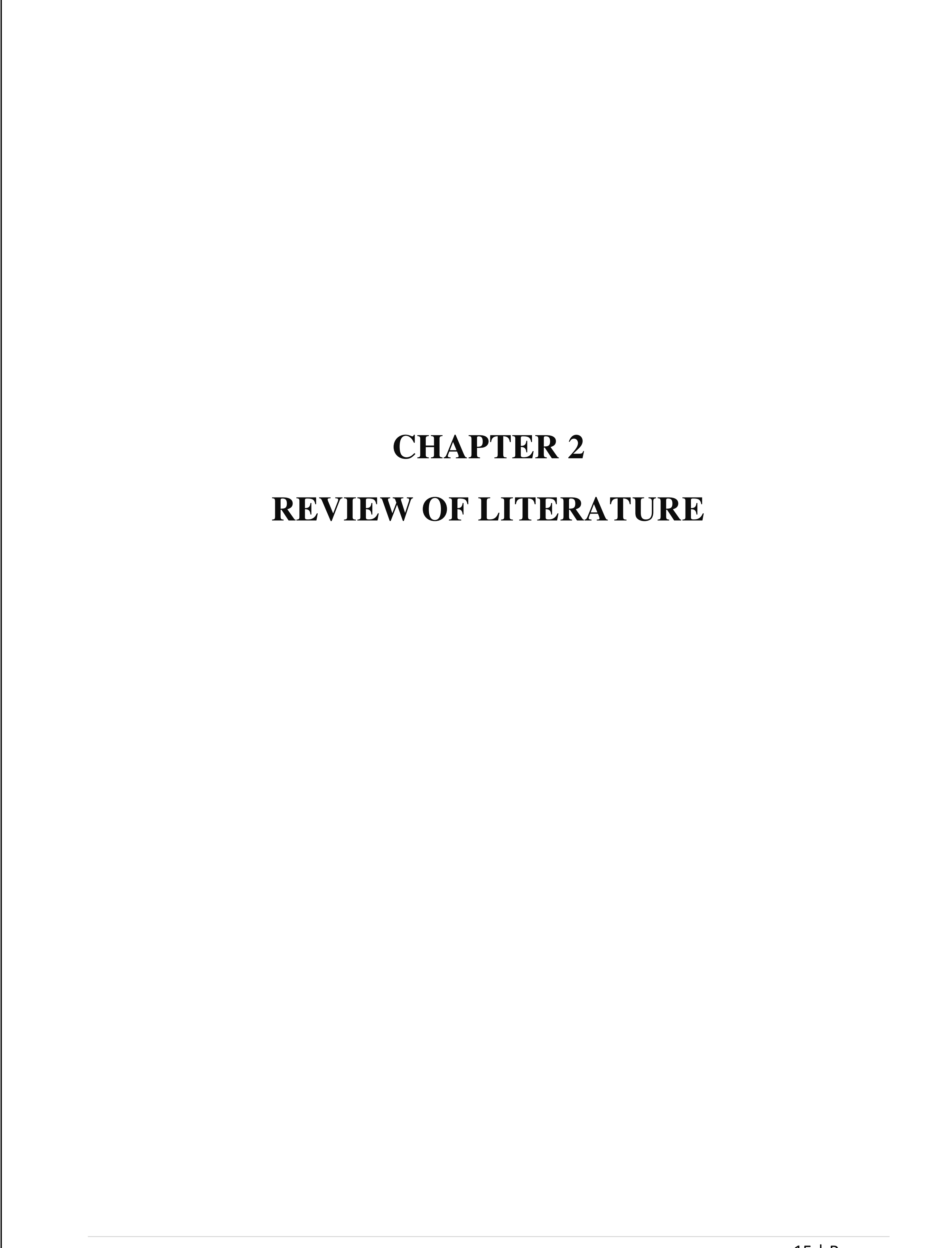
To analyse the performance of the IPOs on the listing day, the required data has been collected from the period January 2010 to December 2014 of the companies which came up with the Initial public offers (IPO).

SOURCES OF DATA:

NSE official website is used to collect list of IPOs for analysis from the period of January, 2010 to December, 2014. Respective company prospectus is used to get details regarding the issue price, issue dates, issue size and oversubscription.

SAMPLE SIZE:

The sample used in this study consists of all Indian firms which went public on National Stock Exchange (NSE) from January 2010 to December 2014. Data is taken of 113 companies for the analysis.



CHAPTER 2
REVIEW OF LITERATURE

1.Madhusoodanan et al (1997) studied both short-term and longterm underpricing. The paper also analyzed the effect of issue size on the level of underpricing and the performance of merchant banks in pricing these issues. In consonance with almost every other study, the authors here found that the underpricing was higher in the short-term.

2.Pandey (2005) made a comparative study between the IPOs issued through the fixed price mechanism and the ones issued through the book build mechanism. The paper also observed the difference in characteristics of the issuers in both mechanisms and compared the performance of these IPOs. The sample chosen comprised 84 IPOs from the period 1999 to 2002.

3.Pandey et al (2009) observed that the previous studies were largely focused on Bombay Stock Exchange. This paper studied the pricing of IPOs listed on the National Stock Exchange. The sample included all the IPOs listed on the NSE during the period 2004 to 2006.

4.Kumar (2009). studied the new book building mechanism in IPO pricing. The book building mechanism was adopted to fix more efficient prices for an IPO. The paper analyzed both the short-term and long-term performances of book built IPOs. The documented results in the paper highlighted that the book built IPOs were underpriced because of the positive listing day returns.

5.Chahine et al (2009). Studied the Building perceived quality of founder-involved IPO firms: Founder's effects on board selection and stock market performance.

6.Chiraphadhanakul et al (2012) suggested that IPOs can be an alternative to short-term investments because of their attractive returns. The sample comprised 111 IPOs listed at the Thailand Stock Exchange from 2000 to 2004. The authors divided the IPOs in their respective industries while studying their performance.

7.Bansal and Khanna (2012) examined the factors which influence an IPO's initial return. The initial return is calculated by way of underpricing. The study used a sample of all Indian firms which went public from April 2000 till 2011 on the Bombay Stock Exchange.

8.Shah et al(2015) studied the listing day performance of 113 IPOs listed on the National Stock Exchange. The authors noted a significantly positive return on the listing day of IPOs. It was noted that the IPOs were initially underpriced. The only notable relationship of the degree of underpricing was found to be with the oversubscription of issue.

9.Kumar (2015) talked about the importance of studying the post issue performance of IPOs, especially their pricing performance. The authors observed that the retail investors participated in the IPO only for listing day gains which hampered their long-term performance.

10. Keshav et.al., (2018) "Impact of Macroeconomic Factors on Indian Stock Market". The objective of the study was to measure the relationship between the stock market and Macroeconomic variables. Secondary data was used to analyze from Jan. 1991 to Dec. 2017. The findings of the study were the relationship with the Indian stock market and all the factors whether positively and negatively affect the movement in the stock market prices. Both the Unemployment rate and average inflation have an inverse.

11. Seshadev Sahoo and Prabina Rajib(2010): conducted study on "After Market Pricing Performance of Initial Public Offerings(IPOs):Indian IPO Market 2002-2006" The study focuses on after market performance of IPOs. The study presents fresh evidence on IPO performances i.e., initial day underprice and long run underperformance for a board set of 92 Indian IPOs. They reported that on average, the IPOs are under-priced to the extent of 46.55 percent at the list price. They also found that high initial day return may be due to the overexpectations of the investors.

12. Himanshu Puri (2012): studied the short-run performance of Indian IPOs, as on the First, Seventh, and thirteenth trading day and he states that IPOs offered positive retunrs on Ist and 7th trading day but subsequently they underperform the market at the end of 30th trading day. He also opines that, investors can make significant positive returns if they go for IPOs and sell them within a period of less than one month.

13. Divya(2013): conducted a study on "performance of Indian IPO's during the financial year 2010-2011." The study focuses on methodology of book –building issue and fixed price issue and factors contributing to the under-pricing or over-pricing of IPO in India. The study concludes that extent of www.eprawisdom.com Vol - 4, Issue- 9, September 2016 83 e-ISSN : 2347 - 9671, p-ISSN : 2349 - 0187 oversubscription of an IPO will determine the first day gains. The oversubscription will lead to larger first day gains for IPOs.

14. Baluja(2013): studied the comparative analysis of listing price performance between graded IPOs in India, she concludes that, IPO grading mechanism has no significant difference of listing price performance of different graded IPOs. She opines that listing performance of the companies

differs just by chance or may be by some other factors such as subscription level, Age, venture capitalist reputation, IPO size etc.

15. Sanjay (2013): studied 432 new IPO issues for the period april 2001 to December 2001. The paper examined important factors that determine short-run underpricing of initial public offerings (IPOs) and impact of IPOs mispricing on investment banks reputation. The paper concludes that 5 variables i.e. number of times an IPO issue subscribed, number of uses of IPOs proceeds, Listing Delay, Industry PE ratio and dummy for companies representing new economies are positively related to the short run initial return on IPOs, while 4 variables, i.e. company size , investors sentiment , investment banks reputation defined in terms of share in IPO proceeds and dummy for private companies IPOs bear a negative relationship with initial.

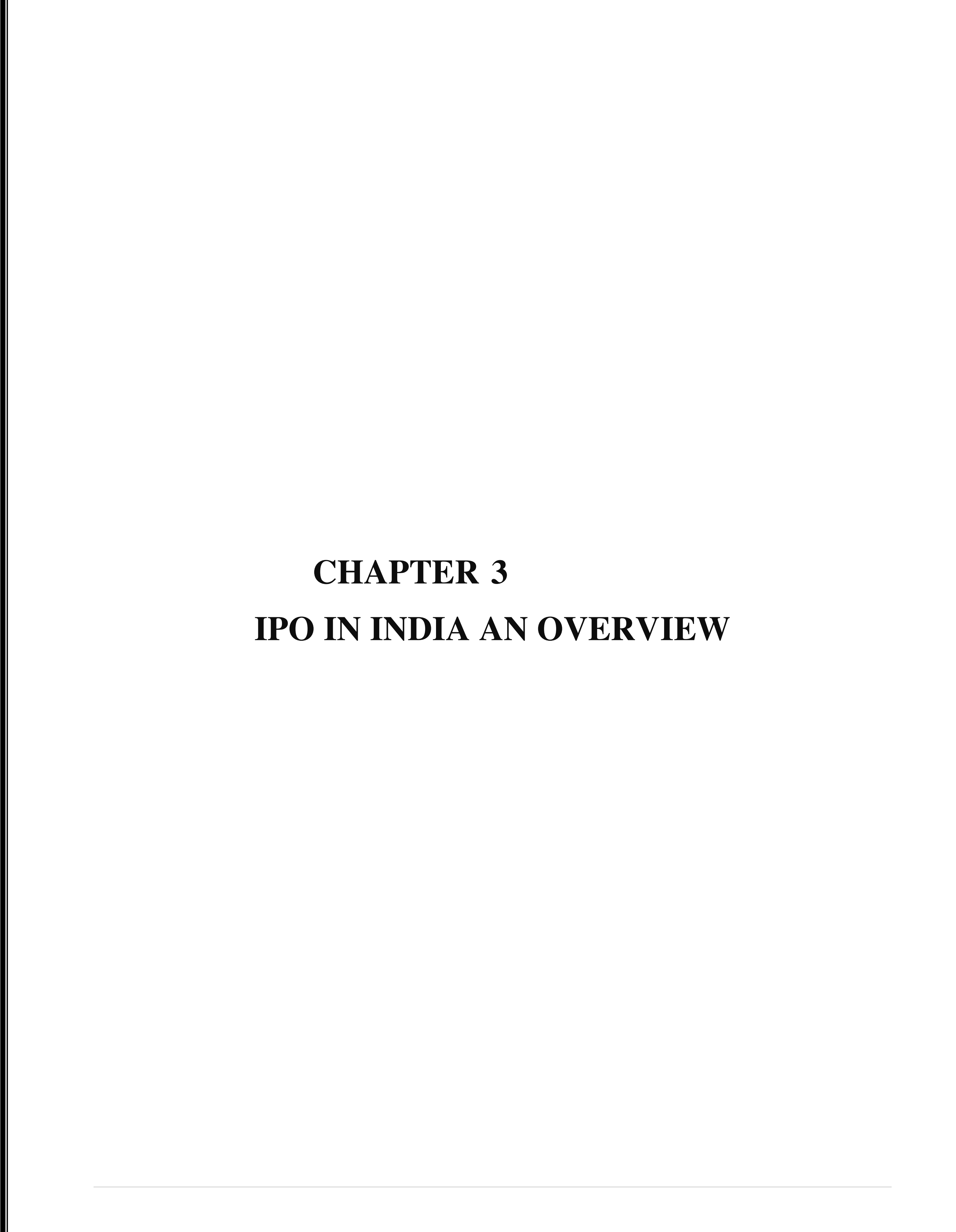
16. Devarajappa et al(2014): Conducted a study on "Post issue Performance of IPOs in India: An Empirical Study". The study focuses on post issue performance of the selected IPOs made during the period from April 2011 to March 2012, and the basis of arriving at issue price. The study concludes that fluctuation depend not only on performance of the company but due to speculations made and external factors.

17. Ramesh et al (2015): Conducted a study on "Performance Analysis of Initial Public offering in Indian Context" the study focuses on Measuring the initial listing performance of IPO for short run, Analyse the price performance of Indian IPOs for the long term, and to examine the extent of under pricing/overpricing of the Indian IPOs. The study concluded that over pricing exists in IPOs listed on NSE in the short as well as long run. The study further states that overpricing is present in short as well as long run. But, is more severe in the long run period i.e. from the day of listing.

18. Prof.Sweety Shah et al (2015): conducted a study on Initial Performance of IPOs in India: Evidence from 2010 to 2014. The study focussed on listing day performance of 113 IPOS from the period January 2010 to December 2015, listed on National Stock Exchange (NSE) India. They found that, there is, on average, significantly positive returns on the listing day. The market adjusted Abnormal Returns of all sample Initial Public Offers (IPO) companies were found to be 7.19%. They also found that IPOs are initially under-priced.

19. Ranjitha et al (2016): Studied the performance of the IPOs during the period 2009 to 2013, The study shows conclusive evidence of initial abnormal returns of a majority of IPOs considered for study. This finding is indicative of the presence of Underpricing of IPOs.

20. Gopalakrishnan et al (2016): studied the performance of IPOs in Indian market, The study examines the performance of the Indian IPOs listed in National Stock Exchange (NSE) during January 2010 to December 2015. Short run price performance of 126 IPOs that entered into Indian capital market in the study period has been examined on listing day and for a time gap of three days, one week, fifteen days, one month, two months and 3 months .The findings reveal that, there exists under-pricing in the Indian Market in the short run. So it is recommended that the investors can participate in IPOs to get returns in the short run.



CHAPTER 3

IPO IN INDIA AN OVERVIEW

The Capital market addresses the "Essential Market" and the "Optional Market. The capital market has two associated and indistinguishable portions, the new backers (the essential market) and stock (auxiliary) market. The essential market is involved by backers for raising new capital from the financial backers by disclosing introductory makes or freedoms issues or offers available for purchase of value or obligation. A functioning auxiliary market advances the development of the essential market and capital development, since the financial backers in the essential market are guaranteed of a nonstop market where they have a choice to sell their ventures.

A corporate may bring capital up in the essential market via an underlying public proposition, privileges issue or confidential position. An Underlying Public Proposition (Initial public offering) is the offering of protections to people in general in the essential market. It is the biggest wellspring of assets with long or endless development for the organization.

An Initial public offering is a significant stage in the development of a business. It gives an organization admittance to assets through the public capital market. An Initial public offering likewise significantly expands the validity and exposure that a business gets. Generally speaking, an Initial public offering is the best way to fund speedy development and extension. Concerning the economy, when an enormous number of Initial public offerings are given, it is an indication of a solid securities exchange and economy.

At the point when the organization makes its most memorable Initial public offering to the general population, the relationship is straightforwardly between the organization and financial backers, and the cash streams to the Organization as its "Offer Capital". Investors accordingly become proprietors of the Organization through their cooperation in the Organization's Initial public offering and have possession privileges over the organization. This is the biggest wellspring of assets for an organization, which empowers the organization to make "Fixed Resources" which will be utilized over the business. The investors of the Organization are allowed to leave their speculation through the optional market

How a First sale of stock (Initial public offering) Works:

Before an Initial public offering, an organization is viewed as private. As a pre-Initial public offering privately owned business, the business has developed with a moderately modest number of investors including early financial backers like the pioneers, family, and companions alongside proficient financial backers like financial speculators or private backers.

An Initial public offering is a major step for an organization as it gives the organization admittance to collecting huge load of cash. This enables the organization to develop and grow. The expanded straightforwardness and offer posting validity can likewise be a consider assisting it with getting better terms while looking for acquired assets too.

At the point when an organization arrives at a phase in its development cycle where it accepts it is full grown enough for the afflictions of SEC guidelines alongside the advantages and obligations to public investors, it will start to promote its revenue in opening up to the world.

Commonly, this phase of development will happen when an organization has arrived at a confidential valuation of roughly $1 billion, otherwise called unicorn status. Be that as it may, privately owned businesses at different valuations with solid essentials and demonstrated productivity potential can likewise fit the bill for an Initial public offering, contingent upon the market rivalry and their capacity to meet posting prerequisites.

Initial public offering portions of an organization are valued through endorsing an expected level of effort. At the point when an organization opens up to the world, the recently possessed private offer proprietorship converts to public proprietorship, and the current confidential investors' portions become worth the public exchanging cost. Share guaranteeing can likewise incorporate extraordinary arrangements for private to public offer proprietorship.

For the most part, the change from private to public is a vital time for private financial backers to trade out and procure the profits they were anticipating. Confidential investors might clutch their portions in the public market or sell a piece or every one of them for gains.

In the mean time, the public market opens up a gigantic chance for a great many financial backers to purchase partakes in the organization and contribute money to an organization's investors' value. The public comprises of anyone with any interest at all in putting resources into the organization.

By and large, the quantity of offers the organization sells and the cost for what offers sell are the creating factors for the organization's new investors' value esteem. Investors' value actually addresses shares possessed by financial backers when it is both private and public, yet with an Initial public offering, the investors' value increments altogether with cash from the essential issuance.

What Is the Initial public offering Cycle?

The Initial public offering process basically comprises of two sections. The first is the pre-showcasing period of the contribution, while the second is the first sale of stock itself. At the point when an organization is keen on an Initial public offering, it will promote to financiers by requesting private offers or it can likewise offer a public expression to produce interest.

The financiers lead the Initial public offering process and are picked by the organization. An organization might pick one or a few financiers to oversee various pieces of the Initial public offering process cooperatively. The guarantors are associated with each part of the Initial public offering a reasonable level of investment, record planning, documenting, promoting, and issuance.

Moves toward an Initial public offering

1.Proposals. Financiers present recommendations and valuations talking about their administrations, the best sort of safety to issue, offering value, measure of offers, and assessed time span for the market offering.

2.Underwriter. The organization picks its financiers and officially consents to endorse terms through a guaranteeing arrangement.

3.Team. Initial public offering groups are framed including financiers, legal advisors, confirmed public bookkeepers (CPAs), and Protections and Trade Commission (SEC) specialists.

4.Documentation. Data with respect to the organization is ordered for required Initial public offering documentation. The S-1 Enlistment Explanation is the essential Initial public offering recording report. It has two sections — the plan and the secretly held recording information.1 The S-1 incorporates primer data about the normal date of the filing.2 It will be changed frequently all through the pre-Initial public offering process. The included outline is likewise modified consistently.

5.Marketing and Updates. Advertising materials are made for pre-promoting of the new stock issuance. Guarantors and leaders market the offer issuance to gauge interest and lay out a last contribution cost. Guarantors can make modifications to their monetary examination all through the promoting system. This can incorporate changing the Initial public offering cost or issuance date as they see fit. Organizations do whatever it takes to meet explicit public offer contribution prerequisites. Organizations should stick to both trade posting necessities and SEC prerequisites for public organizations.

6.Board and Cycles. Structure a governing body and guarantee processes for detailing auditable monetary and bookkeeping data each quarter.

7.Shares Gave. The organization gives its portions on an Initial public offering date. Capital from the essential issuance to investors is gotten as money and recorded as investors' value on the accounting report. In this way, the accounting report share esteem becomes subject to the organization's investors' value per share valuation extensively.

8.Post Initial public offering. Some post-Initial public offering arrangements might be organized. Financiers might make some predefined memories edge to purchase an extra measure of offers after the first sale of stock (Initial public offering) date. In the mean time, certain financial backers might be likely to calm periods.

Benefits and Disservices of an Initial public offering

The essential goal of an Initial public offering is to raise capital for a business. It can accompany different benefits as well as inconveniences.

Benefits

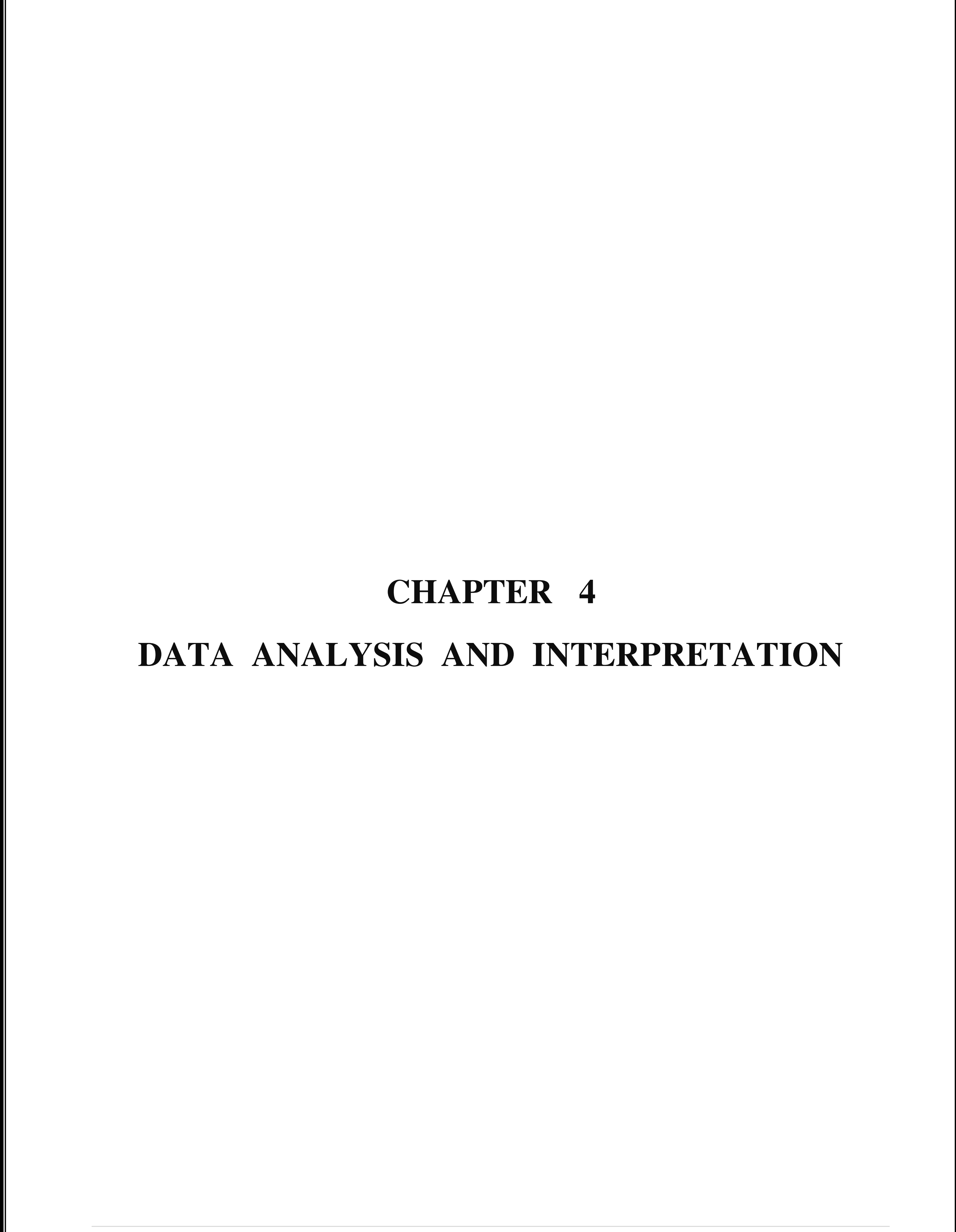
One of the key benefits is that the organization gains admittance to venture from the whole financial planning public to raise capital. This works with simpler obtaining bargains (share transformations) and builds the organization's openness, distinction, and public picture, which can help the organization's deals and benefits.

Expanded straightforwardness that accompanies required quarterly detailing can generally assist an organization with getting more positive credit acquiring terms than a privately owned business. The historical backdrop of starting public contributions (Initial public offerings) follows back to the primary Initial public offering by the Dutch East India Organization in 1602. In any case, the

Indian capital market remained generally immature until the mid twentieth hundred years, when a few Indian organizations started to give offers to the general population.

In 1977, Dependence Businesses Restricted accomplished a noteworthy achievement by giving offers to people in general interestingly. The offers were evaluated at standard worth, and the issue size added up to Rs. 2.82 crore. As India changed its economy and welcome unfamiliar interest during the 1990s, this set out new open doors for organizations in India. This decade saw the rise of a few effective Initial public offerings, remembering the posting of Infosys for 1993, which was the principal Indian organization to list on a US stock trade. The mid 2000s saw a blast in Initial public offering action in India, with many organizations opening up to the world. From that point forward, the Indian Initial public offering market has kept on developing, with an emphasis on further developing straightforwardness and financial backer insurance.

Coming to the present, in the domain of raising money plans through value showcases, the Initial public offering market was a hodgepodge for monetary year (FY) 2022. While many had dreaded a significant accident because of the Coronavirus pandemic, there was recuperation and truth be told great development in 2021. While the primary portion of FY 2021 had a cool reaction because of the pandemic, the third and fourth quarters saw a flood in postings. This energy went on into FY 2022, with 47 issuances contrasted with 26 in FY 2021. This addresses very nearly a multiplying of the quantity of issuances from the earlier year. Nonetheless, 2022 was not all that the market assumptions were set up for. As per the Hold Bank of India Condition of the Economy Report, 2022,[i] simply 17 organizations figured out how to raise a sum of 35,847 crore through mainboard Initial public offerings up to September 2022, including the huge commitment of more than 20,000 crore raised by the Life coverage Partnership of India. Rather than the fundamental board section, the Little and Medium Undertaking (SME) portion presents an alternate situation. The SME fragment encountered a huge rise in September 2022, with 19 organizations effectively raising finances through Initial public offerings. This addresses the biggest number since May 2018. Nonetheless, the primary board section encountered a stoppage, conceivably because of a scope of variables, including expanded unpredictability brought about by international pressures and worldwide monetary strife originating from the circumstance in Ukraine, combined with soaring oil costs, fears of a worldwide downturn, expansion rates, and intensity waves that compromise summer crop



CHAPTER 4

DATA ANALYSIS AND INTERPRETATION

Measure of IPOs Performance:

Consistent with the standard methodology, return of particular day is calculated as the percentage change from the issue price to the closing price on that day in the secondary market.

Ri = (Pi-P0)/P0

Where, Ri = return of i security on listing day, Pi = Closing Price of i security on listing day, P0 = offer price of i security.

Secondly, to calculate index return on listing day,

Mi = (Ii- I0)/I0

Where, Mi = market return on listing day, Ii = closing index at listing day, I0 = closing index at offer day.

Explanation of Independent variables:

Issue Price: Issue price the final offer price offered by the company determined after book building process or fixed price process to the public for subscription of the Initial Public Offer (IPO).

Issue Size: Issue size is the total amount that the issuing company want to raise from Initial Public Offer (IPO). The total issue size is total number of shares offered multiply with the final offer price of the IPO decided by merchant bank.

Oversubscription of IPO: Oversubscription is the number of times the IPO has been subscribed by the various investor categories during the issue offer period.

Market Index Return (Mi): Index return is the absolute change in S & P CNX nifty on the listing day of the IPO and last day of the Issue offer.

Multiple regression analysis has been applied to examine the factors that may explain the underpricing performance of Indian IPOs on listing day. This technique helps in identifying the extent and direction of relationship between the dependent variable and several independent variables. The R square and the adjusted R square generated by it indicates the proportion of variation in the dependent variable explained by the independent variables. On the basis of existing literature, the following OLS regression model has been developed to

find the determinants of performance of IPOs on listing day in India:

$Y1i = \alpha + \beta 1X1 + \beta 2X2 + \beta 3X3i + ... + \varepsilon i$

$\text{Log } Ri = \alpha + \beta 1 \log IP + \beta 2 \log IS + \beta 3 \log OS + \beta 4 \log Mi + \varepsilon i$

Log Ri = Underpricing of ith security

β1 log IP= Issue Price of the firm

β2 log IS= Issue Size of the firm

β3 log OS= Oversubscription of IPO

β4 Mi= Market Index Return (S & P CNX Nifty),

εi= Constant.

Table 2: Sample selection

Criteria	Number of IPOs
Total number of IPOs offered	137
(-) companies not listed on exchange and FPO	24
= Companies listed on NSE	113
(-) Outliers removed from data*	16
= Final sample data taken from companies	97

Source: www.nseindia.com and SPSS 19.0 Version

Outliers removed from data*- number of companies removed from the sample taken as to obtain normality of data for OLS Regression model.

Table 3: Descriptive statistics of Issue price, Issue size, Issue oversubscription and Market return of IPOs Listed in NSE during year 2010 to 2014

Year	Variables	N	Actual N	Minimum	Maximum	Mean	Standard Deviation
2010	Issue price	63	57	11.00	1310.00	202.51	223.59
	Issue size			28.76	2486.35	395.64	505.46
	Oversubscription			.96	93.60	15.21	19.41
	Market Return (Mi)			-.09	.08	.0038	.03
2011	Issue price	30	21	6.00	256.00	99.71	67.78
	Issue size			23.25	1245.00	237.64	329.05
	Oversubscription			1.11	35.21	5.04	8.55
	Market Return (Mi)			-.07	.09	.0000	.056
2012	Issue price	12	11	50.00	1032.00	297.73	314.74
	Issue size			19.00	4155.80	612.20	1198.91
	Oversubscription			.00	40.98	6.41	11.65
	Market Return (Mi)			-.04	.08	.0096	.033
2013	Issue price	3	3	172.00	530.00	304.00	196.64
	Issue size			94.42	950.11	438.31	451.88
	Oversubscription			1.20	11.63	4.83	5.90
	Market Return (Mi)			-.029	-.002	-.02	.015

2014	Issue price	5	5	47	645	228.60	237.60
	Issue size			120.00	351.86	240.19	105.33
	Oversubscription			7.39	59.97	34.60	26.20
	Market Return (Mi)			-.037	.019	-.01	.022

SOURCE:www.nseindia.com and SPSS 19

Table-3 shows the descriptive statistics of issue price, issue size, issue oversubscription of IPO companies and market index return (Mi) listed during 2010 to 2014.

In **2010** data was taken of 63 companies but only 57 companies' data is taken for analysis. The difference between the lowest price 11 and highest price 1310 is huge and average issue price was 202.5. Average issue size was 395.65 crores with minimum issue size 28.76 crores. The average oversubscription was 15.2 times with maximum 93.6 times. The average market return S & P CNX nifty from issue close day to listing day was 0.38% with highest return of 8%.

In **2011** final data was taken of 21 companies. The average issue price is 99.71 with minimum issue price was 6 and highest price 256. The average issue size was 237.64 with maximum issue size 1245 crores. The oversubscription was on average 5 times, with minimum 1 time only. The average market return S & P CNX nifty for the period was 0 %.

In **2012**, 11 companies' data was taken for analysis. In this year also minimum and maximum range is high and average issue price was 297.73. Average issue size was 612.2 crores with minimum issue size of 19 crores. Average oversubscription was 6.4 times with minimum 0 time for one of the IPO. The average market return for that period was around 1 % only.

2013 was slack period for the IPOs and only three IPOs got listed on NSE and minimum issue price was 172 and maximum was 530. Average issue size was 438.31 crores and average subscription was 4.8 times with maximum subscription of 11.6 times of Just dial company IPO. The average market return S & P CNX nifty from issue close day to listing day was negative 1.9%.

In year **2014** total 5 companies' IPO got listed on NSE and maximum issue price was of Monte Carlo company issue. The average issue size was 240 crores for the year and average oversubscription was 34.9 times with maximum 60 times of Sharda Cropchem and 59 times of Snowman Logistic IPO. Average market return for the period was negative 1%.

Table 4 :Average returns of IPO companies during 2010 to 2014.

S. no.	Year	Avg. Initial Raw Return
1	2010	8.30%
2	2011	2.90%
3	2012	3.95%
4	2013	2.14%
5	2014	26.11%

SOURCE:www.nseindia.com and SPSS 19

In above table shows average year wise first day return of the all companies listed in NSE from 2010 to 2014. The return is of only listing day return for the listed IPOs taken from NSE.

Table 5: Regression Result of all sample IPOs during the period 2010-2014.

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
	(Constant)	.042	.152		.274	.784
	Issue price	.012	.028	.041	.425	.672
	Issue size	-.034	.021	-.150	-1.587	.116
	Over subscription	.091	.018	.468	4.922	.000
	Market	.333	.618	.050	.539	.591

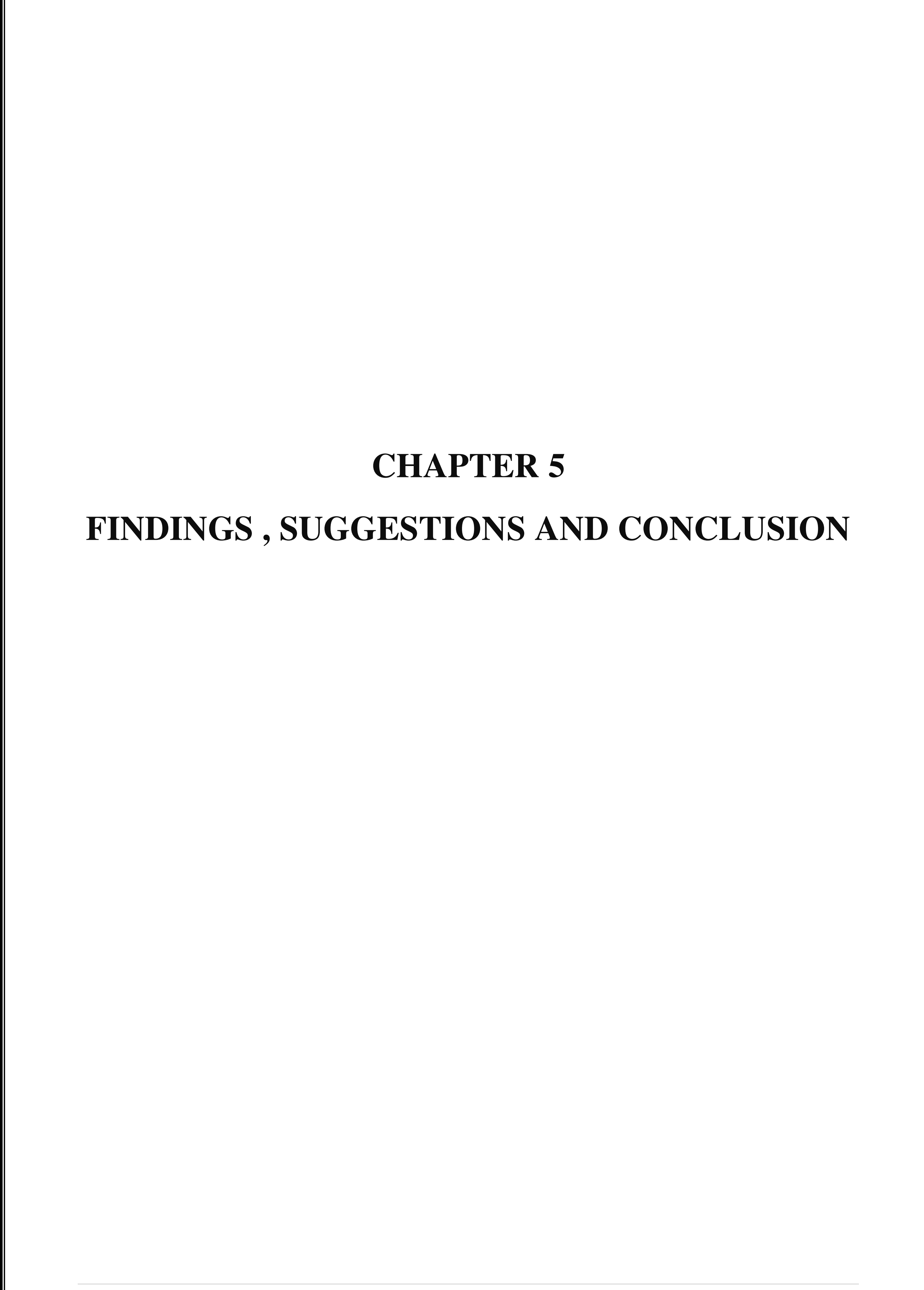
SOURCE:www.nseindia.com and SPSS 19

CHART 5.1:

SOURCE:www.nseindia.com and SPSS 19.

INTERPRETATION:

To test the hypothesis of there is significant difference between several independent variables with the level of INITIAL RETURN; the multiple regression analysis has been used. The above **table-5** shows the regression results of the IPO during the period 2010-2014. From theanalysis it can inferred that independent **variable issue price** did not have any linear relationship with the level of INITIAL performance of an issue. The beta value of issue price was 0.041, with t value of 0.425; indicate that there was no linear relationship between issue price and degree of INITIAL PERFORMANCE. **So it can be concluded that null hypothesis is accepted andthere is no significant association between issue price and INITIAL performance of IPO.** The second independent variable **issue size** was having negative beta value -0.150 with t-value -1.59. So it can be inferred that **there was no linear correlation between issue size and INITIAL RETURN of IPO and null hypothesis cannot be rejected for it.** The significance value of the independent variable **Oversubscription was < 0.05 and coefficient was 0.468 with t- value 4.922 so null hypothesis is rejected and it can be concluded that there is significant relationship between Oversubscription of the issue and INITIAL PERFORMANCE of an IPO**. Higher the oversubscription of an issue means higher the demand for the issue result in higher initial day return of an IPO. **Market index return (S&P CNX Nifty) had co-efficient 0.05 and t- value was 0.539 reveals that there is no relationship between Market Index return and INITIAL RETURN of an IPO and null hypothesis is accepted.**



CHAPTER 5

FINDINGS , SUGGESTIONS AND CONCLUSION

Findings:

•Generally essential issue market has looked descending slanting development as far as number of Initial public offering because of worldwide monetary emergency.

•The all out number of public issues throughout 2010-2014 time span were 113. The absolute worth of Initial public offerings was 63633 crores.

•The typical year wise first day return of the all organizations recorded in NSE from 2010 to 2014 were 8.3 %, 2.9 %, 3.95 %, 2.14 % and 26.11 % separately. Year 2014 has given especially exceptional yields in a portion of the Initial public offerings while the typical return from the all Initial public offerings from 2010 to 2014 has given normal 7 % return.

•regression results uncovers that free factors like Issue value, Issue size

•Market File have no critical connection with introductory day returns of Initial public offering and free factor Issue Oversubscription has straight relationship with undervaluing

suggestions:

- The investigation endeavored to sort out the basic day returns and the factors impacting the degree of underestimating.
- The data for the assessment has been taken of complete 97 Beginning public contributions recorded on NSE between the periods 2010-2014..
- The backslide assessment depict that there was no gigantic association between factors through Issue esteem, Issue size and Market gets back with issue underestimating however the underestimating has straight relationship with the Issue oversubscription.

Conclusion:

It shows the Indian Introductory public contributions are underestimating and the enlightening variable like Issue oversubscription influences it. So monetary benefactors should place assets into Introductory public contributions for transient returns. As the elements Issue esteem, Issue size and Market returns are not showing direct association with beginning day return they are provoked not to rate or assess Starting public contributions considering these variables anyway the variable Oversubscription should be contemplated for the Hypothesis decision.

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