IN-VITRO BIOLOGICAL SCREENING OF ETHANOL EXTRACT OF *Lagerstroemia thorelli* LEAVES

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the Degree of
Bachelor of pharmacy

School of Pharmacy
Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Student Full Name
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Approval

The thesis titled "In-vitro Biological Screening of Ethanol Extract of *Lagerstroemia thorelli* Leaves" submitted by M. M. Rubaiyat Muntasir Meem. ID: 19146003, of, Summer 2022 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

I hereby state that I have fully committed my work according to the rules and regulations of the University policy and this work contains my own original work.

Abstract

The research on *Lagerstroemia thorelli* was performed for evaluating potential medicinal properties of the plant. In order to accomplish this goal, several experiments like antimicrobial property test which included bacterial resistance test and thrombolytic test for potential anticoagulant factor and property findings which required several blood samples from different individuals who contributed in this research study. After completing these experiments evident from various observation that, antimicrobial property test showed some intended results for this plant, which indicates that it contains potential amount of anti-microbial property. However, in this research, the thrombolytic properties were not as satisfactory. These are the initial findings of pharmacological property of this plant. Evidently, on the basis of this research, it can be claimed that this plant *Lagerstroemia thorelli* has the potential to be able to provide good impact in medicines and contribute to humanity.

Keywords: Extraction method, antimicrobial activity, Pharmacological activity, Thrombolytic property, biological property, Zone of inhibition.

Dedication

This project is dedicated to my family and mankind for if there are any possibilities in the future.

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List of Acronyms

ml = millilitre

g = gram mg =

milligram

H_2O = Water

Chapter 1

Introduction

1. Introduction:

Nature provides us with limitless resources for various purposes to contribute to science and society. For instance, plants contain various constituents which has the potential to contribute for the wellbeing of mankind. The past history of plants shows that before there were any actual medicines for a lot of diseases, many plants contained the necessary constituents to heal people. Mainly herbal plant preparations were mostly used to treat many diseases even before the medicinal era. So, basically, the plants are the sole foundation for drug therapy for different diseases. This is why the potential for providing support for the drug discovery process, plants are actually a valid and authentic source for finding necessary constituents and properties which can be beneficial to drug discovery process. This is why searching for different pharmacological properties is necessary for contributing to drug discovery.

Although it can seem like there are an infinite number of plants there, there are really just a limited amount of resources available. “There are around 7.5 million different species of plants, of which approximately 5 million are categorized as "upper plants" and approximately 2.5 million are categorized as "lower plants" (Corlett, 2016)”. More than sixty percent of these plant sources are now being investigated for potential medical applications. They are made from natural herbal derivatives as well as goods that are connected to those herbal derivatives. Natural sources and their derivatives are used in the production of over 80 percent of all sorts of medications, including antimicrobials, anticancer agents, and anticoagulants, amongst others. More than 28 percent of the world's natural resources include compounds that have just recently become accessible(Koni et al., 2022). According to the most current estimates provided by the World Health Organization (WHO), more than 80% of the people rely on various medicinal plants as their main source and method of medical treatment. World Health Organization also stated that there are around 21,000 more or less different plant species that have the potential to be utilized for therapeutic reasons(Sello, 2022).

1.1. A Brief History on Medicinal Plants

Plants have been put to a wide variety of functions, dating back to ancient times, in an effort to both protect people from disease and discover effective treatments for a wide range of illnesses. However, the usage of plants for medical purposes was primarily intuitive rather than for the purpose of doing research on pharmaceuticals directly. In those dark ages, there were not enough resources and information to collect the constituents, and there was a lack of adequate methods to investigate different plants for different ailments. It was a time when there was no light. Not until quite recently did the process of developing drugs involve research on animals and plants. There are currently an infinite number of opportunities for locating new medicinal molecules, thanks to the development of modern approaches to drug discovery and screening processes. However, these discoveries are not so simple to come by because there need to be a set of experiments conducted in order to properly determine the potential, and particular resources are necessary.

Analytical history of medical plants is strongly tied to our ancestors. They were well-versed in the medicinal characteristics of different plants and their essence. This knowledge is intimately related to the analytical history of medicinal plants. Around the year 300 B.C., the Babylonians had a significant amount of knowledge regarding a significant number of vital medicines. One might also find the assertion that "contemporary medicine still uses specific herbs in the same way as the Babylonians(*Ghani, A. (2003) Medicinal Plants of Bangladesh. Asiatic Society of Bangladesh, 2nd Edition, 1-16, 138. - References - Scientific Research Publishing*, n.d.)".

The first known documented reference to the use of plants as medicines was discovered on a clay tablet written in Sumerian and discovered in Nagpur. This tablet dates back around 5000 years. It consists of 12 potions that collectively make reference to more than 250 distinct plants. Some of these plants contain alkaloids, such as poppy, henbane, and mandrake.

It was common knowledge among the ancient civilizations that India was home to a great variety of plants that may be used for medical purposes. The majority of the plants that are used as raw materials in the production of pharmaceuticals and aromatic goods are collected from India's forests, which are the primary repository for a substantial number of medicinal and aromatic plants. The AYUSH system in India has a database that contains the coding for around 8,000 different herbal treatments. The most prominent types of indigenous medical practices are referred to as

Ayurvedic, Unani, Siddha, and Folk (or tribal) Medicine. The Ayurvedic and Unani medical systems are the ones that have grown and been used in India the most over the course of their history.

The ancient Indian Vedas have references to herbal medicine, which is widely available in this nation. India is the origin of many of the plants that are used to make spices, including nutmeg, pepper, cloves, and others.

Throughout the latter part of the 19th century and the early 20th century, there was a substantial potential that the use of medicinal plants as a form of therapy would eventually be phased out. The activity of damaging enzymes, which produces fundamental changes in the drying process of medicinal plants, which in turn results in the therapeutic benefits of medicinal plants, has been written about by a great number of authors as being the cause of the many drawbacks that are associated with the drugs that are obtained from them. depending on the procedure that was used to dry it. During the 19th century, medicinal agents, alkaloids, and glycosides that were isolated in pure form began to replace the medications from which they were initially extracted in increasing numbers. On the other hand, it was quickly realized that if the effects of pure alkaloids were seen more quickly, then the effects of alkaloids as a whole would be seen more completely and would endure longer. At beginning of 20th century, various strategies were proposed with the goal of stabilizing fresh medical plants, particularly those containing unstable therapeutic compounds. In addition, a significant amount of work has been put into researching the development of medicinal plants as well as the optimal growing conditions for them.

Ancient medical professionals and researchers thought that herbs were the answer to treating a wide variety of illnesses and conditions. They investigated this topic in depth and came up with tangible findings on the efficacy of a variety of medicinal herbs and plants. The vast majority of drugs made in this manner do not cause any adverse effects or responses. Because of this, the use of herbal medicine is gaining popularity all over the world at an increasing rate. These medicinal herbs have the potential to treat a wide variety of illnesses that would otherwise be regarded untreatable. The usage herbs for many treatment of illnesses has gained worldwide recognition, and the application medicinal plants is increasing each day(Schulz et al., 2004).

1.2. Available Medicinal Plants in Bangladesh

Bangladesh is a rich resource that has a wide variety of medicinal plants, not all of which have been well researched. The applications of these plants are founded on both historical and contemporary experience, as well as on clinical data; yet, the value of many of them is not supported by any kind of evidence. Their incorporation into conventional medical practice is mostly based on anecdotal evidence and is justified solely by the positive impact they have on patients. “An accurate scientific examination of the pharmacological qualities of these plants, which have been utilized in a variety of various traditional preparations, carries with them an immense amount of potential and promise for the 21st century (Rahman et al., 2001; Ghani, 2003)”.

More than 5,000 angiosperm species belonging to more than 200 groups are native to the subtropical nations that surround Bangladesh. It is currently the ideal season in Bangladesh to sow medicinal plant seeds and tend to their growth. According to "Materia Medica," Chittagong, Dhaka, Rajshahi, and Sylhet, along with other districts of Bangladesh, have an abundance of approximately 5000 different types of herbal remedies. Traditional medicine has been well-known in the nation ever since the very earliest days when it was first practiced there. Because of the favorable conditions for the development of herbal medicine in Bangladesh, the country is able to successfully treat 500 of the 2000 classical disorders. In addition, over the past few years, the use of herbal medicine in the manufacturing sector as well as by large organizations in Bangladesh has seen a substantial surge(Tiralongo et al., 2011). There are now a range of pharmaceutical businesses in Bangladesh that are leaders in the usage of herbal treatments. They may have the misguided belief that being close to nature will not have a negative impact on their health, as the majority of Bangladeshis and tribal people in Bangladesh rely largely on medicinal plants for their primary therapy. They employ various components of the plant, such as the peel, the steam, the fruit, and the flower, in accordance with their religion and belief. Some of the examples are:

Table 1.1 Examples of plants used for treatments

Name of plants	Applied as
Acalypha indica L.	For treating skin diseases.
Piper betel L.	For treating cut injuries.
Carica papaya L.	For treating itches.

1.3. Significance of Medicinal Plants in Drug discovery:

Plants are one of the major sources for drug discovery which contributes a lot in this process. There are a lot of plants which has the potential to become a great source of new drug compound or add up as a supply for alternative source of same components for different drugs. Many plants may contain potential factors and properties of various drugs. But in order for that to be discovered, proper tests should be done to ensure the validity of the plants medicinal use. Most therapeutic agent sources directly come from different available plants and their parts like barks, leaves, roots etc.(Süntar, 2020).

The process of developing medicines from plants requires a multidisciplinary approach that combines methods from the fields of botany, ethnobotany, phytochemistry, and biology. Plants are an important source of novel chemical entities (lead molecules) that may be used in the research and development of medications to treat a wide range of pharmacological targets, such as cancer, HIV/AIDS, malaria, Alzheimer's disease, and chronic pain. Paclitaxel, camptothecin-derived analogues, arteether, galanthamine, and tiotropium, to mention a few, are some of the naturalproduct medications of plant origin that are now being used in clinical practice. Other naturalproduct drugs are currently undergoing Phase II and Phase III clinical studies. Despite the fact that plant-based drug discovery programs continue to provide an important source of new drug leads, numerous challenges are encountered. Some of these challenges include the procurement and authentication of plant materials, the implementation of high-throughput screening bioassays, and the scale-up of bioactive lead compounds. At the same time, there are prospects for India because the country possesses a wealth of genetic resources and traditional knowledge, both of which are essential components for bioprospecting and value addition(Pushpangadan et al., 2018).

Since ancient times, people all throughout the world have had a generally positive attitude toward nature and natural medicines. Even before the identification of diverse bacteria, various components of plants, such as barks, roots, stems, flowers, and seeds, were utilized to treat illnesses or infections caused by germs. Therefore, it is reasonable to think that these plants and herbs have a substantial potential for medical use, as well as an established pharmacological effect.

1.4. Selection of *Lagerstroemia thorelli* as a topic for this project:

At the beginning of this project, it was confusing to find a suitable plant for experimentation. But later on, I was provided with support materials and sources to find it among them. There are some lists of Bangladeshi plants and among them I found *Lagerstroemia thorelli* with no existing articles which was worked on before and since this plant is pretty much available, I thought it would be viable option for researching on this.

1.4.1 About *Lagerstroemia thorelli*:

Lythraceae, the family of Lagerstroemia thorelli is also known to be named as loosestrife family. Another feature of this amazing plant is that it can even tolerate drought conditions which makes it viable for planting at road sides and rural areas due to its tolerance and as such its quite available in Bangladesh in many areas such as areas of Moulovi bazar,Sylhet.

Locally it is called Choto Jarul, a smaller version of the plant Jarul yet there are still differences between the bigger plant and the smaller one in structure, integrity and size of leaves.

A family of flowering plants known as the Lythraceae has 32 genera and roughly 620 species of trees, shrubs, and herbs. Cuphea (275 species), Lagerstroemia (56), Nesaea (50), Rotala (45), and Lythrum are some of the bigger genera (35).

1.4.2 Information on morphology of *Lagerstroemia thorelli*:

This plant is basically the smaller version of Jarul tree but has its own specific morphology that helps to distinguish between the bigger tree and this plant. The wood of this plant is quite easy to penetrate due to its weak cell walls. There are no thorns and latex present in the plant body. Type of leaf is simple as well as the style of it with a length of 10cm and width of 4cm. The leaves have no aroma. The flowers are directly pink in color with maximum size of 2cm. The type and shape of fruits are capsule like and sizes up to 1cm and also possesses brown color.

1.4.3. Plant taxonomy of *Lagerstroemia thorelli*:

Table 1.2: Taxonomy hierarchy of *Lagerstroemia thorelli*

Rank	Scientific name (Common name)
Kingdom	Plantae
Phylum	Spermatophyta
Class	Magnoliopsida (Dicotyledons)
Order	Myrtales
Family	Lythraceae
Genus	*Lagerstroemia*
Species	*Lagerstroemia thorelli*

1.4.4. Pharmacological properties of other species of *Lagerstroemia thorelli*:

There is no available research on the plant *Lagerstroemia thorelli.* So, the possible method to make assumptions about the *thorelli* species is by looking at the other species available such as *Lagerstroemia indica, Lagerstroemia floribunda, Lagerstroemia loudonii*, *Lagerstroemia parviflora* etc. It is known the *Lagerstroemia* family is quite good for antioxidant properties. For example, in *Lagerstroemia speciosa* there is enough anti-oxidant properties available to even consume as tea for diabetic patients. Some articles on *Lagerstroemia speciosa* even some minor signs of antibacterial activities. On this basis the *Lagerstroemia thorelli* can be expected to have some of the signs just like the other species of this family. In the project work there have been some signs of antimicrobial activity, anti-oxidant activity, thrombolytic and cytotoxic as well.

In addition to treating stomach issues, astringent, stimulant, and febrifuge usage was found in the roots of this plant. Diabetes mellitus was treated using tea made from the leaves, and weight reduction was achieved by drinking the tea. Purgatives were made from flowers and barks of trees. A leaf decoction or infusion was utilized in the treatment of inflammation of the kidneys and bladder, dysuria and other urinary dysfunctions, as well as reductions in cholesterol levels, hypertension, and diabetes. By applying a poultice made of the leaves directly to the affected areas,

patients suffering from malaria, headaches, and cracked heeling might get relief from their symptoms(AL-SNAFI, 2019).

1.4.5. Other Related Publication on *Lagerstroemia thorelli:*

Up to now there is not a single publication on the *Lagerstroemia thorelli* plant. There may be some valuable properties which may contribute to the medicinal studies later on. That is why the available properties of this plant needs to be identified.

1.5. Project justification / rationale

The main reason for this project on *Lagerstroemia thorelli* is that there may be some very important properties that reside in the plant which can be rendered very crucial for medicinal purposes. To add more weight to this justification, the other species of the *Lagerstroemia* family has showed some potential activities which has been used as potential medicines since ancient times. Such as *Lagerstroemia speciosa* showed some very impressive pharmacological properties such as, thrombolytic, cytotoxic, antimicrobial, anti-oxidant and some minor other residual properties as well.

1.6. Aim and Objective of the Project

The aim of this project to find and evaluate potential pharmacological properties within the plant extract which might have biological potential.

This project protocol contains the following steps which done with ethanol extract of the *Lagerstroemia thorelli* leaves.

i. Evaluation of antimicrobial activity. ii.
Evaluation of thrombolytic activity.

1.7. Evaluating Antimicrobial Properties:

Microbial infection is one of the leading causes of different diseases all over the world. Many of these infections are directly and indirectly responsible for the deaths of many according to various statistics reports up until now.

Since the 1990s, the field of public health has given increased attention and priority to the problem of newly emerging infectious illnesses. The various governments of the world are now working on contingency plans that will serve as a guide for them in the event of an epidemic; these plans realize that communication would be essential.

Research is often restricted to investigating obstacles to comprehension or instruction in order to promote the production of more effective messages. The study that is now being done on emerging infectious diseases should be expanded such that it also investigates implicit preconceptions here on nature of the issue at hand, in addition to those regarding the concepts of ambiguity, confidence, authority, ethics, and prejudices. This kind of study need to be directed by modern risk communication theory, the emphasis of which has historically been placed on more visibly contested technology and environmental circumstances. This idea would shed light on significant contextual aspects that should be included into communication on developing infectious diseases(Holmes, 2008).

So, to combat the current situations and fight off the growing issues related to infectious diseases what is need to be done is to find components which are able to fend against microbes and for that to happen, the research on antimicrobial screening and evaluation from different sources should be prioritized first. “There are mainly three types of methods which can be used to determine the strength of test samples which are namely i. Disc diffusion, ii. Serial dilution and iii. Biautographic method. However, there is no way to make an educated guess as to how the antimicrobial test will turn out.” When it comes to controlling product growth, several researchers rely on the spatial control region and/or the minimum production. However, the outcomes might be affected by a wide variety of parameters, including production techniques, body mass, the classic counselling

(Bauer et al., 1966) pH, and summertime weather temperatures. In this project, primary main focus is going to be disc diffusion method.

1.7.1 Method of Disc Diffusion (Principle)

Throughout this technique, first the antibiotics are isolated from their sources, which are constrained by a nutrient, and then the formulation of the desired size occurs.

An antibiotic concentration gradient is produced whenever an antibiotic-impregnated disk is put on agar that has previously been infected with the test bacteria. The disk will soak up humidity, and the antibiotics will diffuse outward through the agar medium, forming the gradient. This technique is predicated on the idea that now the antibiotic will absorb moisture and then spread radially outward across the agar medium after it has done so. The potency of an antibiotic is at its maximum adjacent to the edges of the disk, and it gradually decreases as the range from the disk increases. Eventually, it reaches a point when it is no longer prohibitive for the organism, allowing the organism to proliferate unchecked and increasing in numbers. During the incubation phase, a clear area or circle will form surrounding the filter paper disc if the antibiotic is successful in inhibiting the growth of bacteria. This will be the case if the antibiotic is successful in its intended purpose.

In order to achieve poor control, antibiotics (bleaching) and empty layers are used. To hasten the process of biodegradation, we have 24h to heat the plates to 37 degrees Celsius and relocate them. The presence of antimicrobial characteristics, for instance, may inhibit the development of microorganisms in the medium that surrounds the discs, which, in turn, creates a distinct and unmistakable boundary inside the exchange zone. After then, the sample agent's antibacterial activity is evaluated by proper observation the diameter of the circle or ring formed around the filter paper discs (Barry, 1976, Bauer et al., 1966)

Chapter 2

Methodology

2.1. Collection of Plant

Lagerstroemia thorelli was selected because there was no other research on this plant for evaluation of different pharmacological properties. By doing extensive search and depending on the basis of availability of this plant, this particular plant has been selected for analyzing. The leaves of *Lagerstroemia thorelli* was collected in October 2022, from Nabigonj Upazilla.

Table 2.1: Researching on *Lagerstroemia thorelli.*

Name of the Plant	Scientific Name	Family	Part
Choto Jarul (In Bangladesh)	*Lagerstroemia thorelli.*	*Lagerstroemia thorelli.*	Leaves

2.2 Verification of the selected plant

The National Herbarium Bangladesh (NHB), Mirpur, Dhaka, received a sample of the leaves after they were collected for identification and verification. After a few days, the plant's token was gathered and it was determined what kind of plant it was. The provided specimen has the accession number (DACB- 87494) and has been verified by the Bangladesh National Herbarium in Mirpur, Dhaka.

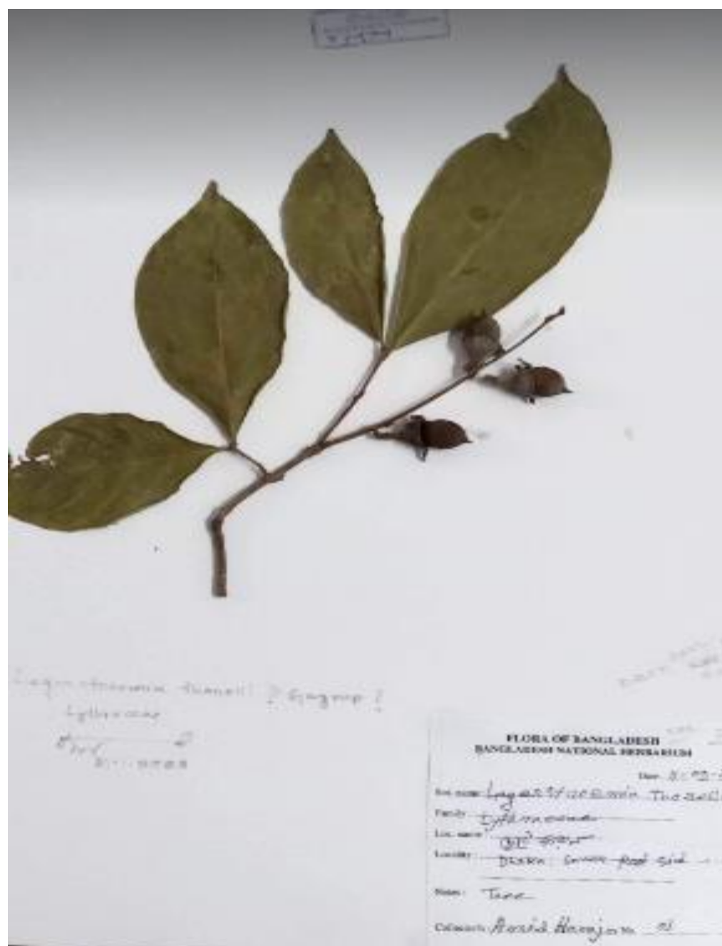

Figure 2.1: *Lagerstroemia thorelli* plant accession number (DACB- 87494) which was collected from the National Herbarium of Bangladesh, Mirpur, Dhaka

2.3. Extraction procedure

Extraction of the medicinal plant has some crucial steps:

The entire extraction procedure can be mainly divided into two parts.

i. Preparation and drying of plant material: It's a two-step part

ii. Extraction process: There are 5 major steps in this part

Preparation and Drying of Plant Materials

i. Crude Plant
ii. Washing Leaves
iii. Drying

Extracting from prepared plant materials

i. Size reduction
ii. Maceration
iii. Filtration
iv. Concentration
v. Drying the plant concentrate
vi. Plant extract.

2.3.1. Preparing the Plant Material

The leaves were properly separated from the stem of the plant and gathered in one place for further processing. Then it was washed with clean water to remove any dust particles to prevent any interference with the testing. The clean leaf was then left to dry up for a day in the sun before being heated in a hot air oven for an hour to complete the drying process. The following phase involved arranging the dried leaves.

2.3.2. Procedure of Plant Extraction

- **Size reduction and weight measuring:**

When the leaves had dried out and became crusty, they were ground with a coarse dust using a powerful grinding machine. Approximately 306 grams of powder were collected, and after that, it was followed by packing the powder in airtight glass containers with the necessary label, and

finally, it was left in a cool, dry, and dark place until further investigation. This was done to avoid cross-contamination, which is why essential steps were taken.

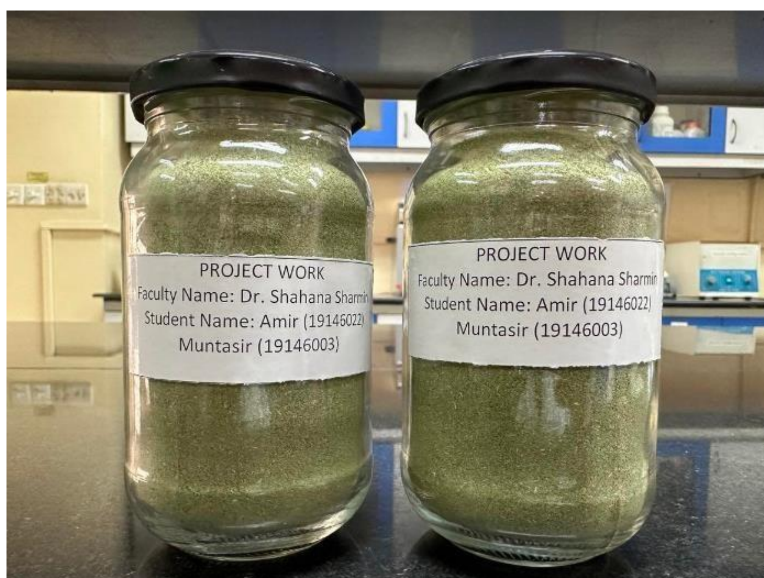

Figure 2.2: Grinded powder of *Lagerstroemia thorelli* leaves.

✞ Extraction of leave powder using solvent

On the basis of types of solvents there are mainly two types of suitable solvents for this task.

i. Aqueous solvents ii.
Organic solvents

For processing the powder properly and efficiently an organic solvent was used in the maceration process. About 1 liter of ethanol was used to soak the 300gm recovered powder from the dried leaves and left to soak well for 2 to 3 days in ambient temperatures of 22 to 25 degrees and it is also occasionally stirred.

Figure 2.3: 2.5L Ethanol for extraction and the powdered sample in jar.

Figure 2.4: Approximately 1L Ethanol mixed with the powder for extraction.

✞ Filtration

After 2 to 3 days of maceration, the contents of the beaker filtered using a cotton and Whatman filter with pore size of 110mm. The cloth was autoclaved for preventing any cross contamination. The filtration was done twice to ensure high level of purity.

✞ Concentration

The collected filtrate was concentrated using a water bath machine with about 50-to-55-degree Celsius temperature until the concentration was thick and viscous enough and all the ethanol have been completely removed. All that remained after the evaporation process is the viscous and concentrated mixture of plat extract which was later collected in a petri dish for further using for different sample testing purposes. After that, the concentrate was allowed to dry for a while in laminar air flow.

Figure 2.5: Evaporation process of the mixture.

Figure 2.6: Final Concentrated product obtained after evaporation.

Table 2.2: The weight of *Lagerstroemia thorelli* ethanol leaves extract after the concentrate was dried.

Initial weight/g (Petri dish without extract)	146.05g
Final weight/g (Petri dish with extract)	163.26g
Weight of extract/g	(163.26-146.05) = 17.21g

The final weight of the finished extract is approximately around 17.21g

2.4. In-vitro analysis of thrombolytic property of *Lagerstroemia thorelli* leaves

For evaluation of thrombolytic property, the following parameters need to be established **Table 2.3: Process parameters for thrombolytic property testing**

Materials	Used as
Plant Extract.	Sample
Clopidogrel (anti-platelet agent)	Positive Standard
Water (H_2O)	Negative Standard

✞ Necessary reagents and materials used for thrombolytic test.

- Blood
- Clopidogrel (anti-platelet agent)
- Distilled water
- Micro centrifuge tube
- Plant extract

✞ Preparation of test sample

For the purpose of preparing the sample for testing After suspending it over the night in 10ml of distilled water, 100 mg of plant extract were totaled. Following that, the soluble supernatant was drained off and given the appropriate filtering treatment.

✞ Preparation of Standard Solution

Clopidogrel is an anti-platelet medication that serves as this experiment's reference standard. Clopidogrel (100 mg) was successfully reconstituted in 10ml of distilled water after being well stirred. This suspension was saved as a stock solution, and then l of the stock solution was used to make the solution that was tested for thrombolytic activity.(Chaudhary et al., 2015)

✞ Preparation of Blood Sample

Blood samples from five healthy human volunteers (n = 5) were obtained after confirming that the conditions were sterile throughout the process. There was no history of anticoagulant treatment. Following the blood donation, one 1ml of blood was injected into the micro centrifuge tubes that had been previously weighed. Following then, clots continued to develop inside the microcentrifuge tubes.

✞ Thrombolytic Property Test Procedure

In the first experiment, a volume of 6ml of venous blood was extracted from each of the volunteer workers. After collecting blood samples from five separate sterile bacteria and allowing them to incubate for 45 minutes at 37 degrees Celsius, the samples were weighed. Following the creation of the clot, all of the micro-tube lines were thoroughly flushed with fresh fluid until they were empty. The weight of the tube that was taken before to the formation of the clot served as the basis for determining the weight of the clot itself. In this particular scenario, 100µl of clopidogrel was utilized as a positive control, and 100µl of distilled water was used as a non-thrombolytic negative control. 100µl of each sample was added from their respective test tubes. All of the micro tubes were incubated for 90 minutes at 37 degrees Celsius to observe the process of clot lysis. After incubation, the released liquid was drained, and the weight of the tubes was measured once more to determine whether or not there was a difference in weight as a result of clot disruption(K et al., 2014). Lastly, the proportion of clot lysis can be broken down as follows:

"Percentage (%) of clot lysis = (wt. of released clot /clot wt.) × 100"

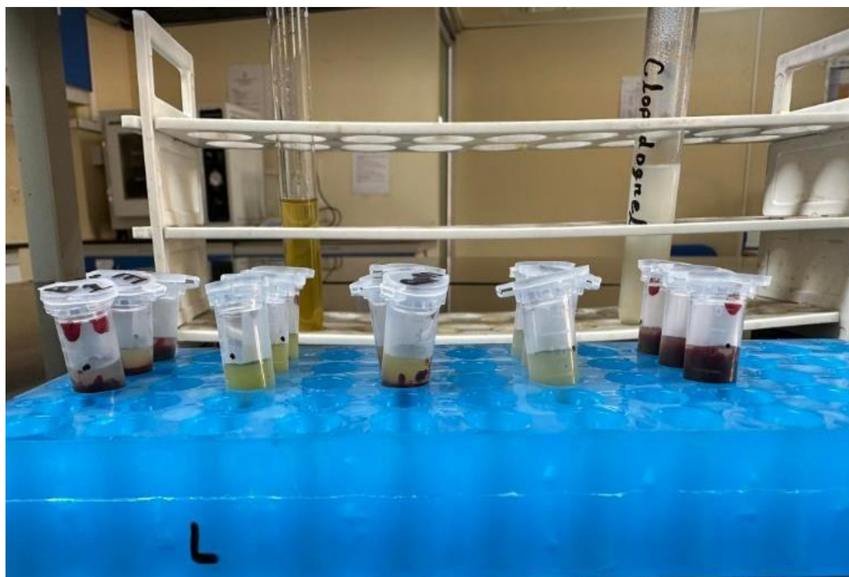

Figure 2.7: Prepared sample, standard and blank for thrombolytic property testing

2.5 Evaluation of Antimicrobial property in *Lagerstroemia thorelli* leaves.

Ethanol extracts of *Lagerstroemia thorelli* is used as test samples for analyzing the
antimicrobial property.

✞ Necessary Apparatus and Reagents for antimicrobial test

i. Filter paper discs ii. Petri-dishes iii. Micropipette iv. Sterile forceps
v. Screw capped test tubes
vi. Autoclave vii. Spirit burner viii. Refrigerator ix. Nose mask and Hand gloves x. Incubator xi. Laminar air flow hood xii. Nutrient Agar Medium
xiii. M.H. Agar

✞ Micro-organisms used for testing

The micro-organisms used were actually nonpathogenic and 2 gram positive and 2 gram negative bacteria were used.

Table 2.4: A list of microorganisms used for antimicrobial tests

Gram positive Bacteria	Gram negative Bacteria
1. *Staphylococcus aureus* 2. *Bacillus subtilis*	1. *Salmonella typhi* 2. *Escherichia coli*

✞ Sterilization procedure

It is a must to perform sterilize all the equipment for testing so that there maybe no cross contamination which may later interfere with the yielding results including beakers, conical flasks, Patri-dishes, cottons and forceps sterilized and kept in aseptic place. UV light was used to sterilize a variety of items, including the tips of micropipette, cotton, forceps, blank discs, and so on. In an autoclave at a temperature of 121 degrees Celsius and a pressure of 15 pounds per square inch, petri dishes and other pieces of equipment were subjected to a 20-minute sterilization process. For controlled experiment this has to be done in a laminar air flow hood. UV light must be turned on at least an hour before working in the controlled environment for testing properly. After the experiment, every piece of equipment was disinfected, and any bacterial strains that had been employed were disposed of properly to prevent any contamination of the surrounding area.

✞ Procedure to performing antimicrobial test

At the beginning, for the purpose of culture preparation, 2.5 g of nutrient broth were dissolved in 100 ml of purified water. Afterwards eight conical flasks, each of which had ten milliliters of broth mixture and had eight distinct bacterial strains added to it, were taken and placed in an incubator at a temperature of 37 degrees Celsius for twenty-four hours. The incubator was constantly shaking. Following a period of twenty-four hours, these conical flasks were taken out of the shaking incubator and stored in a controlled atmosphere. Following that, the agar medium was made by dissolving 7.6 g of M.H. Agar in 200 ml of distilled water. Right after the preparation,
M.H. Agar was placed into Patri-dishes, and these Patri-dishes were left at room temperature so that they might cool down. In the meanwhile, test samples with concentrations of plant extract ranging from 500 mg/ml, 250 mg/ml, 125 mg/ml, 61.5 mg/ml, 31. 25 mg/ml, and 15.625 mg/ml were made and allowed to be soaked into the filter paper discs. Once the M.H. Agar contained in the Patri-dishes had become solid, bacterial strains were inserted into it using cotton bars. Both

streptomycin discs, which served as the standard, and test sample plant extract discs were positioned in the Patri-dishes. After that, these Patri-dishes were placed in an incubator at a temperature of 37 degrees Celsius for a period of twenty-four hours to allow for the growth of bacteria. Once more, 24 hours later, Patri-dishes with various bacterial strains as well as standard and test sample discs were collected, and an observation was made about the inhibitory zone generated by standard and test sample discs(Balouiri et al., 2016).

✞ Determining the zone of inhibition

The antimicrobial ability of the samples can be verified by checking the on-going growth of the microbial growth in the Patri dish from the filter paper disc. If the growth around the sample is inhibited to a certain extent it indicates that the sample is preventing the growth of microbes around the filter paper discs.(Bhargav et al., 2016)

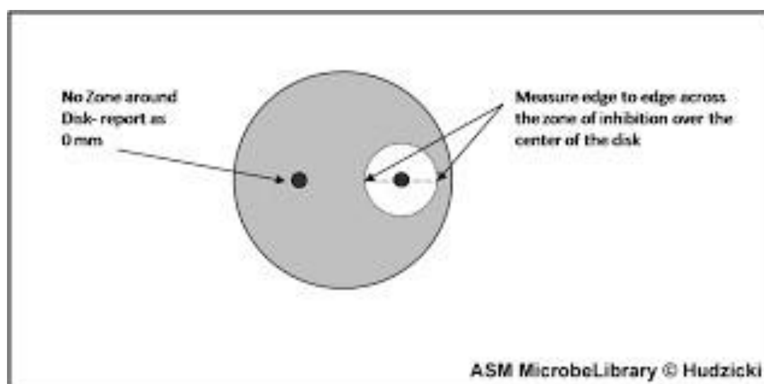

Figure 2.8: Determination of the inhibition zone (Source: asm.org)

Chapter 3 Observation and Results of the test

3.1 Analysis of thrombolytic properties Table 3.1: Results of thrombolytic activity of *Lagerstroemia thorelli*

No. of volunteer	Samples	W1 (Microtube weight in gram)	W2 (Clot + Microtube weight in gram)	W3 (Disruption)	W4 (W3-W1)	W5 (W2-W3)	% of clot lysis

1	Extract (Sample)	0.887	1.51	1.46	0.573	0.05	8.73%
	Clopidogrel (Standard)	0.868	1.53	1.29	0.422	0.24	56.87%
	Distilled Water (Blank)	0.857	1.65	1.62	0.763	0.03	3.93%
2	Extract (Sample)	0.857	1.556	1.513	0.656	0.043	6.55%
	Clopidogrel (Standard)	0.897	1.565	1.328	0.431	0.237	54.99%
	Distilled Water (Blank)	0.853	1.448	1.415	0.562	0.033	5.87%
3	Extract (Sample)	0.876	1.637	1.572	0.696	0.065	9.34%
	Clopidogrel (Standard)	0.85	1.641	1.355	0.505	0.286	56.63%
	Distilled Water (Blank)	0.913	1.601	1.561	0.648	0.04	6.17%
4	Extract (Sample)	0.874	1.401	1.357	0.483	0.044	9.11%
	Clopidogrel (Standard)	0.88	1.448	1.232	0.352	0.216	61.36%
	Distilled Water (Blank)	0.872	1.466	1.349	0.477	0.117	24.53%
5	Extract (Sample)	0.853	1.511	1.448	0.595	0.063	10.59%
	Clopidogrel (Standard)	0.862	1.688	1.368	0.506	0.32	63.24%
	Distilled Water (Blank)	0.88	1.699	1.682	0.802	0.017	2.12%

Here, W1 stands for weight of micro-tube, W2 stands for weight of clot with micro-tube, W3 stands for weight of Clot with micro-tube after clot disruption, W4 stands for weight of Clot after clot disruption, W5 stands for weight of released clot.

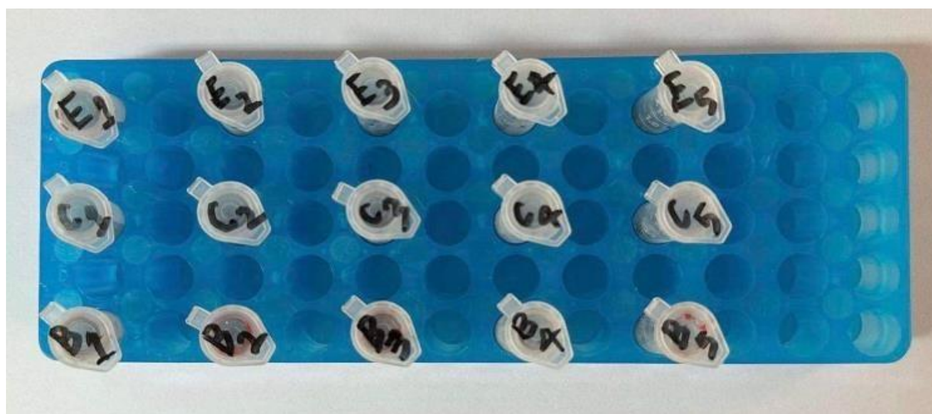

Figure 3.1: Collected blood sample of each volunteer in addition with sample, standard and blank.

From the data obtained, in most cases for each volunteer, the extract showed varying results with the clopidogrel standard which means the *Lagerstroemia thorelli* plant that shows actually has very less significant value as a thrombolytic agent.

3.2 Analysis of Anti-Microbial property of *Lagerstroemia thorelli*

3.2.1. Antimicrobial activity results of *Lagerstroemia thorelli* leaves

In order to conduct an investigation on the antimicrobial properties of an ethanol extract of Lagerstroemia thorelli leaves, various concentrations of the extract were generated, ranging from 500 mg/ml all the way up to 15.625 mg/ml. The area around the filter paper discs helped to determine the power and intensity to resist microbial growth.

Table 3.2 Results of antimicrobial activity of *Lagerstroemia thorelli* on gram positive bacteria.

Name of specimen	Standard	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.125 mg/ml	15.625 mg/ml
Staphylococcus aureus	3.5 cm	1.5 cm	1.5 cm	1.3 cm	1.2 cm	1 cm	0.9cm
Bacillus subtilis	3.3 cm	1.4 cm	1.1 cm	1 cm	0.9 cm	0.7 cm	0.5 cm

Table 3.3 Results of antimicrobial activity of *Lagerstroemia thorelli* on gram negative bacteria.

Name of specimen	Standard	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml	31.125 mg/ml	15.625 mg/ml
Escherichia coli	2.7 cm	1.3 cm	0.9 cm	0.7 cm	0.6 cm	No effect	No effect
Salmonella typhi	2.9 cm	-	-	-	-	-	-

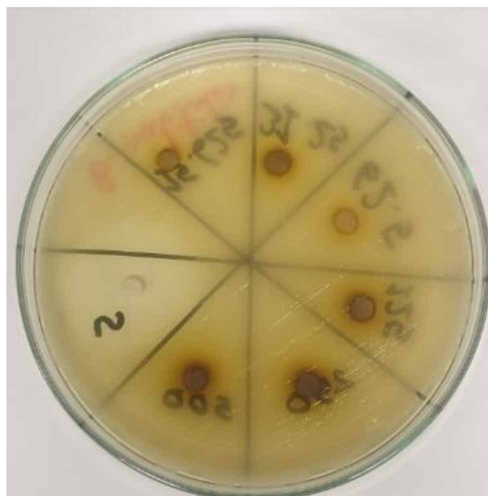

Figure 3.2: Results for *Bacillus subtilis*

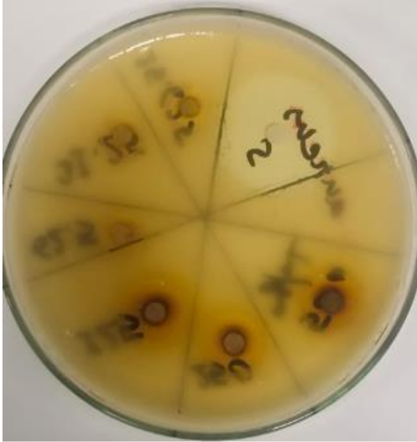

Figure 3.3: Results for *Staphylococcus aureus*

Figure 3.4: Results for *Salmonella typhi*

Figure 3.5: Results for *Escherichia coli*

Chapter 4 Discussion 4. *Lagerstroemia thorelli* Data discussion

4.1. Discussion on the found results and data on *Lagerstroemia thorelli.*

The data resulted from the experiments yielded some good results on antimicrobial activity even though there were not enough promising results on the thrombolytic activity. This may be due to some experimental error; the thrombolytic data was not accurate.

However, the antimicrobial properties looked quite promising as there were some good results on the zone of inhibition. Among the 4 specimens, 3 of them had good zone of inhibition. But on *Salmonella typhi,* there was no effect at all. Si, I believe *Lagerstroemia thorelli* has some good potential to contribute in antimicrobial studies.

4.2. Conclusion

Ethanol extract of *Lagerstroemia thorelli* provided sufficient results from the conducted experiments. It showed a good amount of antimicrobial property from 3 out of 4 test specimens of bacteria (2 gram-positive and 2 gram-negative). The thrombolytic data is quite unreliable at this moment but further investigation may yield some better results. So, I believe that the antimicrobial property has the potential to contribute to healthcare and medicinal studies.

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