Investigation of *in vitro* antioxidant and cytotoxic properties of *Lagerstroemia thorelli* leaf extract

By

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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 (Hons.)

School of Pharmacy
Brac University
September, 2023

Declaration

It is hereby declared that

1. The thesis submitted is my 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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Approval

The thesis titled "Investigation of *in vitro* antioxidant and cytotoxic properties of *Lagerstroemia thorelli* leaf extract" submitted by Imanul Kabir Lihu (19346001), of Spring, 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on September, 2023.

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Ethics Statement

The study does not involve any animal or human trial.

Abstract

Lagerstroemia thorelli belongs to the Lythraceae family and is natively known as choto jarul. This study was undertaken to identify the antioxidant and cytotoxic properties of this plant since no previous reports on its bioactivities were found in a thorough literature search. Antioxidant potential was evaluated using H_2O_2 scavenging activity and total antioxidant capacity (TAC) assays. Cytotoxicity was determined using MTT assay against cervical cancer cell line, HeLa. This plant extract demonstrated strong H_2O_2 scavenging activity with an IC_{50} of 2.22 µg/mL, which was close to that of standard ascorbic acid (IC_{50}=2.84 µg/mL). The TAC was 98.19 ± 3.864 mg AAE/g of dry extract at the highest concentration, 1200 µg/mL. However, the cytotoxicity study on HeLa cell line revealed low cytotoxic activity due to cell line characteristics. Thus, *Lagerstreomia thorelli* can be used in the treatment of free radical induced diseases due to possessing strong antioxidant activity.

Keywords: Antioxidant, Cytotoxic, HeLa, Vero, MTT assay, *Lagerstroemia thorelli*.

Dedication

Dedicated to my parents, younger sister and a good friend for their love and motivation.

Acknowledgement

First and foremost, all praise belongs to Almighty Allah (SWT) for endowing me with patience, knowledge, strength, dedication, good health, and opportunity to complete this project work for my bachelor's degree in pharmacy. Without the Almighty's guidance, I would've not been able to complete this project work.

I want to express my profound appreciation and debt of gratitude to my supervisor, Dr. Raushanara Akter, Professor at the School of Pharmacy, Brac University, for her unwavering support, direction, advice, and invaluable time while I was doing this project. She has also assisted me in finding solutions to all my project-related challenges because of her exceptional teaching abilities. It has been a great privilege and honor to work with her as she is committed and enriched me with her valuable knowledge.

I am also very grateful to Professor Dr. Eva Rahman Kabir, honorable Dean of School of Pharmacy, Brac University for giving me the opportunity and inspiration throughout my Bachelor of Pharmacy (Hons.) journey.

In addition, I want to express my gratitude to Professor Dr. Hasina Yasmin, the Program Director and Assistant Dean of the school of pharmacy, for her encouragement and assistance.

I would like to acknowledge the lab officers at Brac University for their continuous support in the lab. My sincere appreciation to the faculty members at the School of Pharmacy for their divine guidance.

Above all, I am indebted to my parents for their love and support all over my life that has encouraged and assisted me to accomplish my objectives.

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List of Acronyms

gm	Gram
mg	Milligram
µg	Microgram
m	Meter
cm	Centimeter
mL	Milliliter
IC_{50}	Median Inhibitory Concentration
LD_{50}	Median Lethal Dose
AAE	Ascorbic acid equivalent
DMSO	Dimethyl Sulfoxide
R^2	Regression Coefficient
Conc	Concentration
L. thorelli	*Lagerstroemia thorelli*
UV-vis	Ultraviolet Visible

Chapter 1

Introduction

1.1 An overview of the history of medicinal plants

Medicinal plants are defined as plants those have curative qualities or have positive pharmacological effects on human and animal bodies. For medicinal, tonic, purgative, or other health-promoting uses, medicinal plants' roots, leaves, seeds, bark, or other plant parts are employed. Nearly 5000 years ago, in India, China, and Egypt, as well as at least 2500 years ago, in Greece and Central Asia, the first documents of therapeutic herbs were recorded (Ang-Lee, 2001). The earliest known written record of the use of medicinal herbs to prepare medications is found on a slab of Sumerian clay from Nagpur, which dates to about 5000 years ago (Qiu, 2007). Many cultures throughout the world have employed medicinal herbs for thousands of years as a secure therapeutic method. Most civilized countries have created their own Materia Medica, which is a database of information on numerous plants used as medicines. The study of medicinal plants has become one of the oldest sciences in countries like China, India, Egypt, Greece. According to Firenzuoli & Gori (2007) natural remedies made from plants, animals, and minerals are the fundamental means of curing disease in humans. They contain certain qualities, such as synergistic activities. The different plant parts may interact with one another, which may be advantageous for both, damaging for either, or neutralize both of their negative effects. Hard-to-treat diseases like cancer can be significantly improved by chemicals produced from plants. Another quality that distinguishes plant components is their capacity to halt the progression of specific diseases.

1.2 Traditional medicine of Bangladesh

Traditional medical systems including Ayuerveda, Homeopathic remedies, Unani, and Folk medicine have all been used in Bangladesh for a long time. Bangladesh's traditional medical procedures have been used for centuries and are deeply ingrained in the local cultures (Haque et al., 2018). In Bangladesh, an extensive range of medicinal plants exist, and traditional healers claim that up to 1,000 of them can be used to treat patients (Mollik et al., 2010). The list of major medicinal plants in Bangladesh include *Adhatoda vasica, Holarrhena antidysenterica, Aegle marmelos, Mentha spicata, Aloe indica, Nigella sativa, Azadirachta indica, Paederia foetida, Cassia senna, Saraca indica, Cinnamomum verum,Tamarindus indica, Curcuma longa, Terminalia arjuna,Gmelina arborea, T. Belerica, Hemidesmus indicus, T. Chebul* (Saha, 2023b). *Acalypha indica, Adhatoda vasica, Allium sativum, Alstonia scholaris, Ananas sativus, Azadirachta indica, asiatica,Cephalandra indica, Coriandrum sativum, Curcuma longa, Cyperus rotundus, Solanum xanthocarpum, Terminalia arjuna, T. Chebula, Vitex negundo, Zingiber officinalis* are some of the abundantly used medicinal plants of Bangladesh (Saha, 2023b).

1.3 Available medicinal Plants in Bangladesh and their usage

Bangladesh is a part of the Indian subcontinent with extensive array of plant species. The broad range of plants and genetic diversity has helped people in this country to use these components of traditional medicine to treat different health issues. It is an integral part of the Bengali culture. “Demographically” Bangladesh is enriched with natural resources such as, forest resources, biodiversity, minerals, and cultivated crops. Investigation of these natural resources led to the development of the Ayurvedic, Unani, and Homeopathic systems used in this country(Ghani, 2003).

The folk medicine system is used extensively by people in this country and the practitioners of folk medicine are called kavirajes or vaidyas (Rahmatullah et al., 2011).Bangladesh's tribal populations have local medical practitioners who are akin to the kavirajes' folk medicine system. Both kavirajes and tribal medical practitioners used medicinal plants to cure illnesses. The therapeutic techniques were kept inside the family by baidyas and tribal physicians and the expertise was passed down from generation to generation. The kavirajes and tribal healers have therefore been acquainted with the plant species for thousands of years. The estimated number of plant species in Bangladesh is 6500, with 5,700 angiosperm species, woody legumes (68), fiber plants (130), medicinal plants (500), orchids (29), gymnosperms (3), and pteridophytes (1,700) among them (Islam, 2003). Medicinal plants are used to treat or manage a wide range of illnesses, often in the form of extracts, decoctions, juices, powders, pastes, etc. (Rahmatullah et al., 2011).

Table 1: Commonly used medicinal plants in Bangladesh (Bardhan et al., 2018)

Plant species	**Family**	**Local name**	**Traditional uses**
Abutilon indicum L.	Malvaceae	Potari	Gonorrhea and vaginal infections are treated with leaf extract. Antifungal, antimycotic and antidiarrheal activity.
Adina sessilifolia L.	Rubiaceae	Kam gass	Leaf paste used for impetigo, folliculities, minor cellilutis and fungal infections.
Bridelia retusa L.	Euphorbiaceae	Kantakui/ Kantakhasi	Ripe fruits, bark, leaf paste used for impetigo, folliculities, minor cellilutis and fungal infections.
Caesalpinia bonduc L.	Fabaceae	Nata,natai, kokoi, dahara	For urinary tract infections, the plant's aqueous extract is used, along with prepared root, leaves, or tablets made of dried, powdered leaves.
Premna esculenta L.	Verbenaceae	Lalana, Lalong	Pseudomonas aeruginosa, which causes folliculitis, is susceptible to *Premna esculenta L.* employed topically to treat fungus infections.

Urena lobeta L.	Jangli ghagra	Malvaceae	Broad spectrum action is demonstrated by an Urena lobata root extract against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Micrococcus luteus, Escherichia coli, Klebsiella pneumoniae, Shigella dysenteriae,* and *Vibrio cholera*.
Nyctanthes arbor-tristis L.	Shefali, Night jasmine	Oleaceae	Antifilarial qualities are present in flower extract. Leishmaniasis and fungus infections are treated with seed extract.
Holarrhena antidysentrica L.	Bitter oleander	Apocynaceae	Used in dysentery by local people.
Phyllanthus niruri L.	Phyllanthaceae	Bhui Amla or Bhui Amloki	It possesses anti-malarial, anti-viral activity against hepatitis B virus.

1.4 Medicinal plants with antioxidant properties

Bangladesh has a great resource of a wide variety of medicinal plants containing strong antioxidant properties. Medicinal plants with antioxidant properties are used against oxidative stress induced diseases such as, Parkinson's disease, Alzheimer's disease etc. Carotenoids, benzoic acid derivatives, flavonoids, flavones, flavonones, cinnamic acid derivatives, anthocyanins are some of the antioxidants found in the medicinal plants of Bangladesh (Ehsanul & Rahman, 2008).

Table 2: Medicinal plants of Bangladesh with antioxidant properties (Ahmed et al., 2006; Rahman et al., 2006a)

Plant name	**Family**	**Local name**	**Antioxidant Potential**
Cloreodandrum viscosum Vent.	Verbenaceae	Bhat	Strong
Cerebra odollam Gaertin.	Apocynaceae	Dabur	Moderate
Derris ulginosa Benth.	Leguminosae	Kalialata	Strong
Calycopertis floribunda Lamk.	Combertaceae	Guicha lata	High

1.5 Medicinal plants with cytotoxic properties

Natural products are essential in the treatment of cancer since several significant anticancer medicines have been developed from plants, including topoisomerase I inhibitors, taxanes, and vinca alkaloids (Washart, 2014). Many Bangladeshi medicinal plants are traditionally recognized to possess cytotoxic and antitumor effects, and some have a folkloric reputation for being utilized in the treatment of various cancers (Ghani, 2003). There are more than a thousand of plant species with substantial anticancer potential have been identified. In Bangladesh, for the last 2 decades, 64 plants have been studied for its pharmacological activities with some of them investigated for anticancer as well as cytotoxic activity (Akter et al., 2013).

Table 3: Bangladeshi medicinal plants with cytotoxic and anticancer properties (Nutan et al., 1997)

Botanical Name	**Family**	**Availability**
Acacia lebbeck Benth.	Leguminosae	All over Bangladesh
Amorphophalus campanulatus (Roxb.) BI.	Araceae	All over Bangladesh
Daucas carota Linn.	Umbelliferae	All over Bangladesh
Euphorbia tirucali Linn.	Euphorbiaceae	Bangladeshi gardens
Mangifera indica Linn.	Anacardiaceae	All over Bangladesh
Nymphae rubra Roxb.	Nymphaeceae	All over Bangladesh
Xanthum indicum koenig	Compositae	All over Bangladesh

1.6 Reactive oxygen species and free radical production in the body

Radical refers to a molecule containing one or more unpaired electrons. Reactive oxygen species (ROS) refers to highly reactive oxygen metabolites in living organisms due to the result of aerobic activities. Reactive oxygen species (ROS) are typically characterized as by-products of metabolic processes produced by biological systems. Examples of ROS include, superoxide radicals (O2 •), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen (1O2) (Sato et al., 2013). Apoptosis, immunity, differentiation, protein phosphorylation, and the activation of a variety of transcriptional factors are all reliant on proper ROS production and presence inside cells (Rajendran et al., 2014). Free radicals can be produced by both endogenous and exogenous sources. Endogenous free radical production occurs due to ischemia, inflammation, immune cell

activation, infection, cancer, stress, overwork, and aging. Metallic substances, contamination from the environment, radiation, and other factors can produce exogenous free radicals.

1.6.1 Incidence of oxidative stress in various disorders

Oxidative stress is a phenomenon caused by an imbalance between the capacity of biological systems to eliminate certain reactive products and the synthesis and creation of reactive oxygen species (ROS) in cells and tissues. According to Pizzino et al. (2017), although ROS are normally generated as byproducts of oxygen metabolism and have a variety of physiological functions, such as regulating cell signaling, environmental stressors like UV, ionizing radiation, pollutants, heavy metals, and xenobiotics like antiblastic drugs substantially raise ROS production, causing an imbalance that harms cells and tissues. Multiple investigations have shown that, to various extents, oxidative stress can contribute to the development and/or advancement of a number of diseases, including diabetes, cancer, metabolic conditions, atherosclerosis, and heart diseases (Taniyama & Griendling, 2003).

1.6.2 Free radical and oxidative stress-induced harm to the body and its tissues

The potential of proteins and DNA is altered because of the interaction of free radicals and ROS with lipids, which has an impact on cell function and cell damage. Polyunsaturated fatty acids contain homo conjugated double bonds, which are extremely susceptible to chemical reactions mediated by free radicals. The removal of bisallylic hydrogen atoms from such double bonds is facilitated by the low homolytic dissociation energy. Rapid chemical reactions between the resultant radicals and the molecular oxygen generated through these reactions can exacerbate the lipid peroxidation reaction. This results in affecting the ion channels, inactivating the membrane

transport protein or enzyme and further the lipid bilayer might become rigid or more permeable. When a protein is oxidized, sulfhydryl groups are lost through thiol oxidation, mixed disulfides are created, or methionine residues are oxidized to the corresponding sulfoxides (Kehrer & Klotz, 2015).Aldehydes or ketones are produced because of the alteration of amino acids, hydroxy peroxides or ring cleavage in histidine, tryptophan residues leading to cell signal impairing and affecting proteins' framework and functional activities. This leads to a variety of illnesses.

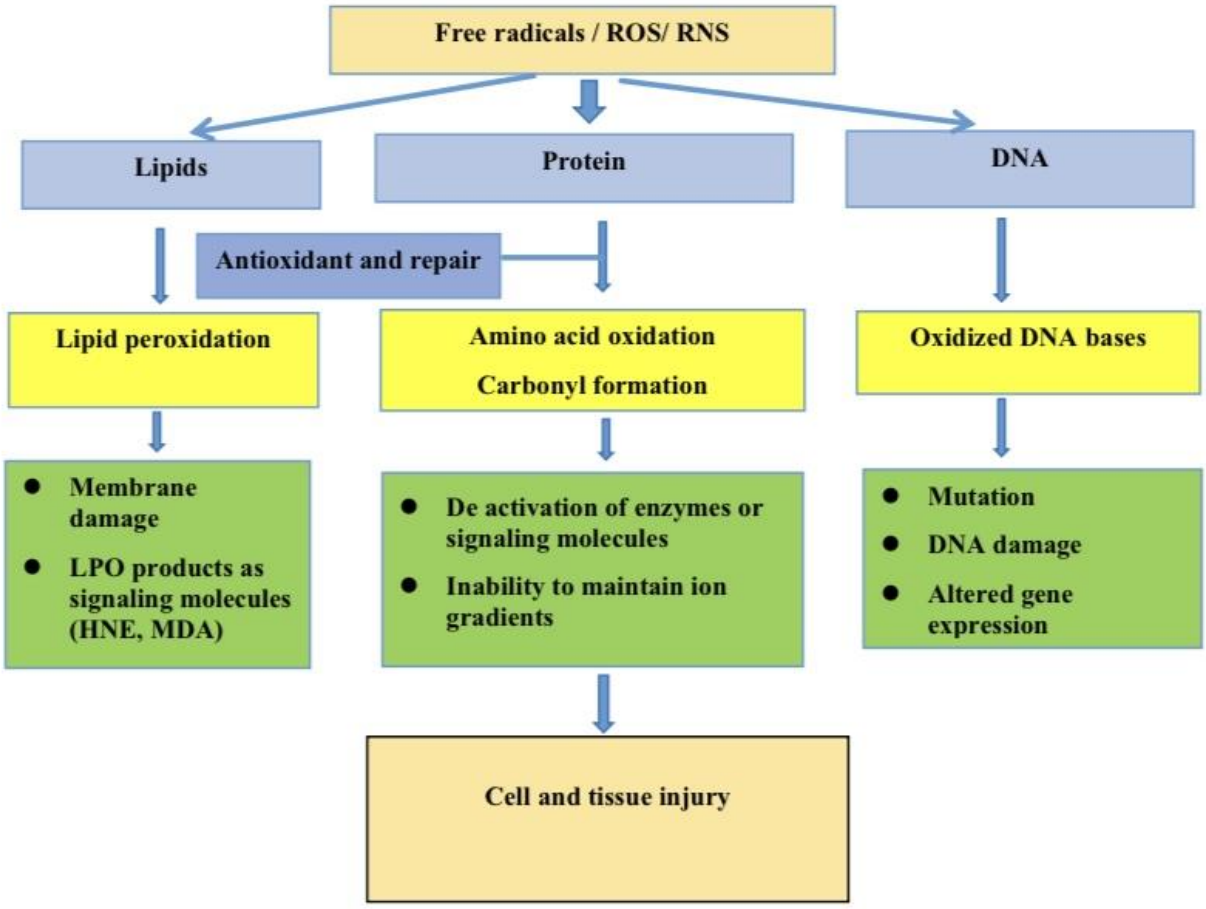

Figure 1: MOA through which free radicals causes cell damage (Kehrer & Klotz, 2015)

Interaction between free radical and DNA affects the gene integrity and gene regulation resulting in detrimental changes in cells. The nucleotide and sugars of DNA are both modified by reactive oxygen species (Cooke et al., 2003). Insufficient DNA repair process led to permanent DNA

damage and cell death. This alters the DNA sequence permanently leading to mutation and transcription errors.

1.7 Free radicals and oxidative stress induced diseases

Due to their reactive qualities, ROS have a high harmful propensity toward bio molecules like lipid, proteins, carbohydrates, and nucleic acids, which can cause cellular damage and even death in extreme circumstances. However, their buildup below dangerous levels have recently been seen as a benefit for plant growth and development, but ROS are clearly poisonous for living cells when produced in excess (Sachdev et al., 2023). Chronic and degenerative diseases, as well as acute pathologies like trauma and stroke, are all brought on by unchecked oxidative stress. It also hastens the aging process in the tissues of the body.

Table 4: Diseases/tissue injuries with free radical component (Kehrer & Klotz, 2015)

Lung	**Nervous system**	**Skin**	**Heart and cardio-vascular system**	**Eye**	**Immune disorders**
Normobaric hyperoxic injury	Alzheimer's disease	Radiation: solar or ionizing	Ischemia/ reperfusion: after infarction or transplant	Retinopathy of prematurity (oxygen)	Rheumatoid arthritis
Bronchopulmonary dysplasia	Amyotrophic lateral sclerosis	Thermal injury	Atherosclerosis	Photic retinopathy	Autoimmune diseases such as, Lupus
Asbestosis, Cigarette smoke	Down's syndrome	Contact dermatitis	Selenium deficiency (Keshan disease)		
Idiopathic pulmonary fibrosis	Myasthenia gravis,	Porphyria,	Hemochromatosis	Cataracts	Inflammation

	Parkinson's disease	Chemicals: Photosensitizers e.g. Tetracyclines			

1.8 Antioxidants

Any substance that significantly slows down or stops the oxidation of an oxidizable substrate when it is present in low concentrations relative to that substrate is referred to as an antioxidant (Halliwell & Gutteridge, 1995). Antioxidants play an important role in maintaining the body defense system against reactive oxygen species.

Classification of antioxidants

The antioxidants are classified according to their solubility, origin, and mechanism of action.

Table 5*: Classification of antioxidants based on mechanism of actions* (Maity et al., 2022)

Antioxidants based on the mechanism of actions			
By preferentially oxidized	**By blocking oxidative chain reaction**	**By synergestic action**	**By chelating the trace amount of heavy metals**
Sodium Bisulphate	BHA	H_3PO_4	EDTA salts
Thiourea	BHT	Citric acid	
Sodium Meta bisulphite	Alpha tocopherol		
Vitamin C			

Table 6: Classification of antioxidants based on origin and solubility (Maity et al., 2022)

Origin		**Solubility**	
Natural antioxidants	**Synthetic antioxidants**	**Water soluble**	**Lipid soluble**
Ascorbic acid	BHA	Glutathione	Carotenes
Citric acid	BHT	Vitamin C	Alpha tocopherol (Vitamin E)
Glutathione	EDTA salts	Lipoic acid	Ubiquinol (Coenzyme Q)
Carotene	H_3PO_4	Uric acid	

1.9 MTT assay for the analysis of *in vitro* cytotoxicity

The MTT assay is a colorimetric technique to identify cytotoxicity or cytostatic activity. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay depends on the measurement of mitochondrial activity by formazan crystal formation from MTT via viable cells (van Meerloo et al., 2011). It is frequently used to assess the *in vitro* cytotoxic effects of medications on cell lines as the total mitochondrial activity is proportional to the number of viable cells in the majority of cell populations.

1.9.1 HeLa cell line (Human cervical cancer)

Table 7: HeLa cell line

Cell line	Morphology	Origin	Species	Ploidy	Characteristics	Supplier
HeLa	Epithelial	Cervix	Human	Aneuploid	G6PD type A	CARS, DU

HeLa cells are human cell cultures that were created ex vivo and are made from cell lines that were removed during a biopsy from living tissue. They are cancerous cells from a strain that have persistently developed ever since their discovery in a cervical carcinoma patient in 1951. As the HeLa cells never expire and continue to multiply because of sustenance, they are considered immortal cancer cells.

1.9.2 Vero cell line

Table 8: Vero cell line

Cell line	Morphology	Origin	Species	Ploidy	Characteristics	Supplier
Vero	Epithelial	Kidney	Monkey	Aneuploid	Viral subtract and assay	CARS, DU

Vero cells are one of the most widely used mammalian continuous cell lines in research, originally isolated from the kidney of an African green monkey in the early 1960s.This cell line has been widely utilized in virology study, as well as in the growth and research on intracellular microorganisms and parasites, evaluation of the molecular effects of drugs, poisons on mammalian cells.

1.10 A description of the plant *Lagerstroemia thorelli*

Lagerstroemia thorelli belongs to the Lythraceae family. It is locally called Jarul or choto Jarul. The plant is habitat to high lands. It is used as an attractive plant in landscapes. The species is prevalent in the Sylhet region of Bangladesh. The Lythraceae family is known as loosestrife and pomegranate family (Xu & Deng, 2017).The family Lythraceae have about 32 genera and 620 species of dicotyledonous flowering plants that are distributed across the tropical and subtropical areas (Xu & Deng, 2017). From India to Northern Australia, Lagerstroemia is spread across China, Japan, and Malesia (Pounders et al., 2017). The plant can be recommended for gardening in urban areas of Bangladesh and Indian sub-continent.

Morphology

Lagerstoremia thorelli, a small to medium-sized tree, ranges from 15 to 35 feet in height. It is a naturalized exotic tree used in roadside plantings and in parks. Lythraceae family has distinguishable characteristics in crumpled petals in the bud. The seed's integument is composed of several layers. It includes mostly seasonal or perennial herbs as well as certain shrubs and trees. *Lagerstroemia thorelli* has been estimated to be originated in the tropical regions of Asia, specifically in the Indian subcontinent (Randhawa & Mukhopadhyay, 1986). It has smooth barks and is thornless. The bark is pale yellowish grey. The leaves of *L. thorelli* are opposite, eliptic and the size ranges from 10.0 Ã— 5.5 cm. The petals of the flowers are pink and have no fragrance at all. The size is around 2 cm. Also, the fruits are capsule, egg shaped, brown in color and 1.3 cm in size. The fruits are surrounded by persistent calyx at the base. The seeds are many, 8 cm long and winged. Characteristics such as attractive fall foliage, good drought tolerance makes crape myrtle ideal for formal and informal landscapes and in urban areas. Native to the Indian subcontinent, the

southeast region of Asia, the northern part of Australia, and portions of Oceania, these flowering plants are cultivated in warmer climates around the world (Pounders et al., 2017).

Fruits and Flowering period

April-September is the fruits and flowering period of *L. thorelli.*

Figure 2: Lagerstroemia thorelli

Taxonomic classification of *Lagerstroemia thorelli*

Table 9: Taxonomic hierarchy of Lagerstroemia thorelli

Rank	Scientific name and Common name
Kingdom	Plantae - Plants
Phylum	Spermatophyta
Class	Magnoliopsida – Dicotyledons
Order	Myrtales
Family	Lythraceae
Genus	Lagerstroemia
Species	*Lagerstroemia thorelli*

1.11 Information on chemical constituents of other species of *Lagerstroemia thorelli*

There haven't been any substantial investigations on *Lagerstroemia thorelli*'s chemical components. According to Al-Snafi (2019)alkaloids, cardiac glycosides, tannins, saponins, sterols, triterpenes, anthraquinones, reducing agents, flavonoids (chalcones, flavanones, and dihydroflavonols), and phenolic glycosides (strosides A–C) are among the chemical components of *Lagerstoremia indica.* Sesamin, β-sitosterol, clauslactone-k, betulinic acid, lingueresinol, are some of the chemical components of *Lagerstroemia floribunda*, along with 23-hydroxyursolic acid, alphitolic acid, dihydro-B-cyclopyrethrosin.(Sikarwar et al., 2016).Ellagitannins, ellagic acid, ellagic acid sulfate, and four methyl ellagic acid derivatives named corosolic acid, gallic acid, 4-hydroxybenzoic acid, 3-O-methyl protocatechuic acid, caffeic acid, p coumaric acid,

kaempferol, quercetin, and isoquercitrin—are the major chemical components of *Lagerstroemia speciosa* leaves (Bai et al., 2008).The Phytochemical constituents of *Lagerstroemia parviflora* include phenols, flavonoids, tannins, saponins, alkaloids, fixed oil, and lipids (Jaisinghani & Magarde, 2022).

1.12 Information on the pharmacological properties of other species of *Lagerstroemia thorelli*

Two most popular species of Lagerstroemia are *Lagerstroemia speciosa* and *Lagerstoremia indica* which are cultivated in warmer region(Egolf & Andrick, 1978). *Lagerstoremia speciosa* has been used to treat diarrhea and diabetes. The Lagerstroemia genus tends to possess good antioxidant, antidiabetic, antimicrobial, and thrombolytic properties. *Lagerstroemia specisoa* leaf extracts have nephroprotective, hepatoprotective and diuretic activity (Sharmin et al., 2018). Aside from anti-inflammatory properties, *Lagerstoremia indica* also has antipyretic, analgesic, anticancer, anti-Alzheimer's, antidiabetic, hepatoprotective, and antithrombin activities (Al-Snafi, 2019). *Lagerstoemia parviflora* is a rare medicinal herb that can be used to treat intestinal constriction and syphilis. The species possess antimicrobial, antioxidant, antitussive, cytotoxic, antiobesity, xanthine oxidase inhibition, antiviral properties (Jaisinghani & Magarde, 2022). Treatment of obesity and hyperglycemia in patients with type 2 diabetes with *L. speciosa* leaf extracts has been effective (Liu et al., 2001). *L. floribunda* possesses antigout activity, antifibrotic, cytotoxic, antibacterial, and antiviral activity (Sikarwar et al., 2016). Also, the plant has been used traditionally to treat stomach problems, lowering blood sugar, and known as slimming tea in Japan.

1.13 Rationale of the project

The plant species *L. thorelli* is a member of the Lythraceae family, and other Lageratroemia species often have good antioxidant, antidiabetic, antibacterial, and thrombolytic activities. For instance, *Lagerstoremia parviflora* has cytotoxic, antiviral, and antioxidant activities. *L. indica* is an analgesic and antioxidant species. *L. speciosa* has significant effects on diabetes. Since the *Lagerstroemia thorelli* species belongs to the same genus Lagerstroemia and share similar chemical components like phenols, polyphenols, tannins, and alkaloids as well as possessing the previously mentioned properties, it is expected that *Lagerstroemia thorelli* will also have an identical biological property. The use of medicinal plants as a treatment for human ailments dates to ancient times. The potential actions and features of the various species of Lagerstroemia in the treatment of diabetes, cancer, obesity, and other health issues have been demonstrated.

A comprehensive review of the literature revealed that this plant has never been the subject of research. Yet, a wide range of earlier studies on species belonging to the same family showed potential bioactivities, which supported and validated the choice of this plant for my current investigation. So, *L. thorelli* was chosen to investigate its cytotoxic and antioxidant properties because cytotoxic properties and antioxidant activity using the selected cell lines and selected method have not been determined previously.

1. 14 Aim and objectives of the project

Aim

The aim of the present study was to evaluate the antioxidant and cytotoxic properties of *Lagerstroemia thorelli* leaves extract.

Objectives

The study had the following objectives:

- To gather data on the previous bioactivity studies of ethanolic extract of *L. thorelli* leaves.
- To assess cytotoxic activity of the selected plant against cervical cancer cell line using MTT test.
- To investigate antioxidant property of selected plant using the H_2O_2 scavenging activity and total antioxidant capacity method.

Chapter 2

Methodology

2.1 Plant material collection and preparation

Dr. Shahana Sharmin, an Associate professor in the Brac University School of Pharmacy, provided the extract of the *Lagerstroemia thorelli* leaf. Previous experiments were done by the project students using the plant extract to analysis its properties. She collected the healthy green leaves of *Lagerstroemia thorelli* from Nabiganj upzila in Habiganj district, Sylhet division, Bangladesh. The specimen was given the accession number DACB-87494. The leaves were then extracted with ethanol.

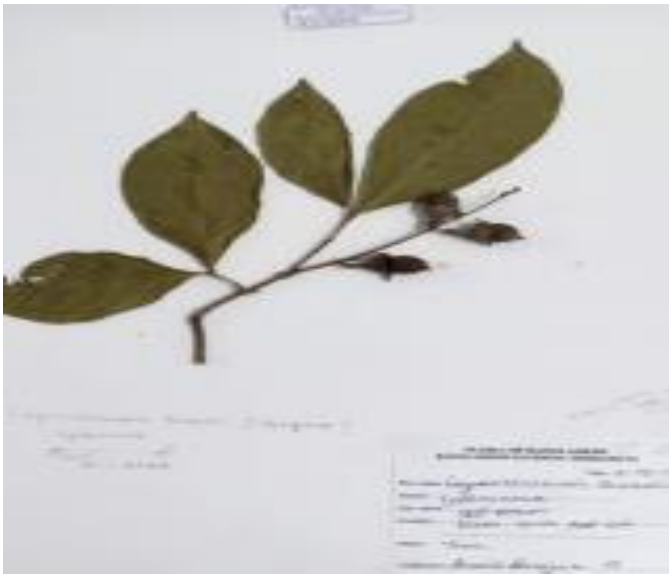

Figure 3: Lagerstroemia thorelli leaves

2.2 The fundamentals of antioxidant study

H_2O_2 scavenging activity assay and the total antioxidant capacity of the plant extract were used to establish the antioxidant study of the medicinal plant.

2.3 H_2O_2 scavenging activity assay

The Nabavi et al., (2008) approach was utilized to determine the H_2O_2 scavenging activity. Using a 40 mM hydrogen peroxide solution in a 50 mM phosphate buffer (with a pH 7.4), the absorbance at 230 nm was detected. Then, 1 mL of sample extract or standard was combined with 2 mL of hydrogen peroxide solution. The absorbance was measured after 10 minutes and compared to a control solution. Phosphate buffer was used to create the blank solution; no hydrogen peroxide was added. Then the formula that follows was used to determine the H_2O_2 scavenging activity:

H_2O_2 scavenge (%)

$$= [(A_{230\ Control} - A_{230\ Sample}) / A_{230\ Control}] \times 100$$

Apparatus and Reagents

Table 10: List of materials and reagents in H_2O_2 scavenging activity assay

Apparatus	Reagents
UV-Vis Spectrophotometer	H_2O_2 solution 40 mM
Test tubes	Phosphate buffer (pH 7.4)
Volumetric flask	Ethanol
Pipette	L-Ascorbic acid
Weighing machine	Leaves extract
	Distilled water

H_2O_2 preparation

4.42 mL of 30% H_2O_2 was dissolved in 50 mL of phosphate buffer (pH 7.4) to make 40 mM concentration of H_2O_2. The solution of H_2O_2 was prepared before the experiment and kept in a dark amber volumetric flask. A fresh solution was prepared each time to perform the experiment.

Phosphate buffer preparation (pH 7.4)

A phosphate buffer containing a pH of 7.4 was prepared by combining 6.8 g of potassium dihydrogen phosphate (KH_2PO_4) and 1.5 g of sodium hydroxide in 1,000 milliliters of distilled water.

Sample preparation for H_2O_2 activity of *L. thorelli*

16 mg of the leaf extract of *L. thorelli* was taken in 64 mL of ethanol to prepare the test sample with the concentration of 250 µg/mL. By repeatedly diluting this stock solution, various solutions with desirable concentration ranging from 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, and 15.63 µg/mL were created. After that, the samples were appropriately marked and then placed in a dry, dark environment.

Standard preparation for H_2O_2 activity of *L. thorelli*

64 mL of ethanol was used to dissolve 16 mg of L-Ascorbic acid, and the standard stock solution had a concentration of 250 µg/mL. Using serial dilution, solutions with concentrations of 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, and 15.63 µg/mL were produced.

Blank Preparation

To make the blank solution, a test tube containing 1.8 mL of phosphate buffer and 0.3 mL of ethanol was added with the appropriate labeling.

Experimental procedure

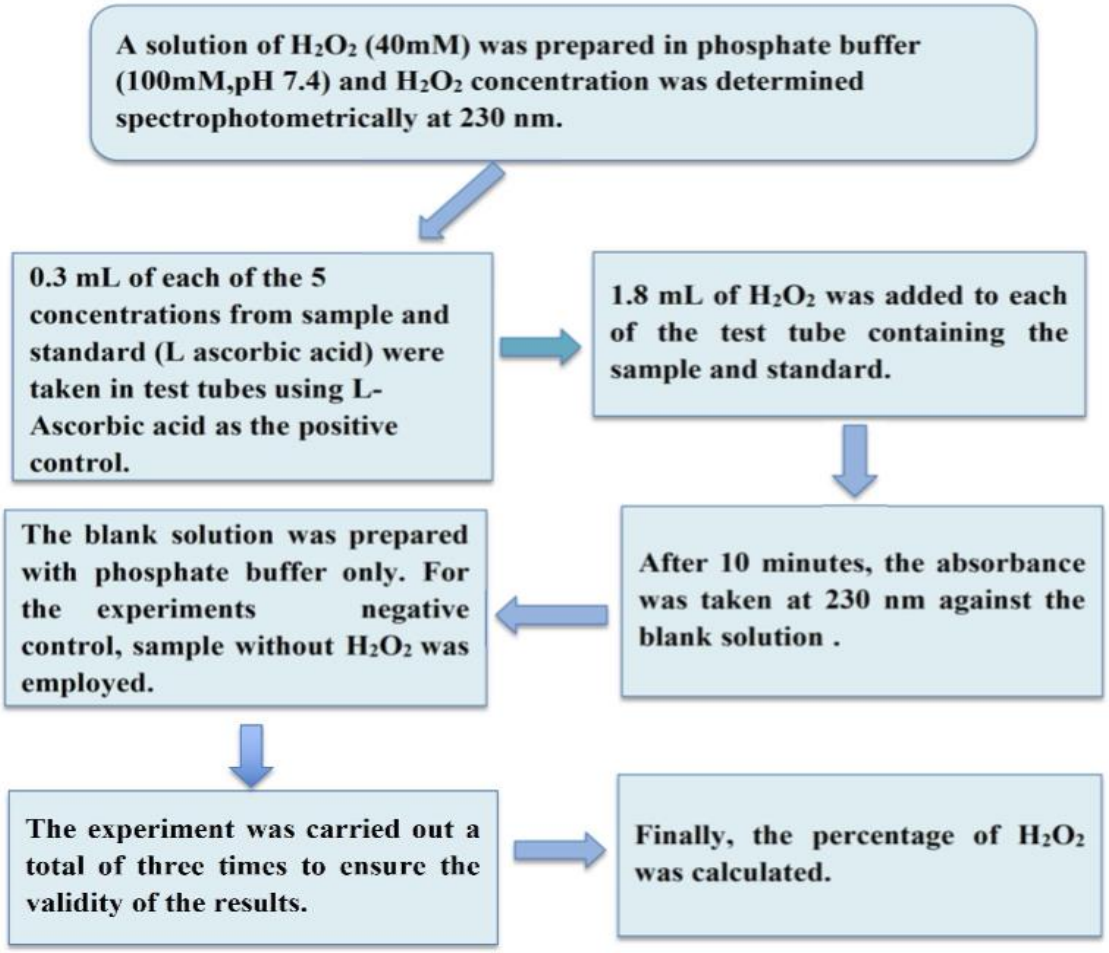

Figure 4: Experimental procedure of H_2O_2 scavenging activity determination

2.4 Total antioxidant capacity study

The phosphomolybdenum method developed by Prieto et al. (1999) also known as the total antioxidant capacity assay, is centered upon the reduction of molybdenum (VI) to molybdenum (V) by the sample, which generates a molybdenum (V)-phosphate complex that turns green at acidic pH levels.

The calibration curve was created using spectrophotometric measurements of absorbance at 695 nm using L-Ascorbic acid as a reference in a number of concentrations.

The total antioxidant capacity of every portion was expressed as ascorbic acid equivalent (AAE) using the equation below.

$A = (c \times V) \div m$

Here, A = Ascorbic acid equivalent (mg of ascorbic acid per gram of dry plant extract).

C = Ascorbic acid concentration (mg/mL).

V = Sample volume (mL)

M = Sample weight (g)

By using the following method, the total antioxidant capacity of *Lagerstroemia thorelli* was determined (Londonkar et al., 2019).

Names of Chemicals and Reagents

Table 11: List of reagents and chemicals for TAC assay

Names of the reagents and Chemicals	Supplier
Ethanol	Active fine chemical Ltd, Bangladesh
Ammonium Molybdate	Active fine chemical Ltd, Bangladesh
Trisodium Phosphate ($Na_3PO_4.12H_2O$)	Merck, Germany
Ammonium Molybdate	Merck, Germany
L-Ascorbic acid	Merck, Germany

Preparation of Sulfuric acid solution

100 mL of 0.6 M sulfuric acid solution was prepared by taking 3.28 mL of 98% concentrated sulfuric acid in a 100 mL volumetric flask, followed by dilution with distilled water up to 100 mL mark.

Preparation of Ammonium Molybdate solution

100 mL of 0.004 M ammonium molybdate solution was prepared by taking 4.49 gram of ammonium molybdate in a 100 mL volumetric flask, followed by dilution with distilled water up to 100 mL mark.

Trisodium Phosphate solution

1.06 g of trisodium phosphate was placed in a 100 mL volumetric flask, and distilled water was added to dilute it to the desired concentration. Thus, 100 mL of 0.028 M trisodium phosphate solution was prepared.

Sample Preparation

To create a stock solution with a concentration of 12 mg/mL, 120 milligrams of *L. thorelli* extract was measured and diluted in 10 mL of ethanol. The sample concentrations of 1200 µg/mL, 600 µg/mL, 300µg/mL, 150 µg/mL, and 75 µg/mL were made by serial dilution of the sample stock solution.

Standard Preparation

To calculate the total antioxidant capacity, ascorbic acid was utilized as the standard.The standard stock solution was created by mixing 10 mL of ethanol with 120 milligrams of ascorbic acid to formulate the standard solution. The standard concentrations of 1200 µg/mL, 600 µg/mL, 300 µg/mL, 150 g µg/mL, and 75 µg/mL were made by sequentially diluting of the standard stock solution.

Blank Preparation

A blank solution was created using 300 µL ethanol and 3 mL reagent solution.

Experimental procedure

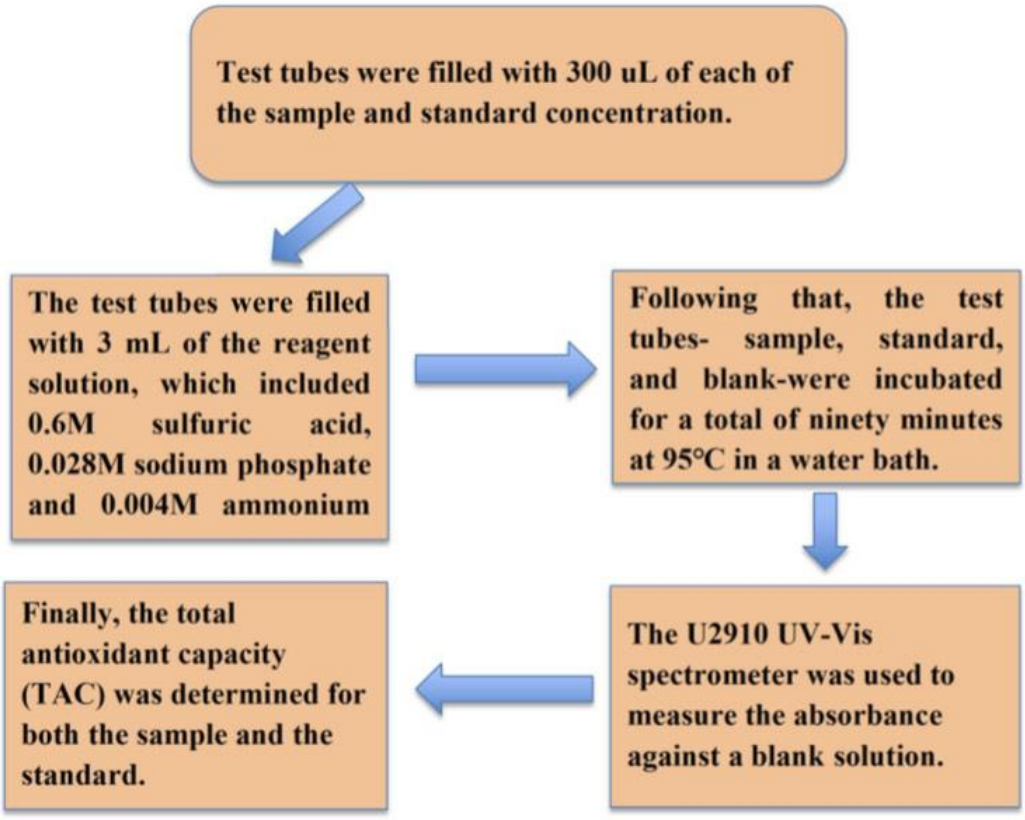

Figure 5: Experimental procedure of Total antioxidant capacity determination

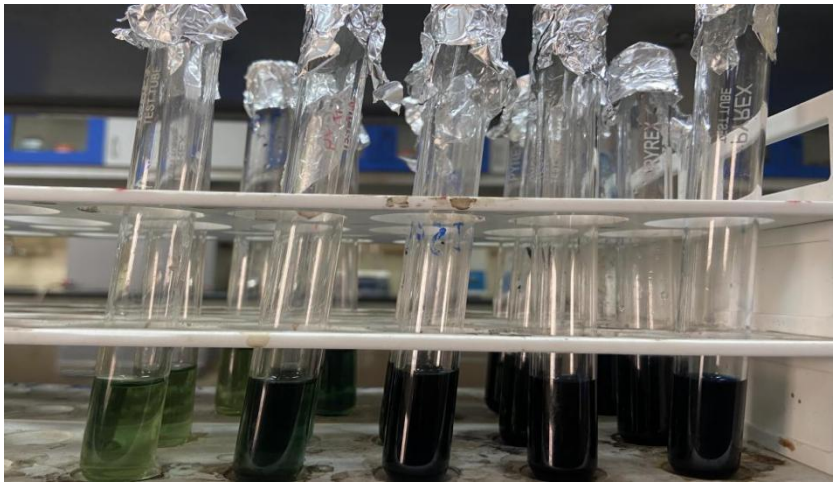

Figure 6: Total antioxidant capacity assay

In the experiment, all the above steps were followed to calculate the total antioxidant capacity of *Lagerstroemia thorelli* and ascorbic acid.

2.5 *In vitro* Cytotoxicity Property analysis

Media

1% penicillin-streptomycin (1:1), 0.2% gentamycin, and 10% fetal bovine serum (FBS) were all contained in DMEM (Dulbecco's Modified Eagles' medium).

Consumables

Table 12: List of consumables for MTT assay

96 well plate	Cell culture media
15 mL tubs	Antibiotics (P+S)
Tips	Gentamycin
Gloves	Serological pipette
Cultural flask	Trypsin

Making of solutions

1 % Penicillin-Streptomycin solution

The antibiotic “penicillin-streptomycin solution”, commonly referred to as pen-strep, is widely employed to eradicate microbial growth in *in vitro* settings (*Penicillin-Streptomycin (10,000*

U/ML), n.d.). Penicillin inhibits peptidoglycan synthesis and streptomycin inhibits protein synthesis leading to bacterial death. Both type of bacteria is susceptible to the Pen-strep. The mixture employed inhibits bacterial growth. To prevent pathogenic bacteria and sustain the sterile environment throughout the experiment, Penicillin-Streptomycin solution was employed in the MTT assay. Each milliliter of the solution contains 10,000 units of penicillin and 10 milligrams of streptomycin.

Dulbecco's Modified Eagles' Medium

Dulbecco's modified eagles' medium is used for tissue culture growth. It contains organic substances such as amino acids, glucose, and (Rohanová et al., 2014). A variety of mammalian cell types can develop in cell culture with the help of the medium DMEM. Cell lines including HeLa, Vero, COS-7, and cells including neurons, smooth muscle cells, primary fibroblasts are cultured in DMEM. A modified DMEM media contains 10% Fetal bovine serum, 1% pencillin-streptomycin solution, and 0.2% gentamycin solution. Addition of vitamins and FBS enhances the organic part of DMEM (Rohanová et al., 2014).

0.2% Gentamycin solution

A 50 mg/mL stock solution of gentamycin was created by dissolving it in 1 mL of deionized water. To sterilize the mixture, a 0.2 filter unit was utilized.

10% Fetal Bovine Serum

Fetal bovine serum (FBS) is frequently used as a growth supplement in cell culture media. These are crucial for both *in vivo* and *in vitro* cell development and maintenance. FBS contains vitamins (especially fat-soluble vitamins like A, D, E, and K), proteins, carbs, lipids, hormones, growth factors, minerals, and trace elements (Francisco Javier Preciado-Gutiérrez et al., 2023). To make 10% FBS, 500 mL of DMEM were mixed with 50 mL of fetal bovine serum (FBS).

Celltiter 96 assay kit

Celltiter 96 aqueous non-radioactive cell proliferation test is a collection of approved reagents that offers a quick and practical way to figure out the cell number in tests for proliferation, cell attachment, chemotaxis, cell death, and cytotoxicity (Protocol, 2001). In the current investigation, a mixture of dye solution was applied to 96-well plates for cell culture. The assay kit was supplied from Promega, (USA).

Preparation of plant extract

4 quantities - 2.5 mg/mL, 2 mg/mL, 1.5 mg/mL, and 1 mg/mL of the leaf extract of *Lagerstoremia thorelli* were used in the experiment. To create the stock solution, 1 mL of Dimethyl sulfoxide (DMSO) solvent and 10 mg of leaf extract were combined to make the mother solution of 10 mg/mL concentration. DMSO was used for diluting the stock solution to 2.5 mg/mL. With DMSO, additional dilution was carried out to create solutions of the required concentrations. After that, the samples were filtered before examining.

Cell culture

Assay plates preparation

Both the cancer (HeLa) and non-cancer (Vero) cell lines were used to prepare the assay plates in DMEM media.

Cells thawing

In cryovials filled with liquid nitrogen, HeLa and Vero cell lines were maintained. When a little amount of ice appeared in the cryovial, it was promptly defrosted by gently spinning it in a water bath set to 37°C. Therefore, beneath a laminar airflow hood, drop by drop, the defrosted HeLa cells and Vero cells were transferred into two different centrifuge tubes containing the DMEM medium. After that, the liquid was gently poured over the cells, and they were placed in culture plates.

Passage of cells

A fresh cell suspension was produced by cell passaging. The cells were transferred to a new medium. FBS was used to rinse the utilized cultured media, and 800 µL of trypsin was added to separate the cells from the upper part of the culture vessels. A microscope was used to look for the detachment after the cells had been cultured. 90% of the cells were detached before 5 mL of DMEM fluid was added to the capillaries and mixed using a pipette. The final step was to blend 1 mL of this mixture with 4 mL of DMEM in a separate vessel, which was then accommodated in an incubator.

Cell harvesting

Trypsin was used throughout the log phase of growth for cell harvesting. The cell was subsequently counted and placed into 96-well plates.

Cell counting

An apparatus named hemocytometer was used to count the total number of cells. The mirror-like surface of the hemocytometer was meticulously cleaned and polished using ethanol and lens paper. On the counting surface, the coverslip was installed. Using Pasteur pipette, an entirely fresh cell suspension was added to the hemocytometer. The surface had simply overflowed due to the adequate suspension that had been given. The counting chamber was then mounted on the microscope stage, and the counting grid was centered after that. A conventional hemocytometer with Neubauer rulings allows for 40X magnification imaging of the full grid. The four large squares' cells were counted, which meant that either cells with upper or left side touching surfaces or those with lower or right-side touching surfaces were counted.

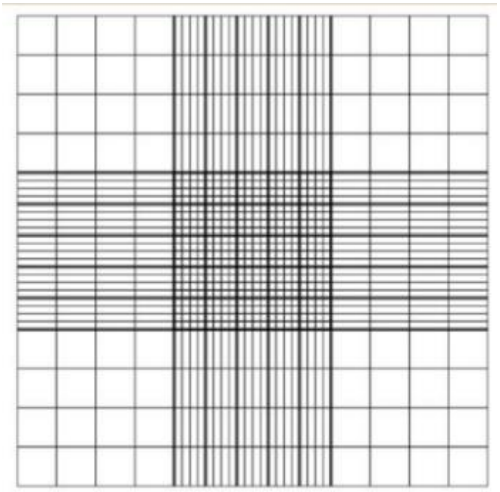

Figure 7: Hemocytometer

Experimental procedure

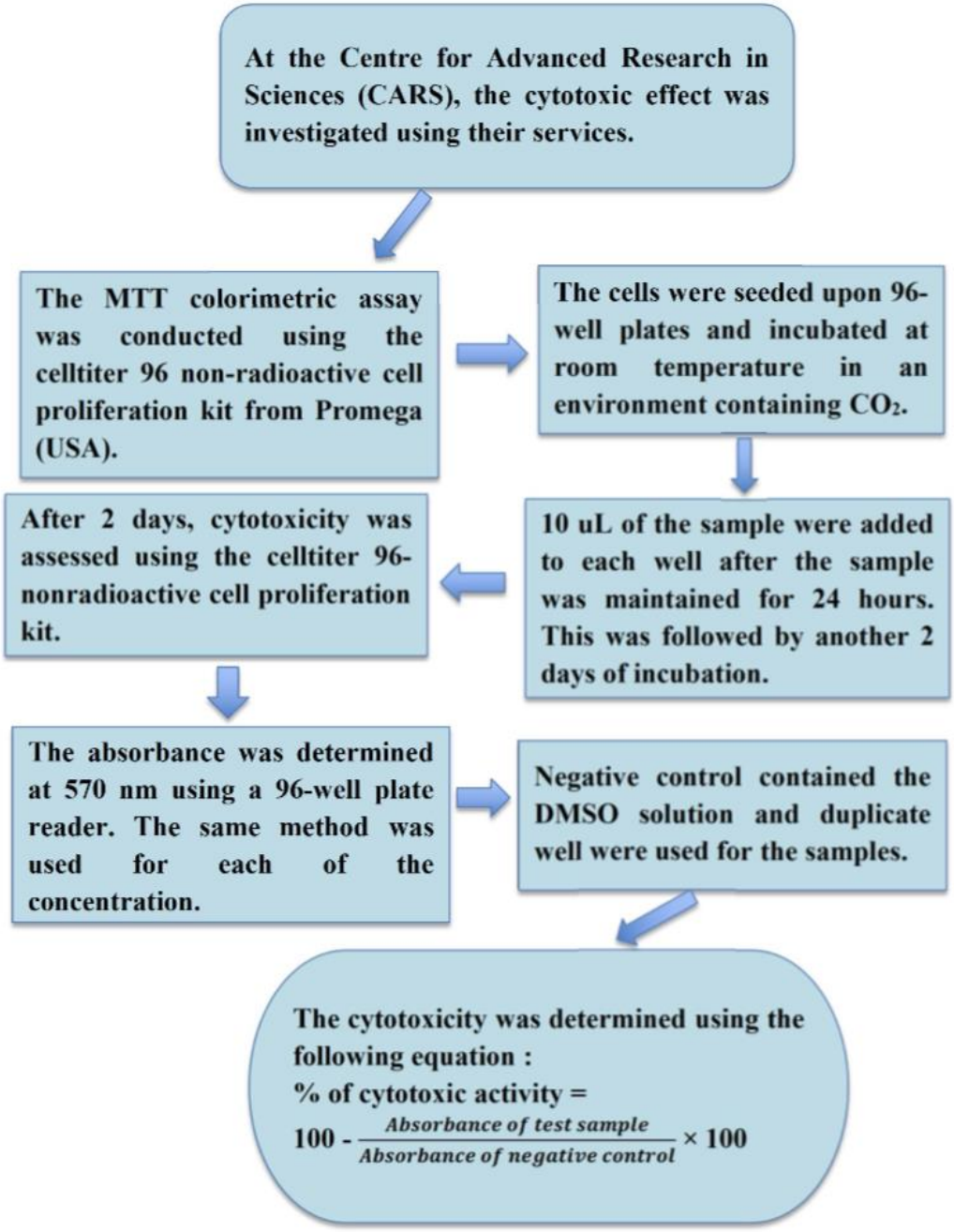

Figure 8: Procedure for cytotoxicity testing

Chapter 3

Results

3.1 Evaluation of H_2O_2 scavenging activity of *Lagerstroemia thorelli*

Using a UV-1280 UV-Vis spectrophotometer from the Shimadzu corporation, the absorbance of the sample and the standard was assessed. At 230 nm, the absorbance of the negative control group was 0.987. The formula "y = mx + c" was applied to calculate the IC_{50} value from the slope of a graph plotting concentration vs percentage (%) of inhibition.

Table 13: Lagerstroemia thorelli IC_{50} Value Calculation

Sample Concentration (µg/mL)	**Absorbance of Sample**	**% of inhibition**	***IC_{50} value***
250	0.834 ± 0.063	15.1 ± 6.44	2.22 (µg/mL)
125	0.376 ± 0.102	61.9 ± 10.38	
62.5	0.304 ± 0.077	69.2 ± 7.84	
31.25	0.209 ± 0.070	78.82 ± 7.24	
15.63	0.109 ± 0.032	88.96 ± 3.28	

% of Inhibition vs Concentration Curve of *Lagerstroemia thorelli*

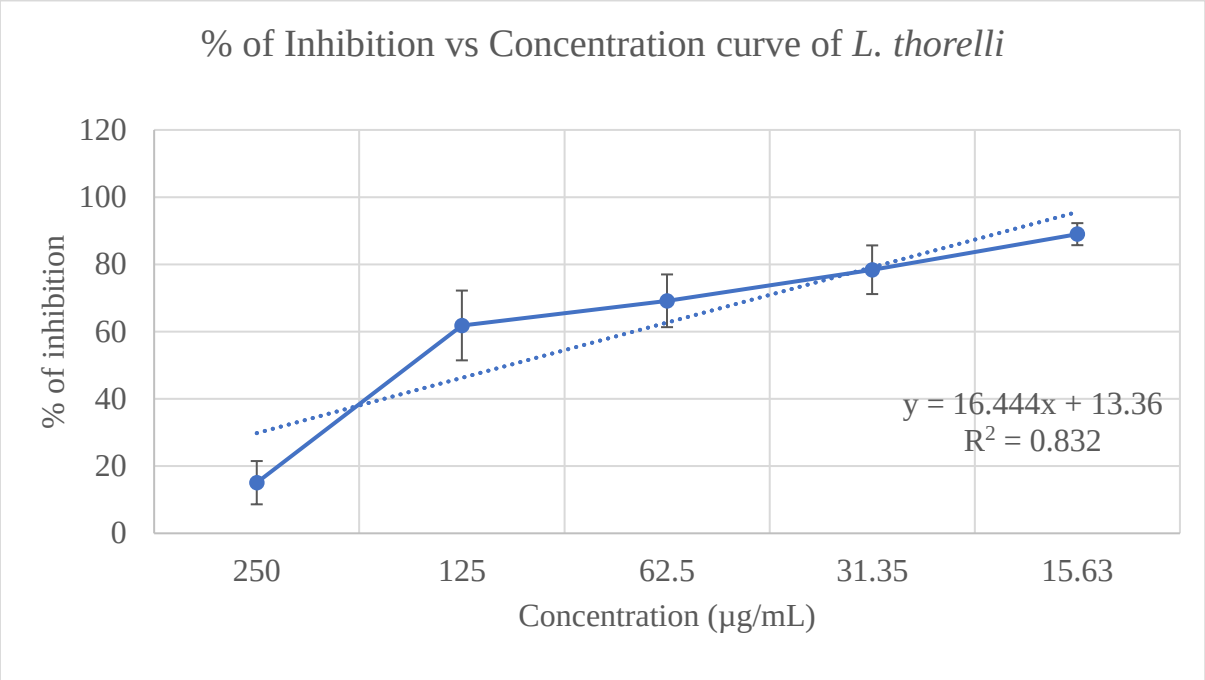

Figure 9: % of Inhibition vs. Concentration Curve of Lagerstroemia thorelli (Sample)

The samples % of inhibition was measured in 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL and 15.63 µg/mL concentrations. From the table, it was clear that as the concentration of the sample dropped, the absorbance dropped correspondingly. However, the H_2O_2 scavenging activity was rising as the concentration fell. Again, it was noted that at the concentration of 15.63µg/mL, the maximum extent of inhibition was observed. The IC_{50} value was calculated using y = 16.444 x + 13.36 from the graph. The sample had an IC_{50} value of 2.22 (µg/mL).

3.2 Evaluation of H_2O_2 scavenging activity of L-Ascorbic acid

Table 14: Ascorbic acid IC_{50} Value Calculation

Standard concentration (µg/mL)	Absorbance	% of inhibition	IC_{50} value
250	0.878 ± 0.057	11.04 ± 5.76	2.84 (µg/mL)
125	0.661 ± 0.019	33.03 ± 1.90	
62.5	0.389 ± 0.078	60.59 ± 7.87	
31.25	0.251 ± 0.018	74.57 ± 1.82	
15.63	0.140 ± 0.019	85.82 ± 1.92	

% of Inhibition vs Concentration Curve of Ascorbic Acid

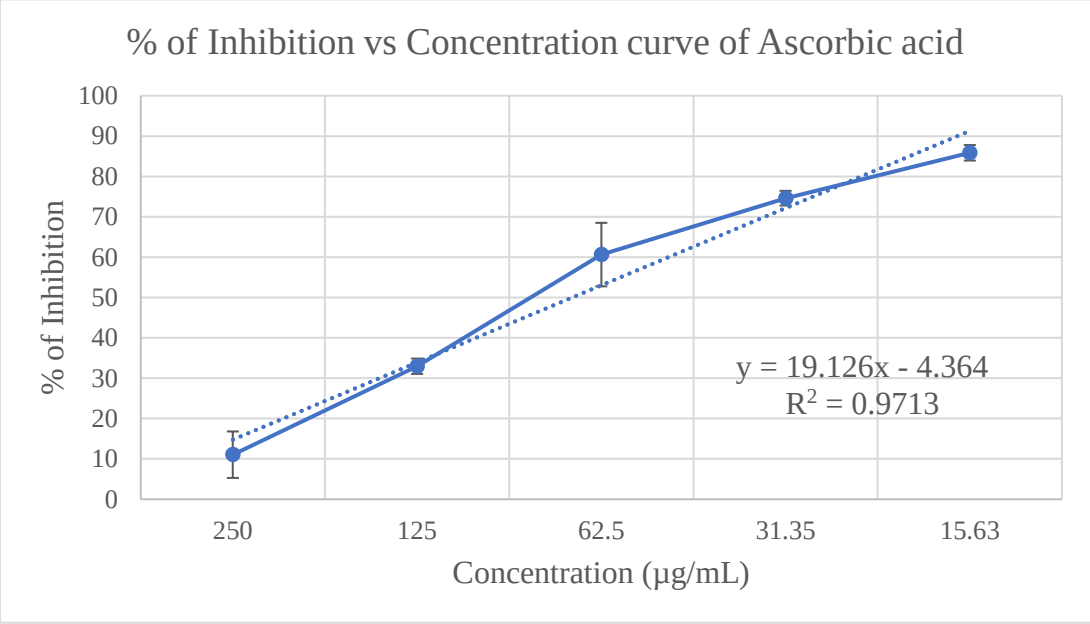

Figure 10: % of Inhibition vs. Concentration Curve of Ascorbic Acid (Standard)

The standard's % of inhibition was measured in different concentrations - 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL and 15.63 µg/mL. According to the table, the standard's absorbance was falling as the concentration was being reduced. On the contrary, the percentage of inhibition raised as the standard solution concentration was dropped. Additionally, at its lowest concentration 15.63 µg/mL, the maximum H_2O_2 scavenging activity was discovered. The IC_{50} value was calculated using y = 19.126 x - 4.364 from the graph. The IC_{50} value found for the standard was 2.84 (µg/mL).

Comparison of the sample and standard

From the obtained data, it was seen that the % of inhibition of H_2O_2 scavenging activity by ethanol crude extract of *Lagerstoremia thorelli* leaves was higher than L-Ascorbic acid corresponding to the concentration.

The sample *L. thorelli* was effective in H_2O_2 free radical scavenging activity from the solutions. The sample's IC_{50} value was 2.22 µg/mL. The standard ascorbic acid's IC_{50} value was 2.84 µg/mL. This implied that, *Lagerstoiremia thorerlli* required a smaller amount to deliver the same antioxidant action as ascorbic acid.

3.3 Study of *Lagerstroemia thorelli*'s total antioxidant capacity

Table 15: L. thorelli leaf extract total antioxidant capacity

Concentration (ug/mL)	Standard's absorbance	Sample's absorbance	TAC present (mg) of ascorbic acid per gram of dry extract in the sample
1200	2.79 ± 0.101	0.971 ± 0.003	98.19 ± 3.864
600	1.426 ± 0.090	0.483 ± 0.002	50 ± 3.139
300	0.63 ± 0.079	0.275 ± 0.014	23.35 ± 2.75
150	0.347 ± 0.021	0.143 ± 0.003	13.23 ± 0.79
75	0.14 ± 0.010	0.08 ± 0.009	5.73 ± 0.373

Interpretation

The antioxidant capacity of *L. thorelli* leaf extract rose along with the increase in different concentrations (75 µg/mL, 150 µg/mL, 300 µg/mL, 600 µg/mL, 1200 µg/mL) with TAC value of 5.73 ± 0.373 mg, 13.23 ± 0.79 mg, 23.35 ± 2.75, 50 ± 3.139 mg, 98.19 ± 3.864 mg of ascorbic acid equivalent (AAE) per gram of dry extract. Additionally, it was seen in the chart that, the antioxidant capacity increased steadily. The antioxidant capacity raised with increase in the concentration. The maximum antioxidant capacity was found at 1200 µg/mL concentration.

Standard Curve of Ascorbic Acid

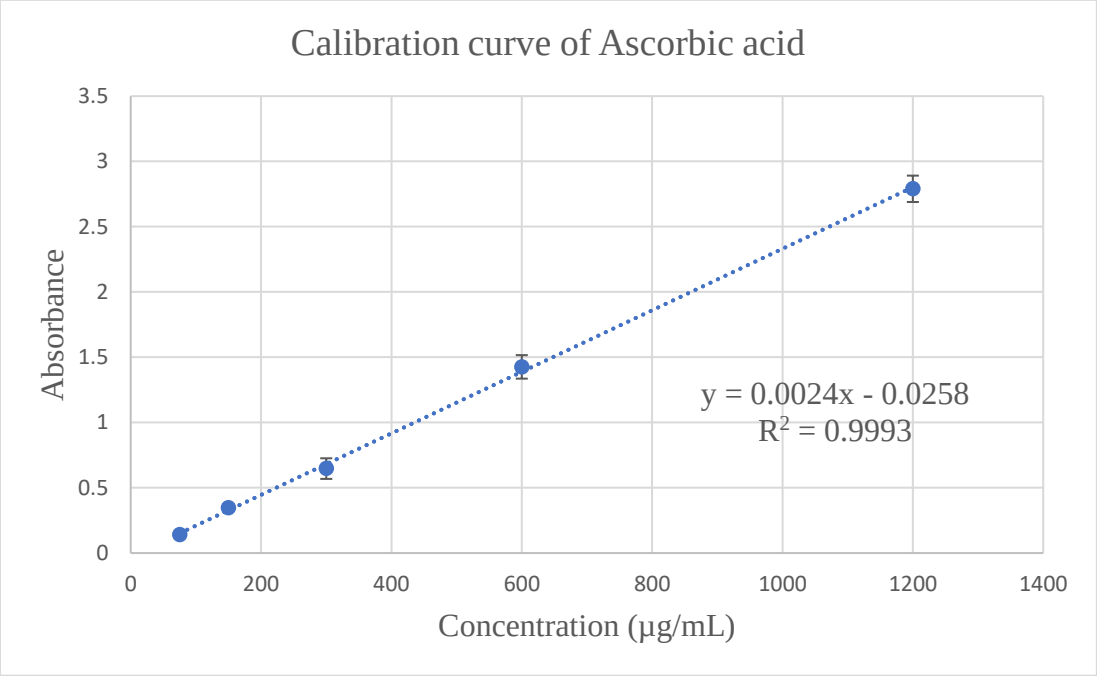

Figure 11: Standard curve of Ascorbic acid for TAC assay

Interpretation

From the above calibration curve, the equation found for ascorbic acid was y = 0.0024x - 0.0258. *L. thorelli's* total antioxidant capacity had been determined using the formula. The concentration and absorbance had a strong correlation, as indicated by the regression coefficient of 0.9993.

Standard Curve of Ascorbic acid and *Lagerstroemia thorelli*

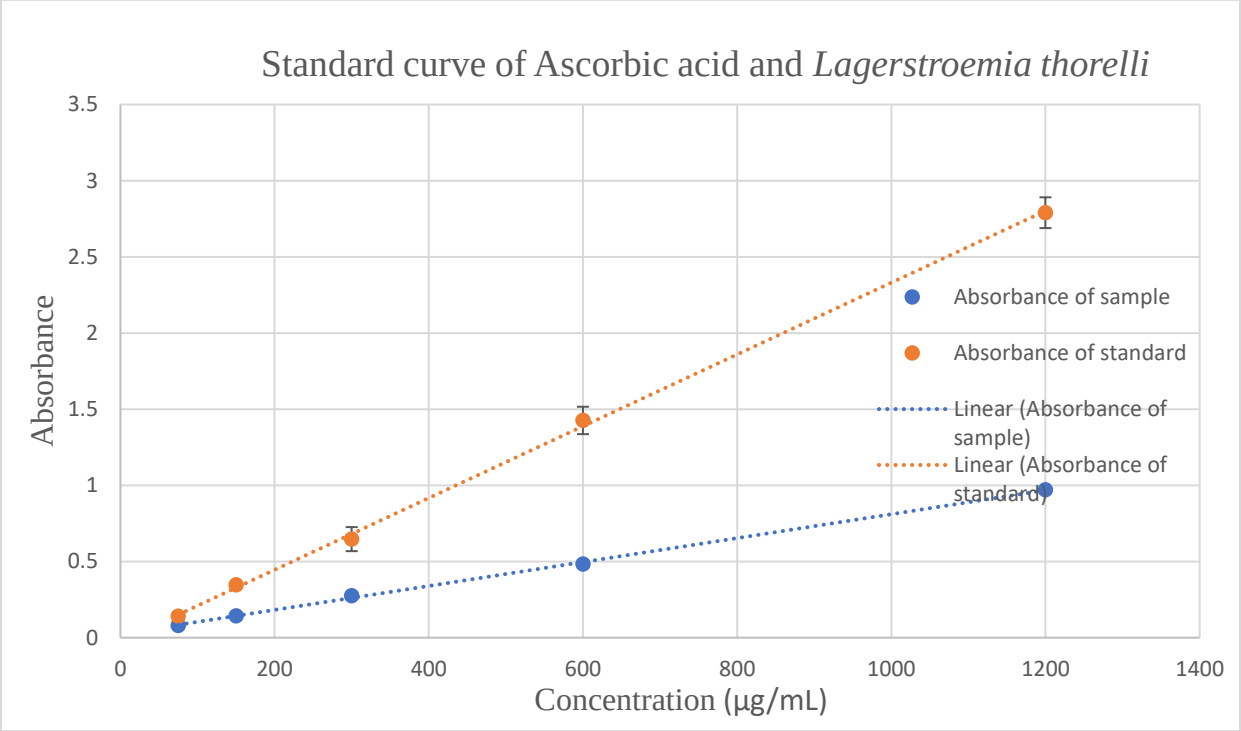

Figure 12: Standard curve of Ascorbic acid and Lagerstroemia thorelli for TAC assay

Interpretation

From Figure 12, it was apparent that the absorbance of the sample and the standard both rose significantly with concentration. Linearity was observed in the graph. To conclude, it can be said, the sample had a good total antioxidant capacity.

3.4 Investigation of *in vitro* cytotoxicity using HeLa cell line

Table 16: Cell viability, and inhibition of HeLa cell growth.

Sample concentration	**% of cell growth inhibition**	**IC_{50} (mg/mL)**
2 % DMSO (Negative control)	0	For HeLa cell line the LD_{50} for sample is 4.077 mg/mL
1 mg/mL	1.22	
1.5 mg/mL	3.76	
2 mg/mL	5.13	
2.5 mg/mL	33	

Interpretation

According to the data obtained from the trinocular microscope about the cells' viability percentage, the HeLa cell line demonstrated the maximum cytotoxicity at 2.5 mg/mL, with 33% of cell death. However, using 1 mg/mL of the sample extract, the least amount of cytotoxicity was observed. With cell death of 3.76 % and 5.13%, low cytotoxicity was seen at concentrations of 1.5 mg/mL, and 2 mg/mL.

Figure A: Dimethyl sulfoxide 2% (negative control)

Figure B: Leaf extract (1mg/mL)

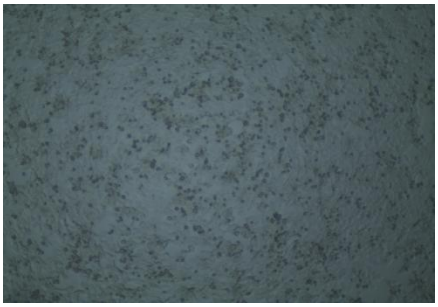

Figure C: Leaf extract (1.5 mg/mL)

Figure D: Leaf extract (2 mg/mL)

Figure E: Leaf extract (2.5mg/mL)

Figure 13: HeLa cell survival at all the concentrations of the leaf extract.

Interpretation

The above images (*Figure 13)* displayed the survival of HeLa cells at all the concentrations of the leaf extract. The percentage of cell viability was 100% at the negative control containing the DMSO (2%) solution *(Figure A).* At 1 mg/mL *(figure B)* concentration, the cell growth suppression percentage was 1.22 % leading to a decrease in the survival of cells. The growth inhibition percentage increased to 3.76 % at 1.5 mg/mL *(figure C)* concentration showing a decrease in the cell survival comparing to the previous concentrations. Again, at 2 mg/mL, *(figure D)* due to an increase in the growth suppression percentage of 5.13% it led to a decrease in number of viable cells. The lowest cell viability was seen at 2.5 mg/mL *(figure E)* with 33 % cell death. Furthermore, the IC_{50} value of sample extract on HeLa cell line obtained was 4.07 mg/mL.

Bar graph of cytotoxic effect of ethanol extract of *Lagerstroemia thorelli* on HeLa cell line

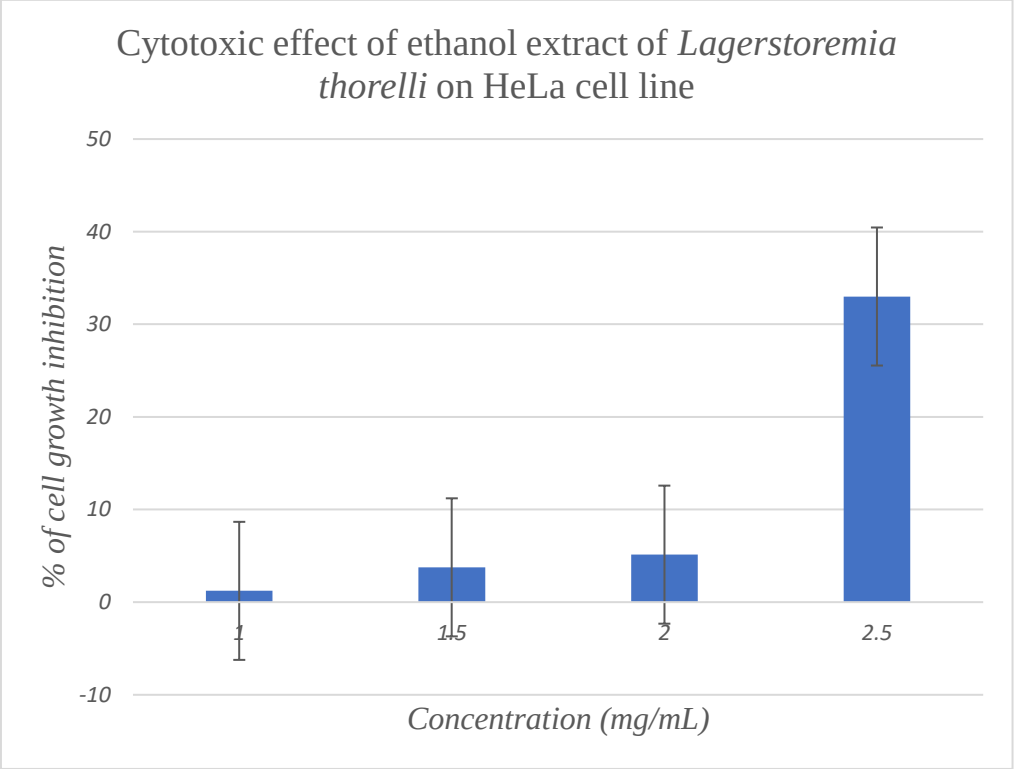

Figure 14: *Cytotoxic effect of ethanol extract of Lagerstoremia thorelli leaf on HeLa cell line*

Interpretation

From the bar chart, it was shown that 1 mg/mL concentration of the leaf extract had the lowest percentage of cell growth inhibition. However, the 2.5 mg/mL concentration of the extract had the lowest percentage of cell viability, and the largest percentage of cell growth inhibition.

3.5 Investigation of *in vitro* cytotoxicity using Vero cell line

Table 17: Cell viability, and inhibition of Vero cell growth.

Sample concentration	**% of cell growth inhibition**	**IC_{50} value**
2 % DMSO (Negative control)	0	For Vero cell line the LD_{50} for sample is 3.32 mg/mL
1 mg/mL	11.08	
1.5 mg/mL	19.8	
2 mg/mL	22.66	
2.5 mg/mL	38.66	

Interpretation

The maximum cytotoxicity was found on the Vero cell line at 2.5 mg/mL, as evidenced by data collected using a trinocular microscope on the percentage of Vero cells that survived. The least amount of cytotoxicity was found at 1 mg/mL concentration of the sample.

After 48 hours of incubation, the maximum cell death was found when the sample extract concentration was at 2.5 mg/mL, or 38.66% cell death. The percentages of inhibition were 11.08 %, 19.8%, and 22.6% respectively at 1 mg/mL, 1.5 mg/mL, and 2 mg/mL concentrations. As a

result, the sample's IC_{50} value exceeded 2.5 mg/mL concentration. The IC_{50} value found was 3.32 mg/mL, preventing 50% of the cell growth.

Figure A: Dimethyl sulfoxide 2% (negative control

Figure B: Leaf extract (1mg/mL)

Figure C: Leaf extract (1.5 mg/mL)

Figure D: Leaf extract (2 mg/mL)

Figure E: Leaf extract (2.5mg/mL)

Figure 15: Vero cell survival at all the concentrations of the leaf extract.

Interpretation

The photos *(Figure 15)* demonstrated Vero cell survival at all the leaf extract concentrations. The viability of cells was 100% in the DMSO (2%) solution *(figure A)* used as the negative control. *At* the concentration of 1 mg/mL *(figure B)*, the percentage of viable cells dropped and demonstrated that 11.08% of cell growth was suppressed. At 1.5 mg/mL *(figure C)* of the leaf extract, the cell survival percentage subsequently decreased to 19.8%. With a 22.66% reduction in cell growth at 2 mg/mL *(figure D)* concentration, the cell survival dropped in accordance with this. At 2.5 mg/mL *(Figure E)* of the leaf extract concentration, the lowest viability of Vero cells was observed. The highest % of cell growth inhibition was 38.66%.

Bar graph of cytotoxic effect of ethanol extract of *Lagerstroemia thorelli* leaf on Vero cell line

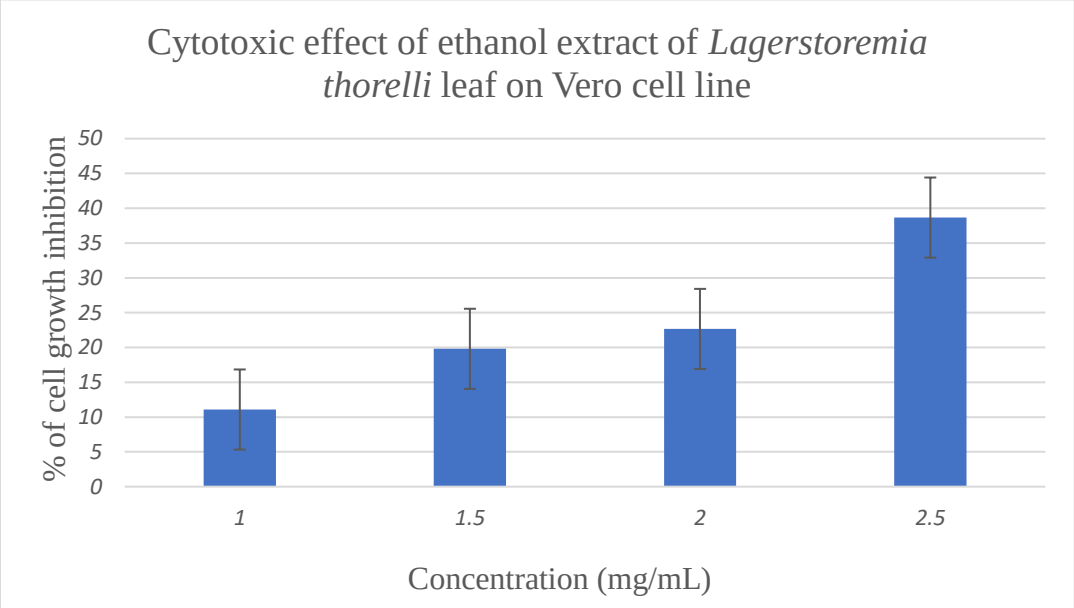

Figure 16: Cytotoxic effect of ethanol extract of Lagerstroemia thorelli leaf on Vero cell line

Interpretation

The bar graph depiction showed that, the minimal degree of cell growth inhibition was at 1 mg/mL concentration with the most cell viability, and the maximal degree of cell growth inhibition occurred at a concentration of 2.5 mg/mL of *L. thorelli* leaf extract with the lowest cell viability.

Chapter 4

Discussion

Lagerstroemia thorelli is a plant that belongs to the Lythraceae family. Lagerstroemia is habitat to the Indian sub-continent (Pounders et al., 2007).The plant had never been studied for its bioactivities, but significant studies have been done on other Lagerstroemia species such as *L. indica, L. speciosa, L. floribunda* etc. Anti-inflammatory, pain relieving, antipyretic, antioxidant, anticancer, antimicrobial, anti-Alzheimer's, antidiabetic, hepatoprotective, and antithrombin properties are all present in *Lagerstormia indica* (Al-Snafi, 2019). *L. parviflora* is a different species that has strong anti-inflammatory, antiviral, antitussive, and antioxidant effects (Jaisinghani & Magarde, 2022).The information gathered from literature study made *L. thorelli* an ideal candidate to be tested for its potential bioactivities. Thus, the present research was conducted to identify the antioxidant and cytotoxic activities of the selected plant. Antioxidant activity of the plant was determined using a qualitative method called H_2O_2 scavenging activity as well as a quantitative method named total antioxidant capacity and its cytotoxic property was evaluated by MTT assay. As there have not been any studies regarding the cytotoxic property of this plant, we attempted to screen for its cytotoxicity on HeLa and Vero cell lines.

H_2O_2 scavenging activity was used to identify the antioxidant property. The IC_{50} value found for the experiment was 2.22 µg/mL, which was comparable to the IC_{50} value for the standard ascorbic acid, which was 2.84 µg/mL. The H_2O_2 scavenging capability of *L. thorelli* has not been the subject of any prior research. However, other species such as, *L. speciosa* has IC_{50} value of 28.00 ±0.16 µg/ml for the hydrogen peroxide scavenging assay method, which compares significantly to ascorbic acid (IC_{50}=187.33 ±3.45 µg/ml) possessing good antioxidant activity (Anil et al., 2010).

So, using this assay method it can be inferred, that *L. thorelli* is a potent scavenger with good antioxidant activity. Plants with high antioxidant potential are utilized to prevent oxidative stress-related illnesses such as, diabetes, atherosclerosis, ischemia, Alzheimer's disease, diabetes related neurodegenerative diseases, retinopathy etc(Kehrer & Klotz, 2015). Thus, it can be inferred that as the plant extract *L.thorelli* scavenge free radicals such as, peroxide, hydroperoxide, etc., this will be effective in preventing the oxidative pathways that lead to degenerative illnesses (Miller et al., 2000).

The total antioxidant capacity was assayed as the antioxidants are used as co-adjuvants to fight oxidative stress. For the assay, TAC found was 98.19 ± 3.864 mg AAE / gram-extract of the sample at the maximum concentration. The lowest concentration showed the presence of 5.73 ± 0.373 mg AAE / gram-extract of the sample. However, from the review it was found that, the total antioxidant capacity of *L. indica* using Ferriccyanide (Fe3+) Reducing Antioxidant Power (FRAP) assay was 3.44 ± 0.27 Trolox/g – extract (Karaboyacı et al., 2022) possessing significant antioxidant activity. Therefore, using ascorbic acid as standard it can be concluded that, *L. thorelli* has good total antioxidant capacity.

MTT assay was used to assess the anticancer property of the sample extract. The IC_{50} value found for HeLa cell line was 4.077 mg/mL and for Vero cell line was 3.32 mg/mL of *L. thorelli* leaf extract. When compared to HeLa and Vero cells, it was found that the *L. thorelli* leaf was more cytotoxic to Vero cells. From the comprehensive review of other species of Lagerstroemia, it was found that *Lagerstroemia speciosa* have low cytotoxic potential. According to (Unno et al. 2004) using the brine shrimp (Artemia salina) lethality test, *L. speciosa* of the ethanol fruit extract demonstrated LC_{50} value at 60 g/mL concentration possessing low cytotoxic activity. However, in human carcinoma cell lines named A549, SK-OV-3, SK-MEL-2, and HCT 15, the species *L.*

indica showed strong cytotoxic actions with IC_{50} of 16.59, 16.64, 17.26 and 8.83 µM for lagerindiol and 6.51, 9.13, 11.38 and 5.87 µM for pterospermin A respectively (Kang Ro Lee et al., 2015). Crude extracts are thought to be the most cytotoxic when their IC_{50} value is less than 30 µg/mL (Suffness & Pezzuto, 1999). Thus, in comparison to the findings from literature articles, *Lagerstroemia thorelli* leaf extract exhibited low cytotoxic activity due to the cell line characteristics.

Chapter 5

Conclusion and Future Perspective

Conclusion

The medicinal plants are an excellent source of medications to treat a variety of ailments. The experiment was carried out to determine the bioactivities of *Lagerstroemia thorelli* leaves extract. According to the study findings, the leaf extract had a strong H_2O_2 scavenging activity. Also, it was observed that *Lagerstroemia thorelli*'s TAC was high. The data obtained from both antioxidant studies were close to the standard L-Ascorbic acid. *Lagerstroemia thorelli* also had concentration-dependent antioxidant capacity showing linearity in the graph. The cytotoxicity study on cervical cancer cell line, Hela revealed low cytotoxic activity due to cell line characteristics. Other cancer cell lines can be used to examine the anticancer effect of *L. thorelli* leaf extract. Therefore, this extract can be used to treat free radical induced diseases such as, cardiovascular disease, neurological disease due to strong antioxidant effect. However, to use the strong antioxidant property of the plant extract against free radical induced diseases, further *in vivo* and clinical trials are required to perform in order to ensure the therapeutic efficacy and safety inside the human body.

Future Perspective

According to research findings on *Lagerstroemia thorelli*'s bioactivities, the plant showed strong antioxidant effect and low cytotoxic activity. Further, investigation can be done to identify its antioxidant properties using enzymatic methods named peroxidase assay, and anthocyanin content assay (Haida & Hakiman, 2019). Future perspectives include:

- Extraction of *L. thorelli* leaves with other solvents such as, methanol, acetone, water to identify the potential variation in pharmacological activities.
- Conducting cytotoxicity test using other cancer cell lines such as, breast, lung and brain cancer.
- Performing *in vivo* studies on the select plant extract to confirm antioxidant activity.
- Investigation of other bioactivities including antiviral, analgesic, antibacterial activities etc.
- Identification of individual compounds responsible for the strong antioxidant activity identified in the current study using HPLC/GC-MS techniques.

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