GCMS-employed characterization of phytoconstituents and cytotoxic activity assessment of *Lagerstroemia thorelli* bark extract

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing a 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 "GCMS-employed characterization of phytoconstituents and cytotoxic activity assessment of *Lagerstroemia thorelli* bark extract" by Rayhan Ahmed (19346073), of Spring, 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on October, 2024.

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Ethics Statement

The study does not involve any animal or human trial.

Abstract

This study explores the cytotoxic properties and chemical composition of the methanol extract derived from the bark of *Lagerstroemia thorelli (L. thorelli);* a medicinal plant historically employed for the treatment of numerous conditions. The extract underwent analysis via Gas Chromatography-Mass Spectrometry (GC-MS) to ascertain its bioactive phytoconstituents where a total 37 phytoconstituents are identified. A variety of chemicals are found based on the area percentage and retention time, such as 13-Docosenamide (Z)-, fatty acid amides, and methyl esters, were found, many of which possess antibacterial, anti-inflammatory, antioxidant, and anticancer activities. The extract's cytotoxic activity was assessed against HeLa cervical cancer cells with the MTT test. A dose-dependent reduction of cell growth was noted, with an IC_{50} value of 1.7856 mg/mL. The highest concentration evaluated (2.5 mg/mL) decreased cell viability by 59.73%, but the lowest concentration (0.0025 mg/mL) demonstrated negligible cytotoxicity (2.87%). The findings indicate that *Lagerstroemia thorelli* bark extract possesses bioactive chemicals with considerable cytotoxic potential, hence endorsing its prospective application in anticancer therapy. Additional research is required to investigate the mechanisms of action, confirm the *in vivo* therapeutic efficacy, and evaluate the safety of this plant extract in clinical applications.

Keywords: Medicinal plant, GC-MS, *Lagerstroemia thorelli*, IC_{50}, Cytotoxicity, MTT assay, Phytoconstituents, Bioactivity, HeLa cells, Anticancer drugs.

Dedication

This work is dedicated to my father for the unwavering support and guidance have shaped my journey and success.

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List of Acronyms

Gm	Gram
Mg	Milligram
µg	Microgram
m	Meter
cm	Centimeter
mL	Milliliter
GC-MS	Gas Chromatography-Mass Spectrometry
IC50	Median Inhibitory Concentration
LD50	Median Lethal Dose
DMSO	Dimethyl Sulfoxide
R^2	Regression Coefficient
Conc	Concentration
L. thorelli	*Lagerstroemia thorelli*
UV-vis	Ultraviolet Visible (Spectroscopy)
HeLa	Cervical cancer cell line
RT	Retention time
MTT	Methyl tetrazolium

Chapter 1

Introduction

1.1 Importance of natural products and medicinal plants in drug discovery.

A medicinal plant has the capacity to treat or cure ailments and have traditionally been employed as medicines within a community or population (Sofowora et al., 2013). Their application precedes recorded history and is one of the most ancient medicinal methods. Since antiquity, plants have been utilized to remedy ailments, promote healing, and maintain health, initially by the Egyptians, Greeks, and Chinese, as well as by indigenous civilizations around (Ang-Lee, 2001). A wide range of ancient methods has been verified by modern scientific studies, and numerous medicinal plants are the source of widely employed pharmaceutical drugs. Despite advancements in synthetic medicine, the use of medicinal plants continues extensively, especially in areas that have restricted access to modern healthcare or within the sphere of complementary and alternative medicine (Ekor, M., 2014).

Lagerstroemia thorelli, a species of the Lythraceae family, highlights the natural products derived from medicinal plants that have historically served as a vital source of bioactive compounds for drug discovery (Newman & Cragg, 2012). The plant's wide phytochemical composition has drawn scientific attention due to its pharmacologically relevant secondary metabolites. It is essential to combine traditional knowledge with modern research methods to speed up the yield of bioactive chemicals from *L. thorelli* compared to other medicinal plants.

Recent investigations of methanol extracts of *L. thorelli* have exhibits significant cytotoxic effects against various cancer cell lines, hence augmenting its oncological potential (Do et al., 2021). GC-MS study of this bark extract showed bioactive phytoconstituents that may be lead compounds for

future pharmacological studies and drug development. These data highlight *L. thorelli's* importance in drug discovery.

1.2 Traditional use of medicinal plants in Bangladesh.

Traditional medicinal plants are those that have been used for thousands of years by different cultures to treat a wide range of illnesses. Many indigenous and traditional medical systems make use of these herbs in their therapies, including Ayurveda, Unani, and Traditional Chinese Medicine. There are several medicinal plants in Bangladesh and other South Asian countries. Nearly 1000 plants with potential therapeutic uses for a range of illnesses are grown here, along with 500 distinct medicinal herbs, 250 of which are used as traditional medicines (Ang-Lee, 2001). The genus Lagerstroemia has historically been employed in traditional medicine to address infections, inflammation, and diabetes (Singh et al, 2024). The examination of *L. thorelli* bark extract in current study enhances our understanding of its pharmacological potential and corroborates its traditional applications. The identification and isolation of potential drug candidates from this medicinal plant are accelerated by the integration of traditional knowledge with advanced scientific techniques such as GC-MS and in vitro bioassays. The following table outlines the traditional usage of various significant medicinal plants found in Bangladesh, including *Lagerstroemia thorelli* in the table 1:

Table 1: Significant medicinal plants found in Bangladesh (Borkatulla et al. ,2023)

Scientific name	**Family**	**Local name**	**Medicinal uses**	**Active compound**
Azadirachta indica	Meliaceae	Neem	treat gum irritation, sores, fever, gingivitis, splenic disorders, tumors, and smallpox.	Nimbin, azadirachtin, nimbolide

Acacia catechu (L.f.	Fabaceae	Khayar	used for intestinal pain and skin diseases.	Catechins, epicatechin, quercetin, tannins
Bacopa monnieri (L.) Pennel	Scorphulariace	Braham	Plant juice is given orally as memory enhancer, diuretic and cardiac tonic.	Bacosides, bacopasaponins, betulinic acid, apigenin
Brassica nigra (L.) Koch.	Brassicaceae	KaloSarisha	Powdered seeds used as a rubefacient and vesicant.	Sinigrin, glucosinolates, allyl isothiocyanate, myrosinase
Centella asiatica (L)	Apiaceae	Thankoni	Root and leaf extracts used dermatological conditions, abdominal tumors, syphilis, leprosy, hemorrhoids, and insect bites.	Asiaticoside, madecassoside, asiatic acid, flavonoids
Clerodendrum inerme	Lamiaceae	Bon Jui	Anticancer, hypotensive, rheumatism, fever	Flavonoids, saponins, diterpenoids, sterols
Enhydra fluctuans Lour	Asteraceae	Helencha	treat inflammation and biliousness.	β-sitosterol, stigmasterol, flavonoids, quercetin, catechins
Ficus benghalenis L.	Moraceae	Bot	cure kidney pain	Tannins, flavonoids, sterols, β-sitosterol, lupeol, leucodelphinidin
Heliotropium indicum L.	Boraginaceae	Hatishur	Leaf juices used for conjunctivitis.	Pyrrolizidine alkaloids (heliotrine, lasiocarpine), flavonoids, tannins
Nyctanthes arbor-tristis L.	Oleaceae	Shefali	bark treat pneumonia, leaves treat rheumatism, flowers reduce inflammation,	

			seeds treat skin conditions.	Nyctanthin, flavonoids, tannins, β-sitosterol, iridoid glycosides
Lagerstroemia thorelli	Lythraceae	Jarul	Traditionally used for diabetes, fevers, and skin problems; recently tested for cytotoxicity.	Flavonoids, tannins, triterpenoids

The table 1 highlights that, numerous medicinal plants have been traditionally used for various therapeutic applications. *Azadirachta indica* (Neem) cures illnesses such as gum irritation, fever, and tumors, while Seeds of *Brassica nigra* (Kalo Sarisha) are utilized topically to enhance circulation. Dermatological conditions and digestive pain are relieved by *Acacia catechu* (Khayar). Thankoni, or *Centella asiatica*, is used to treat abdominal neoplasms and dermatological disorders. *Clerodendrum inerme* is known to have antipyretic and anticancer properties. Choto Jarul, or *L. thorelli*, has been used to treat dermatological and diabetic conditions and has recently been studied for its cytotoxic qualities. It is well known that *Bacopa monnieri* (Braham) can enhance cardiovascular and cognitive function. These plants have a variety of bioactive substances, including triterpenoids, flavonoids, and tannins, which support their medicinal uses (Borkatulla et al., 2023).

1.3 General significance of cytotoxic studies

Cytotoxic studies are scientific investigations that evaluate the capacity of a chemical to kill or inhibit the growth of cells, especially cancerous cells. The study of these factors is essential for understanding the toxicological effects of chemicals on cellular systems which plays a crucial role in the development of pharmaceuticals, especially in the context of cancer treatment (Barile et al., 1993). Cytotoxic studies play a critical role in the fields of drug development, cancer research, toxicology, and environmental health. Cytotoxicity assays assess the ability of drugs to induce cell death or suppress the proliferation of cancer cells. The significance of these studies in the realm of drug development cannot be overstated, particularly concerning anticancer agents like Paclitaxel and Docetaxel, which are classified within the Taxol group. Paclitaxel serves as a significant naturally occurring lead compound. Paclitaxel is primarily sourced from the bark of the slow-growing Western yew, due to its complex and unique chemical structure. (Panchagnula, 1998).

A sample of pacific yew (*Taxus brevifolia*) bark was collected in 1962 in order to screen natural products. Bark extracts had substantial anti-cancer efficacy in early in vitro cytotoxicity assays, such as the KB assay, indicating the presence of a potent bioactive chemical that potentially inhibits the growth of cancer cells. In a way the cytotoxicity study offers critical insights into the impact of chemicals on cell viability, facilitating the identification of new treatments and guaranteeing the safety of compounds prior to their application in clinical environments.

The intercontinental marketing services analysis indicates that the anticancer medicine market in Bangladesh is expanding at an annual rate of 20 percent due to a concerning increase in cancer patients (Ahmed, 2013b). The search for novel anticancer pharmaceuticals is a pressing necessity. Overall cytotoxicity is very necessary in terms of cancer treatment by selective targeting of cancer

cells, also understand the mechanisms of action, understanding chemical toxicity, identification of bioactive compounds.

1.4 Medicinal plants with cytotoxic properties

Natural medicinal herbs have been utilized by humanity for various purposes for many years. Notably, the progress of scientific methods in the past century has made possible the separate extraction of active constituents from diverse plants in pure form for multiple therapeutic applications (Khan et al., n.d.). Phyto molecules originating from plants have contributed to the development of anticancer pharmaceuticals. Numerous chemotherapeutic agents are derived from or are synthetic analogs of phytochemicals. The intricate architectures and biological functions of these phyto molecules are utilized to treat cancer or formulate pharmaceuticals (Newman & Cragg, 2012). In Bangladesh there are many medicinal plants which produce cytotoxic activity. So that, a list of the plants which their cytotoxic activity and active compounds are given below-

Table 2: *Plants with their cytotoxic activity and active compounds*

Scientific name	**Local name**	**Active compound**	**Cytotoxic activity**
Catharanthus roseus	Nayantara	Vincristine, vinblastine	Used for chemotherapy in leukemia and lymphoma.
Azadirachta indica	Neem	Nimbin, azadirachtin, gedunin	cytotoxic effects against various cancer cells.
Swertia chirayita	Chirayta	Swertiamarin, mangiferin	Exhibits cytotoxic effects; used in cancer treatments.
Nigella sativa	Black cumin	Thymoquinone	Induces apoptosis and inhibits proliferation of cancer cells.

Lagerstroemia thorelli	Choto jarul	Triterpenoids, flavonoids, phenolic compounds	cytotoxic effect against cancer cells, causing apoptosis and inhibiting cell proliferation.
Calotropis procera	Akanda	Calotropin, uscharin	Cytotoxic to cancer cells, inhibits tumor growth.
Ocimum sanctum	Tulsi	Eugenol Ursolic acid Rosmarinic acid	Cytotoxic activity against lung, breast, and skin cancer cells.

Numerous medicinal herbs exhibit cytotoxic effects on cancer cells attributable to their bioactive components as we can see in the table 2. *Catharanthus roseus* (Nayantara) has vincristine and vinblastine, which are utilized in chemotherapy for leukemia and lymphoma. *Calotropis procera* (Akanda) and *Ocimum sanctum* (Tulsi) exhibit cytotoxic properties, with substances such as calotropin, eugenol, and ursolic acid, affecting several malignancies. *Azadirachta indica* (Neem) exhibits cytotoxic properties due to substances such as nimbin and azadirachtin. *L. thorelli* (choto jarul) has triterpenoids and flavonoids, demonstrating cytotoxic properties. *Swertia chirayita* (Chirayta) comprises swertiamarin and mangiferin, which are utilized in oncological therapies. *Nigella sativa* (black cumin) contains thymoquinone, which induces apoptosis in cancer cells.

1.5 Overview of common *in- vitro* cytotoxicity assays.

In vitro cytotoxicity assays are essential for assessing the cytotoxic effects of substances on cultured cells, particularly during the screening of possible anti-cancer medicines. These assays assess a compound's capacity to limit cell viability, diminish cell proliferation, or trigger cell death through mechanisms such as apoptosis (planned cell death) or necrosis (uncontrolled cell death) (Freshney, R. I. 2010). Before moving on to *in-vivo* investigations or clinical trials, these assays

mimic the interaction of medications with cancer cells in a controlled environment. The results of these assays provide essential insights into the efficacy and safety of the substances (Fotakis & Timbrell, 2005), several assays, such as MTT, LDH, and Annexin V/PI, are applied depending on the particular research emphasis that is being investigated at.

MTT Assay

The MTT assay is a prominent colorimetric method for assessing the cytotoxic effects of substances on cell lines, providing a measure of cell viability based on mitochondrial function. The assay employs MTT, which is a yellow tetrazolium salt is transformed by mitochondrial enzymes in metabolically active cells into formazan and a purple-colored insoluble product. (Mosmann, 1983). Researchers can evaluate the cytotoxic effects of drugs or plant extracts by measuring purple color intensity by counting the living cell. The MTT assay can also be employed in the *in vitro* evaluation of formulations or extracts, as this case, for example, with the evaluation of the cytotoxic activity of *L. thorelli* bark extract (van Meerloo et al., 2011). This correlation, between the number of active mitochondria and the number of living cells, is important in the study of new anticancer or cytotoxic drugs. However, necrotic cells do not proceed in this way. Upon completion of the incubation period, the formazan crystals are solubilized in a dissolving solution. A spectrophotometer is utilized to measure the intensity of the purple color, which correlates directly with the quantity of viable cells. The assay is commonly utilized for studies on cell proliferation, cytotoxicity testing, and the calculation of IC_{50} values (Denizot & Lang, 1986). The MTT assay, while simple and cost-effective, cannot differentiate between several cell death processes, including necrosis and apoptosis.

In terms of cell line in this paper one cell line is used which is HeLa cell line (Human cervical cancer). The HeLa cell line derives from cervical cancer cells of a human female, notably Henrietta

Lacks, in 1951. It possesses an epithelial shape and is extensively utilized in cancer research. HeLa cells are aneuploid, indicating an atypical chromosome count, a characteristic commonly observed in cancer cells. They demonstrate G6PD type A enzyme activity, a marker valuable for metabolic research. Cells exhibiting aberrant ploidy and accelerated proliferation are crucial for investigating cancer biology. These malignant cells have persisted in growth and reproduction since their isolation, establishing them as the inaugural human cells to be effectively cultured for extended research. HeLa cells are distinguished by their longevity; in contrast to normal cells, they do not experience senescence and can proliferate endlessly under suitable laboratory circumstances. They are crucial for research in oncology, virology, pharmacology, and cellular biology, as they offer a reliable and renewable supply of human cells for scientific investigation (F. A. Khan, 2011). HeLa cells are provided by research organizations, including CARS (Centre for Advanced Research in Sciences) at Dhaka University (DU).

1.6 Natural products potential implications in cancer therapy.

The use of natural products has proven to be effective in the fight against cancer for more than four decades. Microbes and plants that are native to both terrestrial and marine habitats are the primary sources of these chemicals that have been developed successfully (Demain & Vaishnav, 2010). Natural product researchers are interested in *L. thorelli,* a Lythraceae plant with anti-cancer properties. *L. thorelli* bark methanol extract, which has a long history of medical use, exhibits cytotoxic activity, implying cancer therapeutic potential. *L. thorelli* may function as a possible anti-cancer agent due to its diverse bioactive constituents, multi-targeted processes, capacity to address treatment resistance, and reduced toxicity relative to synthetic drugs in cancer treatment. Natural product used in various cancer therapy are shown below in table 4- (Takahashi et al., 2012)

Table 3: Natural products implications in cancer therapy

Plant extracts/ phytoconstituents	**Cancer type**	**Mechanism of action**
Bark of *Taxus brevifolia* (Pacific Yew Tree)	Ovarian, breast, and lung cancers	Paclitaxel prevents microtubule breakdown during cell division, stopping mitosis, which kills cancer cells.
Leaves of *Catharanthus roseus* (Madagascar Periwinkle)	Testicular cancer	The alkaloids inhibit mitosis and cell division by binding to tubulin and preventing microtubule synthesis.
Seeds of *Glycine max* (Soybean)	Breast and prostate cancers	Genistein, a phytoestrogen, inhibits tyrosine kinase and promotes cancer cell death.
Lagerstroemia speciosa (Banaba) Leaves	Breast and liver cancer	Cancer cells undergo apoptosis when corosolic acid activates caspases and inhibits the PI3K/Akt signaling pathway. This route is essential for cell survival, growth, and apoptosis rejection. Inhibiting this mechanism kills cancer cells.
Seeds of *Nigella sativa* (Black Cumin)	Breast, lung, and colon cancers	Apoptosis and cancer cell line inhibition are caused by thymoquinone. It regulates signaling pathways like p53 and NF-κB.

In the table 4, many natural substances which have been studied for anticancer effects. For example, Paclitaxel from *Taxus brevifolia* stabilizes microtubules, limiting cancer cell division (Wani et al., 1971). Vincristine and vinblastine from *Catharanthus roseus* limit cell proliferation by disrupting microtubule formation (Noble, 1990). Lagerstroemia phytoconstituents such corosolic acid, betulinic acid, and flavonoids like quercetin and kaempferol have shown promising anticancer properties. *Lagerstroemia speciosa* leaf corosolic acid induces apoptosis and inhibits the PI3K/Akt pathway, killing breast and liver tumors (Miura et al., 2004).

Thymoquinone from *Nigella sativa* causes apoptosis by targeting p53 and NF-κB pathways (Woo et al., 2012). These phytoconstituents demonstrate the importance of natural cancer treatments.

1.7 Advantages of natural products over synthetic products in cancer therapy.

Natural products have been crucial in the history of anticancer medication discovery, with numerous commonly utilized anticancer medicines derived from natural sources. Irinotecan and paclitaxel originate from plants, actinomycin D and mitomycin C are obtained from bacteria, and bleomycin is extracted from marine species. These chemicals are vital to cancer therapy and are anticipated to remain essential in future treatments due to their efficacy in targeting cancer cells (Huang et al., 2021). Natural cancer treatments provide numerous benefits compared to synthetic medicines. Natural chemicals, sourced from many biological origins, target different cancer pathways, hence improving treatment efficacy and minimizing resistance. They frequently demonstrate reduced side effects and less toxicity, enhancing patient tolerance. Certain natural compounds have superior absorption and bioavailability. Numerous substances have been utilized in traditional medicine, signifying their safety and efficacy. Natural items may enhance conventional treatment and stimulate the discovery of new drugs.

Camptothecin and taxol are the two most effective examples, both identified between the 1950s and 1960s during a campaign undertaken by the National Cancer Institute (NCI) to explore the therapeutic potential of natural products. (Wall, M. E., & Wani, M. C. 1995). Furthermore, they can alter the tumor microenvironment, impede cancer cell proliferation, and provide comprehensive advantages. Nonetheless, additional research is required to validate their safety and efficacy (Demain & Vaishnav, 2010b; Newman & Cragg, 2016c). Moreover, the key to finding and creating a successful cancer treatment is recognizing medicinal plants with strong cytotoxicity.

The search for safer, more selective, and effective natural chemotherapeutic medicines is gaining momentum every day (Akter et al., 2014).

1.8 Phytoconstituents in medical plants and their importance

Phytoconstituents serve as the basis for numerous modern pharmaceuticals and traditional medical practices. They are essential for addressing a range of diseases and conditions owing to their diverse biological effects. Plant extracts include a wide variety of biologically relevant phytochemicals, such as alkaloids, tannins, glycosides, flavonoids, saponins, phytosterols, and resins. These chemicals are spontaneously synthesized by plants and have diverse medicinal effects. Alkaloids possess a fundamental nitrogen atom and are recognized for their antispasmodic, antibacterial, analgesic, and anticholinergic effects. Typical instances comprise atropine, caffeine, quinine, and morphine (Roy, 2017). Flavonoids are secondary metabolites characterized by a phenolic structure. They exhibit antioxidant, anti-inflammatory, anticancer, and enzyme-inhibitory activities, and are significant in the management of disorders such as cancer and Alzheimer's (Panche et al., 2016). Phytosterols are cholesterol analogues included in plant seeds, nuts, and oils that aid in lowering cholesterol levels by obstructing absorption (Leitzmann, 2016). Saponins are amphiphilic chemicals that create complexes with lipids and proteins, predominantly located in legumes and utilized as foam stabilizers (Leitzmann, 2016).

The antibacterial and protective characteristics of tannins are exhibited through their interactions with proteins. These tannins are hydrolysable and condensed. Glycosides are substances made up of a sugar molecule and a non-sugar fragment. These compounds have antiplatelet, anticancer, and antifungal properties (Khan et al., 2020).

1.9 Overview of GC-MS Technology

Volatile substances are quantified through gas chromatography-mass spectrometry (GC-MS), a cutting-edge process for identifying phytochemicals of plants. It combines both mass spectrometry and gas chromatography (GC) separation. The volatile substances are identified by mass spectrometry (MS) with measurement of the analytes masses at specific m/z values, and separated chromatographically through gas chromatography (GC) based on their retention time. In addition, labile substances are suitable for GC-MS analysis e.g., alkaloids, terpenes and volatile oils. However, for non-volatile chemicals derivatization may be required. This method is best for phytochemical profiling in medicinal plant research due to its qualitative as well quantitative results (Sparkman et al., 2012). The system used for identifying the compounds of this mixture both a gas chromatography (GC) method and mass spectrometry (MS). Gas chromatography (GC) which is capable to separate good volatiles molecules from bad ones. Next, mass spectrometry method (MS), which just identifies most critical compounds providing its structure but it cannot perform the separation of compounds mixture. Developed around the mid-1950s, these methods combine so as to allow for both compound separation and compound identification. In GC-MS, a sample vaporized and carried away though the column by an inert gas where chemicals are separated based on difference in their interaction with stationary phase. The compounds are then injected into the MS, where they undergo ionization and fragmentation followed by analysis of their m/z ratio to provide each chemicals identification (Karasek & Clement, 2012). Due to its high sensitivity, specificity and speed of analysis GC-MS is well suited for the identification of compounds in lower concentrations within complex mixtures, which makes it useful in fields such as food safety, forensics and drugs amongst others (Hites, 1997). Liquid Chromatography-Mass Spectrometry (LC-MS) broadens the scope to include polar and thermally unstable analytes

(Kumar & Vijayan, 2014), whilst Nuclear Magnetic Resonance (NMR) offers structural insights but is less efficient with complex mixes (Rule & Hitchen, 2006). Overall, GC-MS stands out for its detailed molecular identification of volatile compounds.

1.10 An overview of the plant *Lagerstroemia thorelli* and its uses

Lagerstroemia thorelli, a member of the Lythraceae family, is locally referred to as "Choto Jarul" and possesses the ability to endure drought conditions. The plant is also located in roadside and rural regions of Bangladesh, including Moulovi Bazar and Sylhet. The Lythraceae family has approximately 32 genera and 620 species of dicotyledonous flowering plants found in tropical and subtropical regions. (Xu & Deng, 2017).

Morphology –

This is the diminutive variant of the "Choto Jarul" tree, a small to medium-sized tree that attains heights of 15 to 35 feet. It possesses distinct morphological characteristics that facilitate the differentiation between the larger and smaller Jarul trees. The weak cell walls of its wood facilitate easy penetration. It possesses smooth bark and is devoid of thorns. The leaf is of a basic kind, measuring 10 cm in length and 4 cm in width, devoid of any aroma. The flower of this plant is pink and reaches a maximum size of 2 cm. It possesses brown, capsule-shaped fruits of 1 cm in size. The fruiting and flowering period of *L. thorelli* occurs from April to September.

Figure 1: *Tree and flower of Lagerstroemia thorelli*

Taxonomy

Table 4: *L. thorelli taxonomy hierarchy*

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

L. Thorelli can be used in Traditional Medicine to address inflammation, fever, and gastrointestinal disorders, as well as for its anti-cancer potential. Methanol extracts from the bark exhibit cytotoxic activity against neoplastic cells, possess antioxidant properties, demonstrate anti-inflammatory effects, show potential in diabetes management, and exhibit antimicrobial activity.

1.10.1 Biological Properties of different parts of the plant *L. thorelli*

The plant *L. thorelli*, popularly referred to as Thorell's crape myrtle, has been investigated for its diverse therapeutic characteristics. Various plant components, such as bark, leaves, flowers, and roots, have been analyzed for their biological activity, which are ascribed to a variety of phytoconstituents (Lihu, 2023). In table 5, it shows that the *L. thorelli* contains a variety of compounds and characteristics that have long been utilized medicinally. The leaves include flavonoids, tannins, and saponins, which have antibacterial, antimicrobial, and anti-inflammatory properties that can be used to treat infections and heal wounds. The blossoms, which are high in anthocyanins, flavonoids, and tannins, have anti-inflammatory and possibly antidiabetic qualities, helping to regulate inflammation and reduce oxidative stress. The bark, which contains flavonoids, phenolic compounds, and tannins, has cancer-fighting potential as well as antioxidant and anti-inflammatory characteristics, making it effective for healing wounds, infections, and fevers. The root, which contains saponins, alkaloids, and tannins, has antifungal and antibacterial qualities and was traditionally used to treat fevers and infections.

Table 5: Different properties of Lagerstroemia thorelli part

Plant part of *L. thorelli*	**Phytochemicals**	**Properties**	**Traditional uses**
leaves	Flavonoids, tannins, saponins	Antibacterial Antimicrobial Anti-inflammatory	Treating infections, Wound healing
Flower	Anthocyanins, flavonoids, tannins	Anti-inflammatory, Antidiabetic effects	Managing inflammation, Oxidative stress relief
Bark	Flavonoids, phenolic compounds, tannins	Cancer therapy, Antioxidant & Anti-inflammatory	Wound healing, cutaneous infections, & antipyretic effects.

Root	Saponins, alkaloids, tannins	Antifungal, Antibacterial	Reducing fever

1.11 Information on chemical constituents of other species of *L. thorelli*.

Chemical ingredients present in various species of the Lagerstroemia genus, which exhibit similarities to *L. thorelli*. These species have been examined for their bioactive chemicals and prospective therapeutic qualities.

Table 6: *Chemical constituents of various species of the Lagerstroemia genus*

Species	**Chemical constituents**	**Properties**
Lagerstroemia thorelli	Flavonoids, Phenolic compounds, Tannins, Saponins	Cytotoxic, antioxidant, Anti-inflammatory
Lagerstroemia speciosa	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. (Bai et al., 2008)	Antidiabetic Antioxidant Anti-inflammatory
Lagerstroemia indica	Tannins: Ellagitannins, gallotannins. Triterpenoids, Phenolic glycosides (strosides A–C) **Al-Snafi (2019)**	Antimicrobial Anti-inflammatory Antioxidant
Lagerstroemia parviflora	phenols, flavonoids, tannins, saponins, alkaloids, fixed oil, and lipids	Antimicrobial, Antioxidant Anti-inflammatory
Lagerstroemia floribunda	Sesamin, β-sitosterol, clauslactone-k, betulinic acid, lingueresinol	Antioxidant, Antidiabetic Anti-inflammatory

Lagerstroemia subcostata	Rutin, quercetin, Gallic acid, Triterpenoids, Saponins	Antimicrobial, Anti-inflammatory, Antioxidant

Table 6 shows Lagerstroemia species' chemical components and characteristics. *L. thorelli* is cytotoxic, antioxidant, and anti-inflammatory due to its flavonoids, phenolic compounds, tannins, and saponins. *Lagerstroemia speciosa* contains antidiabetic, antioxidant, and anti-inflammatory ellagitannins, corosolic acid, and quercetin (Bai et al., 2008). *Lagerstroemia indica* has antibacterial, antioxidant, and anti-inflammatory tannins, triterpenoids, and phenolic glycosides (Al-Snafi, 2019). Phenols, flavonoids, tannins, and alkaloids make *L. parviflora* antibacterial, antioxidant, and anti-inflammatory. *L. floribunda* contains antioxidant, antidiabetic, and anti-inflammatory sesamin and betulinic acid, while *L. subcostata* has quercetin, and triterpenoids.

1.12 Current research gaps

Early research on the GC-MS-based phytochemical characterization and cytotoxic action of *L. thorelli* exhibits some substantial deficiencies. Although numerous studies have identified bioactive compounds by GC-MS, a comprehensive phytochemical profile is still absent, particularly for various plant parts and supplementary analytical techniques such as LC-MS. The few in vitro screenings conducted in cytotoxicity studies lack comprehensive mechanistic insights and in vivo evaluations necessary to validate their potential as therapeutic agents. More importantly, there has been a lack of research on the potential for synergistic interactions between substances and bioavailability. Also, there is not accessible enough research on ecological changes in phytochemical composition on standardizing extraction processes. Not enough clinical studies have been conducted to back up the therapeutic assertions on anticancer effects. Our knowledge of *L. thorelli's* safety profile and therapeutic potential may be greatly improved if these gaps were filled (Leitzmann, 2016).

1.13 Rational/Significance of the project

The lagerstroemia genus is recognized for its diverse species, which contain a variety of bioactive compounds. *L. indica* possesses analgesic and antioxidant properties, but *L. parviflora* exhibits cytotoxic, antiviral, and antioxidant effects. *L. speciosa* possesses significant anti-diabetic properties. Notwithstanding these significant discoveries, *L. thorelli*, a member of the Lythraceae family, has not been subjected to medicinal investigation. *L. thorelli* may possess medicinal potential similar to other Lagerstroemia species due to its tannins, alkaloids, phenols, and polyphenols. In accordance with the conventional application of medicinal flora for health advantages, *L. thorelli* may exhibit comparable biological activity, necessitating more investigation for the treatment of diabetes, cancer, and other medical ailments. A comprehensive literature study reveals that *L. thorelli* has not been previously studied properly. Nevertheless, other investigations on different species within the Lythraceae family have evidenced diverse bioactivities, including anticancer, antioxidant, and antidiabetic effects. The current corpus of research endorses the use of *L. thorelli* for examination. This study intends to characterize the phytochemical compounds and assess the cytotoxic potential of *L. thorelli*, as these aspects have not been previously investigated using specific cell lines and methodologies.

1.14 Aim and objectives of the project

Aim

The aim of the study is to evaluate the cytotoxic activity of the methanol extract of *Lagerstroemia thorelli* and identify the potential bioactive compounds by GC-MS contributing to the cytotoxic effect.

Objectives

Few specific objectives of *L. thorelli*:

1. Identify bioactive compounds using gas chromatography–mass spectrometry (GC–MS)
2. To carry out cytotoxicity testing to ascertain its efficacy in inhibiting cancer cells growth (HeLa cell).
3. The objective of this study is to explore its potential therapeutic application in the treatment of cancer.
4. Contributing to science by offering insights into a medicinally underexplored species.

Chapter 2

Materials and Methodology

2.1 Chemicals and Reagents

During the study, a variety of chemicals and reagents typically are used. Two different test types are conducted in this study: one involves using GC-MS to identify the phytoconstituents of *L. Thorelli* methanol bark extract, and the other involves testing cytotoxic activity of the extract.

Chemical and reagents used in cytotoxic effect analysis-

Table 7: *Chemical and reagents used in Cytotoxic effect analysis*

Chemical/Reagent	**Purpose/Function**
Dulbecco's Modified Eagles Medium (DMEM)	Commonly used culture media for growing cancer cells.
Fetal Bovine Serum (FBS)	Provide nutrients for cell growth.
Antibiotics: Penicillin-Streptomycin	Prevents bacterial contamination in cell cultures.
Trypsin-EDTA	Detach adherent cells from the culture surface during sub-culturing.
MTT[3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]	MTT is transformed into a purple formazan product by metabolically active cells, which is then used in cell viability tests to measure cytotoxicity.
Dimethyl sulfoxide (DMSO)	Dissolves formazan crystals after the MTT assay.
Positive Control: Doxorubicin	Standard chemotherapy agent for comparing cytotoxic effects.

Table 8 highlights the diverse chemicals and reagents employed in the assessment of the cytotoxic effects of substances on cancer cells. Dulbecco's Modified Eagle Medium (DMEM) is a widely

utilized culture medium that facilitates the proliferation of cancer cells by supplying the requisite environment for growth. Fetal Bovine Serum (FBS) is incorporated to provide vital nutrients that facilitate cellular proliferation. Antibiotics, particularly penicillin-streptomycin, are employed to preserve the sterility of cell cultures and avert bacterial contamination. Trypsin-EDTA is utilized to dislodge adherent cells from the culture substrate during sub-culturing, facilitating adequate maintenance and experimentation. The MTT reagent, converted into a purple formazan product by metabolically active cells, is employed in cell viability assays to assess cytotoxicity. Following the MTT experiment, dimethyl sulfoxide (DMSO) is employed to solubilize the formazan crystals for subsequent examination. Doxorubicin is utilized as a positive control, functioning as a conventional chemotherapeutic drug to evaluate the cytotoxic effects of the test compounds in comparison to a recognized cytotoxic agent.

Chemicals and reagents for GC-MS phytoconstituent characterization:

Table 8: *Chemicals and reagents for GC-MS phytoconstituent characterization*

Chemical/Reagent	**Purpose/Function**
Methanol	Used for plant extraction to dissolve phytoconstituents
Helium as carrier gas	Responsible for transporting the vaporized compounds through the column.
Standards for Calibration: Alkane Series Standards, Pure Reference Compounds (alkaloids, flavonoids, terpenoids)	Used for calibration and compound identification.

Table 9 highlights the chemicals and reagents employed in the GC-MS (Gas Chromatography-Mass Spectrometry) procedure for phytoconstituent characterization. Methanol serves as a solvent for the extraction of phytoconstituents from plant material, facilitating the dissolution of chemicals

for analysis. Helium functions as the carrier gas, enabling the conveyance of vaporized compounds across the GC column during separation. Standards, particularly alkane series standards and pure reference compounds (such as alkaloids, flavonoids, and terpenoids), are crucial for calibration and precise identification of phytoconstituents through the comparison of retention durations and mass spectra.

2.2 Plant material collection

The plant's bark powder was collected from Dr. Shahana Sharmin, Assistant Professor at BRAC University, School of Pharmacy. One of her thesis students collected the barks of *L. thorelli* from Nabiganj Upazila in the Habiganj District, Sylhet Division, Bangladesh. The specimen was assigned the accession number DACB-87494. The phytochemicals were subsequently extracted using ethanol.

Figure 2: *Lagerstroemia thorelli bark*

2.3 Preparation of plant extract

The bark powder of *Lagerstroemia thorelli* was collected and then dissolved in a beaker containing 1,000ml of ethanol. The powdered bark was subsequently soaked in ethanol for seven days to allow the compounds to dissolve into the solvent. After dissolve a period of seven days, the mixture was filtered with a suction pump to separate of the ethanol extract from the bark residue. After that, the ethanol was collected and kept it for drying by using a rotary evaporator, which is also known as a rotavapor, after the drying process it led to a development of concentrated plant extract. Following this process and the drying procedure, an authentic plant extract was obtained and it preserved for further testing. The pictures of the mixture and dried extract are showed in figure 3 & 4 accordingly.

Figure 3: Lagerstroemia thorelli bark powder soaked in methanol

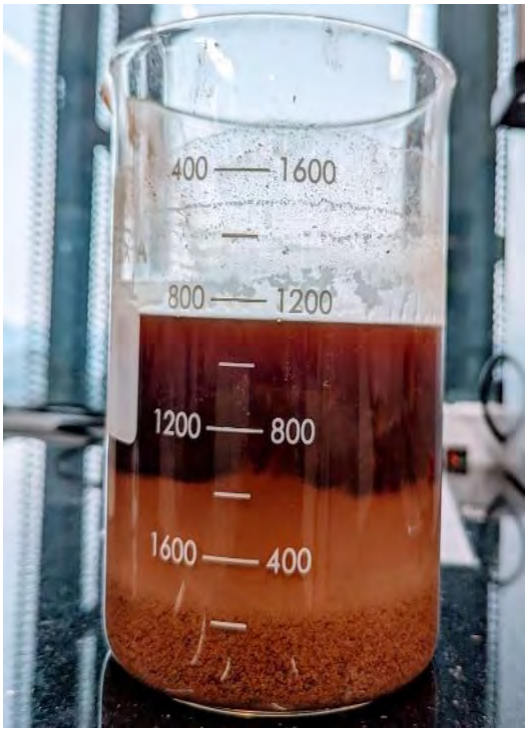

Figure 4: Dried L. thorelli methanol bark extract

2.4 Cytotoxicity property analysis

The MTT test is a well-established method for determining the cytotoxicity of medicinal plant such as *L. thorelli* ethanol bark extract. It is frequently used for evaluating cell survival and cytotoxicity. This assay is also known for its broad application and reliability. In this study the MTT assay is used to evaluate the cytotoxic effects of *L. thorelli* ethanol bark extract on cancer cells (HeLa cell). The aim is to determine the extract's IC_{50} value, indicating the concentration required for 50% inhibition of the survival of cells.

Conducting the MTT assay there are few steps which required for the *L. thorelli* bark extract are described in detailed below:

Preparation of solution: Several solutions for the cytotoxicity and cell culture experiments were prepared. Here 1% Penicillin-Streptomycin solution was used which blocks the formation of cell walls and protein to prevent bacterial contamination. Also to promote cellular growth, 1% penicillin-streptomycin, 10% fetal bovine serum (FBS), and 0.2% gentamicin were added to Dulbecco's Modified Eagle's Medium (DMEM). For the growth FBS provided all the vital nutrients such vitamins, proteins, and growth hormones. The gentamicin stock solution must be sterilized before the use. The promega cell titer 96 Assay Kit was used to measure the survival of cell in 96-well plates.

Preparation of sample- Sample was prepared for using four different concentrations of *Lagerstroemia thorelli* bark extract: 2.5 mg/mL, 0.25 mg/mL, 0.025 mg/mL, and 0.0025 mg/mL. A stock solution of 2.5 mg/mL was created by dissolving 7.5 mg of bark extract in 3 mL of dimethyl sulfoxide (DMSO). Serial dilutions utilizing DMSO were performed to obtain reduced concentrations. The samples went through to filtration before using them in the experiment.

Instrument used- Biological bio safety cabinet (Model: NU-400E, Nuaire, USA), CO_2 incubator (Nuaire, USA), Trinocular microscope with camera (Optika, Italy), Hemocytometer, Microplate spectrophotometer (EPOCH, Winooski, VT, USA)

Consumable used- 96-wall plate, 15ml tubes. Tips, Gloves, Culture flask, Cell culture media, Antibiotics (P+S), Gentamycin, Serological pipette, Trypsin etc.

Procedure- The cytotoxicity of *L. thorelli* bark extract was evaluated at the Centre for Advanced Research in Sciences utilizing HeLa (human cervical cancer cells). The cells were grown in DMEM supplemented with 1% penicillin-streptomycin, 0.2% gentamycin, and 10% fetal bovine serum (FBS). HeLa cells ($2.0x10^4$ cells/100 µL) were inoculated into 96-well plates and incubated at 37°C with 5% CO_2. On the subsequent day, 25 µL of filtered bark extract samples were introduced into each well. Cell viability was evaluated after 48 hours utilizing the cell titer 96 non-radioactive cell proliferation assay kit. Each sample underwent duplicate testing.

Data Analysis- Using this formula given below can calculate the % of cell viability for every extract concentration in comparison to the untreated control.

$$\text{Cell Viability (\%)} = \left(\frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ control\ cells}\right) \times 100$$

Plotting a graph that the dose-response relationship of cell viability (%) versus the concentration of the extract. The IC_{50} value, representing the extract concentration that induces 50% reduction of cell viability, can be determined from the dose-response curve using software like excel.

2.5 Method of GC-MS analysis of *Lagerstroemia thorelli* methanol extract

The GC-MS analysis of *Lagerstroemia thorelli* methanol extract was conducted to detect its phytoconstituents, employing a high-sensitivity GCMS-TQ8040 equipment (Shimadzu, Japan). In the methodology a DAB-5ms non-polar Innowax column was used which is also designed to differentiate a diverse array of phytoconstituents based on the volatility and polarity. At first, at the initial stage the column temperature was at 100°C for 1 minute, then the temperature gradually increased to 300°C over the time period of 20 minutes. This is efficient for the separation of compounds with low and high volatility. A minimum injecting concentration of 0.5 µL was inserted to maximize sample entrance into the system. The helium gas was the carrier gas, and at 1 mL/min the flow rate was set in the split less mode. To achieve a better compound separation the split ratio must be at five. To ensure the sufficient vaporization and detection of the compound, the injector and detector temperature were set at 250 and 230°C, respectively. To fragment the molecules the electron ionization was used at +0.50 kV. Then the mass spectra were taken from 50 to 600 m/z over the period of 40 minutes. This comprehensive study identified the specific compounds from the extract where finding bioactive components which can elucidate its cytotoxic, antioxidant, or other therapeutic characteristics. This method provides a complete chemical profile of this *L. thorelli* methanol bark extract which will help in the understanding of its biological effects.

2.6 Identification and quantification of individual phytoconstituents

To identify and quantify the phytoconstituents in the bark extract of *L. thorelli* the GC-MS analysis was used. First of all, in the retention times the compounds get isolated from the mixture as the compound pass through the gas chromatograph and meanwhile the mass spectra were analyzed to determine molecular weight and structure by generating the mass spectrum. The combination of retention time and mass spectral data identifies and characterizes the extract's phytoconstituents. After that the generated spectra were compared to reference samples were compared with the reference sample available in the Wiley database and National Institute of Standards & Technology (NIST) libraries (ALILOU and AKSSIRA, 2021). In a way, this method identifies a wide range of bioactive compounds which includes amides, glycosides, alkanes, esters, and fatty acids. All the compound has therapeutic qualities such as antibacterial, antioxidant, and anticancer activity.

Chapter 3

Results

3.1 Cytotoxicity of *L. thorelli bark extract* against HeLa cell line

The results of cytotoxic activity from *L. thorelli bark extract* against HeLa cell line is presented in table 9-

Table 9: Cell viability, survival of cell and inhibition of HeLa cell growth

Concentration of samples		**Absorbance @570nm**	**Survival of cell (%)**	**% of cell growth inhibition**	**IC_{50} (mg/mL)**
			HeLa	HeLa	
Control (DMSO)		1.608 ± 0.035	100	0	
Sample	0.0025mg/mL	1.576 ± 0.005	97.13	2.87	
	0.025 mg/mL	1.377 ± 0.088	87.38	12.62	IC_{50} value is 1.7856
	0.25 mg/mL	0.806333 ± 0.019	50.15	49.85	
	2.5 mg/mL	0.683333 ± 0.052	40.27	59.73	

Interpretation-

The table 9 highlights that, the trinocular microscope data indicates that the *L. thorelli* bark extract had the highest cytotoxicity against HeLa cells at the highest concentration of 2.5 mg/mL, resulting in 59.73% cell death. The lowest cytotoxicity was observed at the lowest concentration of 0.0025 mg/mL. At intermediate dosages, % of cell death was 49.85% and 12.62% for 0.25 mg/mL and 0.025 mg/mL concentration respectively, demonstrating a dose-dependent increase in cytotoxicity. The IC_{50} value indicates that *L. thorelli* bark extract has dose-dependent cytotoxicity, with increased concentrations resulting in increased inhibition of HeLa cell growth.

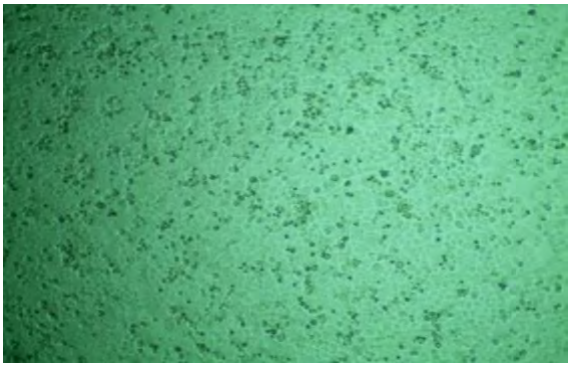

Figure A- DMSO control group

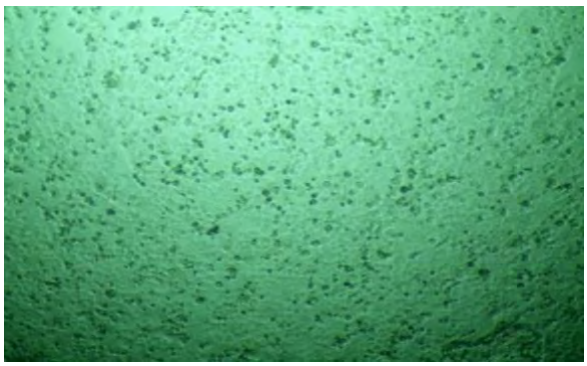

Figure B- Bark extract (0.0025mg/ml)

Figure C- Bark extract (0.025mg/ml)

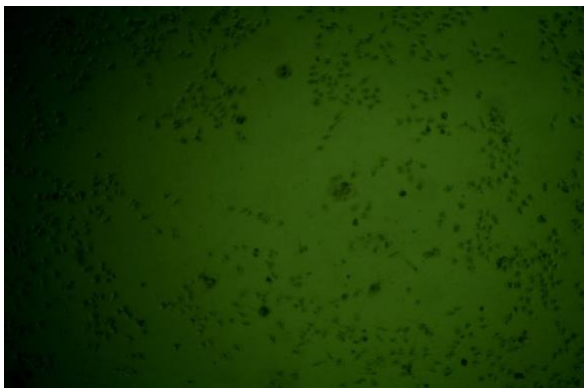

Figure D- Bark extract (0.25mg/ml)

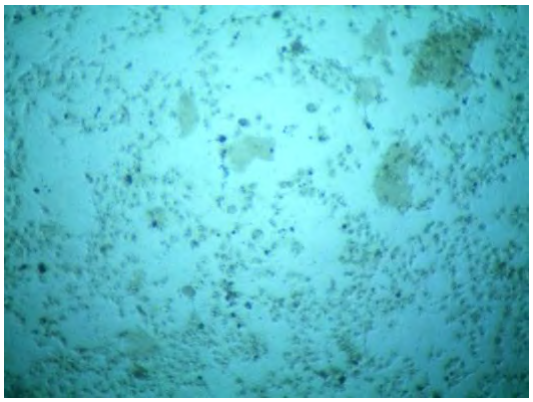

Figure E- Bark extract (2.5mg/ml)

Figure 5: *HeLa cell survival at all the concentrations of the bark extract*

Interpretation - The pictures show HeLa cells survival when exposed to different concentration of *L. thorelli* bark extract. Cell viability was 100% in the negative control (Figure A), which contained only DMSO. The minimum reduction of cell growth was seen at 2.87% at 0.0025 mg/mL concentration (Figure B). The cell growth inhibition percentage increased to 12.62% at a concentration of 0.025 mg/mL (Figure C), which decreased cell survival in comparison to lower concentrations of 0.0025 mg/mL. Cell growth suppression dramatically rose to 49.85% at 0.25 mg/mL concentration (Figure D), which resulted in a notable decrease in viable cells. With 59.73% cell death, the maximum suppression was observed at 2.5 mg/mL concentration (Figure E). The concentration required to inhibit 50% of cell viability was found to be 1.7856 mg/mL, which is the extract's IC_{50} value on the HeLa cell line. The given below bar graph (Figure 6) showing the ethanol bark extract of *Lagerstroemia thorelli's* cytotoxic effects on the HeLa cell line-

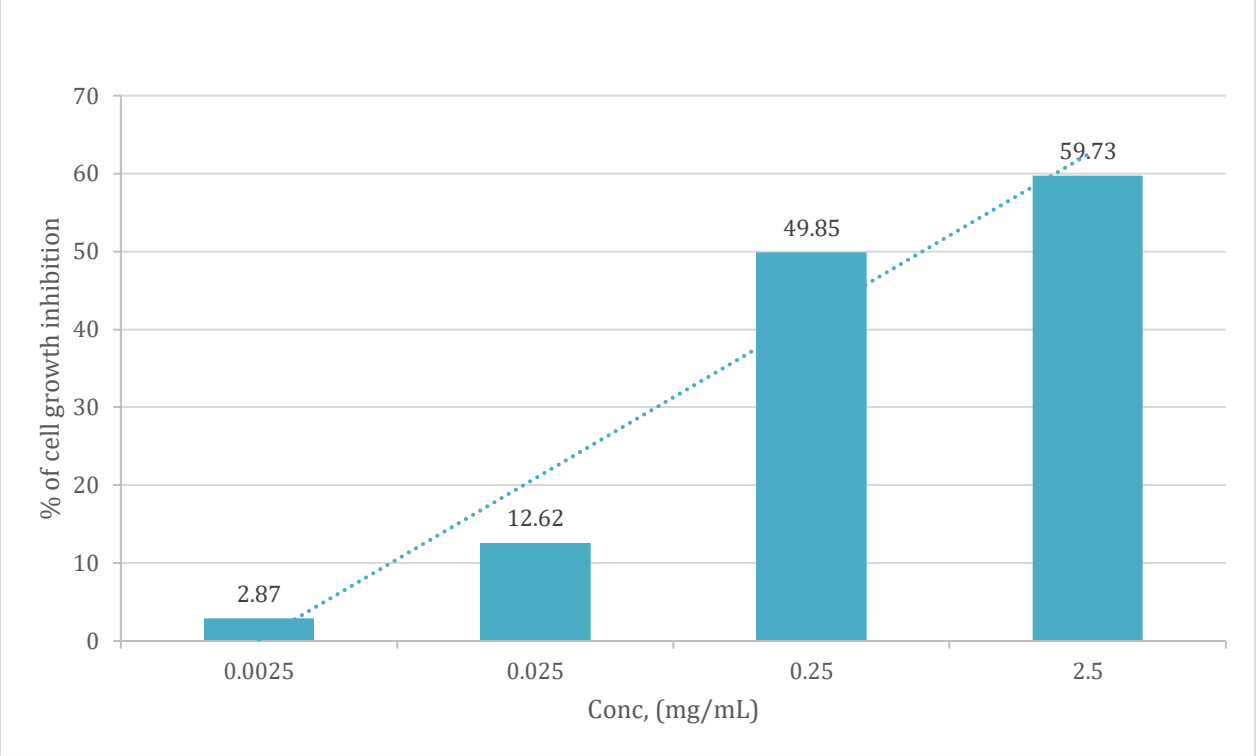

Figure 6: Cytotoxic effect of ethanol extract of Lagerstroemia thorelli bark on HeLa cell line

Interpretation- The figure 6 demonstrates a dose-dependent effect of *L. thorelli* bark extract on cancer cells. At a low concentration of 0.0025 mg/mL, the extract exhibited lowest effects, resulting in the lowest percentage of cell growth inhibition which is 2.87%. In contrast, at the highest concentration of 2.5 mg/mL, the extract caused the highest percentage of cell growth inhibition which is 59.73% and the lowest cell viability, indicating potent cytotoxic activity. The other two concentrations are 0.025mg/mL and 0.25mg/mL, here also the percentage of cell growth inhibition is 12.62% and 49.85% accordingly. The findings indicate that higher concentration of the extract is more efficacious in suppressing cancer cell proliferation, highlighting its potential as an anticancer treatment.

3.2 GC-MS Chromatogram of *Lagerstroemia thorelli* bark extract

The GC-MS chromatogram of *L. thorelli* bark extract offers essential insights into its phytochemical composition by isolating volatile components and identifying them through their mass spectra and retention durations. Essential bioactive components such as fatty acids, phenolic compounds, and saponins were identified, recognized for their cytotoxic activity (Alilou & Akssira, 2021). Significant chemicals, including thymol (a TBDMS derivative) and 13-docosenamide (Z), 6,9-Octadecadienoic acid, methyl ester was discovered, demonstrating potential anticancer effects. These results correspond with cytotoxicity experiments (IC_{50} = 1.7856 mg/mL) performed on HeLa cell lines, providing the therapeutic potential *of L. thorelli*, especially in cancer research. A GC-MS chromatogram of *L. thorelli* bark extract (figure 7, table 11) represents all the phytoconstituents identified by their retention time and mass spectra. The principal characteristics of a GC-MS chromatogram are:

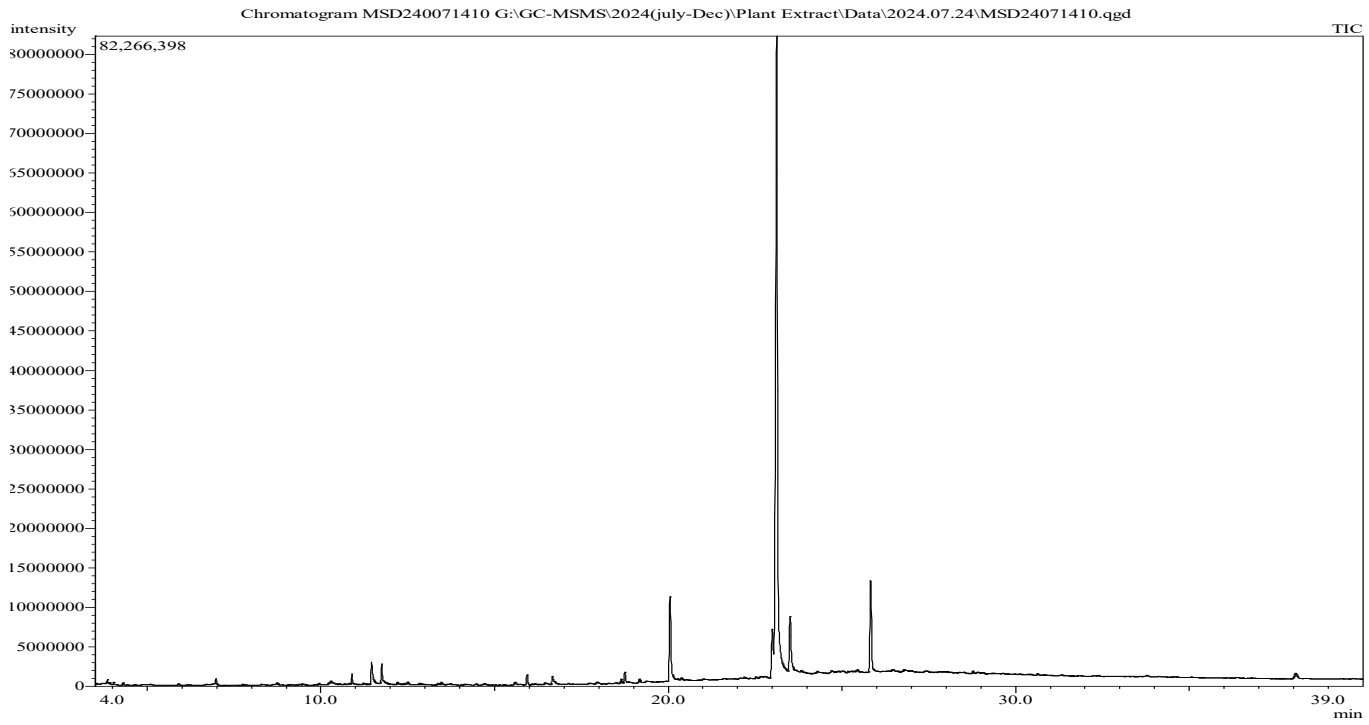

Figure 7- GC-MS chromatogram of methanol bark extract of Lagerstroemia thorelli

3.3 Phytoconstituents of the extract identified by GC-MS analysis

The GC-MS chromatogram of *Lagerstroemia thorelli* bark extract exhibits a prominent peak at 22 minutes, signifying the presence of a significant compound, 13-Docosenamide, (Z)- (C22H43NO). The component contains 64.7% of the total area. Minor peaks can be noticed between 10 and 20 minutes, as well as after 30 minutes, indicating the existence of less frequent compounds. Hexadecanamide was found at around 20minutes where the % of area was 6.61. Also 6,9-Octadecadienoic acid, methyl ester and Octadecanamide found at 22 & 23 minutes respectively where the % of area was 3.81 and 4.71. At 25 minutes another component was found which is Phthalic acid, di(2-propylpentyl) ester and its % of area was 6.83. The smaller peaks are probably terpenoids, phenolic compounds, or alkaloids, whereas the larger peak likely indicates a significant bioactive component, specifically a long-chain fatty acid amide. Mass spectral analysis is essential for comprehensive identification. These compounds may be responsible for the claimed pharmacological actions of the extract.

L. thorelli bark extract often contains a variety of bioactive compounds in the phytoconstituents identified by GC-MS analysis. Here a total 37 Phytochemicals are identified and quantified in the methanol bark extract of *L. thorelli*. Chemical formula and classification, of this GC-MS phytoconstituents are listed below in the table 10:

Table 10: Bioactive compounds identified by GC-MS analysis

SL NO	**R. Time**	**Area %**	**Compound Name**	**Molecular formula**	**Chemical class**
1	3.53	0.09	Thymol, TBDMS derivative	$C_{16}H_{28}Osi$	Phenolic compounds, **S**ilicon compounds.
2	3.8	0.08	3-Furaldehyde	$C_5H_4O_2$	Furan derivatives
3	4.301	0.09	Hexanal dimethyl acetal	$C_8H_{18}O_2$	Acetals

4	9.937	0.09	2-(4'-Methoxyphenyl)-2-(2'-methoxyphenyl) propane	$C_{17}H_{20}O_2$	Aryl propane derivatives
5	10.225	0.16	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	$C_4H_9NO_5$	Nitro alcohols / Nitro diols
6	10.325	0.12	Tridecane, 2,5-dimethyl-	$C_{15}H_{32}$	Alkanes
7	10.807	0.08	Pentadecane	$C_{15}H_{32}$	Alkanes
8	11.219	0.09	Nonane, 3-methyl-5-propyl-	$C_{13}H_{28}$	Alkanes
9	11.463	1.8	Beta -D-Glucopyranoside, methyl	$C_7H_{14}O_6$	Glycosides
10	11.754	1.09	Methyl (methyl 2,4-di-O-acetyl-3-O-methyl-alpha-D-galactopyranoside) uronate	$C_{13}H_{20}O_9$	Uronates
11	11.81	0.15	Eicosane, 2-methyl-	$C_{21}H_{44}$	Alkanes
12	13.368	0.14	Eicosane	$C_{20}H_{42}$	Alkanes
13	13.625	0.11	Hen eicosane	$C_{21}H_{44}$	Alkanes
14	15.932	0.6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Esters
15	16.67	0.68	Tetradecanamide	$C_{14}H_{29}NO$	Amides
16	18.642	0.25	Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$	Esters
17	18.75	0.67	6-Octadecenoic acid, methyl ester, (Z)-	$C_{19}H_{36}O_2$	Esters
18	19.167	0.25	Methyl stearate	$C_{19}H_{38}O_2$	Esters
19	20.053	6.61	Hexadecanamide	$C_{16}H_{33}NO$	Fatty acid amide
20	22.191	0.09	Triacontane, 1-bromo-	$C_{30}H_{61}Br$	Alkyl bromides
21	22.999	3.81	6,9-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	Fatty acid methyl ester
22	23.126	64.7	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	Fatty acid amides
23	23.507	4.71	Octadecanamide	$C_{18}H_{37}NO$	Fatty acid amides
24	23.695	0.21	11-Methyltricosane	$C_{24}H_{50}$	Alkanes
25	24.28	0.14	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	Haloalkanes
26	24.805	0.1	Phytyl, 2-methylbutanoate	$C_{25}H_{48}O_2$	Esters

27	24.885	0.14	Diglycolic acid, heptadecyl neopentyl ester	$C_{26}H_{50}O_5$	Esters
28	25.028	0.17	Trans-2,3-Epoxydecane	$C_{10}H_{20}O$	Epoxides
29	25.168	0.1	2-Methylhexacosane	$C_{27}H_{56}$	Alkanes
30	25.268	0.16	Erythro-7,8-Bromochlorodisparlure	$C_{19}H_{38}BrCl$	Organobromine compounds
31	25.331	0.1	Docosyl nonyl ether	$C_{31}H_{64}O$	Alkyl ethers
32	25.826	6.83	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	Phthalate esters
33	26.45	0.33	Z, Z-6,27-Hexatriactontadien-2-one	$C_{36}H_{68}O$	Unsaturated ketones
34	26.865	0.13	Beta-Alanine, N-methyl-N-(1-methyl-4-nitro-1H-imidazol-5-yl)-, methyl ester	$C_9H_{14}N_4O_4$	Amino acid derivatives
35	26.992	0.11	Tetrapentacontane	$C_{54}H_{110}$	Alkanes
36	27.399	0.13	Nonahexacontanoic acid	$C_{69}H_{138}O_2$	Fatty acids
37	38.053	0.67	Beta-Sitosterol	$C_{29}H_{50}O$	Sterols

In table 10 it highlights that the GC-MS chromatogram of *L. thorelli* bark extract reveals multiple significant components according to their retention times. The most significant peak occurs at 22 minutes, denoting 13-Docosenamide, (Z)-, with a chemical formula of C22H43NO, comprising 64.7% of the entire area, signifying it as the predominant constituent in the extract. Furthermore, Hexadecanamide is detected at 20 minutes, constituting 6.61% of the area. Two further significant chemicals, 6,9-Octadecadienoic acid methyl ester and Octadecanamide, were detected at approximately 22 and 23 minutes, comprising 3.81% and 4.71% of the area, respectively. At 25 minutes, a notable chemical, Phthalic acid, di(2-propylpentyl) ester, was identified, constituting 6.83% of the area. The results indicate that the extract has a combination of primary and secondary bioactive chemicals, with possible therapeutic applications.

3.4 GC-MS evaluates the bioactivity of the detected phytoconstituents.

GC-MS analysis facilitates the assessment of the bioactivity of identified phytoconstituents by determining the chemical components present in the plant extract, which frequently result in different pharmacological effects. Although GC-MS does not directly assess bioactivity, it offers comprehensive insights into the molecular structure and content of phytoconstituents. The bioactivity of GC-MS phytoconstituents are listed below in the table 11 which shows their chemical and biological properties.

Table 11- Compounds with their significant bioactivity

SL NO	Compound Name	Bioactivity	References
1	Thymol, TBDMS derivative	Antimicrobial, Antioxidant, Anti-inflammatory properties. TBDMS enhances stability.	Marchese et al., 2016; Lopes-Lutz et al., 2008
2	3-Furaldehyde	Antimicrobial, Antioxidant, used in flavoring and fragrances.	Wenzel et al., 2010; Shahidi & Zhong, 2015
3	Hexanal dimethyl acetal	Antimicrobial, used in flavoring and food preservation.	No et al., 2017; Özcan et al., 2005
4	2-(4'-Methoxyphenyl)-2-(2'-methoxyphenyl) propane	Antioxidant, Anti-inflammatory.	Atanacković et al., 2011
5	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	Biocidal, Antimicrobial.	Oskoueian et al., 2011
6	Tridecane, 2,5-dimethyl-	Insecticidal, Pheromonal.	Cj et al., 2017

7	Pentadecane	Pheromonal, Antimicrobial.	Bruno et al., 2015
8	Nonane, 3-methyl-5-propyl-	Pheromonal, Plant volatile.	Belmar et al., 2001
9	beta-D-Glucopyranoside, methyl	Antioxidant, Anti-inflammatory, Antimicrobial.	Da Silva et al., 1994; Aubert et al., 2004
10	Methyl (methyl 2,4-di-O-acetyl-3-O-methyl-. alpha. -D-galactopyranoside) uronate	Antimicrobial, Antioxidant, Anti-inflammatory.	Sarkar & Sucheck, 2010
11	Eicosane, 2-methyl-	Antimicrobial, Insect pheromone.	Octarya et al., 2021
12	Eicosane	Antifungal, Pheromone, Plant volatile.	Bhat et al., 2024
13	Hen eicosane	Antimicrobial, Insect pheromone.	Vanitha et al., 2020
14	Hexadecanoic acid, methyl ester	Anti-oxidant, Hypocholesterolemia, Nematicide, Pesticide, Lubricant, Anti-androgenic,	Siswadi & Saragih, 2021; Garrido et al., 2015
15	Tetradecanamide	Antimicrobial, Anti-inflammatory.	Moni et al., 2022
16	Methyl 9-cis,11-trans-octadecadienoate	Antioxidant, Anti-inflammatory.	Muzahid et al., 2022
17	6-Octadecenoic acid, methyl ester, (Z)-	Antioxidant, Anti-inflammatory.	Krishnamoorthy & Subramaniam, 2014

18	Methyl stearate	Anti-diarrheal, Cytotoxic, Anti-proliferative.	Nakaziba et al., 2022; Garrido et al., 2015
19	Hexadecanamide	Antimicrobial, Anti-inflammatory.	Rai et al., 2023
20	Triacontane, 1-bromo-	Insecticidal, Antifungal.	Mohamed et al., 2016
21	6,9-Octadecadienoic acid, methyl ester	Antioxidant, Anti-inflammatory.	Krishnamoorthy & Subramaniam, 2014
22	13-Docosenamide, (Z)-	Antimicrobial, Anti-inflammatory.	Shareef et al., 2016
23	Octadecanamide	Antimicrobial, Anti-inflammatory.	Cheng et al., 2010
24	11-Methyltricosane	Antimicrobial, Insect pheromone.	Saha et al., 2024
25	Tetrapentacontane, 1,54-dibromo-	Insecticidal, Antifungal.	Garrido et al., 2015
26	Phytyl, 2-methylbutanoate	Antimicrobial, Anti-inflammatory.	Ishtiaq et al., 2024
27	Diglycolic acid, heptadecyl neopentyl ester	Antimicrobial, Anti-inflammatory.	Medvedeva et al., 2017
28	trans-2,3-Epoxydecane	Antimicrobial, Insect pheromone.	Courregelongue & Pons, 2024
29	2-Methylhexacosane	Insect pheromone, Antimicrobial.	Spikes et al., 2010; De La Paz Fernández et al., 2010
30	erythro-7,8-Bromochlorodisparlure	Insect pheromone.	Joseph et al., 2016
31	Docosyl nonyl ether	Antimicrobial, Surfactant properties.	Ngangbam et al., 2024

32	Phthalic acid, di(2-propylpentyl) ester	Endocrine disruptor, Anti-inflammatory, orally toxic in Pregnancy	Garrido et al., 2015
33	Z, Z-6,27-Hexatriactontadien-2-one	Antimicrobial, Insect pheromone.	Shin et al., 2006
34	beta. -Alanine, N-methyl-N-(1-methyl-4-nitro-1H-imidazol-5-yl)-, methyl ester	Antimicrobial, Anti-inflammatory.	Ali et al., 2017
35	Tetrapentacontane	Antimicrobial, Insect pheromone.	Taher et al., 2023
36	Nonahexacontanoic acid	Antimicrobial, Anti-inflammatory.	Siswadi & Saragih, 2021
37	beta. -Sitosterol	Cholesterol-lowering, Anti-inflammatory, Anticancer.	Bangar et al., 2022

The table 11 shows a variety of compounds identified, along with their associated bioactivities. Compounds such as thymol TBDMS derivative, beta-D-glucopyranoside, and hexadecanoic acid methyl ester were detected at retention times of 3.53, 11.46, and 15.93 minutes, with relative peak areas of 0.09%, 1.8%, and 0.6%, respectively. These compounds show antimicrobial, antioxidant, and anti-inflammatory properties, making them potential candidates for therapeutic applications. A notable peak at 22 minutes signifies the presence of 13-Docosenamide, (Z)- ($C_{22}H_{43}NO$), comprising 64.7% of the total area, indicating its importance in the extract. Eicosane was detected at 13.36 minutes, recognized for its function as a plant volatile and insect pheromone, indicating its ecological interactions. Other bioactive compounds, such as beta-sitosterol (RT: 38.05, % area: 0.67), exhibit cholesterol-lowering, anticancer, and anti-inflammatory properties, rendering them

significant in disease management. Tetrapentacontane (RT: 26.99, % area: 0.11) and tridecane were identified, exhibiting insecticidal and antifungal properties pertinent to agricultural and pest control applications. The presence of phthalic acid, di(2-propylpentyl) ester raises concerns regarding its endocrine-disrupting properties, highlighting the necessity for caution. The detected compounds exhibit various bioactivities, such as antimicrobial, anti-inflammatory, antioxidant, and insecticidal properties, indicating potential applications in pharmaceuticals, agriculture, and environmental science.

Chapter 4

Discussion

Lagerstroemia thorelli is under investigation for its cytotoxic effects on HeLa cancer cell lines through the MTT assay. This study represents the initial investigation into the cytotoxicity of *L. thorelli*. Other species within the same genus have exhibited a range of bioactivities, including anti-inflammatory, anticancer, antidiabetic, and antioxidant effects. Established cytotoxicity standards indicate that crude extracts exhibit maximum potency when their IC_{50} values fall below 30 µg/mL. The research study examined the cytotoxic effects of *L. thorelli* bark extract on HeLa cells, demonstrating an independent dose-dependent response. The highest concentration of the extract (2.5 mg/mL) caused significant cell death, decreasing cell viability by 59.73%. The bark extract exhibits notable cytotoxic activity. The lowest tested concentration (0.0025 mg/mL) exhibited lower cytotoxicity, resulting in merely 2.87% cell mortality. Intermediate doses demonstrated heightened cytotoxicity, resulting in 12.62% cell mortality at 0.025 mg/mL and 49.85% cell mortality at 0.25 mg/mL concentration. The IC_{50}, or the dose at which 50% of the cells were inhibited, was determined to be 1.7856 mg/mL, signifying the extract's significant anticancer potential against HeLa cells. It appears less potent than the established cytotoxicity benchmark.

Through using the GC-MS analyzer, we were able to characterize and quantify a considerable number of chemicals that are related to fatty acids, fatty acid ester, alkanes, glycosides, esters, and other compounds in our study concerning *L. thorelli*. The GC-MS analysis of the bark extract identified a prominent peak at around 22 minutes, corresponding to 13-Docosenamide, (Z)- ($C_{22}H_{43}NO$), which represented 64.7% of the overall composition. This chemical is a long-chain fatty acid amide, indicating its possible bioactivity. Also, 6,9-Octadecadienoic acid methyl ester

and Octadecanamide, were detected at approximately 22 and 23 minutes, comprising 3.81% and 4.71% of the area, respectively. Additional minor peaks, detected between 10 and 20 minutes and after 30 minutes, are associated with minor chemicals, presumably terpenoids, phenolics, or alkaloids, which may also enhance the bioactivity of the extract. The findings indicate that *L. thorelli* bark extract possesses potent bioactive chemicals, notably 13-Docosenamide, (Z)-, which may account for its significant cytotoxic effects on cancer cells. The dose-dependent escalation in cell mortality, along with the recognized phytoconstituents, substantiates the extract's potential as a natural anticancer drug. The GC-MS analysis a chemical profile of the extract, determine its bioactivity by correlating the detected chemicals with established pharmacological effects, such as anticancer. Biological effects of *L. thorelli* bark extract phytoconstituents indicate therapeutic applications. Thymol, a TBDMS derivative, disrupts microbial membranes and removes free radicals to fight bacteria, viruses, inflammation, and cancer. Hexanal dimethyl acetal and 3-furaldehyde are antioxidants and antimicrobial, whereas 2-(4'-methoxyphenyl)-2-(2'-methoxyphenyl) propane inhibits cancer cell proliferation. Additionally, methyl beta-D-glucopyranoside promotes gut health and has antidiabetic benefits as a prebiotic. For beauty products, Hexadecanoic acid methyl ester and eicosane are used, while pentadecane, nonane, and 3-methyl-5-propyl- are used industrially. A variety of bioactive compounds in the extract suggest medicinal promise in several fields, requiring more study.

Significant variations are seen when comparing the cytotoxicity of *Lagerstroemia thorelli* against various Lagerstroemia species. For example, here *Lagerstroemia indica* has strong antibacterial, anti-inflammatory, anticancer, and antioxidant properties. whereas Triterpenoids, flavonoids, and phenolic acids are among the bioactive components of *L. indica* that are thought to be cytotoxic, meaning that they have a substantial impact on the cancer cells. The IC_{50} values of *L. indica* differ

based on the type of study carried out and often low concentration suggests its high cytotoxicity (Al-Snafi, 2019). Due to the outstanding uses of the plant for antidiabetic and anticancer treatment, this plant was focused remarkably. Similar to corosolic acid, the plant exhibits excellent anticancer activities due to its high cytotoxicity via compounds that yield IC_{50} values in the low µg/mL range. Like the *L. speciosa, L. floribunda* produces compounds as flavonoids and triterpenoids that act as anti-inflammatory and anticancer agents.

L. thorelli had an IC_{50} of 1.7856 mg/ mL in HeLa cells; which is rather a low cytotoxicity. The cytotoxic effect of *L. thorelli* is prominent yet the high IC_{50} value of permit to conclude that a higher concentration of the latter is needed to obtain effects similar to those of the former, thus making the compound less effective. This is the first report on cytotoxicity profile of *L. thorelli* and the extent of efficacy may be due to the characteristic phytoconstituents which showed different behaviors on some specific cancer cell line like HeLa. Other Lagerstroemia species, especially *L. indica, L. speciosa* and *L. floribunda* contain potential anticancer constituents which display higher levels of cytotoxicity than the one seen in the present study with *L. thorelli* extract at higher concentrations only. Further studies might target to identify and quantify the active compounds in *L. thorelli* that can enhance its cytotoxicity and therefore it's utility in cancer therapy.

L. thorelli, a Southeast Asian plant, contains a lot of bioactive compounds that include tannins, flavonoids and triterpenoids most of which show promising anticancer properties. These substances have been linked to a number of biological processes, such as reduced development of cancer cells, suppression of angiogenesis (the formation of new blood vessels in tumors), and apoptosis (programmed cell death). Because *L. thorelli* contains a variety of bioactive components, it may be a source of anticancer medications. However, there is some extent more work that is

required in order to enhance these compounds and obtain them as the potent anticancer drugs. The study of *L. thorelli's* mechanism, activity, and toxicity might contribute to revelation of novel anticancer drugs derived from plants. Pharmacological activity of *L. thorelli* bark extract phytoconstituents exhibit therapeutic potentials. Thymol, a TBDMS derivatives interacts with microbial membranes and neutralizes free radical to kill bacteria, viruses, inflammation and cancer. An antioxidant and antimicrobial activity were noticed in hexanal dimethyl acetal and 3-furaldehyde while 2-(4'-methoxyphenyl)-2-(2'-methoxyphenyl) propane exhibited the ability to stop cancer cell proliferation. Further, methyl beta-D-glucopyranoside establishes beneficial effects on the gut microbiota and acts as an antidiabetic through the prebiotic function. In the cosmetics, ester of Hexadecanoic acid methyl ester and eicosane are used whereas pentadecane, nonan and 3-methyl-5-propyl- are used for industrial usage. That there are a number of bioactive compounds in the extract, this indicates a medicinal potential in several fields, which needs further research.

Chapter 5

Conclusion & Future direction

Conclusion

This project was based on a medicinal plant which is *Lagerstroemia thorelli* for identifying the phytoconstituents and cytotoxic activities against HeLa cells. *L. thorelli* has not been studied earlier and this is the initial towards the research to evaluate the cytotoxic effects of *L. thorelli* against any cancer cell lines for its potential therapeutic use by demonstrating that it has the ability to reduce cell growth at higher concentration. The extract exhibits an IC_{50} value of 1.7856 mg/mL at a higher concentration of 2.5mg/ml which seem to be less potent than the established cytotoxicity benchmark**.** A total 37 phytoconstituents or bioactive compounds was found which are identified by using the phytochemical screening employed by GC-MS analysis. The compounds are including thymol, a derivative of TBDMS, 3-furaldehyde, hexadecanoic acid methyl ester, and other compounds notable which are demonstrating a beneficial effect as anticancer agents. This research demonstrated moderate cytotoxic activity in *L. thorelli* and identified a significant number of compounds.

Future direction

Future research on *L. thorelli* bark extract needs to concentrate on *in-vivo* studies that evaluate its efficacy, toxicity, and pharmacokinetics in living organisms, along with mechanistic studies to better understand the molecular mechanisms involved in its cytotoxic effects. The isolation and purification of active compounds are essential for finding the most effective drugs, while testing across a wider array of cancer cell lines, including breast, lung, and brain cancer, will aid in

assessing selectivity and minimizing potential toxicity. In addition, the usage of different solvents such as methanol, acetone and water for extracting *L. thorelli* leaves might also reveal several pharmacological activities. Further extensive research co-operating with existing pharmaceuticals for investigation of combination therapy might improve effectiveness and deliver methods may increase bioavailability. For medicinal plant's medical applications, the standardization of extraction procedures and the identification of particular components responsible for a variety of activities via the use of GC-MS methods will be absolutely necessary.

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