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GC-MS-employed Phytochemical Characterization and Anticancer, Antidiabetic, and Antioxidant Activity Screening of Lagerstroemia Thorelli

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Lagerstroemia thorelli (*L. thorelli*) is a member of the Lythraceae family and has not been previously researched. Thus, this study aimed to investigate its unexplored potential and identify novel therapeutic prospects. This research evaluated antioxidant, antidiabetic, and cytotoxic potentials along with compound characterization of the ethanolic leaf extract of *L. thorelli*. The antioxidant potential was assessed using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical and hydrogen peroxide (H_2O_2) scavenging assays, total antioxidant capacity (TAC), total phenolic content (TPC), total flavonoid content (TFC) determination, antidiabetic property was assessed using α-amylase inhibition, and the cytotoxic effect was examined on HeLa and Vero cells using MTT colorimetric assay. Chemical characterization was performed using gas chromatography-mass spectrometry (GC-MS). The findings demonstrated strong antioxidant, strong antidiabetic, and moderate cytotoxic activities. Comprehensive phytochemical analysis revealed its abundance in flavonoids, phenols/phenolics, tannins, glycosides, steroids, resin, etc. GC-MS analysis of the *L. thorelli* extract identified 80 important compounds including *cis*-11-eicosenamide, beta-D-glucopyranoside, methyl-, alpha-D-glucopyranoside, methyl-, phthalic acid, gamma-sitosterol, phytol, silicic acid, squalene, butanoic acid, cyclobarbital, etc. which are well-documented for their antioxidant, antidiabetic, and anticancer effects. Thus, it can be inferred that *L. thorelli* could hold new promises in treating diseases like diabetes and free radical-induced conditions, including neurodegenerative diseases.

1. Introduction

Throughout history, plants used for medicinal purposes have played a vital role in healthcare and research worldwide, proving their effectiveness, and generating a market worth over $100 billion.[1] Up to 80% of the world's population, as per the World Health Organization (WHO) reports, relies on traditional medicines, including herbal remedies, for primary care.[2,3] Researchers are increasingly turning to medicinal plants as they serve as an excellent source of bioactive compounds, with approximately 40% of pharmaceuticals on the market today coming from natural sources.[4] Scientists explore the beneficial phytochemicals present in medicinal plants like anthraquinones, glycosides, cardiac terpenoids, phenolic compounds, saponins, alkaloids, tannins, etc., through phytochemical analysis to investigate their therapeutic potential, thereby contributing to the advancement of novel therapeutics.[5]

Reactive oxygen species (ROS) are essential in tissue homeostasis and cell signaling, where it is critical to maintain the delicate harmony between generating and eliminating ROS.[6] The occurrence of any disruption in the state of balance due to excess or a lack of ROS results in the development of diseases. Consequently, modulating strategies become necessary to restore equilibrium and effective disease treatment.[6] Several studies have found a correlation between ROS and the development of carcinogenesis, mutations, and cellular transformation, primarily attributed to DNA, lipid, and protein damage.[7–10] Previous studies have documented that synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have potential carcinogenic and hepatotoxic effects.[11] Therefore, exploring naturally occurring antioxidants from medicinal plants is imperative. Antioxidants present in medicinal plants, such as flavonoids, tannins, and phenolic acids, play a crucial role in neutralizing ROS due to their potential antioxidant properties.[12] Thus, they offer a potentially effective approach for alleviating oxidative stress's deleterious effects.

Cervical cancer stands as the fourth most common malignancy among women worldwide, accounting for 342,000 deaths and 604,000 new cases in 2020.[13] Alarmingly, about 90% of these fatalities and new cases were reported from countries with low- and middle-incomes, underscoring its status as the third most prevalent malignancy type and the fourth

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most common cause of death from cancer in women.[13,14] While effective, the primary chemotherapy, cisplatin, has notable side effects and resistance issues, limiting its clinical use.[15,16] This has driven the pursuit of novel chemotherapeutic agents to enhance cervical cancer treatment, with a focus on reducing patient exposure to side effects through combination therapies. Recognizing these challenges, efforts are being made to explore the use of natural products derived from medicinal plants to reduce side effects and improve treatment outcomes.

The global incidence of type 2 diabetes has substantially increased during the previous three decades, impacting approximately 422 million individuals.[17] By the year 2025, it has been estimated that there will be 1.5 million annual deaths attributed to diabetes and obesity.[17] Therefore, affordable treatment is urgently needed to address this growing concern. Studies have identified several medicinal plants with antidiabetic properties due to their high content of phenol/phenolic compounds, alkaloids, terpenoids, flavonoids, and glycosides.[18,19] Quercetin from onions and resveratrol from grapevines are two examples of these compounds, which have been reported to enhance carbohydrate metabolism and improve insulin secretion.[20] Garlic, turmeric, and rosella flowers have been identified as potential remedies for diabetes.[21] Thus, researchers are increasingly exploring medicinal plants to uncover bioactive compounds for affordable diabetes treatments.

An unexplored species with no prior study, *Lagerstroemia thorelli*, is a perfect candidate for in-depth phytochemical analysis and bioactivity study. *L. thorelli* belongs to the Lythraceae family, falls under the Lagerstroemia genus, and is locally identified as "Jarul" in Bangladesh.[22,23] In this study, we aimed to analyze the antioxidant, cytotoxic, and antidiabetic properties of *L. thorelli* plant extract. It is important to note that no compounds have been identified from this plant as of today. Therefore, we conducted gas chromatography-mass spectrometry (GC-MS)-based phytochemical characterization to identify the individual compounds responsible for contributing to the screened activities.

2. Methodology

2.1. Plant Collection and Authentication

The leaves of *L. thorelli* were collected from Nabiganj Upazila, Habiganj (24.3750°N 91.4167°E.), Bangladesh, in October 2022 and were identified and authenticated by experts at the National Herbarium Bangladesh (NHB), Mirpur, Dhaka (Accession number DACB-87494).

2.2. Preparation of Extract

The cleaned and sun-dried leaves of *L. thorelli* were ground into a coarse powder, yielding an approximate mass of 306 g. For extraction, the powder was soaked in 1 L ethanol for 2–3 days at a temperature between 22–25 °C (room temperature), with intermittent agitation according to the method described by Phrompittayarat et al. with a slight modification.[24] After filtration, filtrate was concentrated in a water bath at 50–55 °C. To prevent cross-contamination, the concentrated extract was transferred to a petri dish and subjected to drying under laminar airflow. A total of 17.21 g extract was produced from 306 g of dry powder and the % yield of extract was 5.62 % w/w. Finally, the extract was stored at 4 °C in the refrigerator.

2.3. Preliminary Phytochemical Screening

Preliminary phytochemical analysis standard protocols were followed with slight modifications to identify the chemical components of the examined extract, including carbohydrates, flavonoids, glycosides, alkaloids, tannins, etc. (Table 1).

Table 1. Preliminary phytochemical screening of *L. thorelli* leaf extract.

Name of the test	Class of compounds	Presence (+)/Absence (−)
Potassium dichromate test, Lead acetate test, and Ferric chloride test	Tannins	+++
Zinc ribbon test, Lead acetate test, and Alkaline reagent test	Flavonoids	+++
Ferric Chloride test, Lead acetate test, and Iodine test	Phenol/ Phenolic compound	+++
Acetic anhydride test and Turbidity test	Resins	++
Hager's test, Mayer's test, and Wagner's test	Alkaloids	–
Molisch's test and Fehling's test	Carbohydrates	++
Libermann Burchard's test and Acetic anhydride test	Phytosterols	–
Borntrager's Test and Modified Borntrager's test	Glycosides	++
Froth test and Olive oil test	Saponins	–
Libermann-Burchard's test and Salkowski's test	Steroids	++

Note: Any phytochemical group is indicated by a "+" when it is present and by a "−" when it is not. Key to bioavailability: (++) ve indicates medium intensity, (+++) ve strong intensity, (+) ve weak intensity, and (−) ve indicates absence.

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2.4. Gas Chromatography-Mass Spectrometry (GC-MS) Employed Phytochemical Characterization of *L. Thorelli* Leaf Extract

GC-MS analysis of *L. thorelli* leaf extract was analyzed using GCMS-TQ8040 instrument (Shimadzu, Japan) with high sensitivity and high performance according to the method described here. A DB-5 ms non-polar column (internal diameter 30×0.25 mm and film thickness 0.25 μm was used. Initially, the column temperatures were kept at 50 °C for 1 minute, then gradually increased to 300 °C for 20 minutes. A volume of 0.5 μL of the extract was injected for analysis. Helium was used as the carrier gas with a flow rate set at 1 mL/min in the spitless mode. The split ratio was 5, and the temperatures of the sample injector and detector were maintained at 250 and 230 °C, respectively. Electrons having an energy of about +0.50 kV were used in electron ionization mass spectrometry. Furthermore, 40 minutes were spent recording mass spectra in the 50 m/z to 600 m/z region.

2.5. Quantification and Identification of Individual Phytoconstituents of *L. Thorelli* Leaf Extract

Identification and quantification of individual phytoconstituents of the *L. thorelli* leaf extract were done by comparing retention indices and mass spectra fragmentation patterns of each compound reference sample available in the Wiley database and National Institute of Standards & Technology (NIST) libraries.[25] Furthermore, the relative proportion of each component was determined by quantifying and relating its % peak area to the overall peak areas.

2.6. Assessment of *In-Vitro* Antioxidant Activity Using DPPH, H_2O_2, TPC, TFC, and TAC Methods

Numerous comprehensive techniques, including the DPPH free radical scavenging test, H_2O_2 scavenging assay, total antioxidant capacity assessment, and determination of the total flavonoid and phenol/phenolic content, were used to assess the antioxidant properties of the extract.

2.7. Evaluation of DPPH Free Radical Scavenging Activity in *L Thorelli* Leaf Extract

The widely employed technique of free radical scavenging with DPPH as mentioned by Rahman et al.,[26] was used to assess the free radical scavenging activity. It is measured through the discoloration of the DPPH solution following adding extract solution at varying concentrations (50–500 μg/mL). Each test tube contained a mixture of 3 mL of ethanol and 0.2 % DPPH, to which 1 mL of sample and the standard solution were added, followed by a 30-minute incubation in the dark. Absorbance was measured at 517 nm using a UV-visible spectrophotometer. This analysis used ethanol as the blank and ascorbic acid as the standard. The percentage of DPPH inhibition (% I) was calculated and plotted against extract concentration to determine IC_{50} values. The DPPH free radical's percent inhibition (% I) was determined using the following equation, **% Inhibition** = $\mathbf{(1\text{-}A/A_o)\times 100}$, where A_o stands for the blank's absorbance (1 mL ethanol + 3 mL DPPH solution), and A denotes the sample's or the standard's absorbance.[27]

2.8. H_2O_2 Scavenging Activity

The H_2O_2 scavenging activity was assessed by following the procedure outlined by Nabavi et al.[28] This method involved measuring the absorbance at 230 nm using a 40 mM H_2O_2 solution in a 50 mM phosphate buffer with a pH of 7.4. Following that, a volume of 2 mL of H_2O_2 was combined with a volume of 1 mL of the sample extract. After a 10-minute incubation time, the absorbance was compared to a blank solution containing only a phosphate buffer. The H_2O_2 scavenging activity was calculated using this formula, $\mathbf{H_2O_2}$ **scavenge (%)** = $\mathbf{[(A_{230\,Control}-A_{230\,Sample})/A_{230\,Control}]\times 100}$, where, A represents the absorbance, $A_{230\,Control}$ is denoted as the absorbance at 230 nm for the control solution and $A_{230\,Sample}$ is the absorbance at 230 nm for the sample solution.

2.9. Assessment of Total Phenolic Content (TPC)

A slightly modified version of the method narrated by Ainsworth and Gillespie (2007) was used to determine the total phenolic content (TPC).[29] The assessment of total phenol/phenolic content utilizes Folin-Ciocalteu's reagent as an oxidative agent and gallic acid solution as the standard as stated by Lamuela-Raventós et al.[30] 2 mL of Na_2CO_3 solution and 0.5 mL of gallic acid standard solutions at different concentrations (25 to 100 μg/mL) were added to test tubes. 0.5 mL of sample solutions were transferred to the test tubes containing Na_2CO_3 solution. Absorbance was measured at 760 nm wavelength after incubation for 20 minutes. A standard curve was generated employing gallic acid as a standard reference to determine the total phenolic content of the test sample. The TPC content of the extract was quantified in terms of gallic acid equivalents utilizing this equation: $\mathbf{C = (c\times V) \div m}$, where, C represents the TPC content, expressed as gallic acid equivalent (GAE), measured in milligrams of gallic acid per gram of dried plant extract, c denotes the gallic acid concentration calculated from the calibration curve (mg/mL), V is the volume of the sample solution in milliliters, and m is the weight of the sample in grams.

2.10. Assessment of Total Flavonoid Content (TFC)

A slightly modified approach derived from the methodology mentioned by Haida and Hakiman was used to assess the TFC of the plant extract.[31] Five different concentrations (100 to 1200 μg/mL) of standard and sample solutions were taken to

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which 3 mL of ethanol was combined, followed by the addition of 200 μL of 10% $AlCl_3$ solution and 200 μL of 1 M CH_3COOK solution. After the mixtures were allowed to rest for 30 minutes following dilution with 5.6 mL of distilled water, the absorbance was measured at 415 nm, with ethanol used as the blank and quercetin as the standard. The TFC was quantified using this formula, **C = (c×V)/m**, where C represents the TFC, given as quercetin equivalent (QE), in milligrams of quercetin per gram of dried plant extract, c denotes the concentration of quercetin calculated from the calibration curve (mg/mL), V is the volume of the sample in milliliters and m is the weight of the sample in grams.

2.10. Assessment of Total Antioxidant Capacity (TAC)

The TAC of *L. thorelli* leaf extract was determined using the previously described method by Pisoschi and Negulescu.[32] 300 μL of the sample and standard solution were added to each test tube, containing 2 mL of the reagent solution mixture (0.6 M sulfuric acid, 0.028 M sodium phosphate, and 0.004 M ammonium). The test tubes were incubated in a water bath at 95 °C for 90 minutes. The absorbance was recorded at 765 nm using a U-2910 UV-Vis spectrometer. The TAC of the extract was quantified in terms of ascorbic acid equivalent (AAE) using this formula, **A = (c×V) ÷ m**, where A represents the ascorbic acid equivalent measured in milligrams of ascorbic acid per gram of dry plant extract, c is the concentration of ascorbic acid (mg/mL), V is the volume of the sample in (mL), and m is the weight of the sample in grams.

2.11. Evaluation of *In-Vitro* Antidiabetic Activity

Kifle and Enyew, 2020, outlined the 3,5-dinitrosalicylic acid (DNSA) process used in the α-amylase inhibition assay.[33] DNSA reduces the yellow-colored DNS through a redox reaction, yielding a red color. Various concentrations ranging from 125–1000 μg/mL of plant extract and standard solutions were prepared and used to assess this activity. Glimepiride was used as a standard and the absorbance of standard and sample solutions was measured using a UV-Vis spectrophotometer (U-2910, Hitachi High Technologies, USA). The formula, **% α-amylase inhibition = (Ao–A1) ÷ Ao×100** was used to calculate the percentage inhibition of α-amylase where A_o represents the absorbance of the control and A_1 is the absorbance of the sample/standard.

2.13. MTT Assay

2.13.1. Cell Culture

The cytotoxicity screening of *L. thorelli* leaf extract was conducted using a human cervical cancer cell line (HeLa, ATCC CCL-2) and a normal cell line, healthy kidney cells from monkeys (VERO, ATCC CCL-81). The cell lines were procured from ATCC, based in Manassas, VA 20108, USA. The cell lines were placed in Advanced DMEM, supplemented with 10% inactivated NBCS and 5 mM L-glutamine, and incubated at 37 °C in a humidified environment with 5% CO_2.[12]

2.13.2. The MTT Colorimetric Assay

To determine the cytotoxicity of *L. thorelli* leaf extract, the MTT colorimetric assay was described and validated by Akter et al. and Uddin et al.[34,35] Cells were placed at a specific density of 1.0×10^4–2.0×10^4 cells/well in 96-well plates and then incubated, allowing them to adhere for 24 hours before being treated with different concentrations (1.0–2.5 mg/mL) of *L. thorelli* extract for 48 hours. Subsequently, the cells were washed, incubated with MTT solution for 2 hours, lysed with dimethyl sulfoxide (DMSO), and absorbance was measured at 560 nm after 45 minutes using a microplate reader (Wallac 1420 Multilabel counter, PerkinElmer). 2% DMSO and cycloheximide functioned as the negative and positive control, respectively. The cytotoxicity was determined with the equation, **% of cytotoxic activity = 100–(Absorbance of the test sample/ Absorbance of the negative control) * 100.**

2.14. Statistical Analysis

Experiments for assessing antioxidant potential (DPPH, H_2O_2, TPC, TFC, and TAC) were repeated and conducted three times to increase accuracy and precision. The MTT colorimetric assay was carried out in triplicates while antidiabetic activity was performed in duplicates. All statistical analyses, as well as the graphs, were done using MS Excel (2013), with all results presented as mean ± SD.

3. Results

3.1. Preliminary Phytochemical Screening of *L. Thorelli* Leaf Extract

The symbol (+) indicates the presence of phytochemicals in one experiment, (+ +) in two experiments, (+ + +) in three experiments, and absence is denoted by (–) (Table 1). The presence of flavonoids, glycosides, phenolic compounds, carbohydrates, tannins, steroids, and resins presence was confirmed by phytochemical screening of *L. thorelli leaf* extract (Table 1). However, the extract was devoid of saponins, phytosterols, and alkaloids.

3.2. GC-MS Employed Identification and Quantification of Individual Phytoconstituents of *L. Thorelli* Leaf Extract

GC-MS analysis of individual constituents of *L. thorelli* leaf extract led to the identification of 80 compounds belonging to different phytochemical classes (Figure 1, Table 2). Based on the

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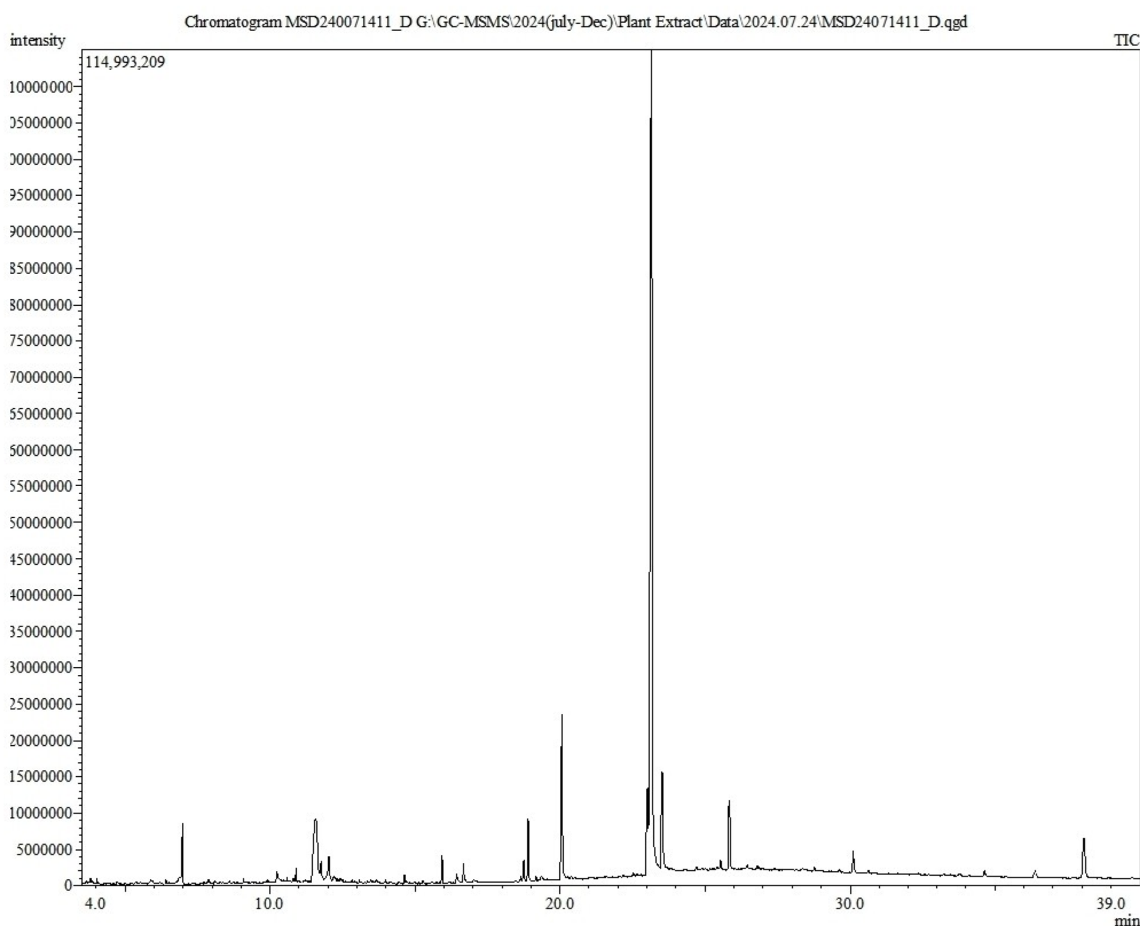

Figure 1. GC-MS chromatogram of *L. thorelli* leaf extract.

% area, the major compounds detected among them were *cis*-11-eicosenamide (49.25 %), hexadecanamide (6.53 %), octadecanamide (5.03 %), beta-D-glucopyranoside, methyl- (4.16 %), alpha-D-glucopyranoside, methyl- (4.04 %), 6,9-octadecadienoic acid, methyl ester (4.03 %), phthalic acid, di(2-propylpentyl) ester (2.8 %), gamma-sitosterol (2.8 %), phytol (2.22 %), silicic acid (1.916), alpha-methyl-l-sorboside (1.74 %), methyl (methyl 2,4-di-O-acetyl-3-O-methyl-alpha-D galactoside uronate (1 %) and squalene (0.87 %). The compounds of *L. thorelli* leaf extract identified through GC-MS analysis exhibit a diverse range of bioactivities that have been studied previously, as summarized in Table 3. These bioactivities include potential antidiabetic, antioxidant, anticancer, antimicrobial, anti-inflammatory, antifungal, neuroprotective, cardioprotective, antihyperlipidemic properties, and many others.

3.3. DPPH Free Radical Scavenging Activity of *L. Thorelli* Extract (LTE)

The antioxidant capacity of LTE was evaluated using DPPH free radical scavenging assay at various concentrations ranging from 500 μg/mL to 31.25 μg/mL, with ascorbic acid (AA) as the standard acid (Table 4). The findings showed an increase in the percentage of inhibition with the increasing concentration of *L. thorelli* extract and the standard ascorbic acid, suggesting a concentration-dependent antioxidant activity. At lower concentrations (between 31.25 μg/mL to 125 μg/mL), the antioxidant activity of the LTE was almost identical to that of ascorbic acid, the standard. The percentage inhibition of LTE showed a significant increase as the concentration rose to 500 μg/mL, compared to the value observed at 31.25 μg/mL. The half-maximal inhibitory concentration (IC_{50}) for LTE was 83.05 μg/mL, while for AA it was 47.55 μg/mL, indicating that LTE requires a comparatively greater concentration to inhibit at the same level as ascorbic acid.

3.4. Hydrogen Peroxide (H_2O_2) Scavenging Activity of *L. Thorelli* Leaf Extract

The hydrogen peroxide (H_2O_2) scavenging activity of the LTE is displayed in Table 5. The antioxidant activity of the LTE was assessed at various doses (31.25 to 500 μg/mL), using ascorbic acid (AA) as the standard. An increasing percentage saw a concentration-dependent antioxidant effect of inhibition of the sample extract at higher concentrations. The sample extract of LTE showed a strong inhibition of 120.07 % at the maximum

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Table 2. Phytoconstituents of *L. thorelli* identified and quantified by GC-MS.

Sl. No.	R. Time	Area %	Compound Name	Molecular Formula	Chemical Class
1	3.525	0.14	1-Gala-l-ido-octose	$C_8H_{16}O_8$	Sugars
2	3.665	0.12	Succinic acid, 3-methoxybenzyl nonyl ester	$C_{20}H_{28}O_4$	Esters
3	3.765	0.11	Furfural	$C_5H_4O_2$	Aldehydes
4	3.823	0.13	3,3-Dimethoxy-2-butanone	$C_8H_{12}O_4$	Ketones
5	3.862	0.06	Silane, methyldiethoxymethoxy-	$C_6H_{16}O_3Si$	Organosilanes
6	3.895	0.05	1,3-Dioxolane-4-methanol, 2-ethyl-	$C_6H_{12}O_3$	Dioxolanes
7	4.046	0.08	1,1,3,3-Tetramethyl-3-(1-methylpropoxy) disiloxan-l-ol	$C_8H_{22}O_3Si_2$	Siloxanes
8	4.737	0.06	6-Oxa-bicyclo [3. l .0] hexan-3-one	$C_5H_6O_2$	Bicyclic ketones
9	5.891	0.13	Arsenous acid, tris(trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	Organoarsenic compounds
10	5.961	0.08	D-Limonene	$C_{10}H_{16}$	Monoterpenes
11	6.095	0.05	Pantolactone	$C_6H_{10}O_3$	Lactones
12	6.413	0.07	7-Octen-2-ol, 2,6-dimethyl-	$C_{10}H_{200}$	Alcohols
13	7.885	0.1	Alpha-Terpineol	$C_{10}H_{18}O$	Terpenes
14	8.868	0.05	Acetic acid, l,7,7-trimethyl-bicyclo [2.2] hept-2-yl ester	$C_{12}H_{20}O_2$	Esters
15	9.096	0.11	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	Phenols
16	9.936	0.07	2-(4′-Methoxyphenyl)-2-(2′-methoxyphenyl) propane	$C_{17}H_{20}O_2$	Alkyl propane
17	10.242	0.62	2-(Isobutoxymethyl)oxiranehexadecanoic acid	$C_7H_{14}O_2$	Oxiranes
18	10.475	0.1	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	Alkanes
19	10.54	0.06	Tetradecane, 3-methyl-	C_5H_{32}	Alkanes
20	10.589	0.12	1 -Undecanol	$C_{11}H_{24}O$	Alcohols
21	10.695	0.07	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	Alkanes
22	10.805	0.12	Beta-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O$	Sugars
23	11.224	0.06	Pentadecane, 7-methyl-	$C_{16}H_{34}$	Alkanes
24	11.55	4.04	Alpha-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	Glycosides
25	11.588	4.16	Beta-D-Glucopyranoside, methyl	$C_7H_{14}O_6$	Glycosides
26	11.756	1	Methyl (methyl 2,4-di-O-acetyl-3-O-methyl-alpha-D-galactoside uronate	–	Glycosides
27	11.895	0.2	Silane, [[(3beta,5alpha,11beta,20 R)-pregnane-3,11,20,21-tetrayl]tetrakis(oxy)]tetrakis[trimethyl	$C_{33}H_{68}O_4Si_4$	Organosilanes
28	12.026	1.74	Alpha-Methyl-l-sorboside	$C_7H_{14}O_6$	Sugar alcohols
29	12.2	0.45	9-methylheptadecane	$C_{18}H_{38}$	Alkanes
30	12.345	0.12	Tridecane, 4-cyclohexyl-	$C_{19}H_{38}$	Alkanes
31	12.434	0.2	1-Ethynyl-3,5-dimethyladamantane	$C_{14}H_{20}$	Adamantanes
32	12.501	0.1	Octadecane, 3-methyl-	$C_{19}H_{40}$	Alkanes
33	12.596	0.04	1-Tetradecanol	$C_{14}H_{30}O$	Alcohols
34	12.836	0.07	Heptadecane	$C_{17}H_{36}$	Alkanes
35	13.084	0.11	Ethanol, 2-(dodecyloxy)-	$C_{14}H_{30}O_2$	Alkyl ethers
36	13.369	0.07	Eicosane	$C_{20}H_{42}$	Alkanes
37	13.472	0.15	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	$C_{10}H_{12}O_3$	Phenolic compounds
38	13.56	0.06	Heptadecane, 4-methyl-	$C_{18}H_{38}$	Alkane
39	13.675	0.15	Dodecanamide	$C_{12}H_{25}NO$	Fatty amide of Lauric acid
40	13.994	0.1	Loliolide	$C_{11}H_{16}O_3$	Benzofuran
41	14.635	0.28	Neophytadiene	$C_{20}H_{38}$	Diterpenes
42	14.718	0.12	1 -(2-Propen-1-yloxy) dodecane	$C_{15}H_{28}O$	Alkyl ether
43	15.262	0.13	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Phytol
44	15.586	0.05	Isopropyl tetradecyl ether	$C_{17}H_{36}O$	Alkyl ether
45	15.642	0.02	Butanoic acid	$C_{10}H_{16}F_3NO_3$	Fatty acid

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Table 2. continued

Sl. No.	R. Time	Area %	Compound Name	Molecular Formula	Chemical Class
46	15.933	0.87	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Fatty acid ester
47	16.435	0.51	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Fatty acid
48	16.666	0.85	Tetradecanamide	$C_{16}H_{34}O_3$	Fatty amide of myristic acid
49	17.035	0.12	Diethylene glycol monododecyl ether	$C_{19}H_{34}O_2$	Fatty acid ester
50	18.646	0.19	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	Fatty acid ester
51	18.75	0.79	8,11,14-Docosatrienoic acid, methyl ester	$C_{23}H_{40}O_2$	Fatty acid ester
52	18.901	2.22	Phytol	$C_{20}H_{40}O$	Diterpene alcohol
53	19.172	0.17	Methyl stearate	$C_{19}H_{38}O_2$	Fatty acid methyl ester
54	19.357	0.19	7-Hexadecenal, (Z)-	$C_{16}H_{30}O$	Fatty aldehyde
55	20.061	6.53	Hexadecanamide	$C_{16}H_{33}NO$	Fatty amide
56	20.275	0.13	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	Fatty acid methyl ester
57	20.715	1.92	Silicic acid	$C_{10}H_{28}O_4Si_3$	Silicon oxoacid
58	22.525	0.18	Methyl myristoleate	$C_{15}H_{28}O_2$	Fatty acid methyl ester
59	23.008	4.03	6,9–0ctadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	Fatty acid ester
60	23.155	49.25	cis-11-Eicosenamide	$C_{20}H_{39}NO$	Fatty amide
61	23.517	5.03	Octadecanamide	$C_{18}H_{37}NO$	Fatty amide of stearic acid
62	23.821	0.09	2-Methylhexacosane	$C_{27}H_{56}$	Alkane
63	24.5.11	0.05	Cyclobarbital	$C_{12}H_{16}N_2O_3$	Barbiturates
64	24.895	0.1	4-Hydroxy-4-methylhex-5-enoic acid, tert.-butyl ester	$C_{11}H_{20}O_3$	Fatty acid ester
65	25.019	0.04	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyllcyclohexan-1- perhydrol	$C_{14}H_{24}O_4$	–
66	25.065	0.05	Bis(3,7-dimethyloct-6-enyl) phthalate	$C_{28}H_{42}O_4$	Phthalic acid ester
67	25.312	0.03	Pregn-4-ene-3,11,20-trione	$C_{32}H_{58}N_2O_6Si_3$	Pregnane derivative
68	25.435	0.19	11-Methyltricosane	$C_{24}H_{50}$	Alkane
69	25.539	0.49	Hexadecanoic acid, 2-hydroxy-l -(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	Fatty acid ethyl ester
70	25.693	0.07	Undec-10-ynoic acid, octadecyl ester	$C_{29}H_{54}O_2$	Fatty acid
71	25.834	2.84	Phthalic acid, di(2-propylpentyl) ester	$C_{26}H_{26}O_4$	Phthalate ester
72	26.72	0.04	Nonadecyl heptafluorobutyrate	$C_{23}H_{39}F_7O_2$	Fatty acid ester
73	26.85	0.12	18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradehydro-16(hydromethyl)-, methyl ester, (15. beta.,16 E) -	$C_{21}H_{28}N_2O_2$	Secoyohimban alkaloid
74	26.984	0.14	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	Dibromoalkane
75	30.116	0.87	Squalene	$C_{30}H_{50}$	Triterpene
76	33.797	0.12	2-Butenal,2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	$C_{14}H_{22}O$	Aldehyde
77	34.351	0.14	4-Pyridinecarbaldehyde 4-methyl-3-thiosemicarbazone	$C_8H_{10}N_4S$	Organo thiocompound
78	34.643	0.24	Vitamin E	$C_{29}H_{50}O_2$	Tocopherol
79	36.38	0.44	Campesterol	$C_{28}H_{48}O$	Phytosterol
80	38.066	2.84	Gamma-Sitosterol	$C_{29}H_{50}O$	Phytosterol

concentration of 500 μg/mL, whereas the standard, AA, showed a little higher inhibition of 142.967%. The LTE's antioxidant activity consistently closely resembled that of AA at lower doses (between 62.5 to 250 μg/mL), indicating similar efficacy in scavenging free radicals at these concentrations. The sample's efficacy was similar to the standard, as evidenced by the close IC_{50} values of 2.84 for AA (standard) and 2.22 for the LTE (sample).

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Table 3. Bioactivity of the identified phytoconstituents studied previously.

Sl. No.	Compound Name	Bioactivity	References
1	1-Gala-l-ido-octose	Memory drug production to prevent dementia	[36]
2	Succinic acid, 3-methoxybenzyl nonyl ester	Potent antioxidant	[37]
3	Furfural	Antiviral, antioxidant, anti-tumor, anti-histaminic, and fungicides	[38]
4	3,3-Dimethoxy-2-butanone	–	–
5	Silane, methyldiethoxymethoxy-	–	–
6	1,3-Dioxolane-4-methanol, 2-ethyl-	Potential antibacterial and antifungal activity	[39]
7	1,1,3,3-Tetramethyl-3-(1-methylpropoxy) disiloxan-l -ol	–	–
8	6-Oxa-bicyclo [3. I .0] hexan-3-one	–	–
9	Arsenous acid, tris(trimethylsilyl) ester	Anti-rheumatic activity and treat skin infections	[40]
10	D-Limonene	Antimicrobial	[41]
11	Pantolactone	–	–
12	7–0cten-2-ol, 2,6-dimethyl-	–	–
13	Alpha-Terpineol	Antioxidant, anti-inflammatory, antimicrobial, anticancer, analgesic, gastroprotective, cardioprotective, neuroprotective, and antidiar-rheal	[42]
14	Acetic acid, l,7,7-trimethyl-bicyclo [2.2]hept-2-yl ester	–	–
15	2-Methoxy-4-vinylphenol	Anti-bacterial	[43]
16	2-(4′-Methoxyphenyl)-2-(2′-methoxyphenyl) propane	–	–
17	2-(Isobutoxymethyl)oxirane	–	–
18	Dodecane, 2,6,11-trimethyl-	Antimicrobial	[44]
19	Tetradecane, 3-methyl-	Potential antimicrobial activity	[45]
20	1 -Undecanol	–	–
21	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	–	–
22	Beta-D-Glucopyranose, 1,6-anhydro-	–	–
23	Pentadecane, 7-methyl-	Antioxidant and antimicrobial	[46]
24	Alpha-D-Glucopyranoside, methyl	Antimicrobial	[47]
25	Beta-D-Glucopyranoside, methyl	–	–
26	Methyl (methyl 2,4-di-O acetyl-3-O-methyl-alpha-D-galactoside uronate	Antimicrobial	[47]
27	Silane, [[(3.beta,5.alpha, 11.beta 20 R)-prenane3,11,20,21tetrayl)[tetrakis(oxy)[tetrakis trimethyl-	–	–
28	Alpha-Methyl-l-sorboside	Antioxidant	[48]
29	9-methylheptadecane	–	–
30	Tridecane, 4-cyclohexyl-	–	–
31	1-Ethynyl-3,5-dimethyladamantane	Treat Alzheimer's disease	[49]
32	Octadecane, 3-methyl-	–	–
33	1-Tetradecanol	–	–
34	Heptadecane	Antifungal and antimicrobial	[50]
35	Ethanol, 2-(dodecyloxy)-	Local anesthetic and sclerosing agent	[51]
36	Eicosane	Antifungal activity	[52]
37	(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	Antifungal activity	[53]
38	Heptadecane, 4-methyl-	Antioxidant and antimicrobial	[46]
39	Dodecanamide	Anti-inflammatory and antimicrobial	[54,55]
40	Loliolide	Neuroprotective, antiapoptotic, anti-inflammatory, antiaging, antidiabetic, antioxidant, antidiarrheal, and anthelmintic	[56–59]
41	Neophytadiene	anti-inflammatory, anti-microbial, antioxidant, antipyretic, and anticonvulsant	[60,61]
42	1 -(2-Propen-1-yloxy) dodecane	–	–

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Table 3. continued

Sl. No.	Compound Name	Bioactivity	References
43	3,7,11,15-Tetramethyl-2-hexadecen-1 -ol	Anti-inflammatory, antibacterial, antioxidant, antitumor, and potent antidiabetic (Inhibit alpha-amylase and alpha-glucosidase)	[62,63]
44	Isopropyl tetradecyl ether	–	–
45	Butanoic acid	Antithyroid, vasoconstrictor, anticancer, anti-inflammatory, gut protective, potential antidiabetic, treat mental health problem (schizophrenia)	[64,65]
46	Hexadecanoic acid, methyl ester	Antioxidant, anticancer, antidiabetic, nephroprotective, anti-inflammatory, and antibacterial	[66–70]
47	n-Hexadecanoic acid	Anti-inflammatory antimicrobial, antioxidant, antiatherosclerotic antiandrogenic anticancer Antitumor Hypocholesterolemic	[71–73]
48	Tetradecanamide	–	–
49	Diethylene glycol monododecyl ether	–	–
50	9,12-Octadecadienoic acid, methyl ester	Antioxidant, antimicrobial, and anti-inflammatory	[74]
51	8,11,14-Docosatrienoic acid, methyl ester	–	–
52	Phytol	Antioxidant, antidiabetic, anticancer, anti-inflammatory, diuretic, antitumor, genotoxic, chemoprotective, antimicrobial, antiprotozoal, histamine release inhibitor, and antimicrobial	[75,76]
53	Methyl stearate	Anti-inflammatory, antidiarrheal, cytotoxic, antiproliferative, and antioxidant	[77–79]
54	7-Hexadecenal, (Z)-	Antiviral, and antimicrobial	[80,81]
55	Hexadecanamide	Anti-inflammatory, anticancer, antitumor, antimicrobial, antioxi-dant, antiatherosclerotic antiandrogenic, and hypocholesterolemia	[82]
56	Silicic acid	Skin disorder, bone health, atherosclerosis, Alzheimer's disease, and immune system enhancement	[83]
57	Heptasiloxane, hexadecamethyl-	Antioxidant, antibacterial, anticancer, and antifungal	[84]
58	Methyl myristoleate	–	–
59	6,9–0ctadecadienoic acid, methyl ester	–	–
60	cis-11-Eicosenamide	Antimicrobial	[85]
61	Octadecanamide	Hypolipidemic	[86,87]
62	2-Methylhexacosane	Anticancer and antidiabetic (Alpha-glucosidase inhibitor)	[88]
63	Cyclobarbital	Anesthetic general, testosterone 17beta-dehydrogenase (NADP+) inhibitor, neurotransmitter antagonist, anticonvulsant, skeletal muscle relaxant, and antiproliferative	[89]
64	4-Hydroxy-4-methylhex-5-enoic acid, tert.-butyl ester	Antimicrobial, anticancer, and Antioxidant	[88,90]
65	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyllcyclohexan-1- perhydrol	–	–
66	Bis(3,7-dimethyloct-6-enyl) phthalate	–	–
67	Pregn-4-ene-3,11,20-trione	Hormone biosynthesis (aldosterone)	[91]
68	11-Methyltricosane	Antioxidant	[73]
69	Hexadecanoic acid, 2-hydroxy-l -(hydroxymethyl)ethyl ester	Hemolytic, antioxidant hypocholesterolemia, pesticidal, and nematicidal	[73,92]
70	Undec-10-ynoic acid, octadecyl ester	Inhibitor of cytochrome P450 4 A1, antioxidant, antifungal, and wound healing activity	[93]
71	Phthalic acid, di(2-propylpentyl) ester	Antimicrobial and anticancer	[94,95]
72	Nonadecyl heptafluorobutyrate	–	–
73	18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradehydro-16(hydromethyl)-, methyl ester, (15. beta.,16 E) -	–	–
74	Tetrapentacontane, 1,54-dibromo-	Hypolipidemic and antioxidant	[73]

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Table 3. continued

Sl. No.	Compound Name	Bioactivity	References
75	Squalene	Antioxidant, antitumor, and antidiabetic	[96,97]
76	2-Butenal,2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	–	–
77	4-Pyridinecarbaldehyde 4-methyl-3-thiosemicarbazone	Anticancer, metal-chelating, and anti-prolative	[98]
78	Vitamin E	Antioxidant, anticancer, antidiabetic, immunomodulator, and anticoagulant	[99,100]
79	Campesterol	Antidiabetic, anticancer, and antitumor	[101,102]
80	Gamma-Sitosterol	Antidiabetic (Increase insulin secretion and inhibit glucogenesis)	[73,103]

Table 4. DPPH free radical scavenging activity of *L. thorelli* extract (LTE).

Conc. (μg/mL)	% of Inhibition by Ascorbic acid (AA) (Mean ± SD)	% of Inhibition by LTE (Mean ± SD)	IC_{50} (μg/mL)
500	96.62 ± 1.022	88.44 ± 2.041	LTE = 83.05 AA = 47.55
250	93.90 ± 0.892	78.49 ± 1.205	
125	90.21 ± 0.621	68.21 ± 0.854	
62.5	75.28 ± 0754	66.29 ± 0.251	
31.25	57.95 ± 0.551	59.06 ± 1.015	

The data was presented as the mean value of duplicate of the triplicate experiments with standard deviation.

Table 5. Hydrogen peroxide (H_2O_2) scavenging activity of *L. thorelli* extract (LTE).

Sample Concentration (μg/mL)	% of inhibition by AA (Mean ± SD)	% of inhibition by LTE (Mean ± SD)	IC_{50} value (μg/mL)
500	142.967 ± 0.019	120.07 ± 0.032	LTE = 2.22 AA = 2.84
250	85.82 ± 0.019	88.96 ± 0.032	
125	74.57 ± 0.018	78.82 ± 0.070	
62.5	60.59 ± 0.078	69.2 ± 0.077	
31.25	33.03 ± 0.019	61.9 ± 0.102	

The data was presented as the mean value of duplicate of the triplicate experiments with standard deviation.

Table 6. Total phenolic content, total flavonoid content, and total antioxidant capacity of *L. thorelli* leaf extract.

Concentration (μg/mL)	TPC present in sample (mg) of Gallic acid per gram of dried extract (Mean ± SD)	TFC present in Sample (mg) of quercetin per gram of dried extract (Mean ± SD)	TAC present (mg) of ascorbic acid per gram of dry extract in the sample
1200	750 ± 4.138	893 ± 0.221	97.916 ± 0.003
800	650 ± 1.617	752 ± 0.127	50.583 ± 0.002
400	470 ± 0.087	575 ± 0.144	22.916 ± 0.014
200	210 ± 0.399	273 ± 0.142	13.08 ± 0.003
100	157 ± 0.076	125 ± 0.175	5.916 ± 0.009

The data was presented as the mean value of duplicate of the triplicate experiments with standard deviation.

3.5. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Total Antioxidant Capacity (TAC) of *L. Thorelli* Leaf Extract

The TPC, TFC, and TAC of *L. thorelli* leaf extract were assessed. The data obtained as the average of three duplicate experiments are summarized in Table 6.

The total phenolic content (TPC) of the LTE at concentrations ranging from 100–1200 μg/mL was determined through absorbance measurements of the standard gallic acid and the sample extract (Table 6). The TPC present in the sample was expressed in milligrams of gallic acid equivalent per gram of dried extract. At the maximum concentration of 1200 μg/mL, the absorbance of the *L. thorelli* leaf extract sample's estimated TPC was 750 ± 4.138 mg/g of dry extract. The data pattern suggested that the TPC in the sample reduced as the extract concentration decreased.

The total flavonoid content (TFC), calculated through absorbance, given in mg of quercetin equivalent per gram of dry extract, is shown in Table 6 for LTE at various doses ranging from 100 to 1200 μg/mL. The trend indicated TFC of the leaf extract of *L. thorelli* varied with concentration. The TFC falls consistently (from 893 ± 0.221 to 125 ± 0.175) as the concentration of the *L. thorelli* (sample) extract dropped (from 1200 to 100), implying a decline in flavonoid content as concentration was lowered.

The total antioxidant capacity (TAC) of the extract at different concentrations (100–1200 μg/mL) is shown in Table 6. TAC values were expressed in mg of ascorbic acid equivalent per gram of dry extract in the sample. As the concentration increased from 100–1200 μg/mL, the TAC in the sample increased from 5.916 ± 0.009 to 97.916 ± 0.003 mg, showing an increase in TAC as the concentration increased.

Overall, the data trend implied a concentration-dependent variation, the higher the extract concentration, the greater the phenolic and flavonoid content, as well as enhanced antioxidant activity.

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3.6. Percentage Inhibition of α-Amylase by Standard (glimepiride) and Sample (*LT*)

The % inhibition of α-amylase by the standard (glimepiride) and sample (LT) at different concentrations (125–1000 μg/mL) is given in Table 7. The data suggested that the α-amylase activity was effectively inhibited by *L. thorelli* in a concentration-dependent manner and increased inhibition was observed with increasing concentrations of *L. thorelli*. It is of note that the inhibitory action of *L. thorelli* (sample) was close to that of glimepiride (standard), highlighting its potential as an antidiabetic medication.

Table 7. Percentage inhibition of α-amylase by standard (glimepiride) and sample (*LT*).

Conc. of Standard (μg/mL)	% α-amylase inhibition of glimepiride (mean ± SD)	% α-amylase inhibition of LT (mean ± SD)
125	84.14 ± 6.109 %	80.1 ± 10.324 %
250	88.49 ± 2.333 %	84.7 ± 6.951 %
500	90.98 ± 2.496 %	88.5 ± 6.088 %
1000	95.36 ± 3.345 %	93.9 ± 3.429 %

The data was presented as the mean value of duplicate experiments with standard deviation.

3.7. Cytotoxic Activity of *L. Thorelli* Leaf Extract Against Cervical Cancer Cells (HeLa)

The percentage inhibition of *L. thorelli* leaf extract was assessed at different concentrations from 1–2.5 mg/mL against healthy monkey kidney cells (Vero) and cervical cancer cells (HeLa). *L. thorelli* leaf extract was found to possess concentration-dependent anticancer activity. DMSO 2% concentration was used as the negative control, showing no impact on cell growth (Figure 1). The inhibition of cell growth of HeLa cells was less at lower concentrations (1 mg/mL, 1.5 mg/mL, and 2 mg/mL) as seen in Figure 2 and Table 8. At maximum concentration, (2.5 mg/mL) of sample (LTE) extract, a notable 75% inhibition of HeLa cells was recorded while Vero cells exhibited only 38.66% inhibition, indicating greater cytotoxic potential of *L. thorelli* at higher concentrations. Furthermore, the IC_{50} for HeLa cells (IC_{50} 2.36 mg/mL), was nearly half of that for Vero cells (IC_{50} 4.66 mg/mL), illustrating a relatively stronger cytotoxic effect on Hela, cervical cancer cells compared to Vero, healthy monkey kidney

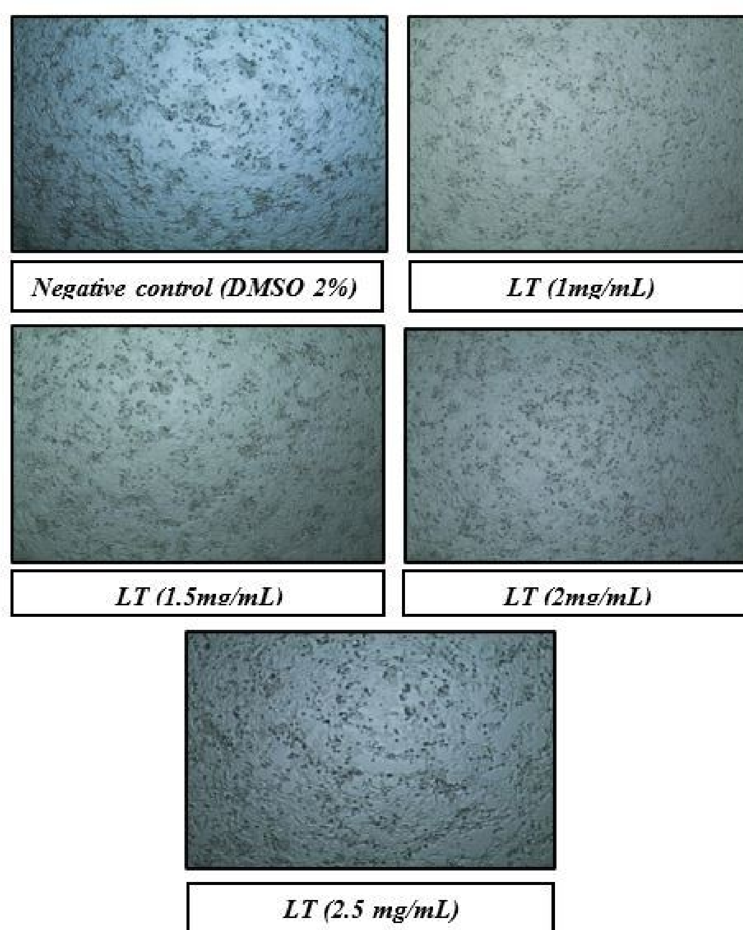

Figure 2. Hella cell survival at all the concentrations of the *L. thorelli* leaf extract.

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Table 8. Cytotoxic activity of *L. thorelli* leaf extract against cervical cancer cells (HeLa) and Vero cells.

Sample concentration	% of cell growth inhibition (Vero) Mean±SD	% of cell growth inhibition (HeLa) Mean±SD	IC_{50} (mg/mL)
2% DMSO (Negative control)	0	0	2.36 mg/mL (HeLa) 4.66 (Vero)
1 mg/mL	1.22±2.031	11.08±3.016	
1.5 mg/mL	3.76±1.640	19.8±2.841	
2 mg/mL	5.13±1.241	22.66±1.642	
2.5 mg/mL	38.66±3.015	75±3.954	

The data was presented as the mean value of triplicate experiments with standard deviation.

cells which indicated less toxicity of *L. thorelli* extract to normal healthy cells (Figure 2 and Table 8).

4. Discussions

Different species of the Lagerstroemia genus have been scientifically investigated to determine their biological and therapeutic activities.[104–109] Among them, the most studied species was *Lagerstroemia speciosa* (*L. spceiosa*) and its antioxidant, hypoglycemic, antibacterial, anti-inflammatory, antidiarrheal, antiobesity, antiviral, antimicrobial, etc. properties have been identified and reported.[104,108] In addition to that, different phytochemicals were isolated and identified from this species.[108] For example, corosolic acid, ursolic acid, amyl alcohol, lageracetal, gallic acid, 4-hydroxybenzoic acid, beta-sitosterol, ellagic acid, 3,3,4-tri-O-methyl ellagic acid, 3-O-methyl-3,4-methylenedioxy ellagic acid, asiatic acid, alphitolic acid, 3,31–di-O-methyl ellagic acid, 3,4,3,4-tetra-O-methyl flavellagic acid, 31, 41-di-O-methyl-3, 4-methylenedioxy flavellagic acid, 3-O-methyl ellagic acid, alanine, alpha amino butyric acid, isoleucine, 6,7-dihydroxy ellagitannin 7, methionine, neolignan, and coumarin have been identified and characterized from *L. speciosa*. From *Lagerostromia floribunda* a wide range of phytochemicals such as ursolic acid, 23-hydroxy ursolic acid, alphitolic acid, sesamin, dihydro-β-cyclopyrethrosin, β-sitosterol, betulinic acid, Clauslactone-K, linguee resinol, etc., have been identified.[104,108]

L. thorelli belongs to the Lythraceae family and has remained scientifically unexplored. This study is the first to investigate its antioxidant, antidiabetic, and cytotoxic effects against cervical cancer cells (HeLa) using ethanolic leaf extract. Additionally, we are reporting some compounds that were identified for the first time from the leaf extract of this plant using GC-MS followed by a preliminary phytochemical analysis of the extract.

Preliminary phytochemical analysis of ethanol leaf extract of *L. thorelli* revealed the presence of flavonoids, phenols/phenolic compounds, steroids, tannins, glycosides, resins, and carbohydrates.[110] However, alkaloids, phytosterols, and saponins were absent in the extract (Table 1). Phytochemicals have been reported to possess great antioxidant potential and provide beneficial effects on human health. Among phytoconstituents mentioned as health benefits providers are primarily flavonoids, iso-flavonoids, phytosterols, phytoestrogens, anthocyanidins, terpenoids, etc.[110] Conversely, in a previous study, preliminary phytochemical analysis of ethanol and aqueous extracts of other species *L. speciosa*, identified the presence of steroids, phenolic compounds, alkaloids, flavonoids, glycosides, terpenoids, saponins, α-amino acids, carbohydrates, starch, organic acids, reducing sugars and tannins in the samples, while cyanogenic glycosides were not detected.[104]

GC-MS analysis of the ethanol extract of *L. thorelli* characterized the individual phytochemicals present in the leaf of this plant. GC-MS analysis enabled the identification of a significant number of important compounds (80 compounds) with diverse chemical natures, including furfural, D-limonene, alpha-terpineol, loliolide, neophytadiene, butanoic acid, silicic acid, β-D-glucopyranoside, 4-pyridinecarbaldehyde 4-methyl-3-thiosemicarbazone, cyclobarbital, pregn-4-ene-3,11,20-trione, phytol, squalene, vitamin E, campesterol, gamma-sitosterol, etc. and all of them possess distinct pharmacological properties (Table 3). It is noteworthy to mention that butanoic acid has been reported to possess antidiabetic, anticancer, anti-inflammatory, antithyroid, gut-protective properties and β-D-glucopyranoside has antioxidant, antidiabetic, anticancer, anti-inflammatory, and analgesic activities.[65,111,112] Moreover, anticancer and antiproliferative effects were reported for alpha-terpineol, phytol, hexadecanoic acid, methyl ester, n-hexadecanoic acid, hexadecanamide, vitamin E, campesterol, squalene, cyclobarbital, 4-Pyridinecarbaldehyde 4-methyl-3-thiosemicarbazone, etc.[89,98,113] Silicic acid has the potential to treat skin disorders, atherosclerosis, Alzheimer's disease, enhance the immune system, and maintain bone health.[83] Pregn-4-ene-3,11,20-trione plays a vital role in aldosterone biosynthesis.[87] In addition to that, a significant number of compounds have been reported previously for their antioxidant activity including furfural, alpha-terpineol, loliolide, neophytadiene, n-hexadecanoic acid, hexadecanamide, phytol, squalene, vitamin E, etc. (Table 3). The findings of many studies have demonstrated the potent antidiabetic effect of multiple compounds namely 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, butanoic acid, phytol, 2-methylhexacosane, squalene, vitamin E, campesterol, and gamma-sitosterol (Table 3).

A literature search on the selected plant divulged no investigations had been carried out on *L. thorelli* to determine its biological and therapeutic activities. Most importantly, previous studies with other species of Lagerstroemia disclosed their strong antioxidant and antidiabetic properties and moderate cytotoxic activity which formed the basis of this study.[104,108] It is worth mentioning that this is the first study of assessing antioxidant, antidiabetic, and cytotoxic activity of *L. thorelli* species.

In the current study antioxidant activity was evaluated using five different comprehensive methods: DPPH free radical scavenging test, H_2O_2 scavenging assay, total antioxidant

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capacity assessment, and determination of the total phenolic and flavonoid content. Concentration-dependent strong DPPH free radical scavenging potential of *L. thorelli* was identified in the current study where the highest DPPH free radical scavenging activity was 88.44±2.041% at the highest concentration of 500 μg/mL and the IC_{50} value was 83.05 μg/mL whereas IC_{50} value of standard ascorbic acid was 47.55 μg/mL. In a previous study, *L. speciosa* methanol flower extract showed excellent DPPH free radical scavenging activity with an IC_{50} value of 41.51 μg/mL and the effect was concentration-dependent.[114] Since the species, plant part, and solvent were different, the antioxidant effect also seemed to be different between the previous study and our study. In another study conducted by Mousa et al. where *L. speciosa* ethanol leaf extract exhibited 97.71% DPPH free radical scavenging at the concentration of 100 μg/mL with an ED_{50} value 10.21±1.33 μg/mL and ED_{50} value of the standard, ascorbic acid was 1.83±1.41 μg/mL and the antioxidant effect was also concentration-dependent.[115] In the present study, *L. thorelli* extract exhibited strong H_2O_2 scavenging activity with an IC_{50} value of 2.22 μg/mL, which was lower than the IC_{50} of standard ascorbic acid (IC_{50}=2.84 μg/mL), and the effect was concentration-driven. Pareek et al. assessed the H_2O_2 scavenging activity of *L. speciosa* hydro-alcoholic leaf extract and found a very strong H_2O_2 scavenging effect with an IC_{50} of 28.00±0.16 μg/mL, which was much lower than ascorbic acid (IC_{50} =187.33±3.45 μg/mL).[116]

TFC, TPC, and TAC measurement assays with *L. thorelli* leaf extract displayed a higher quantity of flavonoids (893±0.221 mg quercetin equivalent/g of dry extract) and phenolics (750±4.138 mg gallic acid equivalent/g of dry extract) in the extract at the concentration of 1200 μg/mL, and 97.916±0.003 mg of ascorbic acid equivalent/g of dry extract was the TAC. The findings indicate that the extract is rich in flavonoids and phenolics. A previous study with *L. speciosa* methanol flower extract also showed that the extract contains a remarkable quantity of phenolics (418.0 mg/g) and flavonoids (50.8 mg/g).[114] A comparison of our study findings with the previous results evident that the leaf extract contained much higher flavonoids and phenolics than the flower extract. Flavonoids are powerful exogenous antioxidants, and they reduce free radicals to generate less reactive oxygen species, and the strong antioxidant potential of flavonoids is associated with their molecular structure, particularly the number and location of hydroxyl groups present in it, resonance, and conjugation effect.[117] Another investigation reported the total phenolic content of aqueous extract of *L. speciosa* where the TPC was found to be 72.3±0.293 mg gallic acid equivalent/100 mg of dry extract and the phenolic compounds have been reported as powerful natural antioxidants due to their hydroxyl groups, which contribute to free radical scavenging and their ability to donate hydrogen.[107] The overall strong antioxidant effect of the *L. thorelli* leaf extract was attributed to the presence of a significant number of compounds in this extract particularly- furfural, alpha-terpineol, loliolide, silicic acid, neophytadiene, n-hexadecanoic acid, hexadecanamide, phytol, squalene, vitamin E, etc (Table 3).

The α-amylase inhibition assay was conducted to assess the antidiabetic property of *L. thorelli* ethanol leaf extract, and this is the first report of the antidiabetic effect of this plant. Carbohydrate metabolism has been reported to increase postprandial glucose levels, and one of the approaches to lowering postprandial glucose levels is the inhibition of carbohydrate digestive enzyme activity.[118] α-amylase is the key enzyme that causes the breakdown of polysaccharides into glucose by catalyzing the hydrolysis of α-1,4-glucan linkages existing in starch, maltodextrins, and related carbohydrates.[119,120] Inhibition of this enzyme activity can prevent the conversion of polysaccharides into glucose molecules and thus, can control the glucose level in the body.[119] Rigorous control of postprandial glucose levels by inhibiting α-amylase activity is crucial in preventing, developing, and treating diabetes. Alpha-amylase inhibitors inhibit the digestibility and absorption of carbohydrates in the gastrointestinal tract, and thus this enzyme can act as a carbohydrate blocker.[119] This research confirmed that *L. thorelli* methanol leaf extract possessed strong α-amylase inhibition activity (93.9±3.429%) which was very close to the standard drug, glimepiride (95.36±3.345%). Our result was in congruence with the finding of a previous study where another species called *L. speciosa* (methanol extract) exhibited very high α-amylase inhibition activity which was 90.82±2.70%. However, ethyl acetate or hexane extract of *L. speciosa* showed low inhibition with values of 54.42±2.36% and 58.50±11.19%, respectively.[121] The effect of solvent plays a crucial role as polar protic solvents like methanol, ethanol, etc. have higher extraction yield compared to non-polar aprotic solvents like ethyl acetate and hexane.[122] Thus, polar protic solvents helped to extract more phytochemicals belonging to diverse classes which showed increased inhibition compared to extract of other solvents. Therefore, the use of ethanol (polar protic solvent) in the experiment ensured a higher yield of extract, causing a strong inhibition of α-amylase by *L. thorelli*, making it a potent contributor as an antidiabetic agent. Natural compounds that act as α-amylase inhibitors include flavonoids, alkaloids, terpenes, iminosugars, and thiosugars.[123] Preliminary phytochemical analysis identified the presence of flavonoids, polyphenols, and carbohydrates that may be responsible for the strong α-amylase inhibition by LTE. GC-MS analysis of LTE identified 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, butanoic acid, phytol, 2-methylhexacosane, squalene, vitamin E, campesterol, gamma-sitosterol, butanoic acid and methyl beta-D-glucopyranoside as its individual compounds, and most importantly, these compounds have been reported to possess antidiabetic effects (Table 3).[65] Therefore, the strong α-amylase inhibition by LTE may be attributed to these compounds. The mechanism of antidiabetic action of 2-methylhexacosane involved inhibition of alpha-glucosidase and inhibition of both alpha-amylase and alpha-glucosidase resulting in potent hypoglycemic effect of 3,7,11,15-tetramethyl-2-hexadecen-1-ol.[88,124] Antidiabetic action of gamma-sitosterol was attributed to increased insulin secretion and inhibition of gluconeogenesis.[103]

Cervical cancer is one of the significant global public health burdens and the leading cause of morbidity and mortality in

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women, including in Bangladesh.[125] Since the treatment cost and drug resistance remain a concern in cancer treatment discovery and development of novel drugs are of utmost importance. Plant-based medicines have huge potential as chemotherapeutics and some plant-derived drugs serve as mainstream therapy for different cancers, for example, paclitaxel for breast cancer, vinblastine, and vincristine for leukemia, and flavopiridol for colorectal cancer.[126] Since no cytotoxic/anticancer effect studies were conducted previously on *L. thorelli* species the current study attempted to screen the anticancer potential of *L. thorelli* against cervical cancer cells (HeLa). The findings of the MTT test against this cell line demonstrated moderate cytotoxicity with an IC_{50} value of 2.36 mg/mL and 75% cell growth inhibition was measured at the highest concentration of 2.5 mg/mL. Compared to HeLa cells this extract demonstrated lower cytotoxicity, 38.66% cell growth inhibition (IC_{50}: 4.66 mg/mL) at the highest concentration of 2.5 mg/mL against healthy monkey kidney cells, Vero. Cytotoxic potentials of ethanolic *L. speciosa* leaf extract induced G1-phase of cell cycle arrest and apoptosis in human hepatocellular carcinoma, HepG2 cells.[127] MTT assay of aqueous ethanolic extract of *L. speciosa* against human lung adenocarcinoma cells (A549) showed a decrease in cell viability of 50.92 ± 0.5% with an IC_{50} value of 841.23 µg/mL at the concentration of 1 mg/mL.[115]

5. Conclusions

This study reported bioactivities and characterization of compounds of *L. thorelli* that have remained unexplored. It primarily focused on the investigations into antioxidant, antidiabetic, and cytotoxic effects of *L. thorelli*, along with the characterization of its compounds, offering a novel perspective on possible pharmacological interventions. The results demonstrated strong antioxidant, strong antidiabetic, and moderate cytotoxic effects of *L. thorelli*. Preliminary phytochemical analysis revealed the presence of different phytochemical classes like flavonoids, phenols/phenolics, tannins, resins, and glycosides which are known to possess important health benefits with strong antioxidant, antidiabetic, and cytotoxic properties. Furthermore, GC-MS analysis of the extract identified 80 compounds and most importantly many of them have been reported previously for their potent antioxidant, antidiabetic, and anticancer effects. Therefore, these compounds may be responsible for the bioactivities detected in this study. The findings from this study indicate that *L. thorelli* could be a promising starting point for developing new medications targeting diabetes and free-radical-induced diseases.

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Declaration of Competing Interests

The authors declare that they have no known competing interests.

Author Contributions

Conceptualization: RA, SHN; Methodology and Experiments: RA, SHN, SH, EST, IKL, AHT, MMRMM, FAR, SS; Writing-Original draft: RA, LMF; Writing-review and editing: RA, SH, FAR. All authors revised the manuscript and approved the final submitted version.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: *Lagerstroemia thorelli* · Antioxidant · Antidiabetic · Cytotoxicity · Phytochemicals · GC-MS

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