

Simarouba glauca leaves as a natural arsenal: GC-MS-based proximate and phytochemical insights with antimicrobial efficacy

Original Article

Abstract:

Simarouba glauca, commonly known as Lakshmi Taru, is a medicinal plant rich in bioactive compounds with diverse therapeutic potential. This study aimed to investigate the phytochemical profile, elemental composition, and antimicrobial efficacy of its leaf extracts. GC-MS analysis of methanol and chloroform extracts revealed the presence of key constituents, including flavonoids, alkaloids, glycosides, and quinic acid. Proximate and elemental analyses confirmed high nutritional content and minimal heavy metal contamination, with all detected metals within WHO safety limits. The antimicrobial activity was evaluated against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, and the chloroform extract exhibited the largest inhibition zones (up to 22 mm). These findings highlight the plant's potential as a safe natural source of antimicrobial agents and dietary supplements. The comprehensive profile of *S. glauca* supports its use in traditional medicine and paves the way for its integration into modern phytopharmaceutical applications.

Key words:

Simarouba glauca, phytochemical analysis, GC-MS, antimicrobial activity, elemental composition

Apstrakt:

Listovi *Simarouba glauca* kao prirodni arsenal: GC-MS zasnovana analiza osnovnog hemijskog sastava i fitokonstituenata

Simarouba glauca, poznata i kao Lakshmi Taru, predstavlja lekovitu biljku bogatu bioaktivnim jedinjenjima sa širokim spektrom terapijskog potencijala. Ova studija imala je za cilj da ispitava fitohemijski profil, elementarni sastav i antimikrobnu efikasnost ekstrakata njenih listova. GC-MS analiza metanolnog i hloroformskog ekstrakta otkrila je prisustvo ključnih sastojaka, uključujući flavonoide, alkaloide, glikozide i kininsku kiselinsku. Proksimalna i elementarna analiza potvrdile su visok nutritivni sadržaj i minimalnu kontaminaciju teškim metalima, pri čemu su svi detektovani metali bili u granicama bezbednosti koje propisuje SZO. Antimikrobnu aktivnost ispitivana je protiv *Staphylococcus aureus*, *Escherichia coli* i *Candida albicans*, a hloroformski ekstrakt pokazao je najveće zone inhibicije (do 22 mm). Ovi nalazi ukazuju na potencijal ove biljke kao bezbednog prirodnog izvora antimikrobnih agenasa i dijetetskih suplemenata. Sveobuhvatan profil vrste *S. glauca* podržava njenu upotrebu u tradicionalnoj medicini i otvara mogućnost za njenu integraciju u savremene fitofarmaceutske primene.

Ključne reči:

Simarouba glauca, fitohemijska analiza, GC-MS, antimikrobnu aktivnost, elementarni sastav

Introduction

Simarouba glauca (abbreviated as *S. glauca*), commonly known as Lakshmi Taru or the paradise tree (Simaroubaceae family), is a tropical plant species native to the rainforests of South America, particularly regions of Brazil, Colombia, and Peru. Known for its diverse medicinal properties (Armour., 1959), this plant has been widely used in traditional medicine to treat a range of ailments, including fever, dysentery, gastrointestinal

disorders, and malaria (Fiaschetti et al., 2011, Jose et al., 2018). Recent studies have focused on its vast pharmacological potential of *Simarouba glauca*, with increasing interest in its bioactive compounds, namely quassinoids (Vieira & Braz-Filho., 2006) and therapeutic efficacy in managing chronic diseases such as cancer, diabetes, and inflammatory conditions significant due to the rich content of bioactive phytochemicals including quassinoids, alkaloids, flavonoids, terpenoids, saponins, and tannins in various parts of this plant (Hussain et al., 2021). As



the therapeutic potential of plant-based medicines becomes more widely recognized, the safety of herbal products has come into focus (Anne et al., 2020). One of the critical safety concerns is heavy metal contamination in medicinal plants, which can pose significant health risks if consumed over long periods. Heavy metals such as lead (Pb), arsenic (As), cadmium (Cd), and mercury (Hg) are often absorbed from soil and water and accumulate in plant tissues. Therefore, it is essential to assess heavy metal levels in *Simarouba glauca* to ensure its safety for therapeutic applications (Anne et al., 2020). Furthermore, evaluating the nutritional elements in the *S. glauca* plant is vital for understanding its potential health benefits, particularly in the context of dietary supplements. A scientific investigation of phytochemicals through GC-MS analysis, microbiological studies, and elemental analysis of *S. glauca* focused on highlighting the need for further research into its medicinal and safety profiles. In particular, research focusing on the leaf extracts of *S. glauca*, which are readily accessible and commonly used in folk medicine, is critical for a better understanding of its pharmacological mechanisms (Niketh et al., 2022).

The objective of this research is to provide a comprehensive evaluation of *Simarouba glauca* leaf extracts through multiple analytical approaches. The phytochemical composition of *S. glauca* leaf extracts will be identified using techniques such as GC-MS (Gas Chromatography-Mass Spectrometry), enabling the detection of volatile and non-volatile compounds and the identification of functional groups. In addition, Energy-Dispersive X-ray Spectroscopy (EDX) will be employed to determine the elemental composition, providing a detailed analysis of the nutritional elements and trace metals present in the leaves. To assess the plant's safety profile, heavy metal analysis will be conducted to measure toxic metal concentrations. Lastly, the antimicrobial activity of *S. glauca* leaf extracts will be evaluated against a variety of fungal and bacterial strains to confirm its traditional use as an antimicrobial agent (Ramasamy et al., 2022).

By combining phytochemical, elemental, and microbiological analyses, this research provides valuable insights into the medicinal efficacy and safety profile of *Simarouba glauca* leaf extracts. The findings of this study will contribute to understanding *S. glauca* as a promising natural resource for pharmaceutical applications while ensuring its safety for human consumption. Furthermore, this research could guide the formulation of standardized herbal products derived from *Simarouba glauca*, thereby advancing its potential in modern medicine and phytotherapy.

Materials and Methods

Collection and authentication of Simarouba glauca leaves

Fresh, healthy leaves of *Simarouba glauca* were collected from the PSGCP Herbal Garden, PSGIMSR Hospital Campus, Peelamedu, Coimbatore, Tamil Nadu, India, and a voucher specimen was prepared and stored at the PSG College of Pharmacy. *Simarouba glauca* (SG) leaves were identified and authenticated (No.: BSI/SRC/5/23/2021/Tech./328) by plant taxonomist Dr. M. U. Sharief, Botanical Survey of India (BSI), TNAU Campus, Coimbatore-641 004. Collected leaves were washed with tap water to remove dust and adherent materials, shade-dried, and pulverized (Preethi Aries MG 216 mixer, 750 watts, green) into coarse powder for the extraction process.

Preparation of Simarouba glauca leaf extracts

Shade-dried, pulverized *S. glauca* leaves were defatted using petroleum ether (40–60 °C), and then extracted with chloroform and methanol using the ultrasonic-assisted extraction technique (Tatke & Rajan., 2016). The resulting *S. glauca* leaf extracts were then concentrated using a rotary evaporator, and the percentage yield of SG leaf extracts was determined. Following GC-MS analysis, the antibacterial activity of the obtained SG leaf extracts was investigated. Additionally, an ICP-OES analysis was performed on leaf powder of *S. glauca* to detect both nutritious and hazardous elements, such as lead, arsenic, mercury, and cadmium (Sundharamoorthy et al., 2025).

Phytochemical analysis of Simarouba glauca leaf extracts

The prepared SG leaf extracts (chloroform and methanol) were subjected to qualitative phytochemical analysis in order to determine the presence of secondary metabolites such as proteins, alkaloids, sterols, terpenoids, flavonoids, glycosides, and tannins as per the standard protocol (Rao et al., 2023).

Proximate analysis of Simarouba glauca

The proximate content of air-dried *Simarouba glauca* leaf powder was determined in accordance with WHO guidelines, including moisture content (LOD), ash value, extractive value, and elemental analysis (WHO., 2007).

Moisture content determination: The standard protocol was followed to determine moisture content or loss on drying, with reference to the air-dried sample (SG leaf) as per the WHO guideline. Two

grams of powdered SG leaf sample were precisely weighed into a petri dish covered with a lid, and the sample was then dried for 1 hour at 110 °C in the hot-air oven. After cooling in a desiccator, it was weighed once again. The amount of air-dried powder was used to calculate the moisture content of the *S. glauca* leaf sample (Schubnell et al., 2020).

Determination of ash value: The ash remaining after ignition of plant material was determined to assess the potency and quality of the herbal drug, with reference to the amount of air-dried crude drug, using three distinct approaches: total ash, acid-insoluble ash, and water-soluble ash (WHO., 2007).

Total ash value: The whole quantity of substance left over after ignition was measured using the total ash method. As per the guideline, 2-4 g of air-dried *S. glauca* leaf material was weighted and ground in a tarred silica crucible. After the material was evenly distributed, the temperature was gradually increased from 450 to 600 °C until the material turned white, indicating complete carbon removal. The sample was then weighed after cooling in a desiccator. If carbon-free ash could not be obtained, the residue was moistened with 2 mL of water, dried in a water bath or on a hot plate, and then re-ignited to constant weight. After allowing the residue to cool in a desiccator for 30 minutes, the weight was measured without delay. The percentage of total ash in mg/g with reference to the air-dried material was calculated.

Acid-insoluble ash value: The residue left after heating the remaining insoluble material and boiling the entire ash in diluted hydrochloric acid is known as acid-insoluble ash. This quantifies the amount of silica, particularly in the form of siliceous earth and sand. 25 mL of dilute hydrochloric acid was added to the total ash, and the mixture was gently boiled for five minutes. Following filtration through ashless filter paper, the sample was thoroughly rinsed with warm water. The filter paper containing the insoluble material was transferred back to the original crucible and ignited to a constant weight. After leaving the residue to cool for 30 minutes in an appropriate desiccator, it was weighed without delay. The amount of acid-insoluble ash (mg) per gram of air-dried substance was subsequently calculated.

Water-soluble ash value: Water-soluble ash value refers to the portion of ash from a substance that dissolves in water, indicating the amount of soluble mineral content and inorganic matter present. Total ash was boiled with 25 mL of water, the insoluble matter filtered and ignited to a constant weight. To calculate the water-insoluble ash value, the weight of the insoluble matter was subtracted from the total ash weight and then the

difference was expressed as a percentage of the original air-dried sample.

Determination of extractive values: Chloroform, methanol, and aqueous soluble extractive values were determined in accordance with the recommended protocols, and the results are documented (Agrawal et al., 2021)

Methanol-soluble extractive value: 5 g of the finely powdered air-dried SG leaf sample was macerated in 100 mL of methanol in a closed flask for 24 hours, with frequent shaking during the first 6 hours. The sample was then allowed to stand for 18 hours before being quickly filtered to prevent solvent loss. 25 mL of the filtrate was dried to constant weight and then evaporated to dryness in an evaporating dish. The methanol-soluble extractive value was calculated with reference to the air-dried drug.

Water-soluble extractive value: In a closed flask, 5 g of air-dried, finely powdered SG leaf sample was macerated with 100 mL of chloroform-water for 24 hours, with frequent shaking during the first 6 hours and standing undisturbed for the remaining 18 hours. The mixture was quickly filtered with precautions to minimize solvent loss. Then, 25 mL of the filtrate was evaporated to dryness in a tarred flat-bottomed evaporating dish, dried at 105 °C to constant weight, and weighed. The percentage of water-soluble extractive value (% w/w) was calculated with reference to the air-dried sample.

Chloroform soluble extractive value: Five grams of air-dried powdered SG leaf sample was macerated in 100 mL of chloroform for 24 hours in a covered flask, shaken regularly for the first 6 hours, and then allowed to stand for 18 hours before being quickly filtered to prevent solvent loss. 25 mL of the filtrate was evaporated to dryness in a tarred, flat-bottomed dish to obtain a constant weight. Chloroform soluble extractive value was calculated with reference to the air-dried material.

Heavy metal analysis of *Simarouba glauca* leaf by the ICP-OES method: The detection of heavy metals is mandatory for all plant samples undergoing research, as the presence of organic and inorganic heavy metals in the SG plant samples may cause many side effects. The ICP-OES (Inductively Coupled Plasma–Optical Emission Spectrometry) is the technique in which the elements/heavy metals present in the SG leaf sample could be determined using Perkin Elmer Optima 5300 DV ICP-OES equipped with a concentric torch with alumina injector, Scott spray chamber, and segmented charge coupled device detector. The observed limits were compared with the normative limits stated in the

WHO recommendation. (Parvathy et al., 2020).

Acid digestion procedure: A dried SG leaf powder sample (0.1012 g) was accurately weighed and transferred into a clean digestion vessel. Then, 8 mL of concentrated nitric acid and 2 mL of concentrated hydrochloric acid were added. The mixture was digested using a microwave digestion system at 180 °C for 30 minutes, after which it was allowed to cool. The digested sample was filtered through Whatman No. 41 filter paper, and the filtrate was made up to volume in a clean 50 mL volumetric flask. The resulting solution was analyzed using an ICP-OES instrument. An ideal blank solution was also prepared (Momen., 2006). The chemical contaminants in medicinal plants can be classified into toxic heavy metals and non-metals, with heavy metal contamination a major concern. Among toxic heavy metals, mercury (Hg), lead (Pb), arsenic (As), and cadmium (Cd) are considered major contaminants.

The heavy metal analysis was performed using a Perkin Elmer Optima 5300 DV ICP-OES equipped with a concentric torch, alumina injector, Scott spray chamber, and a segmented charge-coupled device detector. The following parameters were set: nebulizer flow, 0.61/min (Nebulizer: Gem tip cross flow); radio frequency power, 40 MHz/ 1500 watts; sample introduction, 1.5 mL/min; wavelength range, 165-782 nm; view, axial/radial; auxiliary flow, 1.0 L/min; plasma flow, 15 L/min; equilibration time, 30 sec; a series of multi element standards (1.5 and 10 ppm) were prepared using a 1000 ppm stock solution. The standard was aspirated into an argon plasma operating at 11,000 K, and a calibration graph was constructed using standard concentration (x-axis) and emission intensity (y-axis). Unknown values were obtained by interpolating the emission intensity with the concentration axis. Weight percentages of the elements present in the samples were calculated as follows.

$$\text{Wt \% (g/100g)} = \frac{[\text{Instrument value (ppm)}] \times [\text{Volume in mL}] \times [10^{-4}]}{\text{Weight of SG leaf sample in grams}}$$

The multi-acid digestion technique was used to digest the samples for lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni), and chromium (Cr). As mercury (Hg) and arsenic (As) are volatile, they were digested using a nitric acid-hydrochloric acid-potassium permanganate system before analysis. Lead (Pb), Cadmium (Cd), Arsenic (As), Mercury (Hg), Copper (Cu), Zinc (Zn), Nickel (Ni), and Chromium (Cr) standards were bought from Merck in Germany

and used for constructing the calibration curves for these metals (Mohammed et al., 2017).

Analysis of nutritional elements by EDAX method (Energy-dispersive X-ray spectroscopy): Energy-dispersive X-ray (EDX) microprobe analysis detects the presence of chemical elements in plant tissues as they are viewed under an electron microscope (EM). The detection depends on an element's atomic number and cannot distinguish between ionized, bonded, or free atoms. Standard EDX analyzers can detect elements with atomic numbers 11 (sodium) and higher. The windowless EDX analyzer can detect elements from boron (atomic number 5) upwards, including the major elements present in tissues (carbon, nitrogen, and oxygen). In plant tissues, elements such as sodium, magnesium, potassium, aluminum, silicon, phosphorus, sulfur, chlorine, and calcium are often present at sufficiently high concentrations to be detected and quantified by EDX analysis. Elements at trace concentrations, such as those associated with proteins at enzyme active sites, are usually not detectable.

Semi-quantitative analyses compare spectra or the numbers of X-rays emitted by particular elements to draw limited conclusions about differences in the absolute or relative amounts of elements between specimens or regions of a specimen. Many researchers are involved in the ongoing development of EDX analysis and associated techniques to increase spatial resolution, reduce the risk of detecting trace elements, and accurately quantify composition.

The present study provides information on the basic principles and applications of EDX analysis, along with guidance on some of the technical difficulties encountered with plant tissues. In this technique, at low resolution for qualitative or semi-quantitative analysis of plant specimens, EM-EDX analysis is extremely critical. Normally, plant tissue is dehydrated or frozen before analysis, and is not alive, although some fresh tissues can be analyzed quickly, while they are dehydrating in a vacuum. The techniques for preparing samples for EDX analysis must be specifically designed to avoid artifacts that are common due to element mobility or loss during preparation and sampling. Under this study the relative error of chemical analysis was as follows: if the content of the element was from 1 to 5%, the error was less than 10%; from 5 to 10% - less than 5%; if the content was more than 10%, the error was less than 2% (Goldstein et al., 2003; Scimeca et al., 2018).

GC-MS analysis of *Simarouba glauca* leaf extracts: Prepared *Simarouba glauca* leaf extracts

(chloroform and methanol) were analyzed by GC-MS to identify the chemical constituents. The GC-MS analysis was performed on a combined GC-MS instrument (Agilent 8890: Version: 2022-0823-2154-09279), which provides comprehensive analytical capabilities, allowing users to achieve sensitive, robust, and reliable GC-MS analysis of any sample in a routine setting through an HP-5MS Agilent column with dimensions of 30 m x 250 μ m x 0.25 μ m. The initial flow rate was 1.2 mL/min, the post-run flow rate was 1 mL/min, and the holdup time was 1.2376 minutes. The initial temperature for the GC/MSD transfer (Gas Chromatography/Mass Spectrometry Detector) was set to 280 °C.

This method was designed to analyze phytochemicals through GC-MS. 1 μ L of prepared sample aliquot was injected into the column (Syringe Size: 10 μ L) using an injector with a temperature set at 280 °C, with an air gap of 0.2 μ L. The oven initial temperature was set to 75 °C, with a maximum temperature of around 350 °C and a post-run temperature of 50 °C with a 0.5-minute hold time. The GC program was initiated with a column temperature set to -60 °C to 325 °C (350 °C), a holdup time of 1.2376 min, and a pressure of about 11.367 ps. The average velocity of the column, 40.402 cm/sec, was applied. Helium was used as the carrier gas (1.5 mL/min). The mass spectrometer was operated in EI mode (Electron Energy: 70 eV) with the mass source set at 230 °C for 53 min.

The chromatogram and spectrum of the peaks were scanned three times, and the peaks were observed from 50 to 600 °C at a scanning speed of approximately 1,562 [N=2]. The phytocompounds present in the SG leaf extracts (chloroform, methanol, and aqueous) were identified by matching their mass spectral fragmentation patterns of the respective peaks in the chromatogram with those stored in the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998) library (Shettar et al., 2024).

Evaluation of Antimicrobial Activity:

Antibacterial activity: The antibacterial and antifungal activities of SG leaf extracts were assessed using the Kirby-Bauer method. For antibacterial testing, the bacterial strains of *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) were cultured on agar plates, and well-isolated colonies of similar morphology were selected. These colonies were transferred into a broth medium and incubated at 35 °C for 2-6 hours to achieve sufficient turbidity. The culture turbidity was then adjusted with sterile saline or broth to obtain a suspension containing approximately 1-2 \times 10⁸ CFU/mL. A sterile cotton swab was used

to inoculate nutrient agar plates, ensuring even distribution of the inoculum. After allowing the surface moisture to evaporate, wells of 6 mm diameter were made in the agar, and 30 μ L of leaf extracts at concentrations of 25, 50, 75, 100, and 125 μ g/mL were applied to each well. The plates were incubated at 37 °C for 24 hours, and the zone of inhibition around each well was measured to assess antibacterial activity (Bhalodia et al., 2011; Ramya et al., 2019).

Antifungal activity: For antifungal testing, *Candida albicans* was subcultured on potato dextrose agar plates and incubated at 25 °C for 4-5 days. The fungal inoculum was spread evenly across fresh PDA plates using a sterile swab. After allowing excess moisture to evaporate, disks containing the SG leaf extracts at varying concentrations (25, 50, 75, 100, and 125 μ g/mL) were placed on the plates, which were then incubated at 30 °C for up to 7 days. The inhibition zones around the disks were measured to determine the antifungal activity. Results for both antibacterial and antifungal tests were recorded as the diameter (in mm) of the ZOI (zones of inhibition) for *Staphylococcus aureus* and *Escherichia coli*, and for antifungal activity against *Candida albicans*, respectively (Liu et al., 2002; Berkow et al., 2020).

Results and Discussion

Collection and preparation of *Simarouba glauca* leaf extracts

Healthy fresh leaves of *Simarouba glauca* were procured from the PSGCP Herbal Garden and authenticated by plant taxonomist Dr. M. U. Sharief of the Botanical Survey of India (BSI), TNAU Campus (No.: BSI/SRC/5/23/2021/Tech./328). **Fig. 1(a)** and **1(b)** displayed an image of an SG leaf along with its authenticity certificate. The collected SG leaves were shade-dried and defatted before extraction using an ultrasonic-assisted method with methanol and chloroform. The percentage yields of methanol and chloroform in the resulting SG leaf extracts were determined to be 15.84 \pm 0.03 % w/w and 9.18 \pm 0.01 % w/w, respectively.

Phytochemical analysis of *Simarouba glauca* leaf extracts

The qualitative phytochemical analysis revealed that the SG methanol leaf extract contained proteins, alkaloids, glycosides, flavonoids, terpenoids, and steroids. However, there were no flavonoids, saponins, or carbohydrates in the SG chloroform leaf extract. The results of the observed phytochemical tests are displayed in **Tab. 1**.

Proximate analysis of *Simarouba glauca*

The proximate composition of air-dried *Simarouba glauca* leaf powder, including moisture content (LOD), ash value, extractive value, and elemental analysis, was analysed according to WHO guidelines. The present study's results help to evaluate the potency and quality of the herbal drug relative to the quantity of air-dried crude drug. The quantity of moisture lost during drying was calculated to be $1.12 \pm 0.12\%$ w/w, accounting for the amount of drugs that were air-dried. As indicators of the presence of contaminants in the leaf sample, such as sand, dirt, organic and inorganic residues, and other foreign elements, the total ash value ($7.6 \pm 0.15\%$), water soluble ash value ($10.2 \pm 0.19\%$ w/w), and acid-insoluble ash value ($4.0 \pm 0.13\%$ w/w) have been identified.



B



Fig. 1. (A) *Simarouba glauca* leaf and **(B)** authentication certificate

Table 1. Phytochemical analysis of SG chloroform and methanol leaf extracts

S.no	Phytochemicals Test	SG Chloroform extract	SG Methanol extract
1	Alkaloids	+	+
2	Glycosides	+	+
3	Flavonoids	-	+
4	Terpenoids	+	+
5	Steroids	+	+
6	Saponins	-	-
7	Carbohydrates	-	-
8	Tannins	+	+
9	Proteins	+	+

SG - *Simarouba glauca*, (+) Presence, (-) Absence

The amount of active phytochemical components contained was determined using the extractive values of SG leaves following extraction with the appropriate solvents. Following that, it was calculated using the amount of air-dried crude medicine. Methanol, chloroform, and aqueous SG extracts all contained several phytochemical components, with extractive values of 18.40% w/w, 11.28% w/w, and 5.00% w/w, respectively. The result are shown in **Tab. 2** as a percentage (%) w/w.

Heavy metal analysis of *Simarouba glauca* leaf by the ICP-OES method

Since ancient times, phytometal elements have been used in a variety of pharmaceutical approaches to support and maintain the body's health. Based on our research, the leaf sample of *Simarouba glauca* is a rich source of essential elements, including Fe^{2+} , Zn^{2+} , Cu^{2+} , and Mg^{2+} . In the current investigation, ICP-OES analysis was used to detect the presence of metal ions and heavy metals in the SG leaf sample. Cadmium (0.002 g/mL), lead (0.028 g/mL), nickel (0.085 g/mL), and chromium (0.279 g/mL) were detected, whereas mercury, potassium, and arsenic were below the detection limits (BDL). Other elements, such as copper (0.134 mg/L), iron (1.768 mg/L), magnesium (7.592 mg/L), sodium (0.099 mg/L), zinc (0.072 mg/L), calcium (28.23 mg/L), and aluminum (0.221 mg/L), were also detected. The concentrations of all detected heavy metals (As, Cd, Pb, Ni, and Hg) were found to be within the permissible limits recommended by the World Health Organization (WHO, 1996), as presented in **Tab. 3**.

Table 2. Proximate analysis of *Simarouba glauca* leaf

Physicochemical Parameter	Values (%) w/w
Moisture content determination (LOD at 105°C)	1.12±0.12%
Total ash value	7.6±0.15%
Acid-insoluble ash value	4.0±0.13%
Water soluble ash value	10.2±0.19%
Chloroform soluble extractive value	5.00%
Methanol soluble extractive value	11.28%
Aqueous soluble extractive value	18.40%

Analysis of nutritional elements by EDAX method (Energy-dispersive X-ray spectroscopy)

EDAX analysis provides a fast, high-resolution, and simple method for simultaneously examining the amounts and locations of many different elements within a tissue. It can be used at many different levels of sophistication, although care is always required in specimen preparation and data analysis. From this method, qualitative and semi-quantitative results can be easily obtained, as shown in **Fig. 2** and **Tab. 4**.

GC-MS analysis of *Simarouba glauca* leaf extracts

The combined GC-MS instrument (Agilent 8890: Version: 2022-0823-2154-09279) was used to analyse the phytochemicals in *Simarouba glauca* leaf extracts (chloroform and methanol). The study's findings showed that the SG chloroform leaf extract contained 14 chemical compounds, specifically carbohydrates and amino acids, such as 1,2,3 trihydroxypropane (11.76%), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (2.17%), cyclohexanamine, N-3-butenyl-N-methyl (2.36%), DL arabinose (2.42%), 4H-pyran-4-one,2,3-

Table 3. Elemental/Heavy metal analysis of *Simarouba glauca* leaf powder

Sophisticated Analytical Instrument Facility, IIT Madras, Chennai-36 Perkin Elmer Optima 5300 Dv ICP-OES					WHO permissible limits for heavy metals in plant (mg/kg)
Sample code	Element symbol & Wavelength (nm)	Weight of sample in gm / Volume in mL	Dilution Factor	Concentration	Source: WHO (1996)
Dried Leaf Powder (SG)	Al 308.215	0.1012 g/50 mL	1	0.221 mg/L	
	As 188.979	„	„	BDL	0.05 mg/kg
	Ca 317.933	„	„	28.23 mg/L	
	Cd 228.802	„	„	0.002 mg/L	0.02 mg/kg
	Cr 267.716	„	„	0.279 mg/L	1.6 mg/kg
	Cu 327.393	„	„	0.134 mg/L	10 mg/kg
	Fe 238.204	„	„	1.768 mg/L	
	Hg 253.652	„	„	BDL	0.03 mg/kg
	K 334.532	„	„	BDL	
	Mg 285.213	„	„	7.592 mg/L	
	Na 259.393	„	„	0.099 mg/L	
	Ni 231.604	„	„	0.085 mg/L	10 mg/kg
	Pb 220.353	„	„	0.028 mg/L	2 mg/kg
	Si 251.611	„	„	0.679 mg/L	
	Zn 213.857	„	„	0.072 mg/L	0.60 mg/kg

BDL - Below the Detection Limit

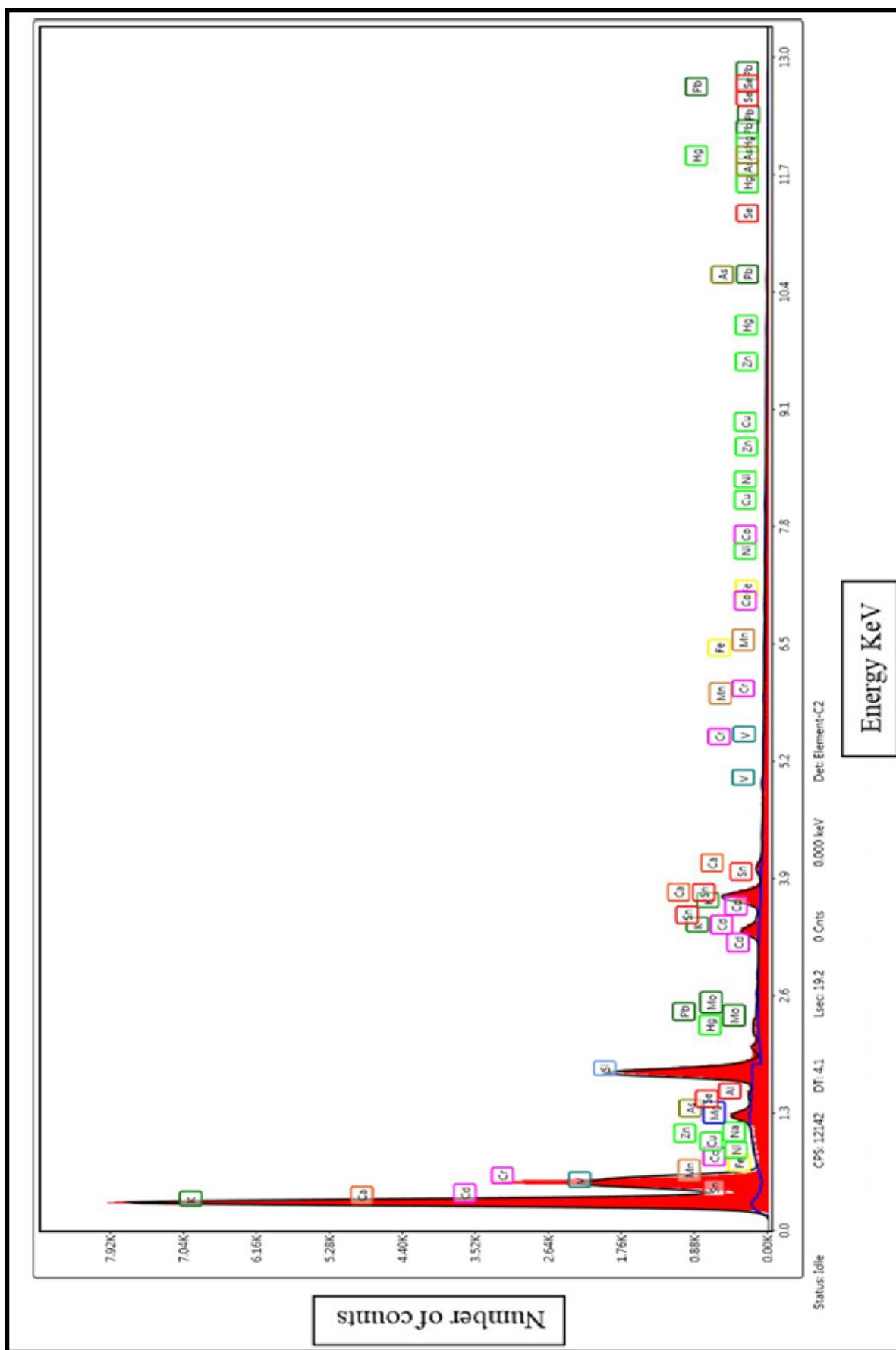


Fig. 2. Elemental analysis of *Simarouba glauca* by EDAX method

dihydro-3,5-dihydroxy-6-methyl (5.40%), 5-hydroxy methyl furfural 1- (1.31%), tetraethyl ammonium nitrate (2.55%), 1,3-diazacyclooctane-2-thione (1.81%), 3-O-methyl-d-glucose (5.23%), 6-deoxy-D-mannono-4-lactone (1.98%), 3-O-methyl-d-glucose (5.23%), lactose (11.72%), 1, 2, 3-benzenetriol (13.67%), 3, 4-altrosan (3.89%), and quinic acid (33.75%). **Tab. 5** shows the detected compounds' chemical formulas and molecular weights, while **Fig. 3** displays their mass spectra.

The SG methanol leaf extract's GC-MS analysis revealed the presence of 12 chemical compounds including 2-ethyl-N-[(2S)-1-methoxypropan-2-yl], 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (1.407%), 6-methylaniline (1.70%), 1,2,3 trihydroxypropane (12.77%), decane (1.76%), 4H-pyran-4-one 2,3-dihydro-3,5-dihydroxy-6-methyl (4.56%), isosorbide dinitrate (1.72%), 3-O-methyl-d-glucose (5.07%), 6-deoxy-D-mannono-4-lactone (4.36%), 1, 2, 3-benzenetriol (13.67%), melezitose (13.91%), D-allose (5.50%), 1,2,3,5-cyclohexanetetrol (1 α ,2 β ,3 α ,5 β) (23.99%). **Fig. 4** displays the mass spectrum, while **Tab. 6** lists the chemical formula and molecular weight of the chemical compounds found in the methanol leaf extract of *Simarouba glauca*.

Evaluation of antimicrobial activity

Simarouba glauca, commonly known as Laxmi Taru or the Paradise tree, belongs to the Simaroubaceae family. The leaf and bark extracts of SG are known to have a bioactive components with considerable medicinal uses. Quassinooids are among the

Table 4. The percentage of nutritional elements present in *Simarouba glauca* leaves

S. No	Elements	Weight (%)	Atomic (%)
1	NaK	0	0.01
2	MgK	4.25	6.4
3	AlK	0.81	1.1
4	SiK	41.99	54.7
5	MoL	3.02	1.15
6	PbM	0.12	0.02
7	CdL	0.26	0.09
8	KK	8.83	10.35
9	SnL	3.41	1.05
10	CaK	22.59	20.62
11	CrK	0.28	0.2
12	MnK	0.68	0.45
13	FeK	0.58	0.38
14	CoK	0.68	0.43
15	NiK	0.81	0.5
16	CuK	1.58	0.91
17	ZnK	0.4	0.22
18	HgL	BDL	0.83
19	AsK	0.02	0.01
20	SeK	3.97	3.97

Table 5. GC-MS analysis of the chloroform leaf extract of *Simarouba glauca*

S. No	Chemical Compound Name	Molecular Formula	Molecular Weight m/z (g/mol)	Retention Time (min)	Peak Area (%)
1	1,2,3-trihydroxypropane	C ₃ H ₈ O ₃	92.09	3.712	11.76
2	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C ₆ H ₈ O ₄	144.12	4.019	2.17
3	Cyclohexanamine, N-3-butenyl-N-methyl-	C ₁₁ H ₂₁ N	167.29	5.619	2.36
4	DL Arabinose	C ₅ H ₁₀ O ₅	150.13	6.769	2.42
5	4H-Pyran-4-one 2,3-dihydro-3,5-dihydroxy-6-methyl	C ₆ H ₈ O ₄	144.12	7.094	5.4
6	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126.11	9.026	1.31
7	Acetic acid, 2,2'[oxybis(2,1-ethanediyoxy)] bis	C ₈ H ₂₀ N ₂ O ₃	192.26	9.32	2.55
8	1,3-Diazacyclooctane-2-thione	C ₆ H ₁₂ N ₂ S	144.24	10.132	1.81
9	3-O-Methyl-d-glucose	C ₇ H ₁₄ O ₆	194.18	10.201	5.23
10	6-Deoxy-D-mannono-4-lactone	C ₆ H ₁₀ O ₅	162.14	11.076	1.98
11	1,2,3-Benzenetriol	C ₆ H ₆ O ₃	126.11	12.633	13.67
12	Lactose	C ₁₂ H ₂₂ O ₁₁	342.3	13.827	11.72
13	3,4-Altrosan	C ₆ H ₁₀ O ₅	162.14	15.002	3.89
14	Quinic acid	C ₇ H ₁₂ O ₆	192.17	17.0865	33.75

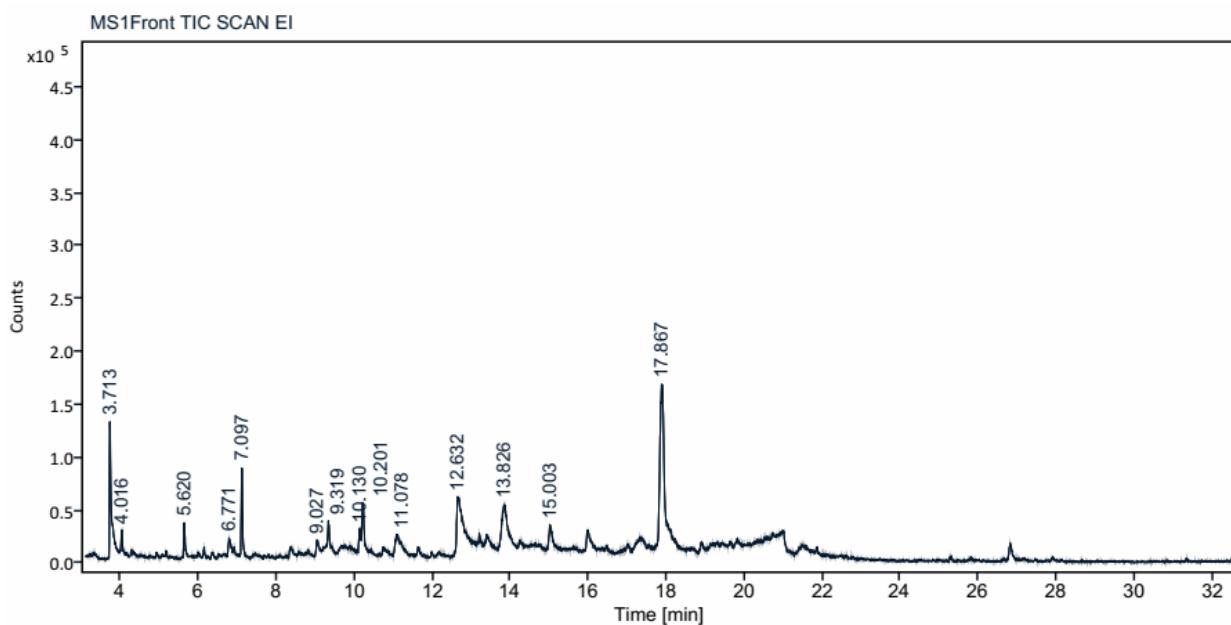


Fig. 3. Gas chromatogram–mass spectrum chromatogram of chloroform leaf extract of *Simarouba glauca*

most important active phytochemicals in the triterpene family, with pharmacological activities such as antibacterial, anticancer, antipyretic, and haemostatic. SG phytochemicals (tannins, terpenoids, alkaloids, phytosterols, and flavonoids) are more effective and less toxic, making them an alternative treatment for common diseases caused by bacterial and fungal species.

Antimicrobial properties of SG leaf extracts (chloroform and methanol) were determined as per the Kirby-Bauer method using *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.

The spherical, Gram-positive bacterium *Staphylococcus aureus* is commonly found on the skin and in the upper respiratory tract. Warm-blooded creatures' lower intestines are frequently home to the rod-shaped, facultatively anaerobic, gram-negative coliform bacteria *Escherichia coli*. Common *E. coli* infections include meningitis in newborns, pneumonia, diarrhoea, bacteremia, and urinary tract infections (UTIs).

The standard drug Teicoplanin (formerly known as teichomycin A2) is a semisynthetic glycopeptide antibiotic with a spectrum of activity similar to that

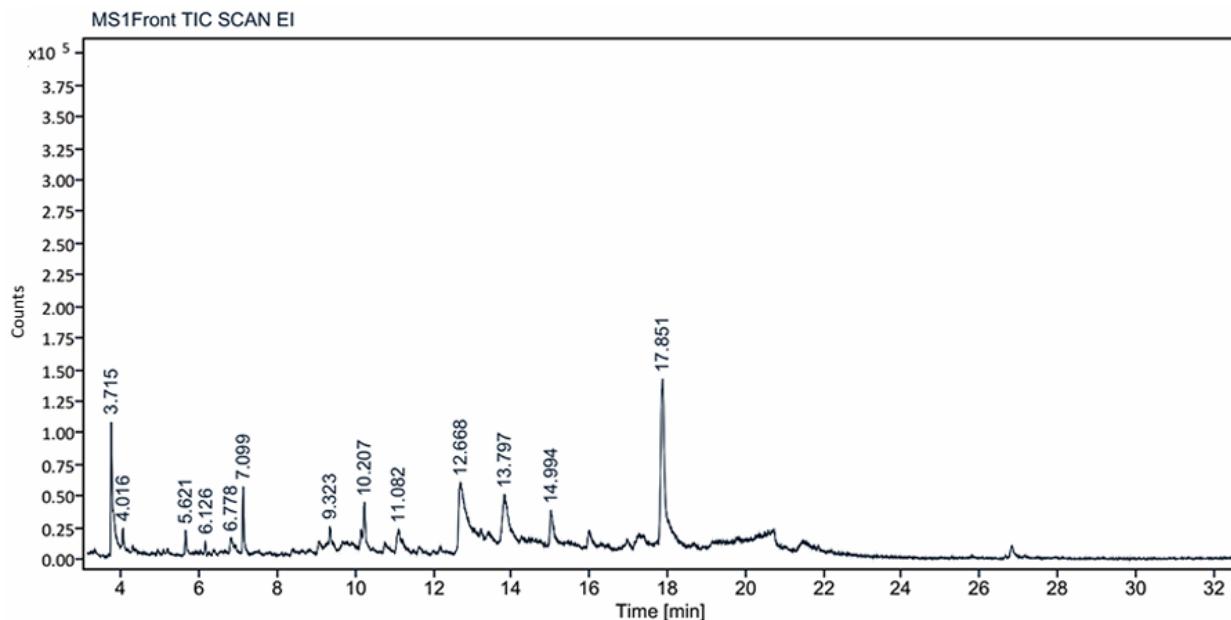


Fig. 4. Gas chromatogram–mass spectrum chromatogram of methanol leaf extract of *Simarouba glauca*

Table 6. GC-MS analysis of the methanol leaf extract of *Simarouba glauca*

S. No	Chemical Compound Name	Molecular Weight m/z (g/mol)	Molecular Weight m/z (g/mol)	Retention Time (min)	Peak Area (%)
1	1,2,3-trihydroxypropane	C ₃ H ₈ O ₃	92.09	3.712	11.76
2	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C ₆ H ₈ O ₄	144.12	4.099	1.4
3	1-Alanine n propargyloxy carbonyl, isohexyl ester 2-ethyl-N-[(2S)-1-methoxypropan-2-yl]-6-methyl aniline	C ₁₃ H ₂₁ NO	207.31	5.619	1.7
4	Decane	C ₁₀ H ₂₂	142.28	6.125	1.76
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C ₆ H ₈ O ₄	144.12	7.1	4.56
6	Isosorbide Dinitrate	C ₆ H ₈ N ₂ O ₈	236.14	9.326	1.72
7	3-O-Methyl-D-glucose	C ₇ H ₁₄ O ₆	194.18	10.207	5.07
8	6-Deoxy-D-mannono-4-lactone	C ₆ H ₁₀ O ₅	162.14	11.082	4.36
9	1,2,3-Benzentriol	C ₆ H ₆ O ₃	126.11	12.67	21.99
10	Melezitose	C ₁₈ H ₃₂ O ₁₆	504.4	13.795	13.91
11	D-Allose	C ₆ H ₁₂ O ₆	180.16	14.996	5.5
12	1,2,3,5-Cyclohexanetetrol, (1 α ,2 β ,3 α ,5 β)	C ₆ H ₁₂ O ₄	148.16	17.852	23.99

of vancomycin, which was used as a positive control. Glycopeptide antibiotics inhibit bacterial cell wall formation by inhibiting peptidoglycan synthesis. They are used to treat multidrug-resistant *Staphylococcus aureus* and enterococcal infections, including UTIs, bacteremia, and infective endocarditis, and rarely cause intra-abdominal infections and meningitis.

Candida albicans is a yeast-like fungus naturally found in oral cavity, skin, and intestines. The overgrowth of yeast can cause infections like candidiasis, which is treated with synthetic antifungal medication (Amphotericin). Both natural and synthetic antibiotics kill or harm bacteria (Atef et al., 2019). A prolonged course of synthetic antibiotic treatment can lead to antibiotic resistance and further damage to the body's immune system. In recent years, the hunt for medications and phytochemicals derived from natural sources has increased.

Traditional healers have long utilized plants to prevent or cure infectious diseases. Plants contain a wide range of secondary metabolites, including tannins, terpenoids, alkaloids, phytosterols, and flavonoids, all of which have antibacterial activities *in vitro*. The current study aimed to identify phytochemicals in SG leaf extracts (chloroform and methanol) through GC-MS analysis and to investigate their potential antimicrobial properties. The SG extract showed variable antibacterial and antifungal activity against the tested bacterial and fungal strains. The largest zone of inhibition was observed for SG chloroform leaf extract against *S. aureus* (19 mm) and *E. coli* (22 mm). In contrast, SG methanol leaf extract showed 15 mm and 19 mm, respectively, compared with the reference drug (23 mm and 25 mm) (Tab. 7 and Fig. 5).

The SG leaf extracts (chloroform and methanol) were found to be efficient in inhibiting the growth of a fungal strain (*Candida albicans*), with inhibition zone sizes of 11-14 mm, 6-12 mm, and 6-10 mm, respectively (Tab. 8 and Fig. 6). The above findings were more susceptible than fungi. Terpenoids, steroids, alkaloids, tannins, and flavonoids, among other active phytochemicals found in SG, are responsible for antimicrobial effects.

Table 7. Antibacterial activity of SG leaf extracts (Chloroform and Methanol)

Antibacterial assay details		Zone of inhibition (mm)									
Bacterial strains name		<i>Staphylococcus aureus</i>					<i>Escherichia coli</i>				
SG leaf extract concentrations (µg/mL)		25	50	75	100	125	25	50	75	100	125
Standard*(Teicoplanin-standard drug)		23 mm					25 mm				
Negative control		0 mm					0 mm				
Sample A & C: SG Chloroform leaf extract		11	11	10	15	19	9	12	12	20	22
Sample B & D: SG Methanol leaf extract		12	17	18	16	15	10	14	15	18	19

*Teicoplanin-TEI30 susceptibility test disc 30 µg

Table 8. Antifungal activity of SG leaf extracts (Chloroform and Methanol)

Antifungal assay details		Zone of inhibition (mm)				
Fungal strain name		<i>Candida albicans</i>				
SG leaf extracts concentrations (µg/mL)		25	50	75	100	125
Control-Amphotericin (standard drug)		18 mm				
Negative control		0 mm				
Sample E: SG Chloroform leaf extract		0 mm	0 mm	11 mm	14 mm	14 mm
Sample F: SG Methanol leaf extract		0 mm	6 mm	9 mm	10 mm	12 mm

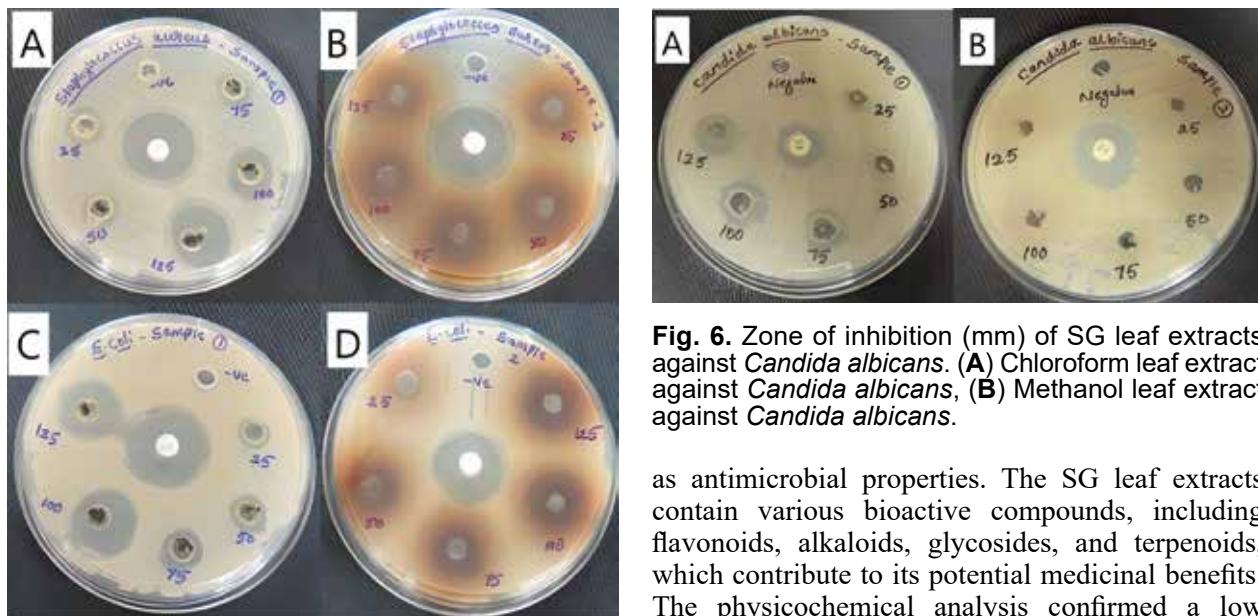


Fig. 5. Zone of inhibition (mm) for SG leaf extracts (A) Chloroform leaf extract against *Staphylococcus aureus*, (B) Methanol leaf extract against *Staphylococcus aureus*, (C) Chloroform leaf extract against *Escherichia coli*, (D) Methanol leaf extract against *Escherichia coli*

Conclusion

In conclusion, the study of *Simarouba glauca* (Lakshmi Taru) leaf extracts revealed significant phytochemical and proximate composition, as well

Fig. 6. Zone of inhibition (mm) of SG leaf extracts against *Candida albicans*. (A) Chloroform leaf extract against *Candida albicans*, (B) Methanol leaf extract against *Candida albicans*.

as antimicrobial properties. The SG leaf extracts contain various bioactive compounds, including flavonoids, alkaloids, glycosides, and terpenoids, which contribute to its potential medicinal benefits. The physicochemical analysis confirmed a low moisture content and notable extractive values, suggesting the presence of active compounds. The results of the heavy metal analysis showed that the leaf extracts remained within the WHO guidelines' acceptable limits. Additionally, the antimicrobial activity showed that the extracts were highly effective against both bacterial and fungal strains. Because SG leaf contains a variety of phytochemicals and nutrients, the chloroform extract exhibited the most potent antibacterial and antifungal activity. These

results position *S. glauca* as a promising natural resource for the development of safe, plant-based antimicrobial agents and nutritional supplements. Future studies focusing on *in vivo* efficacy and formulation development could further harness its potential in modern phytotherapy.

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