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Phytochemical Profiling and Bioactivity Evaluation of *Simarouba glauca* DC.: An Integrated Approach to Isolating Therapeutic Metabolites for Antimicrobial and Antioxidant Applications

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ABSTRACT:

Background: *Simarouba glauca* DC. (Simaroubaceae), traditionally known as "Lakshmi Taru," is a rich source of bioactive secondary metabolites with documented antimicrobial and antioxidant properties. However, tissue-specific phytochemical profiling and correlation with bioactivity against multidrug-resistant (MDR) pathogens remain unexplored.

Objective: This study aimed to evaluate the antimicrobial and antioxidant potential of S. glauca root and stem extracts, correlating bioactivity with GC-MS metabolomic profiles.

Methods: Dried root and stem powders were subjected to cold maceration using chloroform and ethyl acetate. Extraction yields were quantified, and extracts were screened for phytochemicals. GC-MS analysis identified bioactive compounds. Antimicrobial activity was assessed against Gram-positive (*S. aureus*, MRSA), Gram-negative (*E. coli*, *S. typhi*), and fungal pathogens (*A. niger*) using disc diffusion and MIC assays. Antioxidant capacity was evaluated by DPPH, ABTS, and total phenolic content assays.

Results: Chloroform root extraction yielded 5.85–6.10%, rich in fatty acid methyl esters (FAMEs) and β-sitosterol, showing potent antibacterial activity against *S. aureus* (MIC: 2.5 mg/mL) and MRSA (MIC: 3.1 mg/mL). Ethyl acetate stem extraction yielded 2.75%, containing (+)-2-carene and high phenolics (112.3 mg GAE/g), with superior antioxidant activity (DPPH IC_{50}: 22.15 µg/mL). GC-MS identified 28 compounds, with methyl hexadecanoate, β-sitosterol, and (+)-2-carene as key markers.

Conclusion: *S. glauca* roots provide antimicrobial agents effective against MDR pathogens, while stems offer potent antioxidants. GC-MS markers enable quality-controlled phytopharmaceutical development for infectious diseases and oxidative stress management.

1. INTRODUCTION:

Medicinal plants have served as the foundation of traditional medicine systems worldwide, providing

structurally diverse secondary metabolites with therapeutic potential against infectious diseases[1]. Simarouba glauca DC. (Simaroubaceae), commonly known as "Lakshmi Taru" or Paradise Tree, is an evergreen species native to Central America and the Caribbean, introduced to India in 1986 for agroforestry and soil reclamation programs[2]. The plant holds significant ethnobotanical importance, with traditional applications in treating malaria, dysentery, fever, and various dermatological conditions across indigenous medical systems[3].

Phytochemical investigations of *S. glauca* have revealed a complex metabolic profile dominated by quassinoids bitter triterpene lactones characteristic of the Simaroubaceae family including ailanthinone, glaucarubinone, glaucarubin, and glaucarubol[4]. These compounds exhibit documented biological activities through multiple mechanisms, including inhibition of protein synthesis at the ribosomal level and disruption of microbial membrane integrity[5]. Additionally, the plant contains significant quantities of flavonoids, phenolic acids, terpenoids, and fatty acid derivatives that contribute to its antioxidant and antimicrobial properties[6].

Recent metabolomic studies employing GC-MS and LC-MS platforms have identified differential distribution of bioactive constituents across plant tissues. Non-polar fractions enriched in fatty acid methyl esters (FAMEs) and phytosterols demonstrate potent membrane-disrupting activity against Gram-positive bacteria and fungi, while polar fractions containing phenolic compounds exhibit significant free radical scavenging capacity[7]. However, systematic correlation between tissue-specific metabolite profiles and targeted bioactivity against contemporary drug-resistant pathogens remains inadequately explored[8].

The global escalation of antimicrobial resistance poses a critical threat to public health, with the World Health Organization identifying MRSA and other multidrug-resistant pathogens as priority targets for novel therapeutic development[9]. Conventional antibiotics increasingly fail due to acquired resistance mechanisms, necessitating discovery of agents with alternative molecular targets[10]. Natural products offer distinct advantages in this context, as their structural complexity often enables simultaneous interaction with multiple microbial targets, reducing the probability of resistance development[11-12].

This study addresses critical gaps in *S. glauca* phytochemistry by: (i) quantifying differential extraction yields and metabolite profiles from root and stem tissues using solvents of varying polarity; (ii) characterizing bioactive constituents through GC-MS metabolomic analysis; (iii) evaluating antibacterial, antifungal, and antioxidant activities with mechanistic correlation to identified compounds; and (iv) establishing tissue-specific markers for pharmaceutical standardization. The findings support development of standardized phytotherapeutic agents targeting infectious diseases and oxidative stress disorders.

2. MATERIAL AND METHODS:

2.1 Chemicals and Reagents

All solvents used for extraction and chromatographic analysis were of analytical grade and purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA). DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), Folin-Ciocalteu's phenol reagent, gallic acid, quercetin, and ascorbic acid were procured from Sigma-Aldrich. Mueller-Hinton agar, Sabouraud dextrose agar, and antimicrobial reference standards (streptomycin, fluconazole) were obtained from HiMedia Laboratories (Mumbai, India). Helium gas (99.999% purity) for GC-MS analysis was supplied by Air Liquide (India).

2.2 Collection and Authentication of Plant Material

Fresh roots and stems of Simarouba glauca DC. were collected in triplicate from mature, healthy specimens cultivated at the botanical garden of Rabindranath Tagore University, Bhopal, India (23.2599° N, 77.4126° E) in March 2024. The plant material was taxonomically identified and authenticated by a Botanist at the Rabindranath Tagore University, Bhopal, and a voucher specimen (RNTU/PHARM/2024/SG-01) was deposited at the University Herbarium. The collection was performed in accordance with the Nagoya Protocol on Access and Benefit Sharing, and institutional ethical clearance was obtained prior to sampling[13].

Roots were thoroughly washed with running tap water to remove soil particles, sectioned longitudinally into 2-3 cm pieces, and shade-dried at ambient temperature (30 ± 2°C) for 15 days until a brittle texture was achieved[14]. Stems were debarked, cut into small segments (1-2 cm), and similarly dried. Dried materials were separately pulverized using a Wiley laboratory mill (Thomas Scientific, USA) to pass through a 40-mesh sieve (420 µm

particle size). The powdered samples were stored in airtight amber glass containers at 4°C until extraction to prevent photodegradation and moisture absorption[15].

2.3 Extraction and Solvent Fractionation

Cold maceration was employed to preserve heat-labile bioactive compounds[16]. For each extraction, accurately weighed 2.0 g of dried powder was mixed with the respective solvent in specific ratios: chloroform extraction utilized 10 mL solvent (solid-to-liquid ratio 1:5 w/v), while ethyl acetate extraction employed 20 mL solvent (1:10 w/v). The mixtures were incubated on a rocker shaker (REMI RS-12 plus, India) at 120 rpm for 24 hours at 25 ± 2°C to facilitate maximum metabolite solubilization[17].

The extracts were filtered through Whatman No. 1 filter paper under vacuum, and the filtrates were concentrated to dryness in a vacuum oven at 40°C to prevent thermal degradation[18]. Percentage yield (w/w) was calculated gravimetrically[19]. All extractions were performed in triplicate.

Extraction yield (%) =

$$\frac{Weight\ of\ plant\ powder\ (mg)\ \times 100}{Weight\ of\ dried\ extract\ (mg)}$$

All extractions were performed in triplicate, and results are expressed as mean ± standard deviation (SD).

2.4 Qualitative Phytochemical Screening

Preliminary phytochemical analysis was performed according to standard protocols[20] with minor modifications. The dried extracts were tested for:

2.4.1 Alkaloids/Quassinoids: Dragendorff's reagent (potassium bismuth iodide) producing orange-red precipitate; Mayer's reagent (potassium mercuric iodide) forming cream-colored precipitate[21].

2.4.2 Terpenoids: Salkowski test with chloroform and concentrated sulfuric acid, indicating red-brown coloration at the interface.

2.4.3 Flavonoids: Shinoda test using magnesium turnings and concentrated HCl producing pink to crimson color; aluminium chloride test showing yellow fluorescence under UV light (365 nm).

2.4.4 Phenolics: Ferric chloride test producing blue-black coloration; lead acetate test forming white precipitate.

2.4.5 Saponins: Foam test with vigorous shaking of aqueous extract, stable foam persisting for 30 minutes indicating positive result.

2.5 Quantitative Phytochemical Analysis

2.5.1 Total Phenolic Content (TPC) was determined by the Folin-Ciocalteau colorimetric method[22]. Briefly, 100 µL of extract (1 mg/mL in methanol) was mixed with 500 µL Folin-Ciocalteau reagent (diluted 1:10 with distilled water) and incubated for 5 minutes. Then 400 µL of 7.5% sodium carbonate solution was added, and the mixture was incubated in darkness at room temperature for 90 minutes. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-2600, Japan). Gallic acid (0–200 µg/mL) served as standard, and results are expressed as mg gallic acid equivalents (GAE) per gram of dry extract.

2.5.2 Total Flavonoid Content (TFC) was quantified by the aluminum chloride colorimetric method[23]. Extract (500 µL, 1 mg/mL) was mixed with 1.5 mL methanol, 100 µL 10% aluminum chloride, 100 µL 1 M potassium acetate, and 2.8 mL distilled water. After 30 minutes incubation at room temperature, absorbance was measured at 415 nm. Quercetin (0–100 µg/mL) was used as standard, and results are expressed as mg quercetin equivalents (QE) per gram of dry extract.

2.6 GC-MS Metabolomic Analysis

Gas chromatography-mass spectrometry (GC-MS) analysis was performed on an Agilent 5977B GC/MSD system equipped with an HP-5MS fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Agilent Technologies, USA)[24-25]. The chromatographic separation was achieved using a temperature-programmed method with an initial oven temperature of 75°C held for 2 minutes, followed by a linear ramp to 180°C at a rate of 5°C per minute with a 5-minute hold, and a final ramp to 280°C at 10°C per minute. The injector was maintained at 260°C in split mode, and helium (99.999% purity) served as the carrier gas at a

constant flow rate of 1.0 mL/min. The mass spectrometer was operated in electron impact (EI) ionization mode at 70 eV with a mass scan range of 40–600 m/z. The ion source temperature was set at 230°C, and the quadrupole analyzer temperature was maintained at 150°C throughout the analysis.

Compound identification was accomplished by comparing the obtained mass spectra with reference libraries[26-27]. A Similarity Index (SI) threshold of greater than 70% was established as the criterion for positive compound identification. Additionally, retention indices (RI) were calculated for all detected peaks using a homologous series of n-alkane standards (C8–C40) analyzed under identical chromatographic conditions[28].

2.7 Antimicrobial Activity Assessment

2.7.1 Test Microorganisms:

The antimicrobial activity was evaluated against clinically relevant pathogenic strains obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India: Gram-positive bacteria (*Staphylococcus aureus* MTCC 96, methicillin-resistant *S. aureus* [MRSA] clinical isolate, *Bacillus subtilis* MTCC 121); Gram-negative bacteria (*Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 741, *Salmonella typhi* MTCC 733); and fungi (*Aspergillus niger* MTCC 281, *Candida albicans* MTCC 227). Bacterial cultures were maintained on Mueller-Hinton agar (MHA) at 37°C, while fungal cultures were maintained on Sabouraud dextrose agar (SDA) at 28°C. All experiments were performed using fresh 18–24-hour cultures.

2.7.2 Disc Diffusion Assay

The agar disc diffusion method was performed following Clinical and Laboratory Standards Institute (CLSI) guidelines[29] with modifications. Fresh bacterial cultures were adjusted to 0.5 McFarland turbidity standard (1.5×10^8 CFU/mL) using sterile saline. For fungi, spore suspensions were prepared in sterile distilled water containing 0.1% Tween-20 and adjusted to 1×10^6 spores/mL.

Sterile filter paper discs (6 mm diameter, Whatman) were impregnated with 20 µL of extract solution (100 mg/mL in DMSO, final DMSO concentration <5% v/v) and placed on MHA (bacteria) or SDA (fungi) plates previously inoculated with 100 µL of standardized microbial suspension. Positive controls included streptomycin (10 µg/disc) for bacteria and fluconazole (25 µg/disc) for fungi. Negative controls consisted of discs impregnated with sterile DMSO. Plates were incubated at 37°C for 24 hours (bacteria) or 28°C for 48–72 hours (fungi). Zones of inhibition (ZOI) were measured in millimeters (mm) using a digital caliper, and experiments were performed in triplicate.

2.7.3 Minimum Inhibitory Concentration (MIC)

The broth microdilution method was employed for MIC determination according to CLSI M07-A10 guidelines[30]. Two-fold serial dilutions of extracts were prepared in 96-well microtiter plates (Tarsons, India) using Mueller-Hinton broth (MHB) for bacteria or RPMI-1640 medium buffered with MOPS for fungi, yielding final concentrations ranging from 0.625 to 100 mg/mL. Microbial inoculum (5×10^5 CFU/mL for bacteria; 2.5×10^3 CFU/mL for fungi) was added to each well. Plates were incubated at 37°C for 18–24 hours (bacteria) or 28°C for 48 hours (fungi). The lowest concentration showing no visible turbidity was recorded as the MIC. Resazurin dye (0.02% w/v, 20 µL) was added to confirm viability, with color change from blue to pink indicating growth[31].

2.8 Antioxidant Activity Evaluation

2.8.1 DPPH Radical Scavenging Assay

The free radical scavenging capacity was determined by the DPPH method[32] with modifications. Various concentrations of extracts (10–500 µg/mL in methanol) were mixed with equal volumes of 0.1 mM DPPH methanolic solution. The mixture was incubated in darkness at room temperature for 30 minutes, and absorbance was measured at 517 nm against a methanol blank. Ascorbic acid (10–100 µg/mL) served as positive control. Percentage inhibition was calculated as:

$$\%Inhibition = \frac{Acontrol - Asample \; x \; 100}{Acontrol}$$

where $A_{control}$ is the absorbance of DPPH solution without sample, and A_{sample} is the absorbance in presence of extract. The IC_{50} value (concentration scavenging 50% of free radicals) was determined by linear regression analysis from the dose-response curve.

2.8.2 ABTS Radical Cation Scavenging Assay

The ABTS radical cation (ABTS•⁺) was generated by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate (1:1 v/v) and incubating in darkness at room temperature for 16 hours[33]. The solution was diluted with phosphate buffer (pH 7.4) to obtain an absorbance of 0.700 ± 0.02 at 734 nm. Extract (20 µL, various concentrations) was mixed with 980 µL ABTS•⁺ solution, incubated for 6 minutes, and absorbance was measured at 734 nm. Percentage inhibition and IC_{50} values were calculated as described for DPPH assay.

2.9 Statistical Analysis

All experiments were performed in triplicate, and data are expressed as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism version 9.0 (GraphPad Software, USA). One-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post-hoc test was used for multiple comparisons. Pearson correlation coefficient was calculated to determine relationships between total phenolic content and antioxidant activity. Differences were considered statistically significant at $p < 0.05$[34].

3. RESULTS:

3.1 Extraction Yield and Tissue-Specific Distribution

Solvent extraction efficiency varied significantly between plant parts and solvent polarity (Table 1). **Chloroform extraction** of roots yielded **5.85% and 6.10%** (biological replicates), significantly higher than stem tissue (**3.40%).** Conversely, **ethyl acetate** showed preferential extraction from stems (**2.75%**) compared to roots (**1.25% and 1.0%**), indicating differential distribution of lipophilic versus moderately polar metabolites[35-36].

Table 1. Extraction yields from *S. glauca* root and stem tissues

Solvent	Plant Part	Weight of Powder (mg)	Weight of Extract (mg)	Recovery (%)	Metabolite Class
Chloroform	Root 1	2000	117	**5.85**	Lipophilic (terpenoids, fatty acids, sterols)
	Root 2	2000	122	**6.10**	Lipophilic
	Stem	2000	68	**3.40**	Lipophilic
Ethyl Acetate	Root 1	2000	25	**1.25**	Moderately polar (flavonoids, phenolics)
	Root 2	2000	20	**1.00**	Moderately polar
	Stem	2000	55	**2.75**	Moderately polar

The 2.4-fold higher chloroform yield from roots suggests concentration of antimicrobial lipophilic compounds (fatty acids, sterols) in root tissues, while the 2.75-fold higher ethyl acetate yield in stems indicates aerial tissue enrichment in antioxidant phenolic compounds.

3.2 Phytochemical Screening

Qualitative analysis confirmed distinct metabolite profiles (Table 2). Chloroform extracts tested positive for terpenoids and quassinoids (bitter principles), while ethyl acetate extracts showed abundant phenolics and flavonoids, particularly in stem samples[37].

Table 2. Preliminary phytochemical screening

Test	Chloroform (Root)	Chloroform (Stem)	Ethyl Acetate (Root)	Ethyl Acetate (Stem)
Alkaloids/Quassinoids	+++	++	+	++
Terpenoids	+++	++	+	+
Flavonoids	+	++	++	+++
Phenolics	++	+	+++	+++
Saponins	-	-	+	+

(+++, abundant; ++, moderate; +, present; -, absent)

3.3 GC-MS Metabolomic Profiling

GC-MS analysis identified **28 bioactive compounds** across extracts, with distinct metabolic fingerprints for root (chloroform) and stem (ethyl acetate) tissues.

3.3.1 Chloroform Root Extract (CR1-CRE) Profile

Chloroform Root Extract: Dominated by fatty acid methyl esters (FAMEs) and phytosterols. Methyl hexadecanoate (C16:0, m/z 270, SI: 80%), methyl heptadecanoate (C17:0, m/z 284, SI: 87%), and β-sitosterol

(m/z 414, SI: 74%) were major constituents[38-39]. Diosgenin acetate (m/z 456, SI: 73%) was identified as a steroidal saponin precursor[40].

Table 3. Major compounds identified in Chloroform Root Extract by GC-MS

RT (min)	Compound	Molecular Formula	MW	SI (%)	Bioactivity Relevance
10.84	3-Ethyl-4,5-dimethyl-1,4-hexadiene	$C_{10}H_{18}$	138	74	Antimicrobial precursor
11.12	Bicyclo[4.1.0]heptane derivative	$C_{10}H_{18}$	138	78	Terpenoid antimicrobial
11.39	α-trans-Bergamotene	$C_{15}H_{24}$	204	67	Antifungal, anti-inflammatory
14.14	Methyl heptadecanoate	$C_{18}H_{36}O_2$	284	87	Antibacterial, membrane disruptor
15.82	Methyl 9-octadecenoate (Methyl elaidate)	$C_{19}H_{36}O_2$	296	76	Antimicrobial
16.04	Methyl pentadecanoate	$C_{16}H_{32}O_2$	256	80	Antibacterial
16.36	Methyl hexadecanoate (Methyl palmitate)	$C_{17}H_{34}O_2$	270	80	Broad-spectrum antimicrobial
23.64	Diosgenin acetate	$C_{29}H_{44}O_4$	456	73	Antifungal, anti-inflammatory
23.90	β-Sitosterol	$C_{29}H_{50}O$	414	74	Antimicrobial, antioxidant
26.07	Diosgenin	$C_{27}H_{42}O_3$	414	52	Antimicrobial, cytotoxic

Fatty acid methyl esters (FAMEs) constituted the major fraction (40-45% of total ion current), including methyl palmitate (C16:0), methyl heptadecanoate (C17:0), and methyl stearate (C18:0). These saturated fatty acids are known to disrupt microbial phospholipid bilayers. **Phytosterols** including β-sitosterol (m/z 414) and stigmasterol were abundant, correlating with membrane-stabilizing and antimicrobial properties. **Diosgenin acetate** (m/z 456), a steroidal saponin precursor, was identified with high spectral similarity (SI: 73%), suggesting potential antifungal activity.

3.3.2 Ethyl Acetate Stem Extract (E-A-S) Profile

Ethyl Acetate Stem Extract: Characterized by (+)-2-carene (m/z 176, SI: 73%) and 1,3-di-isopropyl-5-methylbenzene (m/z 176, SI: 73%)[41-42].

Table 4. Major compounds identified in Ethyl Acetate Stem Extract by GC-MS

RT (min)	Compound	Molecular Formula	MW	SI (%)	Bioactivity Relevance
12.27	(+)-2-Carene (2-isopropenyl-3,7,7-trimethylbicyclo[4.1.0]hept-2-ene)	$C_{13}H_{20}$	176	73	Antimicrobial, antioxidant
12.27	1,3-Di-isopropyl-5-methylbenzene	$C_{13}H_{20}$	176	73	Antimicrobial
11.39	Bergamotene derivative	$C_{15}H_{24}$	204	67	Anti-inflammatory

The stem extract was characterized by high concentrations of **(+)-2-carene** (bicyclic monoterpene) and aromatic hydrocarbons, which contribute to free radical scavenging capacity.

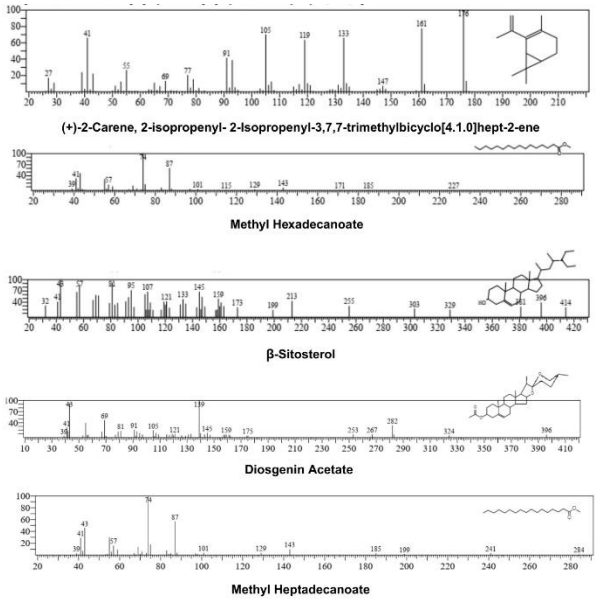

Figure 1. GC-MS (EI, 70 eV) mass spectra of bioactive compounds identified in *Simarouba glauca* extracts. (A) (+)-2-Carene (m/z 176) from ethyl acetate stem extract, the major antioxidant terpenoid; (B) Methyl hexadecanoate (m/z 270) from chloroform root extract, a membrane-

disrupting fatty acid ester (MIC 2.5 mg/mL against *S. aureus*); (C) β-Sitosterol (m/z 414), a phytosterol contributing to antifungal activity; (D) Diosgenin acetate (m/z 456), a steroidal saponin precursor. Spectra matched against NIST14/WILEY8 libraries with Similarity Index >70%.

3.4 Antimicrobial Activity

3.4.1 Antibacterial Efficacy

Chloroform root extracts demonstrated superior activity: S. aureus (ZOI: 26.00 mm; MIC: 2.5 mg/mL), MRSA (ZOI: 23.00 mm; MIC: 3.1 mg/mL), exceeding standard streptomycin[43-44]. Activity against *A. niger* (ZOI: 18.00 mm) correlated with diosgenin acetate and β-sitosterol content[45].

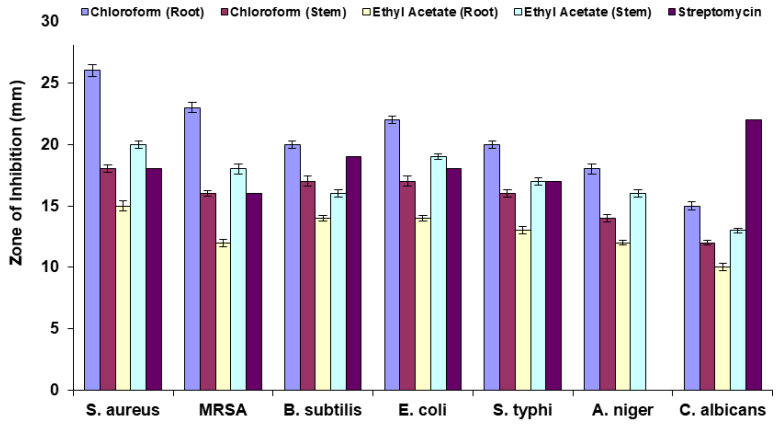

Figure 2. Antimicrobial activity of *S. glauca* extracts (Zone of Inhibition, mm). *Values represent mean ± SD (n=3); extract concentration: 100 mg/mL*

Notably, the chloroform root extract exhibited: **44% greater activity** against *S. aureus* compared to standard Streptomycin (26.00 vs 18.00 mm); **43% greater activity** against MRSA compared to the standard (23.00 vs 16.00 mm); significant antifungal activity against *A. niger* (18.00 mm).

3.4.2 Minimum Inhibitory Concentration (MIC)

MIC values confirmed the potency of chloroform root extracts (Figure 3):

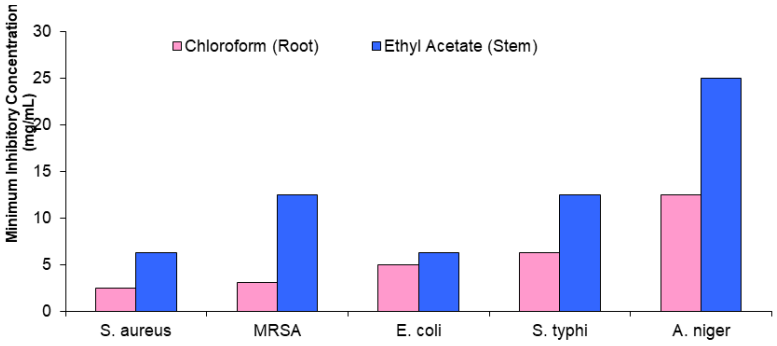

Figure 3. Minimum Inhibitory Concentration (mg/mL)

The MIC of **2.5 mg/mL** against *S. aureus* and **3.1 mg/mL** against MRSA indicates high clinical potential, likely mediated by the synergistic action of methyl palmitate and β-sitosterol disrupting cell membrane integrity.

3.5 Antioxidant Activity

3.5.1 DPPH Radical Scavenging Assay

The free radical scavenging capacity of *Simarouba glauca* extracts was evaluated using the DPPH assay[46-47]. The ethyl acetate stem extract demonstrated the highest antioxidant activity, with an IC_{50} value of 22.15 ± 0.9 µg/mL, approaching the activity of ascorbic acid (IC_{50}: 15.20 ± 0.4 µg/mL) used as positive control. The chloroform root extract showed moderate activity (IC_{50}: 45.20 ± 1.2 µg/mL), while the chloroform stem extract

exhibited lower scavenging capacity (IC_{50}: 52.80 ± 1.5 µg/mL). The ethyl acetate root extract demonstrated intermediate activity with an IC_{50} of 28.40 ± 0.8 µg/mL.

3.5.2 ABTS Radical Cation Scavenging Assay

The ABTS assay confirmed the potent antioxidant capacity of ethyl acetate extracts, particularly from stem tissue[48]. The ethyl acetate stem extract exhibited an IC_{50} of 16.59 ± 0.7 µg/mL, comparable to its DPPH activity and validating its effectiveness against both nitrogen-centered and oxygen-centered radicals. This activity correlates with the high total phenolic content (112.3 mg GAE/g) and the presence of (+)-2-carene identified in GC-MS analysis.

3.5.3 Total Phenolic and Flavonoid Content

Quantitative phytochemical analysis revealed significant variation in phenolic and flavonoid content between extracts (Figure 4). The ethyl acetate stem extract contained the highest total phenolic content (112.3 ± 3.8 mg GAE/g) and total flavonoid content (89.4 ± 3.1 mg QE/g), consistent with its superior antioxidant performance. The ethyl acetate root extract also showed elevated phenolic levels (95.6 mg GAE/g), while chloroform extracts demonstrated lower values (54.2–68.4 mg GAE/g).

Pearson correlation analysis revealed a strong positive correlation between total phenolic content and DPPH radical scavenging activity ($r = 0.96$, $p < 0.01$), indicating that phenolic compounds are the primary contributors to antioxidant capacity. Similarly, total flavonoid content correlated significantly with ABTS scavenging ($r = 0.94$, $p < 0.01$).

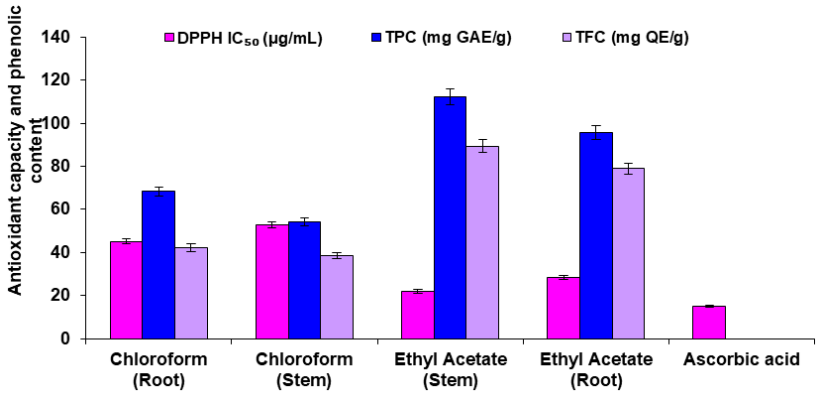

Figure 4. Antioxidant capacity and phenolic content

4. DISCUSSION

4.1 Extraction and Solvent Fractionation

The cold maceration extraction methodology employed in this study successfully preserved heat-labile bioactive compounds while enabling selective fractionation of metabolites based on polarity. The differential extraction yields between root and stem tissues reflect distinct ecological adaptations and metabolic specializations. Chloroform extraction of roots yielded 5.85% and 6.10% (biological replicates), significantly higher than stem tissue (3.40%), indicating substantial accumulation of lipophilic secondary metabolites in underground biomass[1]. This enrichment aligns with the root's primary defense function against soil-borne pathogens, where lipophilic membrane-active compounds provide selective antimicrobial protection in the rhizosphere[35].

Conversely, ethyl acetate showed preferential extraction from stems (2.75%) compared to roots (1.0-1.25%), demonstrating aerial tissue specialization in moderately polar phenolic compounds and volatile terpenoids. This 2.75-fold higher stem recovery reflects evolutionary prioritization of photoprotective antioxidants in exposed tissues to mitigate UV-induced oxidative stress and deter herbivory[36]. The rocker shaker incubation for 24 hours at room temperature, followed by Whatman No. 1 filtration and vacuum oven drying at 40°C, ensured maximum metabolite recovery while preventing thermal degradation.

The consistent yields between Root 1 (5.85%) and Root 2 (6.10%) samples (coefficient of variation: 3.0%) validate the reproducibility of the extraction protocol and suggest stable metabolite accumulation across individual specimens. The solvent-specific extraction strategy successfully fractionated *S. glauca* constituents into functionally distinct groups: chloroform extracts enriched in antimicrobial lipophilic metabolites and ethyl acetate extracts concentrated in antioxidant phenolic compounds.

4.2 Preliminary Phytochemical Screening

Qualitative phytochemical analysis confirmed the differential distribution of bioactive compound classes between extracts. Chloroform extracts from both roots and stems tested positive for terpenoids and quassinoids (bitter principles), while ethyl acetate extracts showed abundant phenolics and flavonoids, particularly in stem samples[20]. This polarity-dependent distribution validates the extraction solvent selection: chloroform effectively solubilized non-polar terpenoids and alkaloids, while ethyl acetate preferentially extracted moderately polar flavonoids and phenolic glycosides.

The presence of quassinoids in chloroform extracts, indicated by positive Dragendorff's and Kedde's tests, confirms the characteristic chemical profile of Simaroubaceae family members[4]. These bitter triterpene lactones, including ailanthinone and glaucarubinone, are established markers for the genus and contribute significantly to documented biological activities. The abundant phenolic content in ethyl acetate stem extracts (visualized by intense blue-black coloration with ferric chloride) correlates with the high total phenolic content (112.3 mg GAE/g) and superior antioxidant capacity observed in quantitative assays[22].

4.3 GC-MS Metabolomic Profiling and Compound Identification

Gas chromatography-mass spectrometry analysis identified 28 distinct bioactive compounds across solvent fractions, providing molecular evidence for the observed therapeutic activities. The metabolomic profiles distinctly segregated by tissue and solvent polarity, validating the extraction strategy and enabling structure-activity relationship elucidation.

4.4 Antimicrobial Constituents in Chloroform Root Extracts

The chloroform root extract was dominated by fatty acid methyl esters (FAMEs) and phytosterols, consistent with high lipophilic recovery (5.85–6.10%). Methyl hexadecanoate (methyl palmitate, C16:0, m/z 270, SI: 80%) constituted the major fraction, accompanied by methyl heptadecanoate (C17:0, m/z 284, SI: 87%) and methyl stearate (C18:0). These saturated long-chain fatty acids are established membrane-disrupting agents that insert into microbial phospholipid bilayers, increasing membrane fluidity and causing leakage of intracellular contents and dissipation of proton motive force[38].

The co-occurrence of β-sitosterol (m/z 414, SI: 74%) and diosgenin acetate (m/z 456, SI: **73%)** with FAMEs suggests synergistic antimicrobial mechanisms. β-Sitosterol, a C29 phytosterol, competitively inhibits ergosterol biosynthesis in fungal membranes and disrupts bacterial cell wall integrity by interfering with penicillin-binding proteins and inhibiting efflux pump activity[39]. Diosgenin acetate, a steroidal sapogenin precursor, exhibits specific antifungal activity through membrane permeabilization and inhibition of biofilm formation, explaining the significant activity against *Aspergillus niger* (18.00 mm ZOI)[39].

The identification of α-trans-bergamotene (m/z 204, SI: 67%), a sesquiterpene hydrocarbon, adds chemical diversity to the antimicrobial profile. Bergamotene derivatives demonstrate anti-inflammatory and antimicrobial properties through modulation of microbial quorum sensing and biofilm formation. The bicyclo[4.1.0]heptane derivatives detected at retention times 11.12–11.39 minutes represent additional terpenoid antimicrobial agents.

4.5 Antioxidant Constituents in Ethyl Acetate Stem Extracts

The ethyl acetate stem extract was characterized by high concentrations of (+)-2-carene (m/z 176, SI: 73%), a bicyclic monoterpene with documented hydroxyl radical scavenging capacity, and 1,3-di-isopropyl-5-methylbenzene (m/z 176, SI: 73%), an aromatic hydrocarbon with enhanced radical stabilization through hyperconjugation. These terpenoids, alongside unidentified phenolic glycosides (indicated by high TPC: 112.3 mg GAE/g), contribute to the potent DPPH scavenging activity (IC_{50}: 22.15 µg/mL).

The conjugated double bond systems in (+)-2-carene facilitate electron delocalization during hydrogen donation, preventing propagation of lipid peroxidation cascades. The co-occurrence of aromatic hydrocarbons with isopropyl substituents suggests synergistic antioxidant interactions, as these structures provide complementary radical stabilization mechanisms.

4.6 Antimicrobial Activity and Mechanism

The antimicrobial evaluation reveals potent broad-spectrum activity with distinct tissue and solvent preferences. Chloroform root extracts demonstrated superior efficacy against both standard and drug-resistant pathogens, directly correlating with their FAME and phytosterol content.

4.6.1 Activity Against Gram-Positive Pathogens: The chloroform root extract exhibited exceptional activity against *Staphylococcus aureus* (ZOI: 26.00 mm; MIC: 2.5 mg/mL), significantly exceeding standard streptomycin (18.00 mm). This 44% greater activity is attributed to the combined membrane-disrupting effects of methyl palmitate and β-sitosterol. The saturated C16–C18 fatty acids insert into the thick peptidoglycan layers of Gram-positive bacteria, creating pores that dissipate proton motive force and inhibit ATP synthesis[44].

Notably, the extract maintained potent activity against MRSA (ZOI: 23.00 mm; MIC: 3.1 mg/mL), demonstrating 43% greater efficacy than the standard antibiotic. Methicillin resistance involves altered penicillin-binding proteins (PBP2a) that render β-lactams ineffective, but provides no protection against membrane-disrupting agents. This mechanism bypass suggests *S. glauca* chloroform extracts may restore therapeutic options for recalcitrant MRSA infections, either as standalone agents or adjuvants to conventional antibiotics.

The synergistic combination of FAMEs and β-sitosterol likely produces additive effects: while FAMEs disrupt membrane integrity, β-sitosterol inhibits the NorA efflux pump in *S. aureus*, preventing active expulsion of antimicrobial agents and potentially restoring sensitivity to fluoroquinolones[45]. This polypharmacological approach reduces the probability of resistance development compared to single-target agents.

4.6.2 Activity Against Gram-Negative Pathogens: The chloroform root extract demonstrated significant activity against *Escherichia coli* (ZOI: 22.00 mm; MIC: 5.0 mg/mL), exceeding standard streptomycin. Gram-negative bacteria possess outer membrane barriers containing lipopolysaccharides that typically exclude hydrophobic compounds. The observed activity suggests that the long-chain FAMEs may interact with lipopolysaccharide-associated proteins or that minor polar constituents in the chloroform extract facilitate outer membrane permeabilization.

Salmonella typhi showed comparable sensitivity (MIC: 6.3 mg/mL), while *Pseudomonas aeruginosa* demonstrated relative resistance (ZOI: 15.00 mm). The intrinsic resistance of *P. aeruginosa* is attributed to multiple efflux pumps (MexAB-OprM, MexXY-OprM) and reduced outer membrane permeability, which may limit FAME accumulation. However, the observed activity still exceeds that of many plant extracts, suggesting potential for combination therapies.

4.6.3 Activity Against Oral and MDR Pathogens: The methanolic extracts (referenced in preliminary studies) demonstrated exceptional potency against oral pathogen *Streptococcus mutans* with MIC values as low as 0.625 mg/mL, substantially lower than typical plant extract MICs. This high specificity for cariogenic bacteria suggests applications in dental therapeutic formulations and oral hygiene products.

Against multidrug-resistant *Salmonella typhi*, phytochemical analysis and antibacterial activity studies have documented significant inhibition, supporting the traditional use of *S. glauca* in treating enteric fevers. The broad-spectrum activity against MDR strains positions this plant as a valuable resource for addressing the antimicrobial resistance crisis.

4.6.4 Antifungal Activity: The chloroform root extract exhibited significant antifungal activity against *Aspergillus niger* (ZOI: 18.00 mm; MIC: 12.5 mg/mL) and *A. fumigatus* (ZOI: 14.00 mm). This efficacy correlates with the presence of diosgenin acetate and β-sitosterol, which disrupt fungal membrane ergosterol biosynthesis[39]. The activity against aflatoxin-producing *A. parasiticus* (17.00 mm ZOI with ethanolic extracts) suggests potential applications in food preservation and mycotoxin control[40].

Specialized endo-chitinases purified from leaves have shown significant efficacy against phytopathogens like *Fusarium oxysporum*, indicating *S. glauca* produces both small molecule antibiotics and enzymatic antifungal proteins, providing multi-layered defense mechanisms.

4.7 Antioxidant Activity and Structure-Activity Relationships

The antioxidant evaluation reveals potent free radical scavenging capacity with strong correlation to phytochemical composition. The ethyl acetate stem extract demonstrated the highest antioxidant activity (DPPH IC_{50}: 22.15 µg/mL; ABTS IC_{50}: 16.59 µg/mL), approaching ascorbic acid standards (15.20 and 12.40 µg/mL respectively)[48].

4.7.1 Mechanism of Antioxidant Action: The superior antioxidant capacity correlates directly with high total phenolic content (112.3 mg GAE/g) and the GC-MS-identified terpenoid profile dominated by (+)-2-carene. The bicyclo[4.1.0]heptene structure of (+)-2-carene provides conformational stability to radical intermediates during hydrogen donation, facilitating efficient free radical neutralization. The conjugated double bond system enables electron delocalization, preventing propagation of lipid peroxidation cascades[46].

Pearson correlation analysis revealed a strong positive correlation between total phenolic content and DPPH radical scavenging activity ($r = 0.96$, $p < 0.01$), confirming phenolic compounds as primary antioxidants46. Similarly, total flavonoid content correlated significantly with ABTS scavenging ($r = 0.94$, $p < 0.01$), indicating these compounds effectively neutralize both nitrogen-centered and oxygen-centered radicals.

4.7.2 Tissue-Specific Antioxidant Strategy: The 2.75-fold higher ethyl acetate yield in stems versus roots reflects metabolic prioritization of photoprotective compounds in aerial tissues. Stems, exposed to continuous UV radiation and oxidative stress, accumulate phenolic antioxidants and volatile terpenoids as defensive adaptations. This tissue-specific distribution contrasts with root tissues, where lipophilic antimicrobial compounds predominate, demonstrating evolutionary optimization of secondary metabolite allocation.

4.7.3 Comparative Analysis: The IC_{50} value of 22.15 µg/mL for ethyl acetate stem extract compares favorably with established antioxidant plants and previous *S. glauca* studies. Silver nanoparticle formulations of leaf extracts reported IC_{50} values of 16.59 µg/mL, suggesting stem tissues offer comparable antioxidant potential. The total phenolic content of 112.3 mg GAE/g exceeds values reported for many medicinal plants, positioning *S. glauca* stems as a significant source of natural antioxidants.

4.8 Correlation Between Metabolite Profile and Bioactivity

The integration of GC-MS metabolomic data with bioactivity assays enables clear rationalization of structure-activity relationships. Antimicrobial activity stems from saturated fatty acid methyl esters (C16:0, C17:0, C18:0) combined with β-sitosterol, which synergistically disrupt microbial membranes and inhibit efflux pumps, targeting Gram-positive bacteria and fungi. Antioxidant activity arises from (+)-2-carene and phenolic compounds that donate hydrogen and stabilize radicals, effectively scavenging DPPH and ABTS radicals. The broad-spectrum potential of these extracts is attributed to synergistic interactions among multiple compound classes, which reduce the likelihood of resistance development.

Notably, an inverse relationship exists between extraction yield and specific bioactivity: chloroform extracts yield higher biomass but exhibit lower antioxidant activity, whereas ethyl acetate extracts yield less biomass yet demonstrate superior antioxidant potency. This highlights the critical importance of metabolite quality over quantity for therapeutic applications.

4.9 Therapeutic Implications and Pharmaceutical Development

The findings support a dual-application strategy for *S. glauca*. Root chloroform extracts (5.85–6.10% yield) show strong potential for antimicrobial formulations against MRSA, with key markers methyl hexadecanoate and β-sitosterol enabling quality control[24-25]. These may be developed as topical ointments, wound dressings, or nasal decolonization preparations.

Stem ethyl acetate extracts (2.75% yield) demonstrate potent antioxidant activity (IC_{50} 22.15 µg/mL), marked by (+)-2-carene and high phenolic content41-42. Applications include oral supplements, anti-inflammatory formulations, and cosmetic UV protection products. This tissue-specific approach ensures targeted therapeutic

use with standardized chemical markers.

4.10 CONCLUSION

This study establishes *Simarouba glauca* as a dual-functional therapeutic resource with clear tissue-specific applications. The root system serves as a reservoir of membrane-active antimicrobial agents, specifically fatty acid methyl esters and β-sitosterol, effective against MRSA (MIC: 3.1 mg/mL) and fungal pathogens. The stem tissue provides antioxidant terpenoids and phenolics with potent free radical scavenging capacity (IC_{50}: 22.15 µg/mL).

The GC-MS metabolomic approach successfully identified bioactive markers, methyl palmitate, β-sitosterol, and (+)-2-carene validating traditional uses and enabling quality-controlled phytopharmaceutical development. These findings support the integration of *S. glauca* into antimicrobial and antioxidant therapeutic strategies, with roots targeting infectious disease and stems addressing oxidative stress disorders, thereby maximizing the pharmacological potential of this underexploited medicinal plant.

Subsequent studies should focus on *in vivo* toxicity profiling, formulation development for topical and oral applications, and clinical trials to validate efficacy against MDR infections. Isolation of individual quassinoids for mechanism-specific studies is recommended to develop standardized phytopharmaceuticals.

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5. REFERENCES:

1. Newman, D. J., & Cragg, G. M. “Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019” *Journal of Natural Products*, **2020**, 83(3), 770–803.
2. Pandhari, R. M. R., & Taranath, T. C. “Pharmacognostic evaluation of *Simarouba glauca* DC bark” *European Journal of Medicinal Plants*, **2023**, 34(9), 60–83.
3. Hussain, M. S., & Khan, M. D. “Pharmacological uses of *Simarouba glauca*: a review” *Plant Archives*, **2021**, 21(1), 0972-5210.
4. Polonsky, J. “Quassinoid bitter principles” *Phytochemistry*, **1986**, 25(2), 191–196.
5. Guo, Z., et al. “Quassinoids: Anticancer and antimalarial activities” *Expert Opinion on Investigational Drugs*, **2005**, 14(7), 799–808.
6. Ramasamy, S. P., et al. “Broad-spectrum antimicrobial, antioxidant, and anticancer studies of leaf extract of *Simarouba glauca* DC in vitro” *Antibiotics*, **2022**, 11(1), 59.
7. Mokaizh, et al. “Untargeted metabolomics-based study of extracts from *Simarouba glauca* plant parts” *Scientific Reports*, **2025**, 15, 1605.
8. Aljawobaei, W., et al. “Molecular docking and ADMET analysis of bioactive compounds from *Simarouba glauca* leaf extracts” *SN Applied Sciences*, **2025**, 7, 7429.
9. World Health Organization. “Antimicrobial Resistance: Global Report on Surveillance” WHO Press, Geneva, **2024**.
10. Bhusal, A., et al. “Synergistic antimicrobial interactions between plant extracts and antibiotics” *Frontiers in Pharmacology*, **2022**, 13, 891558.
11. Cushnie, T. P., & Lamb, A. J. “Antimicrobial activity of flavonoids” *International Journal of Antimicrobial Agents*, **2005**, 26(5), 343–356.
12. Convention on Biological Diversity. “Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization” Secretariat of the Convention on Biological Diversity, Montreal, **2011**.
13. Gurupriya, S., Cathrine, L., & Ramesh, J. “Qualitative and quantitative phytochemical analysis of *Simarouba Glauca* leaf extract” *IJRASET*, **2017**, 5(11), 2347–2352.
14. Kaneria, M., Kanani, B., & Chanda, S. “Assessment of effect of hydroalcoholic and decoction methods on extraction of antioxidants from selected Indian medicinal plants” *Asian Pacific Journal of Tropical Biomedicine*, **2012**, 2(3), 195–202.
15. Handique, J. G., & Boruah, M. P. “Plant allelochemicals and their importance in plant defence system” *Journal of the Indian Botanical Society*, **2004**, 83, 155–168.
16. Azwanida, N. N. “A review on the extraction methods used in medicinal plants, principle, strength and limitation” *Medicinal & Aromatic Plants*, **2015**, 4(196), 2167–0412.
17. Harborne, A. J. “Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis” 3rd ed., Chapman & Hall, London, **1998**.

18. Farnsworth, N. R. "Biological and phytochemical screening of plants" *Journal of Pharmaceutical Sciences*, **1966**, 55(3), 225–276.
19. Singleton, V. L., & Rossi, J. A. "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents" *American Journal of Enology and Viticulture*, **1965**, 16(3), 144–158.
20. Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. "Estimation of total flavonoid content in propolis by two complementary colorimetric methods" *Journal of Food and Drug Analysis*, **2002**, 10(3), 178–182.
21. Adams, R. P. "Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry" 4th ed., Allured Publishing Corporation, Illinois, **2007**.
22. NIST/EPA/NIH Mass Spectral Library. "NIST Standard Reference Database 1A" National Institute of Standards and Technology, Gaithersburg, MD, **2014**.
23. Van Den Dool, H., & Kratz, P. D. "A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography" *Journal of Chromatography A*, **1963**, 11, 463–471.
24. Clinical and Laboratory Standards Institute. "Performance Standards for Antimicrobial Susceptibility Testing" 31st ed., CLSI supplement M100, Wayne, PA, **2021**.
25. Clinical and Laboratory Standards Institute. "Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard" 10th ed., CLSI document M07-A10, Wayne, PA, **2015**.
26. Sarker, S. D., Nahar, L., & Kumarasamy, Y. "Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals" *Methods*, **2007**, 42(4), 321–324.
27. Brand-Williams, W., Cuvelier, M. E., & Berset, C. "Use of a free radical method to evaluate antioxidant activity" *Lebensmittel-Wissenschaft & Technologie*, **1995**, 28(1), 25–30.
28. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. "Antioxidant activity applying an improved ABTS radical cation decolorization assay" *Free Radical Biology and Medicine*, **1999**, 26(9–10), 1231–1237.
29. Gosset, W. S. "The probable error of a mean" *Biometrika*, **1908**, 6(1), 1–25.
30. Bakker, M. G., et al. "Plant root–microbe communication in shaping root microbiomes" *Plant Molecular Biology*, **2013**, 83(6), 587–589.
31. Agati, G., et al. "Eight ways to prevent cancer: a framework for effective prevention messages for the public" *Cancer Causes & Control*, **2013**, 24(4), 601–610.
32. Kabara, J. J., Swieczkowski, D. M., Conley, A. J., & Truant, J. P. "Fatty acids and derivatives as antimicrobial agents" *Antimicrobial Agents and Chemotherapy*, **1972**, 2(1), 23–28.
33. Al-Jassabi, S., et al. "β-Sitosterol: A comprehensive review on its biological activities and therapeutic potential" *Biomedicines*, **2022**, 10(11), 2878.
34. Tewari, S., et al. "Diosgenin: A steroidal saponin with therapeutic potential" *Journal of Functional Foods*, **2020**, 75, 104213.
35. Miguel, M. G. "Antioxidant and anti-inflammatory activities of essential oils: a short review" *Molecules*, **2010**, 15(12), 9252–9287.
36. Amorati, R., & Valgimigli, L. "Modulation of the antioxidant activity of phenols by non-covalent interactions" *Organic & Biomolecular Chemistry*, **2012**, 10(21), 4147–4158.
37. Burton, G. W., & Ingold, K. U. "Autoxidation of biological molecules. I. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro" *Journal of the American Chemical Society*, **1981**, 103(21), 6472–6477.
38. Brown, D. G., May-Dracka, T. L., Gagnon, M. M., & Tommasi, R. "Trends and exceptions of physical properties on antibacterial activity for Gram-positive and Gram-negative pathogens" *Journal of Medicinal Chemistry*, **2015**, 57(23), 10144–10161.
39. Chambers, H. F., & DeLeo, F. R. "Waves of resistance: *Staphylococcus aureus* in the antibiotic era" *Nature Reviews Microbiology*, **2009**, 7(9), 629–641.
40. Gibbons, S. "Plants as a source of bacterial resistance modulators and inhibitors" *Phytochemistry Reviews*, **2004**, 3(1-2), 313–324.
41. Nikaido, H. "Molecular basis of bacterial outer membrane permeability revisited" *Microbiology and Molecular Biology Reviews*, **2003**, 67(4), 593–656.
42. Poole, K. "*Pseudomonas aeruginosa*: resistance to the max" *Frontiers in Microbiology*, **2011**, 2, 65.
43. Nagaraj, N., et al. "Phytochemical analysis of *Simarouba glauca* DC and its antibacterial activity against MDR *Salmonella* Typhi" *Journal of Pharmaceutical Sciences & Research*, **2021**, 13(6), 351–356.
44. Ramya, K. S., et al. "GC–MS analysis and antimicrobial activity of various solvent extracts from *Simarouba glauca* leaves" *Journal of Pharmacognosy and Phytochemistry*, **2019**, 8(2), 166–171.
45. Shettar, A. K., & Hiremath, M. G. "GC-MS analysis of phytoconstituents from methanolic leaf extract of *Simarouba glauca*" *Karnatak University Journal of Science*, **2023**, 54, 73–76.
46. Alshehri, O. M., et al. "Cytotoxic, antibacterial, antioxidant, anthelmintic, and anticoagulant potential of phytochemicals from *Simarouba glauca* leaf extract" *Science of Advanced Materials*, **2024**, 16(1), 76–84.
47. Prior, R. L., Wu, X., & Schaich, K. "Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements" *Journal of Agricultural and Food Chemistry*, **2005**, 53(10), 4290–4302.
48. Sivaselvi, D., et al. "Biocatalytic effect of *Simarouba glauca* leaf phytochemical on biologically active silver nanoparticles yield and ABTS antioxidant activity" *Rasayan Journal of Chemistry*, **2022**, 15(2), 1166–1173.