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Research Article

Exploring the Chemical Diversity and Antimicrobial Potential of *Albizia chevalieri* Leaves Extract through GC-MS and TLC Investigation

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ABSTRACT
Albizia chevalieri has a rich history of use in traditional medicine. Its bark, leaves, and flowers are utilized in treating a range of ailments, including skin conditions, digestive problems, epilepsy, diabetes mellitus, haemorrhoids, asthma, leprosy and gonorrhoea and respiratory issues. A*lbizia chevalieri* leaves were collected, identified, air-dried, pulverized and subjected to cold extraction (maceration) using dichloromethane, and methanol respectively. Antimicrobial activity of the extracts was determined using standard antimicrobial tests against *Bacillus amyloquefaciens, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas auruginosa,* and *Trichophyton rubrum*. The most active extract was thereafter subjected to gas chromatography mass spectrometric analysis. Results of the antimicrobial activity test of the extracts had showed appreciable antimicrobial activity against *Bacillus amyloquefaciens, Staphylococcus aureus, Pseudomonas aeruginosa* and *Trichophyton rubrum* (with MIC values ranging from 250 mg/ml to 125 mg/ml). The results demonstrated significant inhibitory effects, highlighting the potential of the compound as an antimicrobial agent. The GCMS analysis identified 21 compounds from dichloromethane crude extract. Out of these, five (5) compounds were most abundant (revealed >5% peak areas on GC chromatogram)., which were: 1-octadecane 1-iodo, dibutylphthalate, henecosine, oxirintetradecyl and tetrapentacontane. The antimicrobial activity of *Albizia chevalieri* against the pathogenic microorganisms is ascribable to these phytocompounds, thereby proving proof for its ethnomedicinal uses.

Keywords: *Albizia chevalieri;* Antimicrobial; Crude extract; GCMS; Phytochemicals; TLC

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INTRODUCTION

According to studies, humans have been using natural products like plants, animals, microbes, and marine organisms to eradicate and treat various diseases since. It has been estimated that humans have been using plants as remedies for at least 60,000 years.
(Riji *et al.,* 2023). when early humans were searching for food, they occasionally ate poisonous plants, which could cause vomiting, diarrhoea, comas, or other toxic reactions in the body system, and in some cases, death. As a result, early humans had the capacity to develop knowledge and skills about suitable materials and natural treatments (Riji *et al.,* 2023). The World Health Organization (WHO) estimated that due to poverty and lack of access to modern medication, 65-80% of the world's population lives in under-developed nations and relies primarily on plants for treatment.

Albizia chevalieri, also known as the silk tree or mimosa tree, is a species within the Fabaceae family. It is predominantly found in South and Southeast Asia, particularly in countries like India, Bangladesh, Myanmar, and Thailand, subsequently other part of the world. This tree is known for its rapid growth and ability to adapt to various environmental conditions, making it an important species for reforestation and

agroforestry initiatives (Nguyen, 2014). *Albizia chevalieri* has a rich history of use in traditional medicine. Its bark, leaves, and flowers are utilized for treatment of a range of ailments, including skin conditions, digestive problems, and respiratory issues (Ahmed *et al.,* 2019). The tree is also culturally significant in many Asian societies, where it is often planted near homes and temples as a symbol of protection and prosperity. Its presence is believed to bring good fortune and safeguard against negative energies (Hossain *et al.,* 2002).

MATERIALS AND METHODS

Materials and Chemicals

All chemicals used in this investigation were of analytical grade and were obtained from Sigma Chemical Co., St Louis, USA, were obtained from Oxoid Ltd, Wade Road, Basingstoke, Hants, RG24 8PW, UK.

Plant collection and Preparation

The *Albizia chevalieri* leaves were collected at Dutsin-Ma Local Government Area, Katsina state, Nigeria. The leaves were air-dried and ground into fine powder using mortar and pestle in the laboratory. Extraction was carried out through conventional method as described by Fasihuddin *et al. (*2010). This was achieved by soaking the powdered samples in solvent in the order of increasing polarity. A total of 1kg of the powdered sample was extracted using cold soaking method. This was done by soaking the powdered sample material dichloromethane medium polar (with medium polarity) and methanol (more polar). The sample was soaked in dichloromethane in Bama bottles at room temperature for 72 hours. The resulting dichloromethane solution was then filtered using filter paper and the residue was reextracted with fresh dichloromethane for another 72 hours and filtered. The extract was combined and concentrated using the rotary evaporator (model Heidolph Laborota 4000 efficient) under reduced pressure to obtain dichloromethane crude extract. The residues were then re-extracted using similar/ procedure with methanol to obtain methanol, crude extracts, respectively. At the end of the extraction process the dry weight and yield of each crude extract were determined. However, dichloromethane (DCM) extract was used for the study.

Preliminary Phytochemical Screening

A few milligrams of the two different dried extracts were obtained from dichloromethane, and methanol was first dissolved and the various solutions obtained were all subjected to phytochemical screening employing the standard screening test (Trease and Evan, 1996).

Test for Flavonoids

A few drops of concentrated hydrochloric acid were added to a small amount of the extracts of the plant material. Immediate development of a red color indicated the presence of flavonoids.

Test for Tannins

To a portion of the extract, 3-5 drops of ferric chloride was added. A greenish-black precipitate indicated the presence of tannins (Trease and Evan, 1996).

Test for Alkaloids

Few drops of Mayer's reagent were added to 1mL of extract. A yellowish or white precipitate was formed, indicated the present of alkaloids (Trease and Evan, 1996).

Test for Terpenoids

To 5 ml of the extract add 2 ml of chloroform and 3 ml of H_2SO_4, conc., formation of a reddish-brown ring confirmed the presence of terpenoids (Trease and Evan, 1996).

Test for Carbohydrates

Few drops of molish reagent were added to 2 ml of extract later drops of concentrated H_2SO_4 were added along the walls of the test tube. At junction of two liquids, a violet colour ring appeared, indicating that carbohydrate was present.

Test for Anthraquinones

A few ml of H_2SO_4 conc was added to 5 ml of extract, followed by 1 ml of diluted ammonia. The existence of anthraquinones is confirmed by the appearance of rose pink.

Test for Saponins

With a few ml of distilled water, 0.5 mg of extract was quickly shaken. For saponins, the production of foaming is a favourable sign.

Test for Steroids

The presence of steroids is shown by the emergence of red color and yellowish green fluorescence after mixing 2 ml of extract with 2 ml of chloroform and 2 ml of concentrated H_2SO_4, the appearance of red color and yellowish green fluorescence confirmed the presence of steroids

Antibacterial Activity of the Extract (Methods)

Preparation of Inocula

The bacterial strains of *Bacillus* spp., *Pseudomonads spp*., *S. aureus* and *Salmonella* spp. from the culture plates were standardized by matching turbidity of culture to 0.5 McFarland standards which would then be diluted in fresh broth (peptone water) and incubated at 37^{o}C for 24 hours to achieve final inoculums (Ezouberi *et al.,* 2005)

Preparation of Extract Concentrations

One (0.5 g) of the extract were measured using balance and dispensed into the clean and sterile test tube containing 2 ml of distilled water to obtained a concentration of 250 mg/ml, followed by transferring into another containing 1 ml to obtained 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml, and stored for further analysis.

Discs Preparation

Filter paper was punched using puncher and obtained an approximately 6mm diameter, autoclaved at 121^{0}C for 15 minutes and dispensed into each concentration and allowed to absorb for 1 hour

Sensitivity Test Using Disc Diffusion

Thirty-nine grams (39g) of (MHA) were prepared and 0.2 ml of each bacterium was inoculated on to the solidified Mύeller-Hinton agar. The dish was left on bench set (Priya and Deepak, 2007). Discs containing different concentrations of 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml were seeded and Ciprofloxacin would be set as control. The plate was then incubated at 37^{o}C for 24 hours and diameter zone of inhibition was measured.

Determination of Minimum Inhibitory and Minimum Bactericidal Concentration

Dilution tubes methods were used in varying concentration of the liquid medium and the extract in test tubes at 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml, 0.1 ml of the standardized bacterial inoculum in the same tubes. The tubes were then incubated aerobically incubated at 37^{o}C for 24 hours, positive control was equal set up. The tubes with the least growth showed MIC**.** The MIC were then sub-cultured into nutrient agar plates that contain no antibiotic, the lowest concentration of the chemotherapeutic agent that resulted in no growth of the subculture was noted. This refers as MBC of the chemotherapeutic agents (Riji *et al*., 2023).

Thin Layer Chromatography (TLC)

The two extracts were subjected to thin layer chromatography (TLC) using several solvent-system (hexane per ethyl acetate (4:1), hexane per ethyl acetate (5:3) and hexane per ethyl acetate (3:2) to obtain the best solvent system that would give good separation for the compounds suitable for preliminary isolation of the active compounds. The extracts were then spotted on TLC plates and allowed to dry. After drying, the plates were developed in an air-tight chromatographic tank using the perceived solvent system. The developed chromatograms were air dried and visualized; under normal day light using ultra violet light (254 nm & 366 nm).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC–MS analysis of bioactive compounds from the DCM extracts of *Albizia Chevalieri* was carried out using Model 7890A (Agilent Technologies) interfaced with a mass selector detector model 5975°C. The electron ionization was kept at 70eV with anion source temperature of 250°C, using Helium as the carrier gas and HP-5MS (30 mm × 0.25 mm × 0.320 µm) as the stationary phase. The oven temperature was kept at 80°C held for 4 minutes and ramped to 270°C at the rate of 3.5°C/minutes holding for 6 minutes. 1 mg each of the extracts was dissolved in 1ml of acetonitrile. The mixture was vortexed and sieved through 0.4 millipore filter into a 5ml rotavapour flask and dried using rotavapour. 700 µl dichloromethane was added and transferred into screw cap tubes. 1 µl each of the prepared extracts was injected into the column at 300°C. The split mode was employed with a split ratio of 50:1. Relative quantity of the chemical compounds present in each of the extracts of *Albizia Chevalieri* was expressed in percentage based on the peak area produced in the chromatogram.

RESULTS

***Albizia chevalieri* Leaf Extraction Yield**

There is a noticeable difference in efficiency between the extraction yields produced with dichloromethane and methanol. In the study, 16.33 g of extract (8.165% yield) was obtained from the methanol extraction of 200 g of powdered leaves. Only 3.31 g of extract were obtained from dichloromethane extraction of the same quantity (200 g), yielding a 1.655% yield Table 1).

TLC Profile of Dichloromethane Crude Extract

The presence of three different Rf values indicates that the dichloromethane extract contains at least three chemically distinct compounds with varying polarities. The low Rf value (0.10) of D1 suggests a more polar compound, which interacts strongly with the stationary phase. The higher Rf values (0.44 and 0.62) of D2 and D3 indicate less polar or moderately non-polar compounds, which migrate further up the TLC plate. Thin Layer Chromatography (TLC) analysis of the dichloromethane extract revealed three distinct components, labeled D1, D2, and D3: Component D1 travelled 0.5 cm with an Rf value of 0.10; Component D2 travelled 2.2 cm with an Rf value of 0.44 and Component D3 travelled 3.1 cm with an Rf value of 0.62 (Table 2).

The result (Figure 1) showed various spots on the TLC plates for all the extracts when viewed under UV light at 365nm and 254nm. The retention factor (Rf) values of *Albizia chevalieri* extracts were determined.

Phytochemical Screening of *Albizia chevalieri*

The result for the phytochemical screening of *Albizia chevalieri* using two different solvents dichloromethane and methanol is presented in Table 3. The result revealed the presence of active constituents in and seven active constituents in methanolic fraction of the extracts. The active constituents were flavonoids, alkaloids, anthraquinones, saponins, tannins, steroids, triterpenoids and cardiac glycosides. While four active compounds are present in dichloromethane crude extract.

The result showed various spots on the TLC plates for all the extracts when viewed under UV light at 365nm and 254nm. The retention factor (Rf) values of *Albizia chevalieri* extracts were determined (Figure 1).

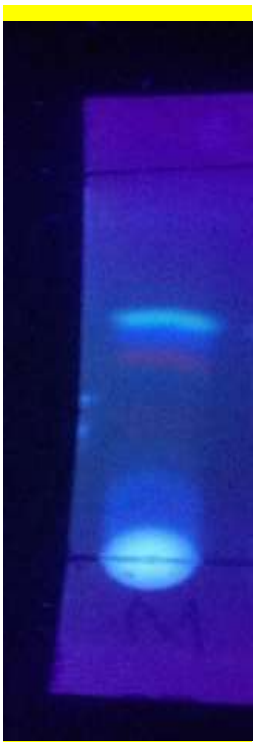
Table 1: Result of Extraction Yield of *Albizia chevalieri*

S/N	Solvents	Quantity of leaves powder (g)	Yield (g)	% Yield
1	Methanol	200	16.33	8.165
2	Dichloromethane	200	3.31	1.655

Table 2: TLC Profile of Dichloromethane and Methanol Crude Extracts Results

Dichloromethane components	Distance travelled by components (cm)	Distance travelled by solvent system (cm)	Retention factor (rf)
D1	0.5	5	0.1
D2	2.2	5	0.44
D3	3.1	5	0.62
D4	4.5	5	0.9
Methanol Components			
M1	1	5	0.2
M2	2.1	5	0.42
M3	2.7	5	0.54
M4	3	5	0.6

TLC of dichloromethane crude extract.



TLC of methanol crude extract.

Figure 1: TLC plates for all the extracts when viewed under UV light at 365nm and 254nm

Table 3: Phytochemical Constituents of *Albizia chevalieri* crude extract

Constituent	Dichloromethane	Methanol
Flavonoids	+	+
Alkaloids	-	+
Anthraquinones	+	+
Saponins	-	+
Tannins	-	+
Steriods	+	+
Triterpenoids	-	+
glycosides	-	+

keys: + = Present - = Absent

The antibacterial efficacy of *Albizia chivalieri* crude extracts (dichloromethane and methanol) against specific bacterial and fungal species is shown in Table 4 as zones of inhibition (mm) at decreasing doses. The zones of inhibition show dose-dependent antibacterial activity as the extract concentration drops. At higher doses (250 mg/ml), both extracts exhibit moderate action; at lower concentrations (≤62.5 mg/ml), they exhibit little to no activity. The greater potency of the conventional medications (ciprofloxacin/fluconazole) over the crude extracts was confirmed by the substantially bigger inhibition zones they produced.

At the lowest tested dose (31.25 mg/ml) for both extracts, Pseudomonas aeruginosa demonstrated inhibition, making it the most vulnerable bacterium. Dichloromethane: 12 → 10 → 10 → 8 mm, Methanol: 14 → 12 → 10 → 7 mm. While at higher concentrations did *Bacillus amyloquefaciens* and *Staphylococcus aureus* exhibit activity, indicating moderate sensitivity. *Klebsiella pneumoniae* showed extremely little susceptibility, showing no action with the methanol extract and inhibition only at 250 mg/ml (dichloromethane extract), indicating considerable resistance at the highest concentration (250 mg/ml) both extracts exhibit mild antifungal activity against Trichophyton rubrum.

The minimum inhibitory concentration (MIC) values for observable microbial growth are displayed in Table 5. MIC values showed low to moderate

antibacterial potency, ranging from 125 to 250 mg/ml, *Pseudomonas aeruginosa* was the most susceptible organism, as evidenced by its lowest MIC (125 mg/ml) for both extracts. MIC values of 250 mg/ml were found for *Bacillus amyloquefaciens, Staphylococcus aureus,* and *Trichophyton rubrum,* indicating that comparatively high doses are required for inhibition. *Klebsiella pneumoniae* demonstrated resistance to both extracts by displaying no detectable MIC (ND). The MIC values of the dichloromethane and methanol extracts were the same, indicating that both solvent extracts may contain the active antimicrobial components.

The MBC/MFC values, which show the lowest concentration needed to kill the bacteria rather than merely stop their growth, are shown in Table 6. The MBC/MFC values matched the MIC values exactly. This suggests that instead of just having bacteriostatic or fungistatic action, the extracts have both bactericidal and fungicidal properties, for *Pseudomonas aeruginosa* once more demonstrated the lowest MBC/MFC (125 mg/ml), indicating a high level of susceptibility. No bactericidal or fungicidal concentration was found, and *Klebsiella pneumoniae* continued to be resistant. In addition, Although Albizia chivalieri crude extracts have broad spectrum antibacterial action, their efficacy varies wi th concentration. *Klebsiella pneumoniae* is the most resistant organism, while *Pseudomonas aeruginosa* i s the most susceptible. The extracts may have micro bicidal effects at effective concentrations based on t he similarity between MIC and MBC/MFC values.

Table 7 presents the Gas Chromatography–Mass Spectrometry (GC–MS) profile of the dichloromethane crude extract, showing the identified chemical constituents, their retention times, molecular formulas, molecular weights, and relative abundance (peak area %).

The extract was found to contain 21 different chemicals. The majority of the chemicals are terpenoids, esters, fatty acid derivatives, and long-chain hydrocarbons, all of which are frequently linked to biological activity. Additionally, retention periods varied from 8.916 to 25.467 minutes, suggesting that both high and low molecular weight molecules were present.

Principal Constituents, with a peak area of 32.38%, tetrapentacontane ($C_{55}H_{112}$) was the most prevalent chemical, indicating that it is the extract's main component. Phytol (5.24%), 1-octadecane-1-iodo (5.10%), and dibutylphthalate (12.44%) all displayed comparatively large peak areas, suggesting a considerable presence. Other noteworthy substances that have been shown in literature to have antibacterial, antioxidant, or anti-inflammatory qualities include henecosine, oxirin tetradecyl, squalene, and eicosatrienoic acid.

The presence of fatty acid derivatives and long-chain hydrocarbons may explain the antimicrobial activity observed earlier in the extract. Phytol and squalene are particularly important as they are known to exhibit antioxidant, antimicrobial, and anti-inflammatory activities. Dibutylphthalate, though sometimes reported as a contaminant, has been documented in several plant extracts and may contribute to antimicrobial effects.

Table 8 highlights the most bioactive compounds selected from the GC–MS profile based on relative abundance and reported biological activity. Tetrapentacontane (32.38%) is the most prevalent chemical, indicating that it might be crucial to the biological effects of the extract, long-chain hydrocarbons have been linked to protective and antibacterial properties in plants. Dibutylphthalate (12.44%) is well-known. for antibacterial and antifungal properties. Its comparatively high concentration points to a significant role in the antibacterial qualities that have been noted. 1-Octadecane-1-iodo (5.10%) the antibacterial activity of halogenated hydrocarbons is frequently improved. Henecosine (4.12%) and Oxirin tetradecyl (4.38%) these substances have membrane-disruptive properties that could prevent the growth of microorganisms. Moreover, the dichloromethane extract's observed antibacterial action has a chemical foundation due to the predominance of these bioactive chemicals. The extract's efficacy against vulnerable bacteria may be explained by the combination or synergistic activities of these chemicals.

The result for the gas chromatography mass spectrophotometry (GC-MS) of the dichloromethane is presented in Figure 2. The result indicated the presence of 22 compounds in the fraction. The result showed that the compound with highest peak area Tetrapentacontane (32.38 %) a followed by Heptadecane with a peak value of Tetracosane (0.83 %).

Table 4: Zone of Inhibition (mm) of various concentrations of *Albizia chivalieri* crude extracts

Extract	Organism	250(mg/ml)	125(mg/ml)	62.5(mg/ml)	31.25(mg/ml)	Ciprofloxacin/Fluconazole
Dichloromethane	*Bacillus amyloquefaciens*	11	ND	ND	ND	31
	Staphylococcus aureus	11	09	ND	ND	24
	Klebsiella pneumonia	09	ND	ND	ND	27
	Pseudomonas auruginosa	12	10	10	08	22
	Trichophyton rubrum	10	ND	ND	ND	21
Methanol	*Bacillus amyloquefaciens*	11	8	ND	ND	31
	Staphylococcus aureus	8	ND	ND	ND	24
	Klebsiella pneumonia	ND	ND	ND	ND	27
	Pseudomonas auruginosa	14	12	10	7	22
	Trichophyton rubrum	10	ND	ND	ND	20

Key: ND= not determined

Table 5: Minimum Inhibitory Concentration (MIC) of *Albizia Chivalieri* Crude Extracts on Test Organisms

Extract	Organism	MIC (mg/ml)
Dichloromethane	*Bacillus amyloquefaciens*	250
	Staphylococcus aureus	250
	Klebsiella pneumonia	ND
	Pseudomonas auruginosa	125
	Trichophyton rubrum	250
Methanol	*Bacillus amyloquefaciens*	250
	Staphylococcus aureus	250
	Klebsiella pneumonia	ND
	Pseudomonas auruginosa	125
	Trichophyton rubrum	250

Table 6- Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of *Albizia chivalieri* Crude Extracts on Test Organisms

	Organism	MBC/MFC (mg/ml)
Dichloromethane	*Bacillus amyloquefaciens*	250
	Staphylococcus aureus	250
	Klebsiella pneumoniae	ND
	Pseudomonas aeruginosa	125
	Trichophyton rubrum	250
Methanol	*Bacillus amyloquefaciens*	250
	Staphylococcus aureus	250
	Klebsiella pneumoniae	ND
	Pseudomonas aeruginosa	125
	Trichophyton rubrum	250

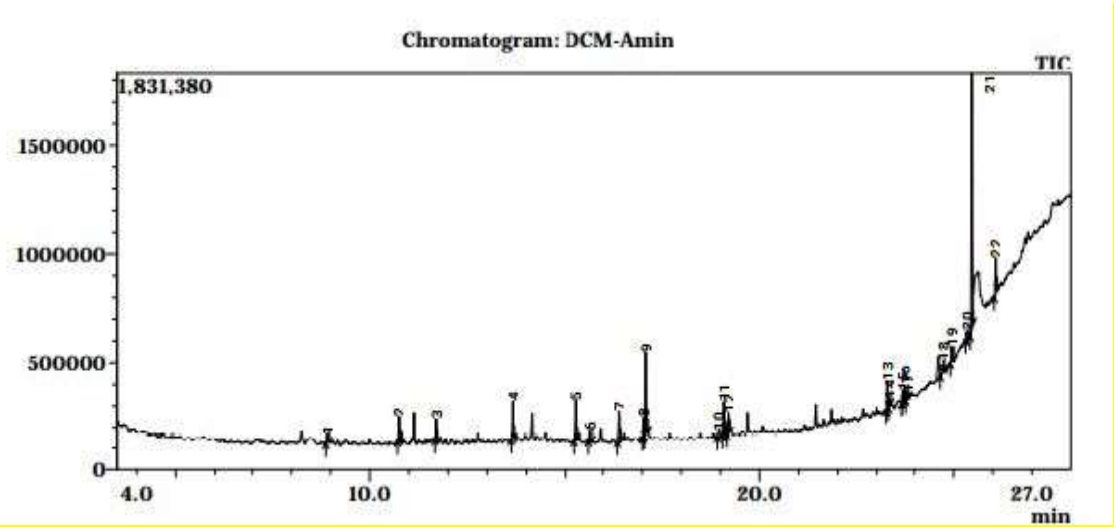


Figure 2: Chromatogram of Gas Chromatography - Mass Spectrometric Result

Table 7: Compounds identify from Gas Chromatography - Mass Spectrometry (GC-MS) of Dichloromethane Crude Extract

Peak	R-Time (min)	Name of Compound	Molecular Formula	Molecular Weight	Peak Area %
1	8.916	Dodecane 4,6 di methyl	$C_{14}H_{30}$	168	1.43
2	16.750	2- propenamide N,N di ethyl	$C_7H_{15}NO_3$	101	1.45
3	11.699	Heptadecane	$C_{17}H_{36}$	204	2.18
4	13.657	Henecosine	$C_{21}H_{44}$	192	4.12
5	15.312	Oxirin tetradecyl	$C_{16}H_{32}O$	204	4.38
6	15.661	1-octa decyne	$C_{18}H34$	204	1.74
7	16.419	1-octa decane 1 iodo	$C_{18}H_{37}I$	28	5.10
8	17.045	Heneicosine	C_{21H44}	258	2.30
9	17.093	Dibutylphthalate	C20H32O2	336	12.44
10	18.950	11'14'17 eicosatrienoic acid	$C17H_{34O2}$	204	1.36
11	19.091	Phytol	C18H38	97	5.24
12	19.208	Heptadecane 8 methyl bus (2, ethylhexyl) phthalate	C24H38	288	2.72
13	23.266	Heptadecane 8 methyl	$C80H_{38}$	964	3.06
14	23.322	Tetracosane	C9H36	304	0.83
15	23.665	Cyclononasiloxaneoctate	$C32H_{36}$	4668	1.46
16	23.738	Ditriacontane	C_9H3_6	384	2.69
17	23.825	Cyclononasiloxaneoctate	$C8H_6O4$	468	1.02
18	24.739	1,3 benzene di carboxylic acid	$C_8H_6O_4$	182	2.21
19	24.956	Tetratetracontane	$C_{44}H_{90}$	542	2.32
20	25.353	Squalene	$C_{30}H_{50}$	361	1.30
21	25.467	Tetrapentacontane	$C_{55}H_{112}$	671	32.38

Table 8: Most Bioactive Compounds Identify from Gas Chromatography- Mass Spectrometry (GC-MS) of Dichloromethane Crude Extract

Peak	R-Time (min)	Name of Compound	Molecular Formula	Molecular Weight	Peak Area %
9	17.093	Dibutylphthalate	$C_{20}H_{32}O_2$	336	12.44
4	13.657	Henecosine	$C_{21}H_{44}$	192	4.12
5	15.312	Oxirin tetradecyl	$C_{16}H_{32}O$	204	4.38
7	16.419	1-octadecane 1 iodo	$C_{18}H_{37I}$	28	5.10
21	25.467	Tetrapentacontane	$C_{55}H_{112}$	671	32.38

DISCUSSION

Methanol crude extract had the highest yield, followed by the DCM extract, which was lower. These findings suggest the number of polar molecules (such as saponins and flavonoids, glycosides) removed from the plant's leaves may have been the highest, subsequently DCM was used to extract molecules of intermediate polarity, such as anthraquinone, tannins, and phenolic acids. From the result, the highest yield was obtained from methanol extract, which could be attributed to presence of high polar substance in the plant material.

Crude dichloromethane and methanol extracts of *Albizia chevalieri* demonstrated antimicrobial activity in agar diffusion assays: maximal zones were 12–14 mm against *Pseudomonas* spp. and 9–11 mm against *Staphylococcus* and *Bacillus* at 250 mg/mL, while standard drugs (ciprofloxacin/fluconazole) produced zones of 22–31 mm. The activity was concentration dependent where measured, but limited diffusion of lipophilic constituents in agar may underestimate potency. Phytochemical screening of related *Albizia* spp. indicates a high prevalence of saponins, flavonoids and phenolics, which could mediate membrane-disruptive or enzyme-inhibitory mechanisms; fractionation and MIC determinations are therefore required to identify active compounds and determine true antimicrobial potency.

Broth microdilution assays identified MICs of 125–250 mg/mL for dichloromethane and methanol crude extracts of *Albizia chevalieri* against the tested bacteria and *Trichophyton rubrum*. These values indicate weak antimicrobial potency in crude form compared with typical antibacterial agents (which act at µg/mL) and even many active plant fractions (often <4 mg/mL). Literature reports for *A. chevalieri* are variable: some studies observed MICs in the single-digit mg/mL range for aqueous or fractionated extracts, while others report higher values for crude extracts — differences attributable to plant part, extraction method, assay protocol, and readout. Follow-up fractionation, standardized broth microdilution with colorimetric endpoints, and cytotoxicity assays are recommended to (i) confirm activity, (ii) concentrate active constituents, and (iii) determine whether safer, lower-MIC fractions can be obtained

The GC–MS analysis of *Albizia chevalieri* leaf extract revealed a diverse array of bioactive phytoconstituents, encompassing hydrocarbons, fatty acid derivatives, terpenoids, phthalates, siloxanes, and sterols. These compounds are known to contribute synergistically to antimicrobial and pharmacological activities. Several aliphatic hydrocarbons, including dodecane 4,6-dimethyl (1.43%), heptadecane (2.18%), henecosine (4.12%), heneicosine (2.30%), tetracosane (0.83%), ditriacontane (2.69%), and tetrapentacontane (32.38%), were identified. Long-chain alkanes are often reported as plant surface constituents with protective functions against microbial invasion and oxidative stress (Sermakkani and Thangapandian, 2012). Moreover, n-alkanes such as heptadecane and tetracosane have been associated with antibacterial and antifungal activities, potentially contributing to the observed antimicrobial effects (Nandhini *et al.,* 2020). The dominance of tetrapentacontane (32.38%) suggests a structural and defensive role, which may also enhance biofilm inhibition. The extract contained 11',14',17'-eicosatrienoic acid (1.36%), a polyunsaturated fatty acid with established antimicrobial and anti-inflammatory properties (Das, 2006). Fatty acids are known to disrupt microbial cell membranes, thereby impairing cellular integrity. Additionally, compounds like oxirin tetradecyl

(4.38%) and 1-octadecane-1-iodo (5.10%) may exhibit surfactant-like or antimicrobial activities due to their amphiphilic nature (Desbois and Smith, 2010). The identification of phytol (5.24%), a diterpene alcohol, highlights the pharmacological relevance of the extract. Phytol is reported to possess antimicrobial, antioxidant, and anticancer properties (Islam, 2018). Similarly, squalene (1.30%), a triterpene and precursor of sterols, is well documented for its antioxidant, chemopreventive, and membrane-stabilizing activities (Spanova and Daum, 2011). These terpenoid constituents may enhance the therapeutic potential of A. chevalieri. Compounds such as dibutyl phthalate (12.44%) and heptadecane-8-methyl bis(2-ethyl hexyl) phthalate (2.72%) were also detected. Dibutyl phthalate has been frequently isolated from plants and microbial metabolites, and it is known to exhibit antibacterial, antifungal, and insecticidal activities (Roy *et al.,* 2006). Although phthalates are sometimes considered environmental contaminants, their consistent detection in phytochemical screenings suggests both endogenous and exogenous origins. Their strong antimicrobial activity supports their contribution to the extract's bioactivity.

The GC–MS analysis also revealed 1,3-benzene dicarboxylic acid (2.21%), an aromatic acid ester derivative. Such aromatic compounds are known to exert antimicrobial and antioxidant effects through free radical scavenging and enzyme inhibition (Jain, *et al.,* 2011). Cyclononasiloxaneoctate (1.46%) was also identified, which is commonly reported in plant GC–MS spectra. Though often considered an artifact from column bleeding or solvents, siloxanes can act as surface protectants in plants and may play a minor role in antimicrobial defence (Kumar *et al.,* 2010). The chemical profile indicates that *Albizia chevalieri* leaves are rich in hydrocarbons, fatty acids, terpenoids, and esters, many of which have been linked with antimicrobial, antioxidant, and therapeutic functions. The predominance of tetrapentacontane, dibutyl phthalate, phytol, and squalene suggests they may contribute significantly to the observed antimicrobial activities. The presence of diverse bioactive metabolites provides a pharmacological rationale for the traditional use of *A. chevalieri* in treating infections and supports further research into its bioactivity through compound isolation and mechanistic studies.

Phytochemical screening is a crucial step in understanding the bioactive potential of plant species, including their pharmacological and therapeutic properties. The methanol crude extract of *Albizia chevalieri* has been analyzed for various phytochemicals, and the results indicate the presence of important bioactive compounds, including saponins, triterpenoids, glycosides, tannins, anthraquinones, steroids, flavonoids, and alkaloids. These compounds are associated with a variety of biological activities, and their presence suggests that *Albizia chevalieri* may possess medicinal properties. Saponins are a group of naturally occurring compounds with soap-like properties due to their ability to form stable foam when mixed with water. They are widely distributed in the plant kingdom and are known for their biological activities, including antimicrobial, anti-inflammatory, and immune-modulatory effects (Sasidharan, 2011). Saponins have also been found to have anti-cancer properties by inhibiting cell proliferation and inducing apoptosis (Wadood, 2013). In addition to these properties, saponins can help reduce cholesterol levels and possess hepatoprotective effects. The positive test for saponins in *Albizia chevalieri* suggests that the plant may be valuable for treating infections and inflammatory conditions. Triterpenoids are a large group of compounds derived from triterpenes, which are built from six isoprene units. They are often found in plants and have demonstrated various pharmacological properties, including anti-inflammatory, antimicrobial, hepatoprotective, and anticancer effects (Santos, 2019). Triterpenoids have been shown to modulate cell signaling pathways, protect against oxidative stress, and have neuroprotective potential. Their presence in *Albizia chevalieri* indicates that this plant may have therapeutic potential for inflammation-related disorders and liver diseases, as well as anticancer activity.

Glycosides are compounds that consist of a sugar molecule bound to a non-sugar aglycone. They are involved in a wide range of biological activities, such as antimicrobial, anti-inflammatory, and anticancer properties (Barrett, 2016). Cardiac glycosides, in particular, are known for their role in the treatment of heart conditions by improving cardiac output. Glycosides can also enhance the absorption of nutrients and exhibit laxative effects, especially when

derived from anthraquinones. The positive presence of glycosides in *Albizia chevalieri* suggests that it may offer potential therapeutic applications, including antimicrobial and anti-inflammatory effects.

Tannins are polyphenolic compounds with significant astringent properties. They have been shown to possess a wide range of biological activities, such as antimicrobial, antioxidant, and anti-inflammatory effects (Saha, 2014). Tannins are often used in traditional medicine to treat gastrointestinal disorders, including diarrhea, because of their ability to bind proteins and other macromolecules. Tannins also exhibit potential antidiabetic properties by inhibiting digestive enzymes. The presence of tannins in *Albizia chevalieri* suggests that it could be beneficial for managing inflammation, oxidative stress, and gastrointestinal disturbances. Anthraquinone are aromatic compounds with diverse biological activities, including laxative, antimicrobial, anticancer, and antiviral effects (Ali, 2019). These compounds are often used in traditional medicine to relieve constipation, but their other therapeutic activities make them valuable for a range of health conditions. For instance, anthraquinones have been shown to inhibit the growth of various cancer cells by interfering with the cell cycle and inducing apoptosis (Choi, 2013). The positive test for anthraquinones in *Albizia chevalieri* indicates that it may have applications as a laxative and anticancer agent. Steroids are a group of naturally occurring organic compounds that possess a common four-ring structure. They are involved in numerous biological functions, including anti-inflammatory, immune-modulatory, and anticancer effects (Varela, 2019). Steroids can also have a role in lipid metabolism, contributing to cardiovascular health. In addition, plant steroids have been shown to possess neuroprotective and antidiabetic properties. The presence of steroids in *Albizia chevalieri* suggests that this plant could be useful for treating inflammation, metabolic disorders, and potentially even as an adjunctive treatment in cancer.

Flavonoids are a diverse group of polyphenolic compounds that are known for their potent antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Gul *et al.,* 2016). Flavonoids can scavenge free radicals, reduce oxidative stress, and protect cells from damage, thus contributing to the prevention of chronic diseases, including cardiovascular diseases and cancer. They also play a role in modulating immune responses and improving vascular health. The presence of flavonoids in *Albizia chevalieri* suggests that it may have significant antioxidant and anti-inflammatory benefits, as well as potential applications in the prevention of diseases linked to oxidative damage. Alkaloids are nitrogen-containing compounds that are often associated with potent pharmacological effects, such as analgesic, antimicrobial, and antidiabetic activities (Berman, 2012). Alkaloids can influence neurotransmitter systems, making them useful in the treatment of neurological disorders. Some alkaloids are also known for their anticancer and anti-inflammatory effects. The positive presence of alkaloids in *Albizia chevalieri* suggests that it may contain compounds that can be used to manage pain, inflammation, and possibly even neurological conditions. The methanol crude extract of *Albizia chevalieri* shows the presence of several important phytochemicals, including saponins, triterpenoids, glycosides, tannins, anthraquinones, steroids, flavonoids, and alkaloids. Each of these compounds is associated with a variety of pharmacological activities, such as anti-inflammatory, antimicrobial, antioxidant, anticancer, and hepatoprotective effects. The positive results from the phytochemical screening support the potential of *Albizia chevalieri* as a source of therapeutic agents for managing a wide range of diseases, including infections, inflammation, cardiovascular diseases, cancer, and metabolic disorders. Further research, including isolation and characterization of individual bioactive compounds, is essential to fully understand the therapeutic potential of this plant.

Saponins are also considered beneficial for lowering blood cholesterol levels and boosting the immune system. The presence of saponins in the DCM extract of *Albizia chevalieri* suggests that the plant may have therapeutic potential for treating infections and inflammatory conditions (Sasidharan *et al.,* 2011).The presence of triterpenoids in the DCM extract of *Albizia chevalieri* indicates that the plant may possess important pharmacological activities related to inflammation and oxidative stress (Santos *et al.,* 2019). The presence of glycosides in *Albizia chevalieri* suggests its potential use in treating infections and reducing inflammation, as well as possibly offering cardiovascular benefits (Barrett *et al.,* 2016).The

presence of tannins in the DCM extract of *Albizia chevalieri* suggests that the plant may have beneficial effects for managing inflammation and gastrointestinal disorders (Saha *et al.,* 2014).The presence of anthraquinones in *Albizia chevalieri* indicates that it may have applications in gastrointestinal health, as well as potential anticancer properties (Ali *et al.,* 2019).

The absence of steroids, flavonoids, and alkaloids in the dichloromethane extract suggests that these compounds may not be present in significant concentrations, or they may not be extracted by dichloromethane. Steroids and flavonoids are known for their anti-inflammatory and antioxidant activities (Varela *et al.,* 2019; Gul *et al*., 2016), while alkaloids are typically associated with analgesic, antimicrobial, and neuroactive properties (Berman *et al.,* 2012). The lack of these compounds in the DCM extract does not rule out their presence in other parts of the plant or in extracts prepared with different solvent. The dichloromethane extract of *albizzia chevalieri* contains several bioactive compounds such as saponins, triterpenoids, glycosides, tannins, and anthraquinones, which suggest that the plant may have antimicrobial, anti-inflammatory, and antioxidant properties. The absence of steroids, flavonoids, and alkaloids in the DCM extract could be due to the limitations of the solvent or the specific chemical profile of the plant. Saponins are known for their surfactant properties and have been associated with antimicrobial, anti-inflammatory, and cholesterol-lowering activities. Their presence in *albizzia achevalieri* suggests potential therapeutic applications in treating infections and metabolic disorders also triterpenoids, a subclass of terpenoids, are bioactive compounds with anti-inflammatory, anticancer, and hepatoprotective properties. Their high concentration in the methanol extract indicates significant medicinal potential (Eze *et al.,* 2023)

The phytochemical composition of *Albizia chevalieri* in methanol crude extract supports its traditional use in treating ailments such as malaria, diabetes, diarrhea, and dysentery. Additionally. The antioxidant properties of flavonoids and tannins make it a candidate for managing oxidative stress-related conditions. The antimicrobial activities of alkaloids and saponins suggest its use against bacterial and fungal infections. The presence of glycosides and steroids indicates potential applications in cardiovascular health. Studies have shown that terpenoids are the most abundant phytochemicals in *Albizzia chevalieri,* followed by steroids, saponins, tannins, flavonoids, glycosides, alkaloids, and anthraquinones. This distribution suggests that terpenoids might play a dominant role in the plant's bioactivity. The methanol crude extract of *albizia chevalieri* contains a rich diversity of secondary metabolites with significant pharmacological potential. These findings provide a scientific basis for its traditional medicinal uses and warrant further studies to isolate specific compounds for drug development.

CONCLUSION

The exploration of *Albizia chevalieri* leaf extract through GC-MS and TLC investigation has unveiled its rich chemical diversity and promising antimicrobial potential, its extracts may serve as a source of natural antimicrobial agents, particularly against multidrug-resistant bacteria. The plant's bioactive compounds support its traditional use in medicine for treating various ailments. The antimicrobial and antioxidant properties of *A. chevalieri* extracts make them suitable for use in cosmetics and as food preservatives. Further studies are needed to isolate and characterize the specific bioactive compounds responsible for the antimicrobial activity. Evaluating the efficacy and safety of *A. chevalieri* extracts in clinical settings will be crucial for potential therapeutic applications.

The present investigation demonstrated that *Albizia chevalieri* leaves harbor a rich diversity of bioactive phytoconstituents, as revealed by GC–MS and TLC analyses. The GC–MS profiling identified a wide array of compounds, including fatty acids, terpenoids, alkaloids, and phenolic derivatives, many of which are reported to possess antimicrobial, antioxidant, and therapeutic activities. Complementary TLC analysis further confirmed the presence of secondary metabolites such as flavonoids, tannins, and saponins, which are consistent with the plant’s ethnomedicinal applications.

The antimicrobial assays revealed that the leaf extracts exhibited varying degrees of inhibitory activity against selected bacterial, supporting the traditional use of *A. chevalieri* in managing infectious diseases. These findings highlight the plant’s potential as a natural source of antimicrobial agents and

provide a scientific basis for its incorporation into phytomedicine and drug discovery efforts.

Overall, this study contributes to the growing body of knowledge on the pharmacological importance of *Albizia* species. However, further work is recommended, including bioassay-guided fractionation, isolation of lead compounds, mechanistic studies, and in vivo evaluations, to fully validate and harness the therapeutic potential of *Albizia chevalieri*.

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