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Phytochemical and Antimicrobial Assessment of *Dalbergia afzeliana* Stem Bark Extracts for its Ethnomedicinal Utility

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ABSTRACT

Dalbergia afzeliana, a tropical hardwood of the Fabaceae family, is widely used in West and Central Africa as ethnomedicine for treating inflammatory conditions, cardiovascular disorders, cough, diabetes, gonorrhea, leucorrhea, and infections of the liver, kidneys, and bladder. This study investigated the phytochemical composition, antimicrobial activity, and GC–MS profile of stem bark extracts prepared by cold maceration with methanol and distilled water. Phytochemical screening revealed alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, triterpenoids, and anthraquinones, with triterpenoids (3.00%) most abundant. Methanol extracts showed strong activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus flavus* (20–24 mm inhibition zones; MIC 2.5 mg/mL), while aqueous extracts exhibited moderate activity (16–18 mm; MIC 5 mg/mL). GC–MS identified 29 bioactive compounds, including lupeol, stigmasterol, squalene, phytol, coumarin, parietin, and hexadecenoic acid. These findings validate the ethnomedicinal use of *D. afzeliana* and highlight its potential as a source of novel therapeutic agents.

Keywords: Antimicrobial activity, *Dalbergia afzeliana*, ethnomedicine, GC–MS, phytochemicals.

INTRODUCTION

Plants have historically been central to medicine, serving as sources of remedies for a wide range of diseases [1, 2]. Medicinal plants, more specifically, remain vital sources of therapeutic agents, phytochemicals, such as alkaloids, flavonoids, tannins, saponins, and terpenoids widely recognized for their pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial, anticancer, and immunomodulatory activities [1–3]. Phytochemical and antimicrobial analyses not only validate traditional remedies but also provide new opportunities to combat drug-resistant pathogens [4].

Dalbergia afzeliana, native to tropical Africa, is traditionally used for inflammatory conditions, cardiovascular support, and infections [5]. Dalbergia species show well-documented therapeutic properties, phytochemical profiles and biological activities: *D. sissoo* is rich in isoflavones with antioxidant and anti-aging potential, *D. odorifera* contains neoflavonoids and dalbergiones with anti-inflammatory and cytoprotective effects, while *D. melanoxylon* and *D. cultrata* yield prenylated isoflavanones and neoflavonoids with strong antimicrobial and anticancer activity**.** [1, 6-8]. However, *Dalbergia afzeliana* remains relatively unexplored, in spite of ethnomedicinal reports of its use in treating inflammatory conditions, cardiovascular issues, cough, gonorrhea, and organ infections [5,6].

This study aims to assess the phytochemical composition and antimicrobial activity of *D. afzeliana* stem bark extracts to provide phytochemical and antimicrobial evidence supporting its medicinal utility. Cold maceration stembark extract of methanol and water are subjected to phytochemical screening, GC-MS analysis and antimicrobial assessment as a means of assessing ethnomedicinal claims on the plant.

MATERIALS AND METHODS

Plant Collection and Extraction.

The plant materials, *D. afzeliana stems,* were collected in October 2024 from various sites in a nearby forest of Aieje Village in Edumoga District, Okpokwu Local Government Area, Benue State. The plant material was identified and herbarium samples were deposited at the Herbarium (Voucher number 025190) of the Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.

About 2.5 kg of the plant material (Plate 1) was shade-dried and ground into powder for cold maceration extraction**.** Cold maceration was performed using methanol and distilled water. Extracts were concentrated and stored for analysis. Ground plant material was distributed into nine glass jars, to which enough methanol to cover each sample completely was added. The extraction process was facilitated by frequent agitation within three days. The extract was separated from the marc using a muslin cloth and further filtered using Whatman filter paper No.3. The combined extract was concentrated and left to dry to constant weight. [1, 2, 9]

Plate.1: Plant parts of *D. afzeliana*

Phytochemical Screening.

Qualitative and quantitative analyses were conducted on the extracts for glycosides, tannins, flavonoids, saponins, anthraquinones, alkaloids, steroids, and triterpenoids using the standard procedures as reported by Harborne [1], and Trease and Evans [10].

Antimicrobial Assay

Microbes for antimicrobial activity tests (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus flavus*, and *Candida albicans*) were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria. Exactly 0.1 g of the extract was weighed and dissolved in 10 mL of dimethyl sulfoxide to obtain a concentration of 10 mg/mL. This was the initial concentration of the extract used to determine its antimicrobial activities. The antibacterial and antifungal sensitivity tests by zones of inhibition (ZOI), the minimum inhibitory concentration (MIC), and the minimum bactericidal/fungicidal concentration (MBC/MFC) of the extracts were determined respectively by the Agar Well diffusion method,

Agar Dilution Method and the Subculture Method [11]. Sparfloxacin and Fluconazole were used respectively, as positive antibacterial and antifungal controls, while Dimethyl sulphoxide (DMSO) was used as a negative control.

Prepared agar plates were seeded evenly with 0.1 mL of standard inoculum of test microorganism using sterile swabs. With a standard cork-borer, 6 mm diameter wells were punched on the media, and inoculated with 0.1 mL solution of the extract at 10 mg/mL. Incubation was done at $37^{O}C$ for 24 hours for bacteria, and $30^{O}C$ for 1-7 days for fungi. Diameters of the clear zone of inhibition around each well were measured. For MIC, agar media were prepared to different concentrations with two-fold serial dilutions of the extracts in test tubes, and inoculated with 0.1 cm^3 of test microbes in normal saline. The incubation cycles were repeated. The lowest concentrations that show no visible growth according to Mc-Farland turbidity scale (1.5×10^8 CFU/mL) was identified. For MBC and MFC, samples from MIC plates with no visible growth were transferred onto fresh agar, incubated and checked for colony growth. The lowest concentration at which no colonies grow, confirming bacterial death was identified as MBC/MFC. [11, 12]

GC–MS Analysis

Methanol and aqueous extracts were analyzed for bioactive compounds using a GC–MS (Perkin–Elmer GC Clarus 500 system) comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC-MS running time was 36 minutes. Turbo-Mass Gold-Perkin Elmer mass detector was used, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2. [13]. Compounds were identified by combining their unique gas chromatographic retention times with their mass spectral fragmentation patterns, which were then referenced against digital libraries. The percentage compositions are calculated from the normalized peak areas and used to express the relative abundances of compounds in the samples. [13,14]

RESULTS AND DISCUSSION

Phytochemical Composition

Table 1 shows the phytochemical profile of methanol and aqueous extracts of *Dalbergia afzeliana*. Triterpenoids (3.00%) were most abundant, alongside alkaloids, flavonoids, tannins, saponins, steroids, and anthraquinones. The methanol extract posted positive results for nearly all tested classes except flavonoids and glycosides.

Methanol, a polar organic solvent, demonstrated a greater ability to extract a broader range of phytochemical groups compared to water, as evidenced by positive results for steroids, triterpenoids, tannins, saponins, anthraquinones, and alkaloids, whereas the aqueous extract only tested positive for saponins and alkaloids. This pattern aligns with existing literature suggesting that methanol effectively solubilizes both moderately polar and non-polar phytochemicals, thereby improving extractability of diverse compounds [15,16].

Table 1: Quantitative and Qualitative Phytochemical Screening of *D. afzeliana* stem bark extracts

Phytochemical	Quantitative Screening	Qualitative Screening	
	Quantity (%)	Methanol Extract	Aqueous Extract
Steroids	0.232%	+	_
Triterpenoids	3.0025%	+	_
Glycosides	N	_	_
Tannins	0.1647%	+	_
Saponins	0.2341%	+	+
Anthraquinones	0.6232%	+	_
Alkaloids	N	+	+
Flavonoids	N	_	_

Key: (+) : Indicated; (-) Not Indicated, (N): Not detected or Negligible

The aqueous extract exhibited a markedly narrower phytochemical profile, detecting only saponins and alkaloids, reinforcing methanol's superior extraction efficiency for a wide range of bioactive. The selective solubility of water likely limited the detection of less polar compounds such as triterpenoids and anthraquinones in the aqueous extract.

Triterpenoids (3.00%) are known for anti-inflammatory and anti-ulcer properties [17], often associated with cardiac and hepatoprotective functions [18]. Anthraquinones (0.62%) exhibit notable antimicrobial and laxative effects [19] while Saponins (0.23%), reportedly possess immune-modulating and anti-inflammatory roles [20]. Tannins (0.16%) are compounds with antioxidant and wound healing potentials [21], whereas steroids (0.23%) are often linked to anti-inflammatory and hormonal effects. Alkaloids detected qualitatively (despite quantitative tests being negative, suggesting the need for further confirmation via more sensitive techniques like HPLC) are supported by literature for antimicrobial and anticancer activities [22].

The presence of triterpenoids, tannins, and alkaloids in the methanol extract provides a biochemical basis for many of the ethnomedicinal claims associated with *D. afzeliana*, including its use in treating inflammation, ulcers, liver disorders, and microbial infections. For instance, the high triterpenoid content aligns with hepatoprotective and anti-ulcer applications [15, 23].

Antimicrobial Activity

As shown in Table 2, methanol extracts showed strong inhibition (20–24 mm) against all tested pathogens, with MIC values as low as 2.5 mg/mL. The aqueous extracts showed moderate activity (16–18 mm; MIC 5 mg/mL).

Table 2: Antimicrobial activity of *D. afzeliana* extracts

Pathogen	Methanol Extract ZOI (mm)	MIC (mg/mL)	Aqueous Extract ZOI (mm)	MIC (mg/mL)	Positive control ZOI(mm) Sparfloxacin/ Fluconazole	Positive Control(DMSO
S. aureus	22	2.5	–	–	34	0
E. coli	21	2.5	16	5.0	R	0
P. aeruginosa	20	2.5	–	–	30	0
C. albicans	24	2.5	18	5.0	32	0
A. flavus	23	2.5	17	5.0	R	0

Key: R=Resistant

From the Zones of inhibition (mm) of extracts of *Dalbergia afzeliana* against selected bacteria and fungi, methanol extract consistently exhibited stronger antimicrobial activity across all tested organisms. This is clearly evident in the grouped bar chart displayed in Figure 1.

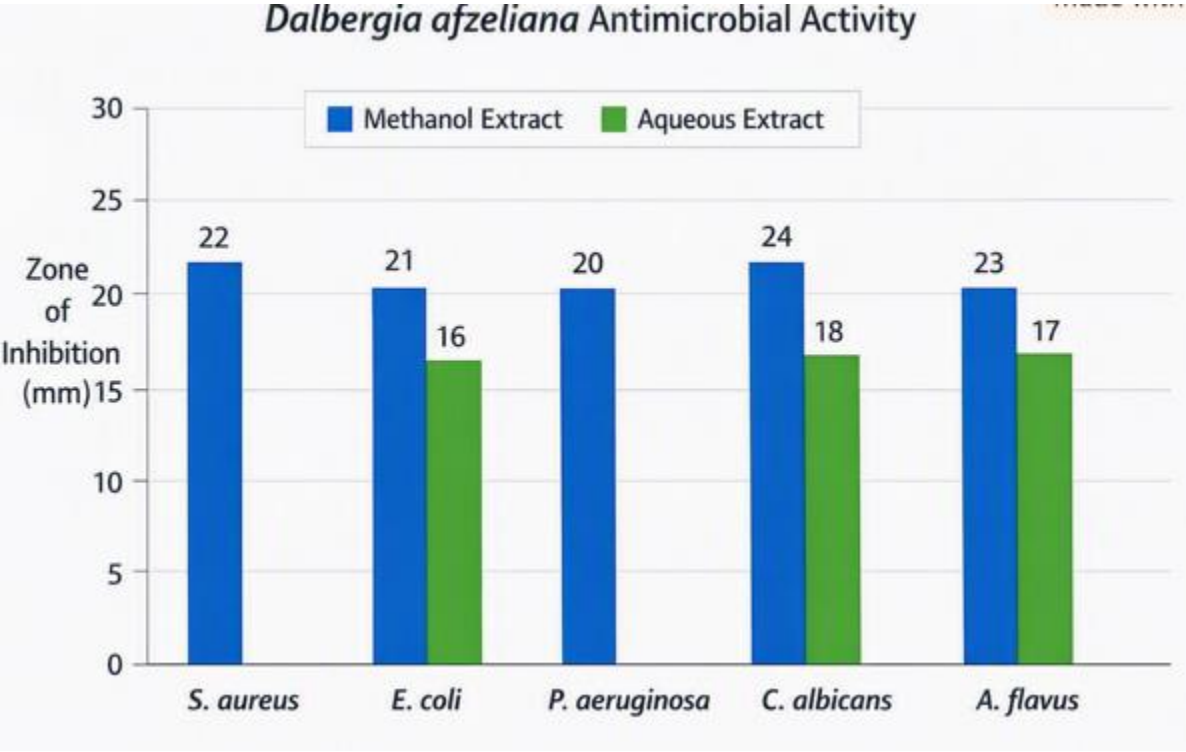

Fig 1: Grouped bar chart of activity of methanol and aqueous extracts across all tested organisms.

GC–MS Profile

The GC-MS Chromatogram for methanol and aqueous stem bark extract of *D. afzeliana* are shown in Figure 2 and Figure 3 while the GC-MS analysis for both solvents is shown in Table 3. The percentage compositions are calculated from the normalized peak areas and used to express the relative abundances of compounds (in percent and ppm) in the two samples. [13,14].

The combined GC–MS analysis revealed a complex mixture of 29 mostly bioactive compounds with distinct profiles between the methanol and aqueous extracts.

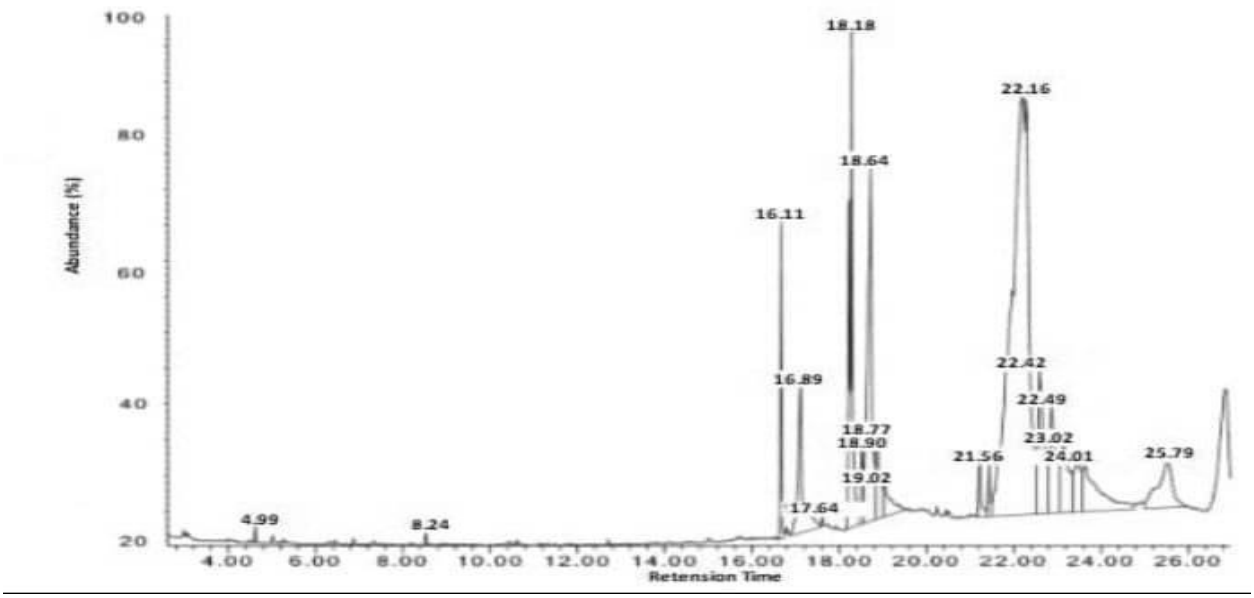

Figure 2: GC-MS Chromatogram for methanol stem bark extract o*f D. afzeliana*

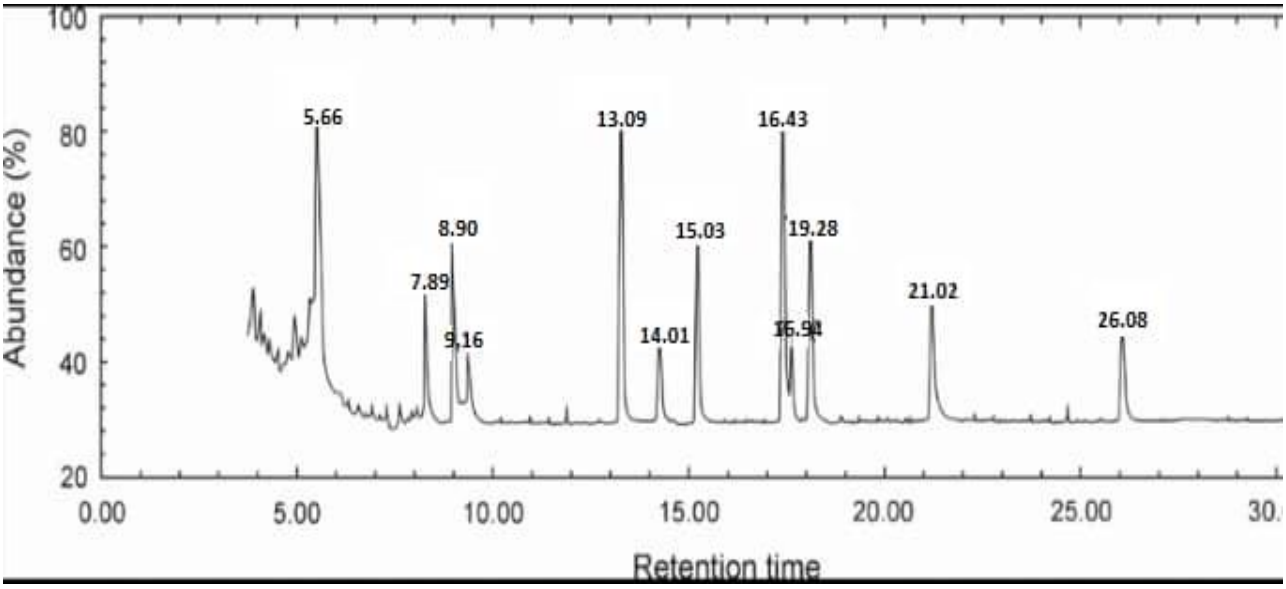

Figure 3: GC-MS Chromatogram for aqueous stem bark extract o*f D. afzeliana*

Table 3: GC-MS- Analysis for Methanol and Aqueous Stem Bark Extracts of *D. afzeliana*

Retention Time (min)	Compound	Formula	Molecular Weight	Peak Area (%)	Quantity (ppm)	Extract Type
4.99	2-Amino-1-propanol	C_3H_9NO	75.11	0.39	3.9	Methanol
5.66	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.13	6.27	62.7	Aqueous
7.89	Homovanillyl alcohol	$C_8H_{10}O_3$	154.17	2.70	27.0	Aqueous
8.24	3-Amino-2-ethylbutanoic acid	$C_6H_{13}NO_2$	131.17	0.34	3.4	Methanol
8.90	Methylparaben	$C_8H_8O_3$	152.15	3.30	33.0	Aqueous
9.16	2,3-Dihydrobenzofuran	$C_8H_{10}O$	122.16	1.92	19.2	Aqueous
13.09	Coumarin	$C_9H_6O_2$	146.14	6.08	60.8	Aqueous
14.01	3,7,3′,4′-Tetrahydroxyflavone	$C_{15}H_{12}O_6$	288.25	2.02	20.2	Aqueous
15.03	1-Thioflavone, 7-methoxy-	$C_{16}H_{12}O_2S$	268.33	3.31	33.1	Aqueous
16.11	2-Oxo-Butanoic acid	$C_4H_6O_3$	102.00	9.32	93.2	Methanol
16.43	Parietin	$C_{16}H_{12}O_5$	284.27	6.10	61.0	Aqueous
16.89	Benzene methanol, 2-aminopropox	$C_{10}H_{15}NO_2$	181.23	4.65	46.5	Methanol
16.94	Naphtho[2,3-b]furan-4,9-dione, 2-isopropyl-	$C_{13}H_{10}O_3$	214.22	2.03	20.3	Aqueous
17.64	2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206.30	1.01	10.1	Methanol
18.18	L-Alanine, methyl ester	$C_4H_9NO_2$	103.13	13.11	131.1	Methanol
18.64	2-Isocyanato-Propane	C_4H_7NO	85.10	11.36	113.6	Methanol
18.77	5-Nitro-2,4-Pyrimidinedione	$C_4H_3N_3O_4$	157.09	3.97	39.7	Methanol
18.90	Palmitic acid	$C_{16}H_{32}O_2$	256.42	3.56	35.6	Methanol
19.02	Phytol	$C_{20}H_{40}O$	296.54	2.20	22.0	Methanol
19.28	4-Methyl-2-[5-(thiophen-2-yl)pyrazol-3-yl]pyridine	$C_{10}H_{10}N_2S$	190.27	3.32	33.2	Aqueous
21.02	10,11-Dihydro-10-hydroxy-2,3-dimethoxydibenz[b,f]oxepin	$C_{15}H_{14}O_4$	258.27	2.66	26.6	Aqueous
21.56	16-Hentriacontanone	$C_{31}H_{62}O$	450.81	2.58	25.8	Methanol
22.16	Lauroscholtzine	$C_{20}H_{22}NO_4$	340.40	12.36	123.6	Methanol
22.42	Corydine	$C_{20}H_{23}NO_4$	341.40	4.71	47.1	Methanol
22.49	Lup-20(29)-ene-3-one	$C_{30}H_{48}O$	424.70	4.32	43.2	Methanol
23.02	Lupeol	$C_{30}H_{50}O$	426.72	3.56	35.6	Methanol
24.01	Stigmasterol	$C_{29}H_{48}O$	412.69	2.58	25.8	Methanol
25.70	Squalene	$C_{30}H_{50}$	410.71	2.46	24.6	Methanol
26.08	1,7,7-Trimethyl-3-phenethylidenebicyclo[2.2.1]heptan-2-one	$C_{15}H_{20}O$	216.32	2.05	20.5	Aqueous

Methanol fraction yielded a chemically diverse array of secondary metabolites, including alkaloids, triterpenoids, esters, fatty acids, alcohols, and phytosterols, compounds known for antimicrobial, antioxidant, and anti-inflammatory properties [24–26]. Among these, L-alanine methyl ester (13.11%) was the most abundant compound. This amino acid derivative is recognized for its antimicrobial potential, primarily through interference with microbial protein synthesis [27]. Another major constituent, 2-isocyanato-propane (11.36%), an isocyanate derivative, exhibits strong antimicrobial activity by targeting microbial enzymes and compromising cellular integrity [28]. The isoquinoline alkaloid Lauroscholtzine (12.36%) demonstrated notable antibacterial and antifungal properties, particularly against *Staphylococcus aureus* and *Candida albicans* [29]. Similarly, Corydine (4.71%)**,** another bioactive alkaloid, has been reported to possess antimicrobial efficacy alongside central nervous system activity [30].

The triterpenes Lupeol (3.56%) and Lup-20(29)-ene-3-one (4.32%) further enhance the therapeutic profile of the extract; Lupeol is a well-characterized anti-inflammatory and antimicrobial agent effective against Gram-negative bacteria such as *E. coli* and fungi like *C. albicans* [31]. Additionally, phytosterols such as Stigmasterol (2.58%) and antioxidants including Phytol (2.20%**)** and Squalene (2.46%) contribute to the extract's pharmacological potential by imparting anti-inflammatory, antibacterial, and wound-healing properties [32]. The predominance of these compounds explains the strong antimicrobial activity observed in the methanol extract, particularly against *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*, and *A. flavus*, with inhibition zones ranging from 20–24 mm and MIC values as low as 2.5 mg/mL.

The distilled water extract, while less chemically diverse, contained several notable flavonoids, phenolics, and coumarins. The major compound, 4H-pyran-4-one, 2,3-dihydroxy-6-methyl- (6.27%), is a flavonoid precursor known for its antioxidant potential, contributing to anti-inflammatory and wound-healing activities. Coumarin (6.08%), another abundant compound, has long been recognized for its antifungal activity, especially against *Candida* species and skin pathogens. Other bioactives included Parietin (6.10%), an anthraquinone pigment with proven antifungal activity, and Methylparaben (3.30%), a well-established antimicrobial agent widely used in pharmaceutical formulations. Additionally, 1-thioflavone, 7-methoxy- (3.31%) and Homovanillyl alcohol (2.70%) are known to exhibit antibacterial and antioxidant effects.

Although the aqueous extract demonstrated limited antibacterial activity—active only against *E. coli*, *C. albicans*, and *A. flavus*—the presence of coumarin, parietin, and methylparaben likely accounts for the antifungal activity observed in vitro. This profile aligns with the plant's traditional use in managing skin infections and inflammatory conditions, suggesting that the aqueous extract may preferentially concentrate polar compounds with antioxidant and antifungal properties.

Overall, the comparative GC–MS results confirm that methanol extraction yields a broader and more potent spectrum of antimicrobial compounds, while aqueous extraction favors antioxidant and antifungal constituents. These findings substantiate the ethnomedicinal claims surrounding *D. afzeliana* and highlight its potential as a source of novel therapeutic agents for infectious and inflammatory diseases.

Dalbergia afzeliana Versus *other Dalbergia* Species

The phytochemical and antimicrobial results for *Dalbergia afzeliana* align with the broader pharmacological potential observed in other members of the *Dalbergia* genus, yet highlight unique features that underscore its novelty. For instance, *Dalbergia sissoo* has been extensively studied for its anti-inflammatory and antipyretic properties, with flavonoids and tannins identified as key bioactive compounds [33].

D. afzeliana demonstrated a higher abundance of triterpenoids (3.00%), suggesting a distinct phytochemical profile that may contribute to its strong antimicrobial activity. Also, *Dalbergia latifolia* is known for antimicrobial and antioxidant activities, largely attributed to its rich polyphenolic content [34], while *D. latifolia* emphasizes flavonoids. However, *D. afzeliana* showed significant triterpenoid and alkaloid presence, broadening the spectrum of potential therapeutic compounds.

Dalbergia odorifera has been reported to contain sesquiterpenes and flavonoids with cardiovascular protective effects [35, 36]. Interestingly, *D. afzeliana* shares ethnomedicinal claims for cardiovascular support but demonstrates antimicrobial potency against pathogens such as *C. albicans* and *A. flavus*, which are not commonly highlighted in *D. odorifera* studies. Also, *Dalbergia melanoxylon* (African blackwood) is valued for its timber but has limited pharmacological data.

The current findings on *D. afzeliana*, therefore, fill a critical gap by providing evidence of bioactivity in an African *Dalbergia* species, supporting conservation and medicinal exploitation.

The GC–MS profile revealed compounds such as L-Alanine methyl ester, Lauroscholtzine, and 2-Isocyanato propane as major constituents, which are not commonly reported in other *Dalbergia* species. This unique chemical fingerprint suggests that *D. afzeliana* may serve as a novel source of antimicrobial and anti-inflammatory agents. The strong inhibition zones (20–24 mm) and low MIC values (2.5 mg/mL) against both bacterial and fungal pathogens further distinguish it from related species, which often show moderate activity.

These findings validate the ethnomedicinal use of *D. afzeliana* and highlight its potential as a candidate for drug discovery. The comparative analysis emphasizes that while other *Dalbergia* species have established pharmacological roles, *D. afzeliana* offers unique phytochemical and antimicrobial evidence that strengthens its novelty and relevance in addressing antimicrobial resistance.

CONCLUSION

This study investigated the phytochemical composition and antimicrobial activity of *D. afzeliana* stem bark extracts for evidence supporting its medicinal utility. This study confirms the phytochemical richness and antimicrobial potency of *D. afzeliana* stem bark extracts, validating its ethnomedicinal use. The presence of bioactive compounds supports its potential as a source of novel therapeutic agents. Further research should focus on isolation and characterization of individual compounds for drug development.

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