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HPLC analysis and antimicrobial activity of *Vitex altissima* leaves extracts

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Abstract

The exploration of ethnomedicinal plants for phytochemical and pharmacological properties is crucial for developing novel therapeutics for chronic diseases. *Vitex altissima*, known as the Peacock chaste tree, is a significant member of the Verbenaceae family, is widely utilized in traditional medicine. This study investigates the phytochemical composition and antibacterial properties of *V. altissima*, a plant recognized for its medicinal use by various indigenous communities, including the Malayali tribes of Servarayan hills. The study involved extracting phytochemicals from the leaves of *V. altissima* using hexane, ethyl acetate and methanol through soxhlet extraction. The extracts were analyzed for secondary metabolites, including carbohydrates, proteins, amino acids, steroids, glycosides, alkaloids, tannins, phenolics, flavonoids, terpenoids, saponins, anthraquinones, oils and resins, diterpenes, phlobatannins and coumarins. Quantitative assessments of phenolic compounds, flavonoids and alkaloids were performed. Column chromatography was employed to fractionate the methanolic extract, resulting in 12 distinct fractions. These fractions were screened for antibacterial activity against *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Staphylococcus aureus* using the agar well diffusion method. The minimal inhibitory concentration (MIC) was determined through a 2-fold serial dilution technique. High-performance liquid chromatography (HPLC) was utilized to identify the polyphenolic compounds responsible for antibacterial activity. Phytochemical screening revealed a diverse array of bioactive compounds in the extracts, with methanol extracts showing the highest total phenolic content (218 ± 7.21 mg GAE/g of extract) and significant antibacterial activity. The ethyl acetate extract exhibited the highest total flavonoid content (40.67 ± 6.65 mg QE/g of extract). Among the fractions, the column fraction from the chloroform-methanol gradient (VACF) demonstrated superior antimicrobial activity, particularly against *S. mutans* and *P. aureginosa*. The MIC values for VACF were 427 µg/mL for *P. aeruginosa* and 400 µg/mL for *S. aureus*, indicating potent antibacterial properties. HPLC analysis identified key polyphenolic compounds, including p-coumaric acid, ferulic acid and elagic acid, as the primary contributors to the antibacterial activity. The presence of these compounds aligns with the observed antimicrobial efficacy and highlights the potential of *V. altissima* extracts as a source of natural antibacterial agents.

Keywords

Vitex altissima; antimicrobial; polyphenols; HPLC

Introduction

Conducting phytochemical studies and pharmacological investigations on important ethnomedical plants can aid in the creation of new drugs that possess the ability to treat chronic illnesses effectively. Numerous contemporary pharmaceuticals are sourced from isolated compounds of plants that have been utilized in traditional medicine. The utilization of plants as a whole or as medicinal substances is expected to persist as a reliable and cost-effective substitute for conventional therapeutic agents (1).

Vitex altissima, commonly known as Peacock chaste tree, is a member of the Verbenaceae family. The plant's geographic range encompasses the Indo-Malaysian region, specifically Bangladesh, India, Indonesia, Myanmar, Papua New Guinea and Sri Lanka. It is a large, woody tree that is 20 m high. This plant is explicitly utilized for treating wounds, skin allergies, leprosy, oral or lingual ulceration, anorexia and cardiac ailments. The plant leaves manage wounds, skin allergies, snake bites and arthritis. *V. altissima* is acknowledged by diverse indigenous communities and used in traditional medicinal practices.

Vitex altissima is widely used among the Malayali tribes of Servarayan hills to treat various ailments. Various new iridoids have been identified from the ethyl acetate extractions of *V. altissima* leaves (2). A previous study revealed that the ethanolic extract of *V. altissima* contains the phytoconstituents n-Hexadecanoic acid, 9, 12-Octadecadienoic acid and Squalene (3). Previous studies have confirmed the antibacterial properties of *V. altissima.* However, there has been no identification of the specific polyphenolic compounds responsible for this antimicrobial activity through bioassay-guided fractionation.

Polyphenols exhibit antibacterial activity by altering bacterial cell structure, morphology and metabolism, primarily due to their hydroxylic groups that interact with various sites of the bacterial cell (4). Phenolic acids, a subgroup of polyphenols, demonstrate antimicrobial effects against both Gram-positive and Gram-negative bacteria, though their potency varies with species, strains and their chemical structure, particularly the number and position of benzene ring substituents. Their mechanisms include extracellular pH reduction (5), membrane hyper acidification, increased membrane permeability and leakage of cellular constituents such as nucleic acids, proteins and ions (6). High concentrations of phenolic acids have shown efficacy against various *Lactobacilli* strains (4). Gram-negative bacteria are affected at the cytoplasmic level, where phenolic acids disrupt membrane integrity and protein function, leading to increased permeability and potassium efflux. Hydroxycinnamic acids (e.g., ferulic acid) and hydroxybenzoic acids (e.g., gallic acid) exhibit specific minimum inhibitory concentrations against bacteria like *E. coli* and *S. aureus* (7). Additionally, non-membrane actions, such as the binding of p-coumaric acid to bacterial DNA, further illustrate the diverse antibacterial strategies of phenolic acids (8).

Plant extracts contain different phytochemicals, such as alkaloids, tannins, terpenes and saponins. These phytochemicals can be separated based on their different polarities. The study employed column chromatography for separation in conjunction with thin-layer chromatography. The column chromatography technique is employed to isolate impurities and achieve purification of biological mixtures. The method commonly isolates active molecules and extracts metabolites from various samples (9). The process of bioassay-guided isolation is considered highly effective as it carefully examines and selects the most significant samples, thereby simplifying the process of isolating pure and active compounds (10). Given the traditional medicinal use of *V. altissima*, our study involved the implementation of column fractionation on the methanolic extract. We aimed to identify the polyphenols responsible for the observed antibacterial activity, utilizing high-performance liquid chromatography.

Materials and Methods

Chemicals

All the chemicals and reagents used were of analytical grade. Solvents including chloroform, methanol, ethyl acetate, hexane, chloroform and silica gel G and silica gel (60-120) were procured from Merk Specialities Pvt. Ltd., DMEM culture media, FBS and antibiotic solutions were purchased from Hi Media, India.

Preparation of plant extract

The specimens of *V. altissima* leaves were procured from the Kollam district and authenticated by the Department of Botany at TKM College of Arts and Science, Kollam, Kerala, India and deposited with specimen number KOLIV2016AJ01. After 3 rounds of washing with running tap water and once with sterile distilled water, the plant material was subjected to shade drying. Subsequently, the substance was pulverized to a coarse powder consistency and securely contained within tightly sealed containers. The plant leaves were subjected to air-drying and pulverized into a coarse powder. The present study involved the preparation of *V. altissima* hexane extract (VAHE), *V. altissima* ethyl acetate extract (VAEA) and *V. altissima* methanol extract (VAME) through the utilization of the soxhlet extraction method with solvents arranged in ascending order of polarity. The concentrated extracts from the rotary evaporator were stored at 4 °C.

Phytochemical analysis

Qualitative analysis

Presence of secondary metabolites in the various leaves extracts *V. altissima* was determined (11). The qualitatively detected parameters included carbohydrate, protein, aminoacid, steroid, glycoside, alkaloid, tannin, phenolics, flavonoid, terpenoid, saponin, anthraquinone, oil and resin, diterpene, phlobatannin and coumarin.

Quantitative estimation of phenols

The quantitative analysis of phenols was carried out using Folin-Ciocalteu reagent following a slightly modified

method (12). 1 mg of the crude extract were made up to 1 mL with distilled water, mixed with Folin-Ciocalteu reagent (1:1 with H_2O) followed by 20 % sodium carbonate solution. The solution was incubated for 40 min and optical density was measured at 725 nm. From the standard curve of gallic acid, the phenolic content was obtained using linear equation and expressed in mg/g gallic acid equivalent (GAE) of dry extract.

Quantitative estimation of flavonoids

The content of total flavaonoids in the *V. altissima* leaves extract was determined by the standard method (13).

Quantitative estimation of alkaloids

The total alkaloid present in *V. altissima* leaves was determined by using Dragendorff's reagent as it was described (14).

Fractionation of crude extract

The methanolic extract of *V. altissima* leaves was fractionated using column chromatography to yield multiple fractions. Silica gel slurry with a mesh size of 60-120 was packed into a column measuring 72 cm in length and 3 cm in diameter. The methanolic extract was mixed with silica gel and loaded into a column after being dissolved in a solvent. Different solvent mixtures, such as n-hexane-ethyl acetate and chloroform-methanol, were utilized for elution (15). The eluent used for the experiment was initially 100 % hexane. Ethyl acetate was added gradually and continuously to increase the polarity. The column was also eluted using a mobile phase consisting of chloroform-methanol gradients (100:0 to 0:100, v/v) with a gradual increase in polarity. A total of 72 fractions were obtained through the process of column fractionation. Fractions with similar TLC profiles, as determined by Rf values, were categorized together. As a result, twelve fractions were obtained and tested for antibacterial activity. The fraction (VACF) obtained from the elution process using a mixture of chloroform and methanol in a ratio of 85:15 exhibited the highest level of antimicrobial activity.

Determination of the antimicrobial activity

The agar well diffusion method assesses the antimicrobial activity of *V. altissima* crude extract and fractions. *E. coli* (ATCC 259222), *K. pneumoniae* (ATCC 13883), *P. aeruginosa* (ATCC 27853), *S. mutans* (MTCC 890) and *S. aureus* (ATCC 25923) were cultured on 20 mL Muller Hinton Agar Medium. Wells with a diameter of 10 mm were punched and subsequently filled with varying concentrations of the sample (250 µg/mL, 500 µg/mL and 1000 µg/mL). The plates were incubated at 37 °C for 24 h. The inhibition zone diameter measurement indicates antibacterial activity. Streptomycin served as the positive control.

Minimal inhibitory concentration (MIC)

The minimum inhibitory concentration refers to the lowest concentration of a substance that effectively ceases the observable growth of a bacterium. The two-fold serial dilution method determined MIC. The stock inoculum growth was adjusted to a 1 % McFarland standard. The broth dilution assay was conducted in a 96-well microtiter plate. 100 µL of the diluted (2 times) organism suspensions were added to each well in the plate, resulting in a final volume of 200 µL. The extracts were dissolved in DMSO to a final concentration of 10 mg/mL and added in increasing concentrations of 62.5, 125, 250, 500 and 1000 µg/mL to the wells. The samples were then incubated overnight at room temperature. A control well was kept with organism alone. Visual inspection and optical density measurements at 630 nm were used to observe and quantify the growth, employing an ELISA plate reader. The OD was measured right after the visual reading. The growth inhibition for the test wells at each extract dilution was determined by the formula:

Percentage of inhibition = (OD of control - OD of test)/ (OD of control) × 100

Identification of polyphenols by HPLC

The polyphenols content in the extract were estimated using high-performance liquid chromatography. The column fraction of *V. altissima* extract and standard compounds (1 mg/mL) was infused into the HPLC system filtering through 0.45 µm PTFE filter. The analysis was conducted on a prominence UFLC system containing LC-20AD system controller, phenomenex Gemini C18 column (250 × 4.6 mm, 5 µm), a column oven (CTO-20A), a Rheodyne injector (USA) with a loop of 20 µL volume and a diode array detector (SPD-M20A). The mobile phase used was, solvent A; methanol-acetic acid - water (10:2:88, v/v) and solvent B; methanol - acetic acid - water (90:2:8, v/v) with gradient program 0-15 min 15 % B, 16 -20 min 50 % B, 21 -35 min 70 % B, 36 -50 min 100 % B and finally the column was regenerated in 10 min. The injection volume was 20 µL and the flow rate was kept at 1 mL/min. The column was kept at room temperature and fractions eluted were examined at 280 nm. Retention time of standard peaks and sample peaks were compared. Analytical data collection was done using LC Lab solutions.

Statistical analysis

The data were obtained in triplicate values and presented as mean ± standard deviation.

Results

Phytochemical screening

Phytochemical screening of hexane, ethyl acetate, methanol extract of *V. altissima* leaves exhibited the presence of various phytochemical such as phenolic compounds, flavonoids, alkaloids, steroids, saponins, terpenoids, glycosides as summarized in Table 1. Although flavonoid, alkaloid phenolic compound and carbohydrates are present in all fractions, existence of protein, glycoside, tannin, terpenoid etc. was only in methanolic extract. Coumarin and diterpenes were present in both ethyl acetate and methanolic extract. There is a scientific basis for using the genus *Vitex* as a valuable natural resource for developing novel drugs and employing disease treatment, as bioactive compounds present in the *Vitex* plant matrix, such as iridoids, diterpenoids, ecdysteroids and flavonoids, have been shown to have antibacterial, antioxidant, anti-inflammatory, antifungal and anticancer properties (16).

Table 1. Qualitative analysis of *Vitex altissima* leaves

Phytochemicals	Inference		
	VAHE	VAEA	VAME
Carbohydrate	+	+	+
Protein	-	-	+
Aminoacid	-	-	-
Steroid	+	-	+
Glycoside	-	-	+
Alkaloid	+	+	+
Tannin	-	-	+
Phenolics	+	+	+
Flavanoid	+	+	+
Terpenoid	-	-	+
Saponin	+	-	+
Anthraquinone	-	-	-
Oil and resin	-	-	-
Diterpene	-	+	+
Phlobatannin	-	-	+
Coumarin	-	+	+

(+) Presence (-) Absence

Quantitative estimation of phenolics, alkaloids and flavonoids.

Polyphenols, which are secondary metabolites and serve as natural antioxidants play an important role in plant growth and development. The wide range of functions includes anticancer activity, anti-inflammatory activity, inhibition of lipid peroxidation and antiaging properties Flavonoids and phenolic compounds are comprised of double aromatic rings which are connected by a component of 3 carbon atoms. This group includes flavonols, flavones, isoflavones, flavanones, flavanonols, anthocyanidins and tannins (17).

The total phenolic content (TPC) in *V. altissima* extracts was quantified using gallic acid equivalents (GAE). The findings indicate that the methanol extract had the highest TPC (218 ± 7.21 mg GAE/g of extract), whereas the hexane extract had the lowest content (55.67 ± 4.04 mg GAE/g of extract), as presented in Table 2. Phenolic compounds, abundantly present in various plant species, exhibit considerable potential as antioxidants that can mitigate oxidative stress-induced cellular damage and associated chronic ailments. According to recent research, there exists a positive correlation between the phenolic content and antioxidant activity (18). TPC and TFC are correlated with distinct biological functionalities. Furthermore, the elevated antioxidant activity evident in plants is ascribed to the collective impacts of flavonoids and phenolic compounds. Due to their natural origin and low toxicity, Phenolic compounds exhibit potential as viable molecules for preventing numerous life-threatening diseases (19).

According to research findings, the hydroethanolic extract obtained from the blossoms of *Anthyllis vulneraria* L. demonstrated the most elevated total polyphenols and flavonoids. Furthermore, the extract above exhibited the highest antioxidant potency and superior antibacterial effectiveness compared to alternative extracts (20). The phenolic content of extracts from *V. altissima* leaves exhibits a significant and proportional correlation with antioxidant capacity.

The present study reports the total alkaloid range (TAC) of the leaves of *Vitex altissima* extracted using hexane, ethyl acetate and methanol solvents. The TAC values obtained for the hexane, ethyl acetate and methanol extracts were 131.00 ± 2.08 mg AE/g, 118.30 ± 4.41 mg AE/g and 79.00 ± 3.79 mg AE/g respectively. The hexane extract exhibited a notable quantity of alkaloids, while the methanol extract displayed comparatively lower alkaloid content. The study reports the total flavonoid content (TFC) regarding quercetin equivalents (QE). The results indicate that the ethyl acetate extract exhibited the highest flavonoid content (40.67 ± 6.65 mg QE/g of extract) compared to the hexane extract (20.67 ± 5.03 mg QE/g of extract) and methanol extract (29 ± 5.29 mg QE/mg of extract) in the current evaluation.

Antimicrobial activity

The presence of antimicrobial activity was determined through the measurement of inhibition zones. The column fractions of VAME were primarily screened for their antimicrobial activity. Antimicrobial activity was reported to be highest in fraction 12 (VACF) from the chloroform-methanol column as indicated in Fig. 1 and Fig. 2. The determination of the antimicrobial activity of VAME and VACF was conducted, and the results are displayed in Table 3 and Table 4 respectively. Research has demonstrated that the methanol extract exhibits a greater capacity for extracting antimicrobial compounds from plants and exhibits superior antimicrobial properties compared to aqueous extracts (21). The results indicate that VACF showed a slightly higher level of antimicrobial activity against *S. mutans* and *P. aureginosa*. Specific residuals can rationalize the observed dissimilarity in the crude extract

Table 2. Phytochemical contents of leaves of *Vitex altissima*

Extract	Phenols (mg GAE/g extract)	Flavonoids (mg QE/g extract)	Alkaloids (mg AE/g extract)
Hexane (VAHE)	55.67 ± 2.33	20.67 ± 2.91	131.00 ± 2.08
Ethyl acetate (VAEA)	181.70 ± 6.09	40.67 ± 3.84	118.30 ± 4.41
Methanol (VAME)	218.00 ± 4.16	29.00 ± 3.05	79.00 ± 3.79

Each value represents the mean ± SEM of three independent experiments.

GAE - gallic acid equivalents, QE - quercetin equivalents, AE - atropine equivalents

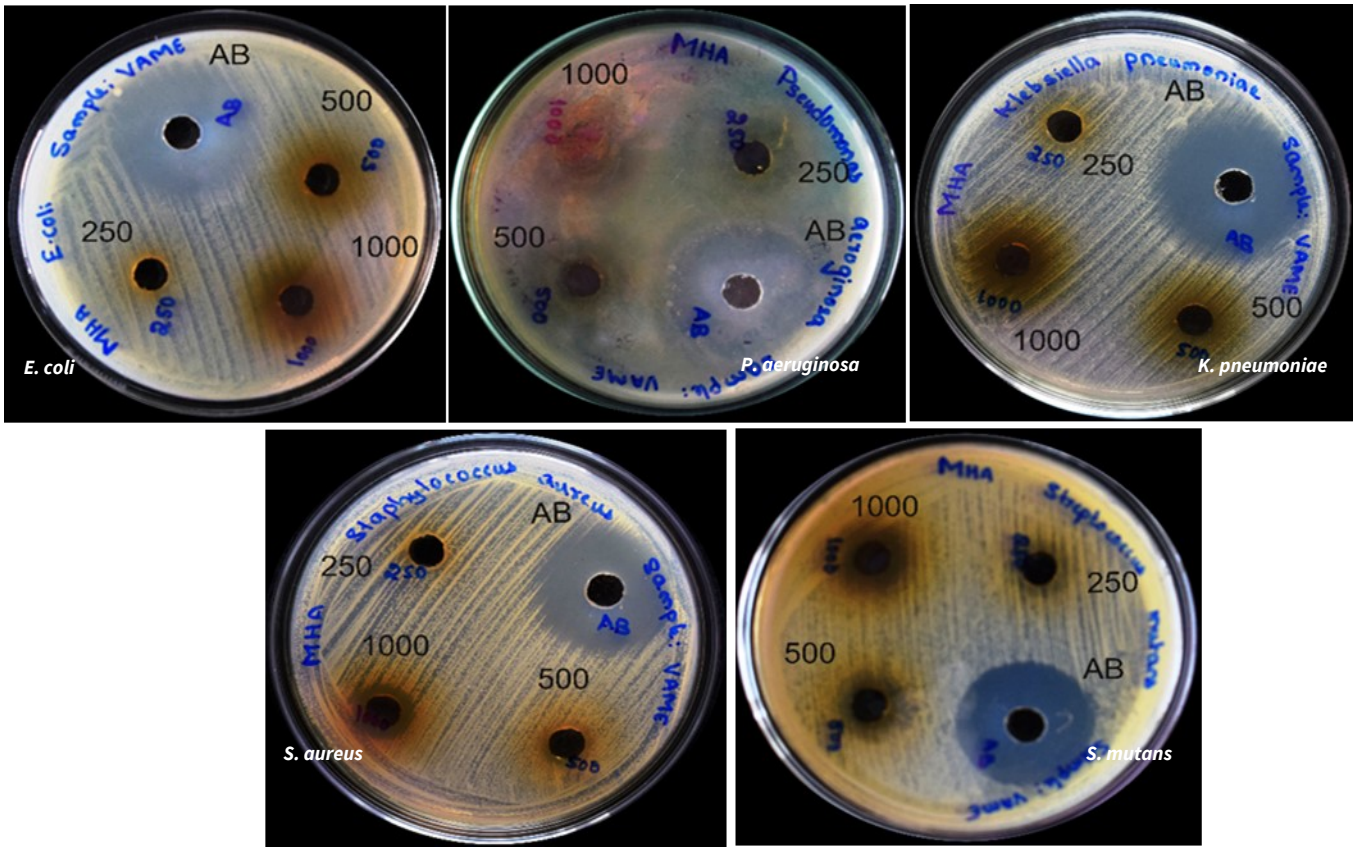

Fig. 1. Antimicrobial activity of *Vitex altissima* methanolic extract (VAME) at different concentrations (250, 500, 1000 µg/mL) by agar well diffusion method against *E. coli*, *P. aeruginosa, K. pneumoniae, S. aureus, S. mutans.* AB – antibiotic streptomycin 100 µg/mL.

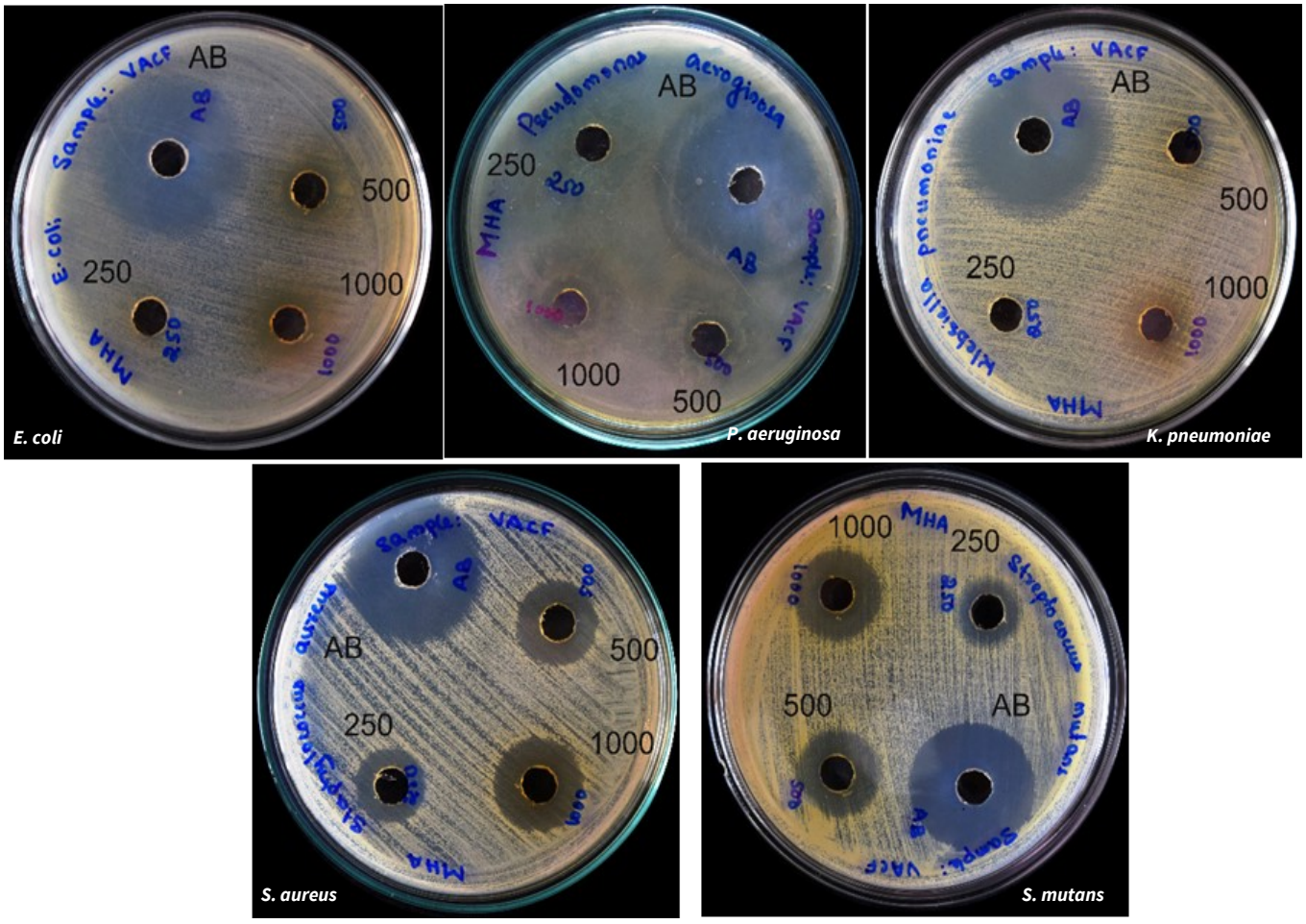

Fig. 2. Antimicrobial activity of *Vitex altissima* column fraction (VACF) at different concentrations (250, 500, 1000 µg/mL) by agar well diffusion method against E. *coli*, *P. aeruginosa, K. pneumoniae, S. aureus, S. mutans.* AB – antibiotic streptomycin 100 µg/mL).

Table 3. Antimicrobial activity of *V. altissima* methanolic extract (Inhibition zones in mm)

Organism	Inhibition zones (mm) VAME (µg/mL)			Streptomycin
	250	500	1000	100
E. coli	N	11	12	29
Pseudomonas aeruginosa	N	10	14	29
Klebsiella pneumoniae	N	10	12	32
Staphylococcus aureus	6	12	19	30
Streptococcus mutans	N	10	12	27

N- No zone of inhibition, VAME – *Vitex altissima* methanolic extract

Table 4. Antimicrobial activity *Vitex altissima* column fraction (Inhibition zones in mm)

Organism	Inhibition zones (mm) VACF (µg/mL)			Streptomycin
	250	500	1000	100
E. coli	N	10	13	29
Pseudomonas aeruginosa	N	13	16	29
Klebsiella pneumoniae	N	N	11	32
Staphylococcus aureus	6	11	16	30
Streptococcus mutans	7	15	18	27

N- No zone of inhibition, VACF – *Vitex altissima* column fraction

obstructing the direct interaction between phytochemicals and microorganisms. The VAME extract exhibited a higher antimicrobial efficacy against *S. aureus*, consistent with previous research findings. Studies showed that *V. negundo* contains numerous polyphenolics and has high antibacterial activity against *E. faecalis* with a zone of inhibition of 23 mm, followed by *S. gordonii* and *K. kristinae* with 11 mm (22). The observed result can be ascribed to the discharge of a high concentration of phytochemicals that exhibit solid antibacterial characteristics.

The determination of the minimal inhibitory concentration (MIC) of VAME and VACF on *P. aureginosa* and *S. aureus* was conducted using the two-fold serial dilution method. The results of this experiment are presented in Table 5. The VAME extract demonstrated a MIC of 503 µg/mL against *P. aeruginosa*, a Gram-negative bacterium and 531 µg/mL against *S. aureus*. At the same time, VACF indicated a decreased concentration for *P. aeruginosa* (427 µg/mL) and *S. aureus* (400 µg/mL).

A previous study found that the ethyl acetate extract of *V. altissima* was significantly more effective against *K. pneumonia* (15 mm) and *A. tumefaciens* (14 mm) than the conventional antibiotic chloramphenicol (23). Using *V. altissima* plant extract in synthesizing SnO_2 nanoparticles with green methods has resulted in remarkable antibacterial properties against pathogenic bacterial strains, as determined by the agar well diffusion method. The leaf extract of *V. altissima* functions as a reducing agent for forming SnO_2 nanoparticles with effective biological properties (24). The antioxidant capacity of phenolic compounds is closely connected to their antimicrobial properties. These properties are achieved through various mechanisms, including radical scavenging, interaction with cell membranes, induction of oxidative stress and disruption of metabolic processes. This dual functionality emphasizes the significance of their chemical structure in producing wide-ranging antimicrobial effects.

Table 5. Minimum Inhibitory Concentration MIC (µg/mL)

Organism	VAME	VACF
Pseudomonas aeruginosa	503	427
Staphylococcus aureus	531	400
Streptococcus mutans	700	380

VAME- *V. altissima* methanolic extract, VACF – *V. altissima* column fraction

HPLC of *Vitex altissima* extracts

High-performance liquid chromatography was used to analyze the polyphenol concentration in the column fraction (VACF). The retention times of Gallic acid, Catechol, Chlorogenic acid, Syringic acid, P-coumaric acid, Elagic acid, Myercetin, Cinnamic acid, Quercetin, Kaempferol and Apigenin were 7.608, 12.126, 23.280, 25.132, 27.924, 28.392, 31.106, 31.704, 35.855, 37.19 and 38.109 respectively. Polyphenols in the extract were identified by retention time. The HPLC profile revealed that the VACF contains an abundance of polyphenols. The 3 most prevalent polyphenolic substances were p-coumaric, ferulic and elagic. Myercetin and quercetin concentrations were observed to be lower. Ferulic acid is regarded as the best antioxidant because the body readily absorbs it and possesses many biological activities, such as antibacterial, anticancer and anti-inflammatory properties (25). Phenolic acids, such as p-coumaric acid and ferulic acid, inhibit bacterial enzymes, cause ion leakage and introduce protons into the cell, interfering with bacterial metabolism. The antibacterial activity of ferulic and gallic acids against pathogenic microorganisms was also reported (26). LC/MS/MS analysis of *Vitex agnus-castus* seed ethanol extracts revealed phenolic compounds like vanillic acid, luteolin, fumaric acid, quercetin, caffeic acid, 4-hydroxybenzoic acid, salicylic acid, kaempferol, butein, ellagic acid, resveratrol, catechin hydrate, phloridzin dehydrate, naringenin and antibacterial activity against *Escherichia coli, Staphylococcus aureus* and *Salmonella typhimurium* (27). Fig. 3 depicts the chromatogram of the standard mixture. In Fig. 4, identification and quantification were accomplished by comparing the retention data and spectra of sample components to those of standards. Ferulic acid is a phenolic

acid derivative in the hydrocinnamic class. The compound's pharmacological activities are attributed to its catechol ring and cinnamic groups. Ferulic acid exhibits dual antioxidant and prooxidant properties. In addition, ferulic acid shows antibacterial activity against Gram-negative and Gram-positive bacteria (28). A study observed that ferulic acid have antibacterial action against *E. coli, P. aeruginosa, S. aureus* and *Listeria monocytogenes* with specified MIC and MBC values. Ferulic acid showed antimicrobial activity against tested bacteria, with MIC of 100 µg/mL for *E. coli, P. aeruginosa, S. aureus* and *L. monocytogenes* (7).

According to a study, p-coumaric acid has better antibacterial action against *Shigella dysenteriae*, with a MIC of 10 mg/mL. Experimental data suggests that p-coumaric acid killed pathogenic bacteria strain by causing irreversible membrane permeability changes, limiting cytoplasmic macromolecule maintenance and inhibiting cellular functions by binding to DNA (8). A recent investigation has successfully identified and substantiated the antibacterial properties of ellagic acid against highly significant and extensively resistant pathogenic bacteria, including MRSA, *P. aeruginosa* and *E. coli* (29).

The study results indicate that the investigated *V. altissima* extracts contain many phenolic chemicals. Further investigation is necessary to understand how these chemicals affect the antimicrobial response of the extracts and any possible interactions that might arise.

Conclusion

The results of the present study identified the hexane, ethyl acetate and methanol extracts of *V. altissima* leaves as an abundant source of active compounds, some of which were extracted and recognized for the first time. The extract of

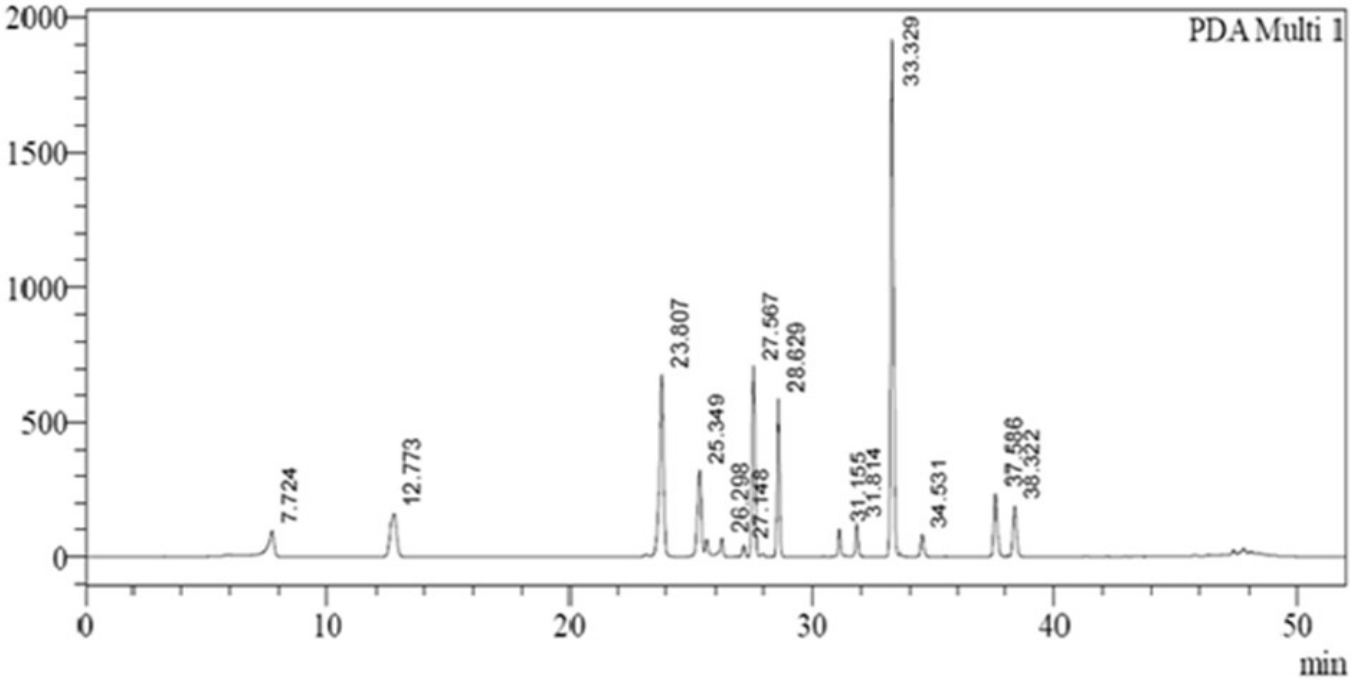

Fig. 3. Chromatogram of standard mixture Gallic acid (7.724), Catechol (12.77), Chlorogenic acid (23.807), Syringic acid, (25.34), P-coumaric acid (27.567), Ferulic acid (28.629), Elagic acid (31.155), Myercetin (31.1814), Cinnamic acid (33.329), Quercetin (34.531), Kaempferol (37.586), Apigenin (38.322).

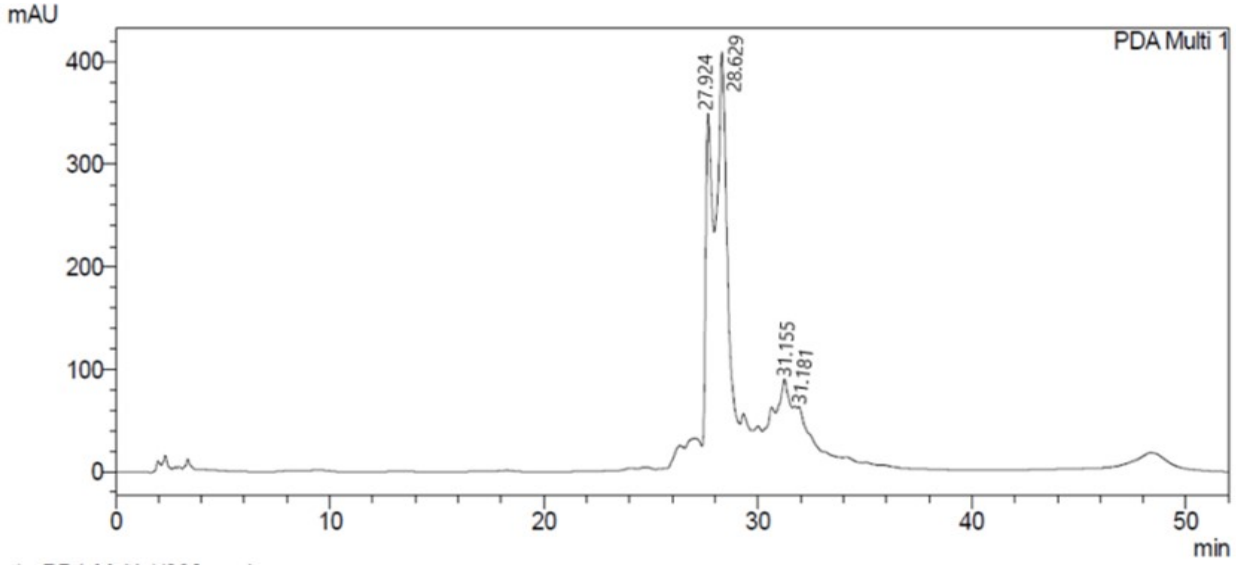

Fig. 4. HPLC profile of polyphenols present in *Vitex altissima* column fraction (VACF) p-coumaric acid (27.924), Ferulic acid (28.629), Elagic acid (31.155), Myercetin (31.181).

leaves in methanol was the most significant source of phenolic acids. Regarding antibacterial activity, methanol extracts were most effective against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, while the column fraction of methanolic extract was more effective against *Streptococcus mutans*. Phenolics dominated the column fraction, including ferulic acid, elagic acid and p-coumaric acid. Additional molecular research is required for clinical applications to elucidate the mechanism and identify other antimicrobial active components.

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Authors' contributions

AA: Conceived and designed the experiments; Performed the experiments; Writing-original draft AR: Conceptualization; supervised the conduct of the study; analyzed and interpreted the data; Writing-review and editing, BT: Data analysis and approval of the manuscript, analyzed and interpreted the data, Data curation, Writing-review and editing. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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