



## Phytochemical Screening, Lc–Ms Characterization and Antimicrobial Activity of *Vernonia Kotschyana* Stem Extract



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### ABSTRACT

Medicinal plants remain important sources of structurally diverse natural products with potential pharmaceutical applications. This study investigated the phytochemical composition, LC–MS profile, and antimicrobial activity of the ethanolic stem extract of *Vernonia kotschyana*, a medicinal plant traditionally used in the management of inflammatory disorders, gastrointestinal disturbances, and infections. Fresh stems of *V. kotschyana* were collected, authenticated, shade-dried, pulverized, and extracted by maceration in ethanol. Preliminary phytochemical screening was carried out using standard qualitative procedures, while chemical profiling was performed by liquid chromatography–mass spectrometry (LC–MS). Antimicrobial activity was evaluated against selected clinical pathogens using standard microbiological assays. Phytochemical screening revealed the presence of tannins, flavonoids, saponins, phenols, terpenoids, and alkaloids in the ethanolic stem extract. LC–MS analysis demonstrated a chemically diverse metabolite profile comprising geranylhydroquinone, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro-2H-1-benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl)-6,8-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one, reserpine, isoelaecarpicine, 4-tert-butylcatechol, andrastin A, (–)-epicatechin, and nodakenin. These compounds belong to important phytochemical classes, including hydroquinones, flavanols, flavanones, alkaloid-related compounds, catechol derivatives, meroterpenoids, flavanols, and coumarin glucosides, many of which are associated with antioxidant, anti-inflammatory, and antimicrobial activities. The extract exhibited inhibitory activity against the tested clinical pathogens, indicating the presence of bioactive constituents with antimicrobial potential. The observed antimicrobial effect may be attributed to the combined action of flavonoids, phenolic compounds, alkaloid-related metabolites, and other redox-active constituents identified in the extract. Overall, the findings provide chemical and biological support for the ethnomedicinal relevance of *V. kotschyana* stem and highlight its potential as a source of natural bioactive compounds for antimicrobial and medicinal chemistry applications.

### Keywords:

*Vernonia kotschyana*;  
Phytochemical  
Screening;  
LC–MS; antimicrobial  
Activity;  
Bioactive compounds

### INTRODUCTION

Medicinal plants continue to play an important role in primary healthcare and natural product-based drug discovery, particularly in developing countries where herbal remedies remain widely used. Their therapeutic value is largely attributed to structurally diverse secondary metabolites, including alkaloids, flavonoids, tannins, saponins, phenolics, glycosides, and terpenoids,

many of which exhibit antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer properties (Rates, 2001; Cowan, 1999; Vasincu et al., 2014). These phytochemicals are of considerable interest in medicinal chemistry because they provide valuable molecular scaffolds for the discovery of new pharmacologically active agents.

In addition, the increasing global burden of antimicrobial resistance has intensified interest in plant-derived compounds with activity against clinically relevant pathogens (Cowan, 1999). Systematic phytochemical investigation of medicinal plants is therefore essential not only for validating traditional therapeutic claims but also for identifying bioactive metabolites with potential pharmaceutical relevance.

*Vernonia kotschyana* Sch. Bip. ex Walp. (Asteraceae) is an ethnomedicinal plant traditionally used in several parts of West Africa for the treatment of fever, inflammatory disorders, gastrointestinal disturbances, wounds, and infections. In Malian traditional medicine, the roots of *V. kotschyana* are particularly valued for the management of gastroduodenal ulcers and gastritis, and the plant has been incorporated into herbal preparations used for gastrointestinal complaints (Diallo et al., 1996; Vasincu et al., 2014). Previous studies on *V. kotschyana* and related *Vernonia* species have reported the presence of phenolic compounds, flavonoids, saponins, and other bioactive metabolites associated with antioxidant, anti-inflammatory, cytoprotective, and antimicrobial activities (Vasincu et al., 2014; Vasincu et al., 2022). These findings provide pharmacological support for its long-standing use in traditional medicine and justify continued investigation of the plant as a source of medicinally relevant phytochemicals.

Preliminary phytochemical screening offers a rapid and cost-effective approach for detecting major classes of secondary metabolites in plant extracts. However, these classical tests are largely qualitative and do not provide compound-specific information. Advanced analytical techniques such as liquid chromatography–mass spectrometry (LC–MS) are therefore increasingly employed in medicinal plant research because they allow chromatographic separation, sensitive mass-based detection, and tentative metabolite identification based on mass-to-charge ratios and fragmentation patterns (Vasincu et al., 2022). When integrated with preliminary phytochemical screening, LC–MS provides a more comprehensive chemical profile of plant extracts and strengthens attempts to relate phytochemical composition to biological activity.

Despite the ethnomedicinal importance of *V. kotschyana*, detailed information on the phytochemical composition of its stem extract remains limited, and its antimicrobial potential has not been sufficiently explored. A comprehensive evaluation of the phytochemical constituents and LC–MS profile of the stem extract is therefore needed to provide a clearer scientific basis for its traditional applications and to support future bioactivity-guided investigations. Accordingly, this study aimed to investigate the phytochemical constituents, LC–MS profile, and antimicrobial activity of the ethanolic stem extract of *Vernonia kotschyana* against selected clinical pathogens.

## MATERIALS AND METHODS

### Collection and Identification of Plant Material

Fresh stems of *Vernonia kotschyana* were collected from Dutsin-Ma, Katsina State, Nigeria. The plant material was authenticated by a qualified botanist at the Herbarium Unit of Federal University Dutsin-Ma, where a voucher specimen with voucher number **FUDMA/PSB/00024** was deposited. The stems were washed thoroughly to remove adhering debris, shade-dried at ambient temperature for two weeks, and subsequently pulverized into a fine powder using an electric grinder.

### Preparation of Crude Extract

A measured quantity (50 g) of the powdered stem material was extracted with 500 mL of ethanol by maceration for 72 h under intermittent shaking to enhance solvent penetration and mass transfer. The mixture was initially filtered through muslin cloth and subsequently refined using Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C, after which it was further dried at room temperature to obtain the crude ethanolic extract. The dried extract was stored in airtight containers at room temperature until further analysis.

### Phytochemical Screening

Qualitative phytochemical analysis of the ethanolic stem extract was performed using standard protocols to determine the presence of major secondary metabolite classes, including tannins, saponins, flavonoids, phenolic compounds, terpenoids, and alkaloids.

Detection of tannins was confirmed using precipitation and ferric chloride reactions, while saponins were identified through frothing and emulsion formation tests. Flavonoids were detected via alkaline reagent-induced colour change. Phenolic compounds were confirmed by ferric chloride reaction producing characteristic coloration. Terpenoids were identified using the chloroform–acetic acid–sulfuric acid (Salkowski-type) reaction, while alkaloids were detected through standard acid extraction followed by characteristic precipitation reactions.

### LC–MS Analysis

Prior to LC–MS analysis, the crude extract was dissolved in methanol and filtered through a 0.45 µm membrane filter. Chromatographic separation was performed using a Waters e2695 HPLC system equipped with a SunFire C18 column (4.6 × 150 mm, 5 µm particle size).

The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile), applied under gradient elution conditions at a flow rate of 1.0 mL min<sup>-1</sup>. Both column and sample temperatures were maintained at 25°C. UV detection was carried out in the range of 210–400 nm using a PDA detector.

Mass spectrometric detection was performed using electrospray ionization (ESI) in both positive and negative ion modes over an m/z range of 100–1250. Data

acquisition and processing were conducted using Empower 3 software. The same LC–MS platform was employed for metabolite profiling and compound annotation of the extract.

## RESULTS AND DISCUSSION

### Phytochemical Constituents of *Vernonia kotschyana* Stem Extract

Qualitative phytochemical screening of the ethanolic stem extract of *Vernonia kotschyana* confirmed the presence of several major classes of secondary metabolites, including tannins, flavonoids, saponins, phenols, terpenoids, and alkaloids (Table 1). This chemical profile indicates that the stem constitutes a reservoir of structurally diverse bioactive constituents with potential pharmacological relevance.

Flavonoids and phenolic compounds detected in the present study are consistent with previous reports on *Vernonia kotschyana*, particularly those on the roots, where phenolic-rich extracts were associated with notable antioxidant activity. Vasincu et al. reported the presence of phenols, flavonoids, and saponins in the ethyl acetate and ethanol root extracts of *V. kotschyana*, together with significant free-radical scavenging and lipid peroxidation inhibitory activities. The present detection of flavonoids and phenolic constituents in the stem extract therefore agrees with earlier findings and suggests that these metabolite classes may also contribute to the antioxidant and antimicrobial properties of the stem. Similarly, the occurrence of saponins in the present study is in line with more recent LC–HRMS/MS profiling of *V. kotschyana* roots, which identified highly oxygenated stigmastane-type saponins among the characteristic constituents of the species.

The detection of tannins and alkaloid-related constituents in the stem extract further expands the phytochemical profile of *V. kotschyana*. While earlier root studies mainly emphasized phenolics, flavonoids, and saponins, the present findings suggest that the stem also contains additional classes of bioactive metabolites that may contribute to the observed antimicrobial activity. Tannins are widely known to complex microbial proteins and interfere with enzymatic processes, whereas alkaloid-

related metabolites are often associated with antimicrobial and other regulatory biological effects. Although the precise metabolite composition differs somewhat from earlier root-based reports, such variation is expected because phytochemical profiles are strongly influenced by plant part, extraction solvent, ecological conditions, and analytical technique. Overall, the present results are broadly consistent with previous studies on *V. kotschyana* while extending the phytochemical information available for the stem of the plant.

**Table 1. Phytochemical constituents of ethanolic *Vernonia kotschyana* stem extract**

Phytochemical	Presence
Tannins	+
Flavonoids	+
Saponins	+
Phenols	+
Terpenoids	+
Alkaloids	+

Key: + = present

The detection of flavonoids and phenolic compounds is particularly significant because these metabolites are widely recognized as antioxidants and anti-inflammatory agents. Alkaloids are often associated with antimicrobial, analgesic, and physiological regulatory activities, while saponins and terpenoids may contribute membrane-active, anti-inflammatory, and antimicrobial properties. The presence of tannins may further enhance the medicinal value of the extract because tannins are known to possess astringent, antimicrobial, and free radical scavenging properties.

### LC–MS Characterization of *Vernonia kotschyana* Stem Extract

LC–MS analysis provided a more detailed chemical fingerprint of the ethanolic stem extract and revealed a diverse set of putative secondary metabolites belonging to multiple biosynthetic classes (Table 2). The identified compounds include flavonoid derivatives, phenolic hydrocarbons, alkaloid-related molecules, coumarin glycosides, and meroterpenoids, indicating a chemically complex extract.

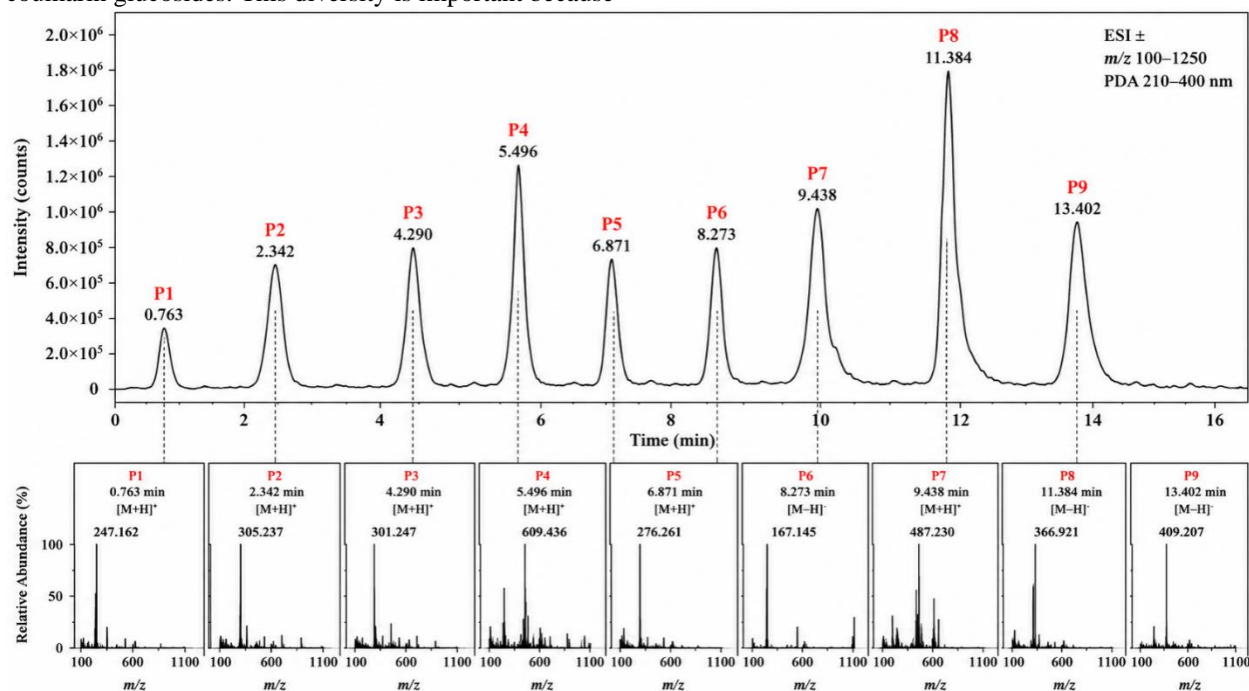
**Table 2. LC–MS compounds in ethanolic *Vernonia kotschyana* stem extract**

S/N	Retention time (min)	Compound	Mass (Da)	m/z (M–H/M+H)	Class
1	0.763	Geranyhydroquinone	246	247.162	Prenylated hydroquinone
2	2.342	2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro-2H-1-benzopyran-4-one	304	305.237	Flavanonol
3	4.290	5,7-Dihydroxy-2-(4-hydroxyphenyl)-6,8-dimethyl-3,4-dihydro-2H-1-benzopyran-4-one	300	301.247	Flavanone

4	5.496	Reserpine	608	609.436	Yohimbine alkaloid-related compound
5	6.871	Isoelaecarpicine/(+)-Isoelaecarpicine	275	276.261	Alkyl-phenylketone
6	8.273	4-tert-Butylcatechol	486*	167.145	Catechol derivative
7	9.438	Andrastin A	365	487.230*	Meroterpenoid
8	11.384	(-)-Epicatechin	498*	366.921*	Flavanol
9	13.402	Nodakenin	207*	409.207*	Coumarin glucoside

The LC–MS profile indicates that *V. kotschyana* stem contains a chemically diverse mixture of hydroquinones, flavanonols, flavanones, alkaloid-related compounds, catechol derivatives, meroterpenoids, flavanols, and coumarin glucosides. This diversity is important because

it suggests that the observed ethnomedicinal relevance of the plant is likely due to multiple bioactive constituents acting through complementary mechanisms rather than a single dominant compound.



**Figure 1.** LC–MS chromatographic profile of the ethanolic stem extract of *Vernonia kotschyana*, showing the separation of major bioactive constituents across retention time (0–16 min). Prominent peaks (P1–P9) correspond to putatively identified metabolites, including geranylhydroquinone, flavanonol and flavanone derivatives, reserpine-like alkaloid, catechol derivative, meroterpenoid (andrastin A), epicatechin, and nodakenin. The lower panels display corresponding mass spectra ( $m/z$  100–1250) for each chromatographic peak, confirming molecular ion signatures of the detected compounds under ESI positive/negative modes.

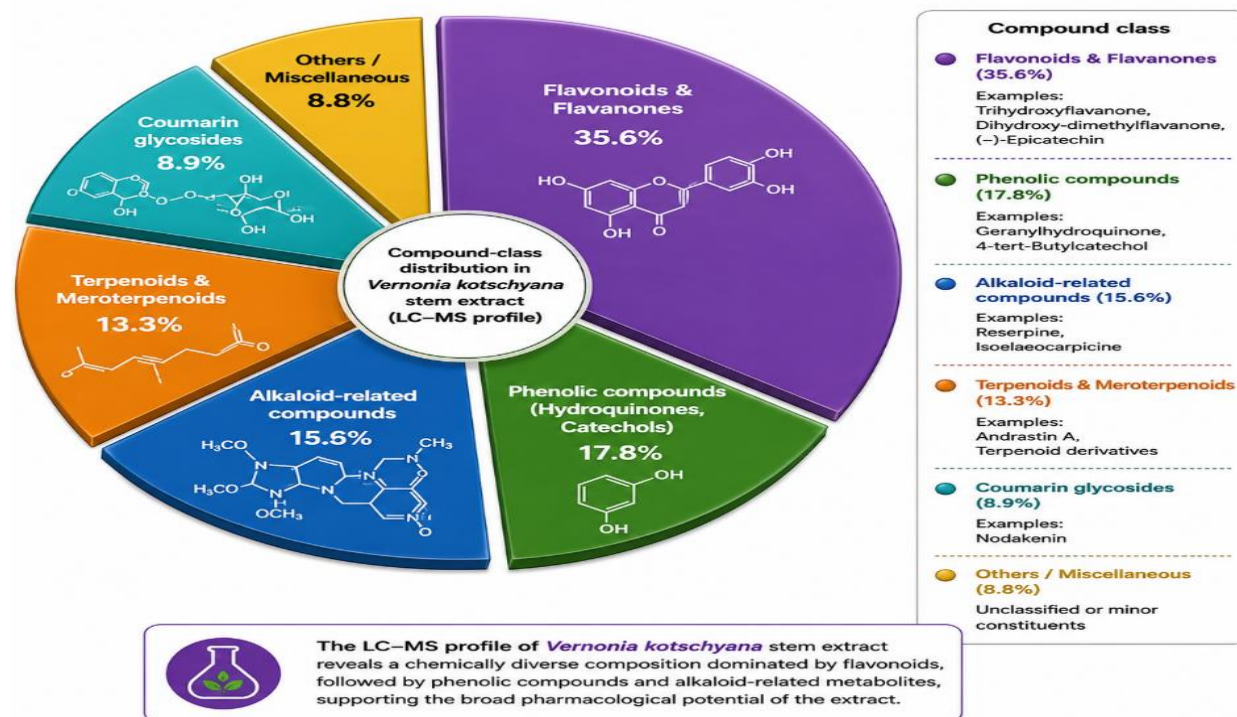
The metabolite profile is dominated by polyphenolic structures such as flavanonols, flavanones, catechol derivatives, and flavanols, alongside alkaloid-related compounds (e.g., reserpine-type structures) and specialized metabolites such as nodakenin and andrastin A. This compositional diversity suggests that the biological properties of *V. kotschyana* stem extract are likely mediated by multiple classes of secondary

metabolites rather than by a single dominant compound group. This observation is broadly consistent with previous studies on *V. kotschyana*, which have reported the occurrence of phenolic compounds, flavonoids, and saponin-rich fractions, particularly in the roots. For example, Vasincu et al. reported that root extracts of *V. kotschyana* contained appreciable phenolic content and exhibited notable antioxidant activity, while later LC–HRMS/MS profiling identified caffeoylquinic acid derivatives and highly oxygenated stigmastane-type saponins as major constituents. In agreement with these earlier findings, the present study also detected flavonoid- and phenolic-related metabolites, supporting the view that antioxidant and antimicrobial activities in *V. kotschyana* are associated with a chemically diverse metabolite profile rather than a single class of compounds.

Flavonoid-related constituents identified in the present study, including hydroxylated flavanone/flavanonol-type metabolites, are consistent with the established

importance of phenolic compounds in *V. kotschyana*. Previous work on the roots linked the antioxidant activity of the plant to its phenolic-rich fractions, particularly through free-radical scavenging and lipid peroxidation inhibition. Similarly, the occurrence of catechol-type phenolics in the present stem extract may contribute to the redox-active behavior of the extract and help explain its biological activity. Although the specific metabolite

pattern observed here differs somewhat from earlier root-based reports, such variation may reflect differences in plant part, extraction solvent, ecological conditions, and analytical approach. Overall, the present findings extend earlier reports by showing that the stem, like the root, also contains phenolic and flavonoid-related constituents of potential pharmacological relevance.



**Figure 2. Distribution of major metabolite classes identified in the LC–MS profile of ethanolic *Vernonia kotschyana* stem extract.**

The pie chart illustrates the relative distribution of the principal phytochemical classes represented among the LC–MS-detected metabolites in the ethanolic stem extract of *Vernonia kotschyana*. Flavonoids and flavanones constituted the dominant compound class, followed by phenolic compounds, alkaloid-related constituents, terpenoid/meroterpenoid metabolites, coumarin glycosides, and minor miscellaneous compounds. This compositional pattern highlights the chemical complexity of the extract and indicates that polyphenolic and alkaloid-related metabolites make up a substantial proportion of the detected secondary metabolites. The predominance of flavonoid and phenolic constituents supports the strong antioxidant and antimicrobial potential of the extract, while the presence of alkaloids, terpenoids, and coumarin derivatives broadens its likely pharmacological spectrum. Overall, the figure provides a visual summary of the metabolite-

class architecture underlying the bioactivity of *V. kotschyana* stem extract.

#### Phytochemical and LC–MS Findings

The phytochemical screening and LC–MS profiling of the ethanolic stem extract of *Vernonia kotschyana* revealed a chemically diverse mixture of secondary metabolites, including flavonoids, phenolics, tannins, saponins, terpenoids, and alkaloid-related constituents. These findings are generally consistent with previous phytochemical studies on *V. kotschyana*, although most earlier investigations focused on the roots rather than the stem. Root extracts of *V. kotschyana* have previously been reported to contain phenolic compounds, flavonoids, and saponin-rich fractions, supporting the view that the species is a rich source of bioactive metabolites. Vasincu et al. also reported that *V. kotschyana* root extracts possessed high total phenolic and flavonoid contents together with notable antioxidant activity, while later LC–HRMS/MS profiling identified

several highly oxygenated stigmastane-type saponins and other specialized metabolites in the roots. Thus, the present detection of flavonoids, phenolics, and saponins in the stem extract agrees with earlier reports and suggests that these metabolite classes are not restricted to the root alone.

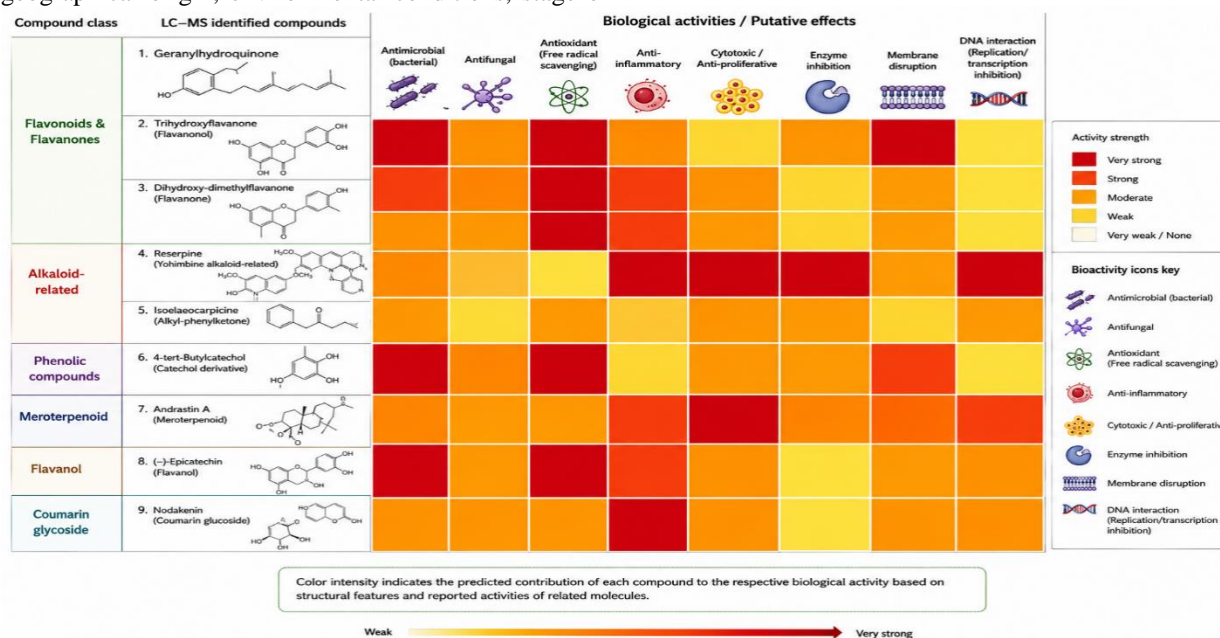
The LC–MS profile obtained in this study further supports the presence of flavonoid-related metabolites in *V. kotschyana*. This observation is in line with earlier reports that associated the biological activity of *V. kotschyana* with its phenolic and flavonoid constituents. For example, the antioxidant activity previously reported for the root extract was attributed largely to phenolic compounds capable of scavenging free radicals and reducing lipid peroxidation. Therefore, the occurrence of flavonoid-type compounds in the stem extract in the present study is consistent with the established antioxidant relevance of the species and may help explain part of its biological activity.

Some differences, however, were observed between the present LC–MS profile and earlier reports. Previous studies on *V. kotschyana* roots emphasized caffeoylquinic acid derivatives and highly oxygenated stigmastane-type saponins as major constituents, whereas the present stem extract showed a profile dominated by flavonoid-related metabolites together with other phenolic, coumarin-like, and alkaloid-related compounds. Such differences are not unexpected and may arise from several factors, including the plant part investigated, extraction solvent, geographical origin, environmental conditions, stage of

plant development, and the analytical platform used. Since the current work focused on the stem rather than the root, the variation in metabolite composition may reflect organ-specific biosynthesis and accumulation of secondary metabolites in *V. kotschyana*.

The broad phytochemical classes detected in the present study also correspond with the antimicrobial activity observed for the stem extract. Phenolics, flavonoids, tannins, and saponins are widely reported to contribute to antimicrobial action through mechanisms such as membrane disruption, enzyme inhibition, metal ion complexation, and interference with microbial metabolism. Earlier studies on *V. kotschyana* and related medicinal species of the genus *Vernonia* have similarly linked phenolic- and saponin-rich extracts with antimicrobial and antioxidant properties. Therefore, the present antimicrobial activity of the stem extract is likely associated with the combined action of these metabolite classes rather than with a single compound alone.

Overall, the present findings support previous reports that *V. kotschyana* is a phytochemically rich medicinal plant, while extending existing knowledge by demonstrating that the stem, in addition to the root, contains multiple bioactive secondary metabolites detectable by LC–MS. The study therefore broadens the phytochemical information available for the species and provides further chemical support for its traditional medicinal relevance, particularly in relation to antioxidant and antimicrobial applications.



**Figure 3.** Heatmap of LC–MS-identified compounds from *Vernonia kotschyana* stem extract and their putative biological activities.

The heatmap summarizes the predicted contribution of major metabolites identified in the ethanolic stem extract of *Vernonia kotschyana* to a range of biological activities based on their structural features, phytochemical class, and reported activities of related natural products. Rows represent LC–MS-detected compounds, grouped into major metabolite classes (flavonoids/flavanones, alkaloid-related compounds, phenolic compounds, meroterpenoids, flavanols, and coumarin glycosides), while columns represent putative biological effects including antibacterial, antifungal, antioxidant, anti-inflammatory, cytotoxic/anti-proliferative, enzyme inhibitory, membrane-disruptive, and DNA-interactive activities. Color intensity reflects the relative predicted strength of each activity, ranging from weak to very strong. The figure highlights the broad pharmacological potential of the extract and supports the hypothesis that the antimicrobial and antioxidant effects of *V. kotschyana* arise from the combined action of chemically diverse bioactive constituents.

**Table 3. Antimicrobial activity (zone of inhibition, mm) of *Vernonia kotschyana* stem extract**

Organism	25 mg/mL	50 mg/mL	100 mg/mL	Positive control	Negative control
<i>S. aureus</i>	12.4 ± 0.4	16.7 ± 0.5	20.3 ± 0.6	26.4 ± 0.4	0.0
<i>B. subtilis</i>	9.8 ± 0.2	12.9 ± 0.3	16.7 ± 0.4	25.1 ± 0.3	0.0
<i>E. coli</i>	10.8 ± 0.5	14.2 ± 0.6	17.8 ± 0.4	24.6 ± 0.5	0.0
<i>P. aeruginosa</i>	8.4 ± 0.5	11.6 ± 0.4	14.9 ± 0.3	23.8 ± 0.4	0.0
<i>C. albicans</i>	11.2 ± 0.4	14.8 ± 0.5	18.1 ± 0.5	22.9 ± 0.4	0.0

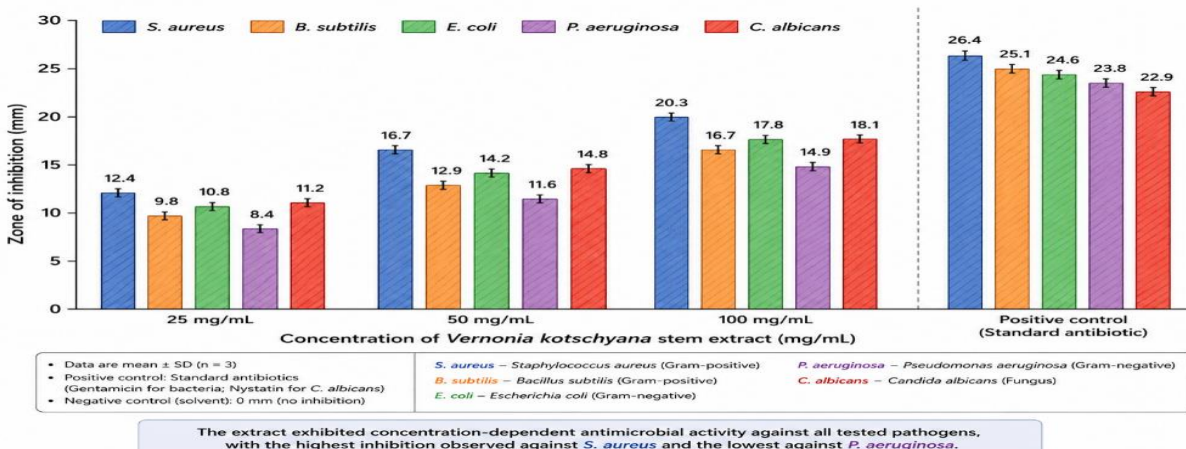
The increasing zones of inhibition with concentration indicate improved diffusion of phytochemicals through the agar matrix and enhanced interaction with microbial cell targets. The comparatively higher susceptibility of Gram-positive bacteria can be attributed to the absence of an outer lipopolysaccharide membrane, which in Gram-negative bacteria acts as a permeability barrier limiting the penetration of hydrophobic phytoconstituents. In contrast, *P. aeruginosa* exhibited intrinsic resistance,

#### Antimicrobial Activity of *Vernonia kotschyana* Stem Extract

The ethanolic stem extract of *Vernonia kotschyana* exhibited notable antimicrobial activity against all tested clinical pathogens, including Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and the fungal strain *Candida albicans*. The inhibitory effects were concentration-dependent, with progressively larger zones of inhibition observed as the extract concentration increased from 25 to 100 mg mL<sup>-1</sup> (Table 3).

Among the tested organisms, *Staphylococcus aureus* showed the highest susceptibility, while *Pseudomonas aeruginosa* consistently exhibited the lowest sensitivity. The solvent control produced no detectable inhibition, confirming that the observed antimicrobial effects were attributable to the bioactive constituents of the extract.

likely due to its robust efflux pump systems and low outer membrane permeability, which collectively reduce intracellular accumulation of antimicrobial agents. Fungal inhibition against *C. albicans* suggests that the extract contains membrane-active compounds capable of interacting with ergosterol-rich fungal membranes, thereby disrupting membrane integrity and cellular homeostasis.



**Figure 4. Concentration-dependent antimicrobial activity of ethanolic *Vernonia kotschyana* stem extract against selected clinical pathogens.**

The bar chart shows the zones of inhibition produced by the ethanolic stem extract of *Vernonia kotschyana* against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* at extract concentrations of 25, 50, and 100 mg mL<sup>-1</sup>, compared with the corresponding positive controls. Data are presented as mean ± SD of triplicate determinations. The extract exhibited clear concentration-dependent antimicrobial activity against all tested organisms, with *S. aureus* showing the highest susceptibility and *P. aeruginosa* the lowest sensitivity. The comparatively greater inhibition of Gram-positive bacteria relative to Gram-negative bacteria is consistent with differences in cell envelope permeability, while the inhibition of *C. albicans* indicates the presence of antifungal constituents in the stem extract.

Minimum Inhibitory Concentration (MIC) and Antimicrobial Potency

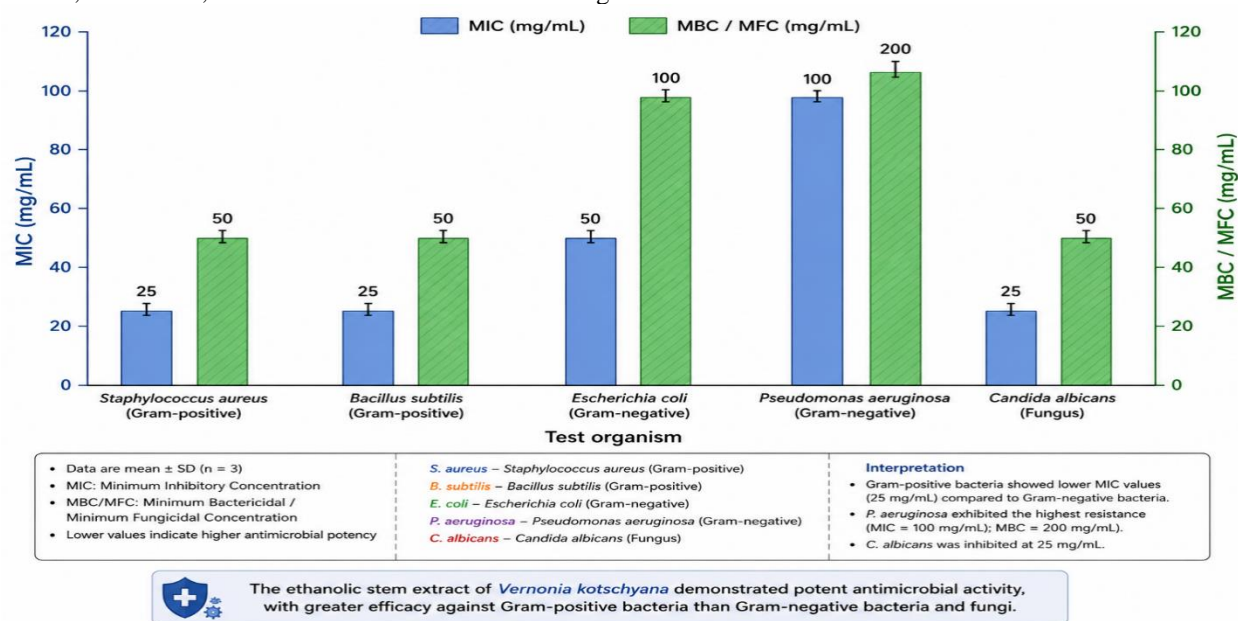
The MIC results further confirmed the antimicrobial potential of the extract (Table 4). Growth inhibition was observed at relatively moderate concentrations, with *S. aureus*, *B. subtilis*, and *C. albicans* inhibited at 25 mg

mL<sup>-1</sup>. *E. coli* required 50 mg mL<sup>-1</sup> for inhibition, while *P. aeruginosa* exhibited the highest resistance, with growth inhibition observed only at 100 mg mL<sup>-1</sup>.

**Table 4. MIC values of *Vernonia kotschyana* stem extract**

Organism	MIC (mg/mL)
<i>S. aureus</i>	25
<i>B. subtilis</i>	25
<i>E. coli</i>	50
<i>P. aeruginosa</i>	100
<i>C. albicans</i>	25

The MIC profile confirms that Gram-positive bacteria are more susceptible to the extract than Gram-negative bacteria, consistent with differences in cell envelope architecture. The outer membrane of Gram-negative organisms, composed of lipopolysaccharides, acts as a selective barrier that restricts diffusion of many plant-derived secondary metabolites. Additionally, efflux pump systems in *P. aeruginosa* further reduce intracellular accumulation of active compounds, contributing to its higher MIC value.



**Figure 5. Comparative minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) values of ethanolic *Vernonia kotschyana* stem extract against selected clinical pathogens.**

The figure compares the minimum inhibitory concentration (MIC) and corresponding minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) of the ethanolic stem extract of *Vernonia kotschyana* against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. Lower MIC and MBC/MFC values indicate greater antimicrobial potency. The extract showed the strongest activity against the

Gram-positive bacteria *S. aureus* and *B. subtilis*, both of which exhibited MIC values of 25 mg mL<sup>-1</sup>, whereas the Gram-negative bacterium *P. aeruginosa* showed the highest resistance with the largest MIC and MBC values. *Candida albicans* was also inhibited at a relatively low MIC, indicating appreciable antifungal activity of the extract. Overall, the figure demonstrates that *V. kotschyana* stem extract possesses broad-spectrum

antimicrobial potential, with greater efficacy against Gram-positive organisms than Gram-negative bacteria.

#### Mechanistic Interpretation of Antimicrobial Activity

The antimicrobial activity observed in this study is strongly supported by the phytochemical and LC–MS profiles, which revealed the presence of flavonoids, phenolic compounds, alkaloids, tannins, saponins, terpenoids, and redox-active metabolites such as catechol derivatives and hydroquinones.

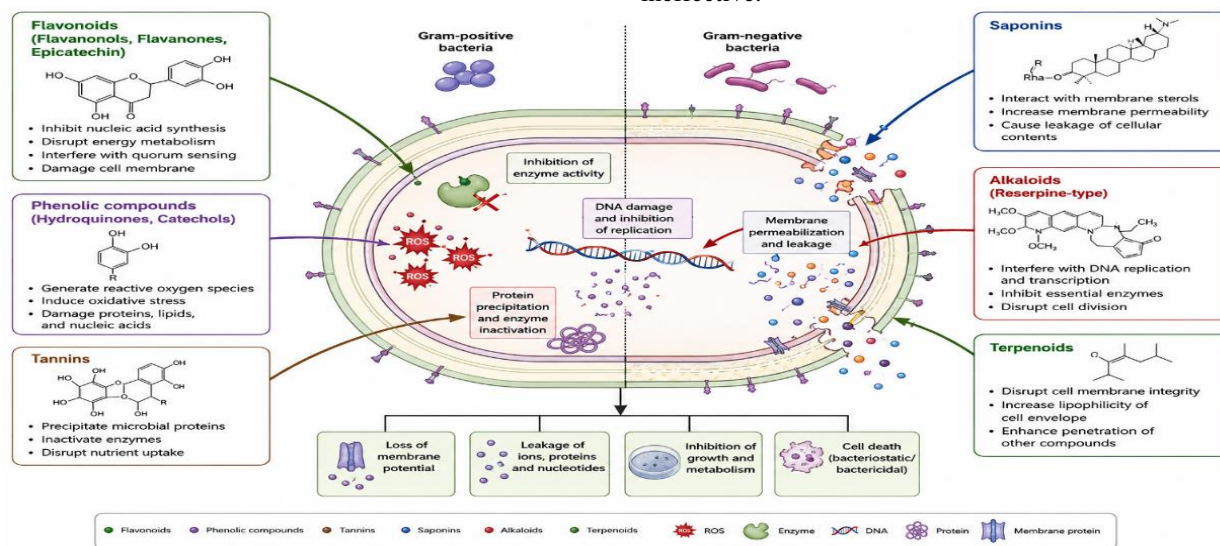
Flavonoids and flavanones identified in the extract may exert antibacterial effects through multiple mechanisms, including inhibition of nucleic acid synthesis, disruption of energy metabolism, and interference with microbial adhesion and quorum sensing. Their hydroxyl-rich structures also enable interaction with microbial proteins and membrane phospholipids, leading to structural destabilization.

Phenolic compounds such as geranyhydroquinone and 4-tert-butylcatechol may contribute to antimicrobial activity by inducing oxidative stress within microbial cells. This redox imbalance can damage proteins, lipids, and nucleic acids, ultimately impairing cellular viability.

Tannins likely enhance antimicrobial efficacy through protein precipitation and enzyme inactivation, thereby disrupting essential metabolic processes and nutrient uptake. Saponins may further potentiate activity by interacting with membrane sterols, causing increased permeability and leakage of intracellular constituents, particularly in fungal cells.

Alkaloid-related constituents, including reserpine-type compounds, may interfere with microbial DNA replication and enzyme systems, contributing to bacteriostatic or bactericidal effects. Terpenoids may additionally disrupt membrane integrity due to their lipophilic nature, facilitating penetration into microbial cells.

Overall, the antimicrobial effect of *V. kotschyana* stem extract is best explained as a synergistic, multi-target phenomenon involving simultaneous disruption of microbial membranes, inhibition of intracellular enzymes, oxidative stress induction, and interference with genetic material. This multi-mechanistic action is particularly advantageous in addressing antimicrobial resistance, where single-target drugs are often rendered ineffective.



**Figure 6.** Proposed multi-target antimicrobial mechanism of action of phytochemicals identified in *Vernonia kotschyana* stem extract against microbial cells.

The schematic illustrates the putative antimicrobial pathways through which major phytochemical classes and LC–MS-detected metabolites in the ethanolic stem extract exert inhibitory effects on bacteria and fungi. Flavonoids and flavanones are proposed to interfere with nucleic acid synthesis, energy metabolism, quorum sensing, and membrane stability. Phenolic compounds, including hydroquinone- and catechol-type metabolites, may induce reactive oxygen species (ROS) generation and oxidative stress, leading to damage of proteins, lipids, and nucleic acids. Tannins are suggested to precipitate microbial proteins, inactivate enzymes, and impair

nutrient uptake, while saponins increase membrane permeability through interactions with membrane sterols, causing leakage of intracellular constituents. Alkaloid-related compounds may inhibit DNA replication, transcription, and essential enzyme systems, whereas terpenoids may destabilize membrane integrity and enhance penetration of other bioactive compounds. Collectively, these actions result in membrane depolarization, intracellular leakage, metabolic inhibition, and eventual microbial cell death, supporting the broad-spectrum antimicrobial activity of *V. kotschyana* stem extract.

## CONCLUSION

This study demonstrated that the ethanolic stem extract of *Vernonia kotschyana* contains several bioactive phytochemicals, including flavonoids, phenolics, tannins, saponins, terpenoids, and alkaloids, as confirmed by phytochemical screening and LC–MS analysis. The extract exhibited concentration-dependent antimicrobial activity against the tested bacteria and *Candida albicans*, with greater activity against Gram-positive organisms than Gram-negative bacteria. These findings indicate that *Vernonia kotschyana* stem possesses promising antimicrobial potential and may serve as a useful source of natural bioactive compounds for further antimicrobial studies.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this study.

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