



Research Article

Phytochemical, Antioxidant, and Antimicrobial Screening of *Vernonia amygdalina* Leaf Extract from Socotra, YemenKhlood A. A. H. Almakhzoum^{1*}, Khalil M.A. Qasem¹ and Hatem A. M. Saleh²¹ Department of Chemistry, Faculty of Education, Aden University, Aden, Yemen.² Department of Chemistry, Faculty of Science, Aden University, Aden, Yemen<https://doi.org/10.47372/uajnas.2025.n1.a03>

ARTICLE INFO	Abstract
Received: 01/09/2025 Accepted: 20/10/2025	<i>Vernonia amygdalina</i> Delile, commonly known as bitter leaf, is a widely recognized medicinal plant in Africa but remains largely underexplored in Yemen. This study aimed to investigate the phytochemical profile, antioxidant capacity, and antibacterial activity of the methanolic leaf extract of <i>V. amygdalina</i> collected from Socotra Island. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, cardiac glycosides, phenols, and steroids. Quantitative estimation demonstrated a high total flavonoid content (238.4 ± 4.2 mg QE/g) and substantial total phenolic content (122.6 ± 3.1 mg GAE/g). Antioxidant evaluation using the DPPH radical scavenging assay showed strong free radical inhibition, with 84.7% activity at 400 μ g/mL and an IC ₅₀ value of 96.4 μ g/mL, while the ferric reducing antioxidant power assay indicated strong electron-donating potential (OD ₇₀₀ = 0.732). Antibacterial testing against clinical isolates showed concentration-dependent inhibition. MIC values were 50 mg/mL for <i>Staphylococcus aureus</i> and <i>S. epidermidis</i> , while Gram-negative bacteria (<i>E. coli</i> , <i>K. pneumoniae</i> , and <i>P. aeruginosa</i>) exhibited higher MIC values (100 mg/mL) with MBCs exceeding 200 mg/mL. These findings support the traditional use of <i>V. amygdalina</i> and highlight the Socotra accession as a promising, locally accessible source of bioactive compounds with antioxidant and antibacterial potential. Further work should isolate active constituents and assess in vivo efficacy and safety.
Keywords: <i>Antibacterial activity;</i> <i>Antioxidant; Phytochemicals;</i> <i>Socotra;</i> <i>Vernonia amygdalina.</i>	

1. Introduction

Medicinal plants have long been recognized as prolific reservoirs of bioactive compounds that underpin traditional healthcare practices worldwide and have continually inspired modern drug discovery programs [1,2]. Ethnobotanical surveys estimate that up to 80% of the global population relies on botanical remedies for primary healthcare, particularly in low- and middle-income countries (LMICs), where access to conventional pharmaceuticals is limited [3]. These plants often contain diverse secondary metabolites, such as phenolic acids, flavonoids, alkaloids, terpenoids, saponins, and sesquiterpene lactones, which exert pharmacological effects ranging from free radical scavenging to antimicrobial action [1, 3]. The escalating burden of chronic diseases and antimicrobial resistance has renewed interest in exploring traditional medicinal flora as a

sustainable and cost-effective source of new therapeutic agents [2]. Consequently, systematic phytochemical and bioactivity screening remains essential for validating ethnomedicinal claims and identifying novel lead compounds for pharmaceutical development [3]. In this context, recent reviews have highlighted the multitarget antimicrobial mechanisms of plant extracts, including membrane disruption, biofilm inhibition, efflux pump interference, and nucleic acid synthesis inhibition, thereby underscoring their potential against drug-resistant pathogens [4].

Vernonia amygdalina Delile commonly known as “bitter leaf” is a perennial shrub of the family Asteraceae that is indigenous to tropical Africa but is now cultivated and consumed in various regions as both food and medicine [3, 5]. Ethnomedicinally, *V. amygdalina* leaves are used to manage ailments, including fever, gastrointestinal disorders,

*Department of Chemistry, Faculty of Education, Aden University, Aden, Yemen
E-mail address: kqasem1981@gmail.com

diabetes, hypertension, malaria, and helminthiasis [3, 5]. Phytochemical studies have consistently revealed that *V. amygdalina* is rich in flavonoids (e.g., luteolin, apigenin, quercetin), sesquiterpene lactones (e.g., vernolide, vernodalin), alkaloids, terpenoids, saponins, and tannins [1, 3, 5]. These compounds collectively contribute to the plant's pharmacological profile, with in vitro and in vivo studies reporting antioxidant, antimicrobial, antidiabetic, anticancer, anti-inflammatory, and hepatoprotective activities [6-8]. Recent comprehensive reviews have further confirmed its broad pharmacological spectrum, highlighting newly isolated compounds such as vernoamyoside A and B, and their antioxidant, antibacterial, and anticancer activities [1].

Yemen faces severe socioeconomic and public health challenges exacerbated by prolonged armed conflict. The deterioration of healthcare infrastructure, scarcity of diagnostic and therapeutic resources, and widespread poverty have contributed to the persistence and spread of infectious diseases, both of which lack effective vaccines or treatments and those transmitted through contaminated food and water [9-12]. Vulnerable populations, such as children, the elderly, and immunocompromised individuals, are disproportionately affected, and antimicrobial resistance is a growing concern [13- 15]. In such circumstances, the scientific validation of accessible medicinal plants with proven therapeutic potential, such as *V. amygdalina*, could provide cost-effective and culturally acceptable interventions to help mitigate disease burdens in resource-limited settings.

Comparative analyses of *V. amygdalina* extracts from different geographical origins have demonstrated substantial variability in phytochemical content and bioactivity, which is often influenced by environmental factors, soil composition, and extraction protocols. For example, Ethiopian polar leaf extracts showed strong DPPH radical scavenging activity ($IC_{50} = 17.4\text{--}27.9\text{ }\mu\text{g/mL}$), correlated with high total phenolic (85–112 mg GAE/g) and flavonoid (32–48 mg QE/g) contents [16]. Nigerian methanolic extracts achieved $IC_{50} \approx 20.6\text{ }\mu\text{g/mL}$ and exhibited notable antibacterial effects against *Staphylococcus aureus* and *Escherichia coli* (zones of inhibition: 12–20 mm at 100 mg/mL) [17,18]. In contrast, Ghanaian aqueous and hydroalcoholic extracts showed moderate radical scavenging ($IC_{50} \approx 60\text{--}80\text{ }\mu\text{g/mL}$) but superior α -glucosidase inhibition ($IC_{50} \approx 48\text{ }\mu\text{g/mL}$), reflecting differences likely driven by solvent polarity and edaphic conditions [19, 20]. These findings emphasize the need for region-specific investigations to capture local chemotypic variations [16, 21].

Recent experimental work confirmed significant antibacterial activity of ethanol and ethyl acetate extracts of

V. amygdalina against Gram-positive bacteria, with phenolic content reaching 154.7 mg GAE/g [22]. Similarly, Samuel et al, (2025) reported that novel metabolomic insights linking environmental stress factors to differential accumulation of flavonoids and sesquiterpene lactones, underscoring the importance of local adaptation [23].

Despite extensive research on *V. amygdalina* from West and East Africa, no published studies have characterized the phytochemical and bioactivity profiles of *V. amygdalina* leaves grown on Socotra Island, Yemen. Socotra's distinctive climatic zones, from arid coastal plains to humid mountainous regions, could significantly influence the biosynthesis and accumulation of secondary metabolites [1]. Studying *V. amygdalina* from this unique environment not only fills an important knowledge gap, but may also reveal novel phytoconstituents and bioactivities relevant to both local and global health contexts. This aligns with recent calls for bioprospecting underexplored habitats to identify unique chemotypes with potential for antimicrobial drug development [24].

Based on this background, we hypothesize that the unique environmental conditions of Socotra Island may influence the biosynthesis of secondary metabolites in *V. amygdalina*, resulting in distinct phytochemical profiles and, consequently, unique antioxidant and antimicrobial activities compared to accessions from other regions.

Accordingly, the present study aimed to perform a comprehensive screening of *V. amygdalina* leaves collected from Socotra Island, Yemen, focusing on (i) qualitative and quantitative phytochemical profiling, (ii) in vitro antioxidant activity using DPPH and complementary assays, and (iii) antimicrobial efficacy against representative bacterial pathogens. By integrating chemical and biological evaluations, this study aimed to expand the pharmacopeia of *V. amygdalina*, contribute to natural product drug discovery, and inform practical health strategies in Yemen and similar resource-limited settings.

2. Materials and Methods:

2.1 Plant Material Collection and Authentication

Fresh leaves of *Vernonia amygdalina* were collected during the summer of 2024 from Socotra Island, Yemen. The plant material was authenticated by Professor Hassan M. Ibrahim, Professor of Plant Taxonomy, Department of Life Sciences, Faculty of Science, Sana'a University. The leaves were washed thoroughly with distilled water to remove debris, shade-dried at ambient temperature (25–28 °C) for approximately two weeks. The dried leaves were subsequently ground into a fine powder using a sterile electric grinder (Panasonic; Model MX-EX1511), and the resulting particle size was standardized to approximately 1 mm using a sieve. The powdered material was stored in airtight containers at 4 °C until further use.

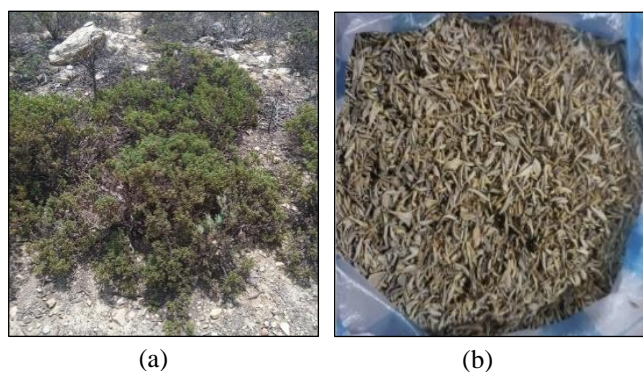


Figure 1. *Vernonia amygdalina*: (a) plant growing in its natural habitat on Socotra Island; (b) shade-dried leaves prepared for extraction

2.2 Preparation of Methanolic Extract

A known weight of 100 g of powdered leaf material was extracted with 1000 mL of 70% methanol (plant-to-solvent ratio 1:10 w/v) using the maceration technique with continuous agitation on an orbital shaker at 150 rpm for 24 h at room temperature (25 ± 2 °C). The extraction process was repeated three times to maximize yield. The mixture was filtered through Whatman No. 1 filter paper, and the resulting filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator. The crude extract was weighed, and the extraction yield (%) was calculated as $(\text{Weight of dried extract} / \text{Initial weight of plant material}) \times 100$. The dried material was stored at 4 °C in amber-colored glass vials. For biological assays, the extract was reconstituted in 10% dimethyl sulfoxide (DMSO), and working solutions were prepared at concentrations of 25, 50, 100, and 200 mg/mL [25].

2.3 Phytochemical Screening

2.3.1 Qualitative Analysis

Preliminary phytochemical screening of the methanolic extract was performed using standard qualitative procedures to identify the major classes of secondary metabolites. The presence of alkaloids was confirmed by Mayer's and Dragendorff's tests, whereas flavonoids were detected using the aluminum chloride (AlCl_3) colorimetric test. Tannins and phenolic compounds were detected using the ferric chloride (FeCl_3) assay, and saponins were identified using the froth test, which relies on the persistence of foam formation. Terpenoids were detected using Salkowski's test, whereas cardiac glycosides were confirmed using the Keller–Killiani reaction. Steroids were identified by the Liebermann–

Burchard reaction, and sesquiterpene lactones were verified using Kedde's reagent test. The results demonstrated that the methanolic extract of *Vernonia amygdalina* contains a wide spectrum of secondary metabolites with potential pharmacological relevance [26].

2.3.2 Quantitative Estimation

In addition to qualitative detection, the extract was subjected to quantitative estimation of its phenolic and flavonoid content. The total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent method, with results expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g). The total flavonoid content (TFC) was estimated using the aluminum chloride colorimetric method, and the values were expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE/g). These quantitative assays provide a more precise evaluation of phenolic and flavonoid levels that contribute to the antioxidant and antimicrobial properties of the extract [27, 28].

2.4 Antioxidant Assays

The antioxidant capacity of the extract was evaluated using free radical scavenging and reducing power assays. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was conducted by preparing extract solutions with concentrations ranging from 50 to 400 $\mu\text{g/mL}$. Absorbance was measured at 517 nm after incubation, and the percentage inhibition was calculated in comparison with the control. The half-maximal inhibitory concentration (IC_{50}) values were determined from dose–response curves. In addition, the ferric reducing antioxidant power (FRAP) assay was performed to assess the electron-donating ability of the extract by measuring the reduction of Fe^{3+} to Fe^{2+} at 700 nm [29–31].

2.5 Antibacterial Activity

The antibacterial activity of the extract was determined using the agar well diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines with minor modifications. Five medical bacterial isolates were tested: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The isolates were obtained from hospital laboratories in Sana'a, Yemen, and identified using standard microbiological and biochemical methods. Bacterial suspensions were adjusted to a turbidity of 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL, and were spread evenly onto Mueller–

Hinton agar (MHA) plates. Wells of 6 mm diameter were bored aseptically into the agar, and 100 μ L of each extract concentration (25, 50, 100, and 200 mg/mL) was introduced into separate wells. Plates were pre-incubated at room temperature for 30 min to allow diffusion, followed by incubation at 37 °C for 24 h. Ciprofloxacin (5 μ g/mL) was used as the positive control and 10% DMSO served as the negative control. The antibacterial effect was evaluated by measuring the diameters of the inhibition zones in millimeters [25, 32, 33].

2.6 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC values of the extracts were determined directly from the results of the agar diffusion assay at the tested concentrations. MIC was defined as the lowest concentration of the extract that produced a visible inhibition zone against the test organisms. To establish the MBC, samples were taken from the margins of the inhibition zones at different concentrations and subcultured onto fresh MHA plates. The MBC was defined as the lowest concentration at which no bacterial growth occurred after incubation. This approach allowed for the determination of MIC and MBC values consistent with the tested concentration range (25–200 mg/mL) [34, 35].

2.7 Data Presentation

All assays were performed in triplicates. The results are expressed as mean \pm standard deviation (SD). No further statistical analyses were applied beyond the descriptive presentation of the mean and variability.

3. Results and Discussion

3.1. Preliminary Phytochemical Screening

Preliminary phytochemical screening of *Vernonia amygdalina* leaf extract from Socotra revealed the presence of several classes of bioactive compounds with notable pharmacological relevance (Table 1). Alkaloids, flavonoids, and saponins were detected in abundant amounts, whereas tannins, terpenoids, phenols, and sesquiterpene lactones were present at moderate levels; in contrast, steroids and cardiac glycosides were observed only in trace quantities. The predominance of polar and semi-polar metabolites such as flavonoids, phenols, and saponins can be attributed to the use of 70% methanol as the extraction solvent in combination with shaker-assisted maceration. Hydroalcoholic solvents are well documented for their ability to solubilize a wide range of phenolic and glycosidic compounds, while gentle extraction conditions help preserve

thermolabile metabolites, such as glycosides. This solvent-dependent profile aligns with previous studies on *V. amygdalina* conducted in Africa and Asia, where methanolic extracts consistently yielded higher levels of flavonoids and phenolics than aqueous or non-polar extractions. For example, Tunasamy et al. [36] and Tura et al. [22] reported similarly elevated levels of flavonoids and phenolic compounds, which were strongly correlated with radical scavenging and antibacterial activities. The abundant saponins in the socotra extract further support its traditional application as an antimicrobial agent, since saponins are known to compromise bacterial membrane integrity. The moderate presence of tannins and terpenoids corresponds with previous phytochemical screenings that attributed astringent and anti-inflammatory activities to these classes. Taken together, these findings not only reinforce the chemical richness of *V. amygdalina* but also provide the first systematic documentation of its phytochemical profile in the Yemeni context, where such investigations have been scarce.

Table 1. Preliminary Phytochemical Constituents of *Vernonia amygdalina* Leaf Extract from Socotra

Phytochemical Class	Presence in Socotra Sample	Method Used (Qualitative Assay)
Alkaloids	+++ (abundant)	Mayer's and Dragendorff's reagent tests
Flavonoids	+++ (abundant)	Aluminum chloride (AlCl ₃) colorimetric test
Tannins	++ (moderate)	Ferric chloride (FeCl ₃) test
Saponins	+++ (abundant)	Froth (foam) test
Terpenoids	++ (moderate)	Salkowski's test
Cardiac Glycosides	+ (trace)	Keller–Killiani test
Phenols	++ (moderate)	Ferric chloride (FeCl ₃) test
Steroids	+ (trace)	Liebermann–Burchard reaction
Sesquiterpene Lactones	++ (moderate)	Kedde's reagent test

3.2. Total Phenolic and Flavonoid Contents

Quantitative determination of the total phenolic and flavonoid content of the *V. amygdalina* leaf extract from Socotra revealed substantial levels of these phytochemicals (Table 2). The extract exhibited 122.6 ± 3.1 mg GAE/g of phenolics and 238.4 ± 4.2 mg QE/g of flavonoids. These values corroborate the preliminary phytochemical screening (Table 1), in which flavonoids were identified as abundant and phenols as moderate. The unusually high flavonoid content recorded here exceeds the values reported in comparable studies of African accessions, where Ethiopian and Nigerian extracts typically yield 32–48 mg QE/g and 20–40 mg QE/g, respectively [16–18]. Such elevated flavonoid accumulation in the Socotra sample may reflect unique edaphic and ecological conditions that influence secondary metabolism. Since flavonoids and phenolics are known to act as major contributors to antioxidant and

antimicrobial activities, the richness of these compounds in the Socotra accession provides a plausible biochemical basis for its bioactivity [37]. Additionally, recent reviews emphasized that flavonoids and phenolics are key drivers of antioxidant and antimicrobial activity through multitarget mechanisms [4].

Table 2. Total phenolic and flavonoid contents of *Vernonia amygdalina* leaf extract from Socotra.

Parameter	Observed Value
Total Phenolic Content (mg GAE/g)	122.6 ± 3.1
Total Flavonoid Content (mg QE/g)	238.4 ± 4.2

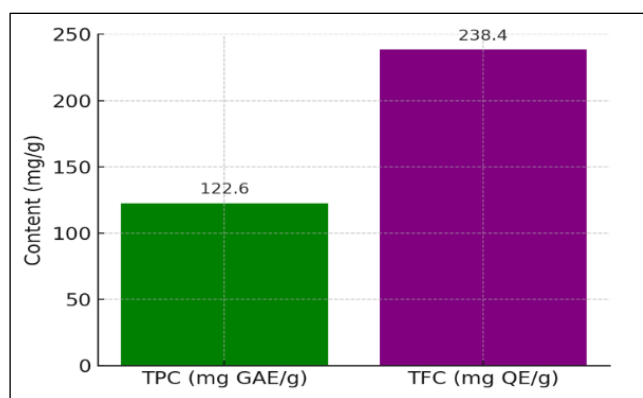


Figure 2. Total phenolic content (TPC) and total flavonoid content (TFC) of *V. amygdalina* leaf extract.

3.3. Antioxidant Assays

The antioxidant potential of the socotra extract was further evaluated using free radical scavenging and reducing power assays (Figures 1–3). In the DPPH radical assay, the extract showed 84.7 ± 2.0% inhibition at 400 µg/mL with an IC₅₀ of 96.4 µg/mL, demonstrating considerable radical quenching activity, albeit weaker than that of standard antioxidants such as ascorbic acid (IC₅₀ < 30 µg/mL). The FRAP assay confirmed its reducing capacity, with an absorbance of 0.732 at 700 nm. These values are consistent with the elevated phenolic and flavonoid content reported in Table 2, underscoring their contribution to the observed antioxidant activity.

When compared with earlier studies, Ethiopian polar extracts exhibited stronger DPPH scavenging (IC₅₀ = 17.4–27.9 µg/mL) alongside phenolic contents of 85–112 mg GAE/g and flavonoid contents of 32–48 mg QE/g [16]. Nigerian methanolic extracts likewise demonstrated potent activity (IC₅₀ ≈ 20.6 µg/mL) and correlated antibacterial effects [17,18]. In contrast, aqueous and hydroalcoholic extracts of Ghanaian displayed moderate radical scavenging

(IC₅₀ ≈ 60–80 µg/mL) but superior α-glucosidase inhibition (IC₅₀ ≈ 48 µg/mL) [19,20]. Relative to these studies, Socotra extract showed markedly higher flavonoid content but comparatively weaker radical scavenging, suggesting that chemotypic variation, soil composition, and extraction methodology likely shape the balance of bioactivity [21].

Table 3. Antioxidant assay parameters of *Vernonia amygdalina* leaf extract from Socotra.

Parameter	Observed Value
DPPH Scavenging Activity (% at 400 µg/mL)	84.7 ± 2.0
IC ₅₀ (µg/mL)	96.4
Ferric Reducing Antioxidant Power (OD at 700 nm)	0.732

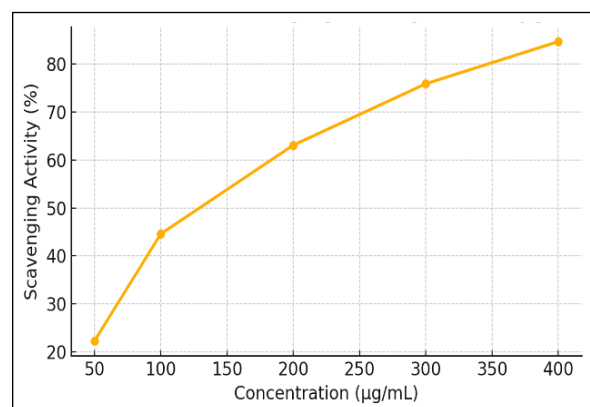


Figure 3. DPPH radical scavenging activity of *V. amygdalina* extract at different concentrations (50–400 µg/mL).

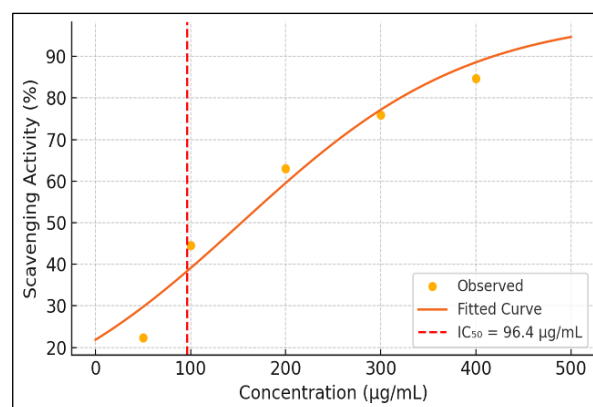


Figure 4. IC₅₀ determination of *V. amygdalina* extract based on the fitted dose–response curve (50–400 µg/mL).

3.3. Antibacterial Activity

The antibacterial activity of *Vernonia amygdalina* leaf extract was evaluated across a concentration range of 25–200 mg/mL (Table 3, Figure 4). A clear dose–response

Table 3. Antibacterial activity of *Vernonia amygdalina* leaf extract at different concentrations (simulated example)

Test Organism	25 mg/mL	50 mg/mL	100 mg/mL	200 mg/mL	MIC (mg/mL)	MBC (mg/mL)
<i>Staphylococcus aureus</i>	9.2 ± 0.3 mm	12.8 ± 0.4 mm	15.2 ± 0.4 mm	17.5 ± 0.5 mm	50	100
<i>Staphylococcus epidermidis</i>	10.1 ± 0.3 mm	13.9 ± 0.5 mm	16.1 ± 0.6 mm	18.3 ± 0.6 mm	50	100
<i>Pseudomonas aeruginosa</i>	7.0 ± 0.2 mm	9.8 ± 0.3 mm	12.8 ± 0.3 mm	14.6 ± 0.4 mm	100	>200
<i>Escherichia coli</i>	6.5 ± 0.2 mm	8.7 ± 0.3 mm	11.5 ± 0.2 mm	13.4 ± 0.3 mm	100	>200
<i>Klebsiella pneumoniae</i>	6.8 ± 0.2 mm	9.1 ± 0.3 mm	12.0 ± 0.3 mm	14.0 ± 0.4 mm	100	>200

relationship was observed, with inhibition zones increasing proportionally with the concentration. Among the tested organisms, *Staphylococcus epidermidis* and *Staphylococcus aureus* were the most susceptible, exhibiting inhibition zones of 16.1 ± 0.6 mm and 15.2 ± 0.4 mm, respectively, at 100 mg/mL, with MIC values of 50 mg/mL and MBC values of 100 mg/mL. In contrast, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were less sensitive, requiring higher concentrations (≥ 100 mg/mL) to achieve significant inhibition, with MBC values not reaching 200 mg/mL. These findings are consistent with previous reports by Ijeh and Ejike [38] and Tunasamy et al. [36], who also observed stronger susceptibility in gram-positive bacteria than in gram-negative bacteria, likely because of differences in cell wall structure and permeability. Minor variations between our results and earlier studies may also reflect differences in environmental growing conditions affecting phytochemical composition, the extraction solvents and protocols applied, or the specific microbial strains tested.

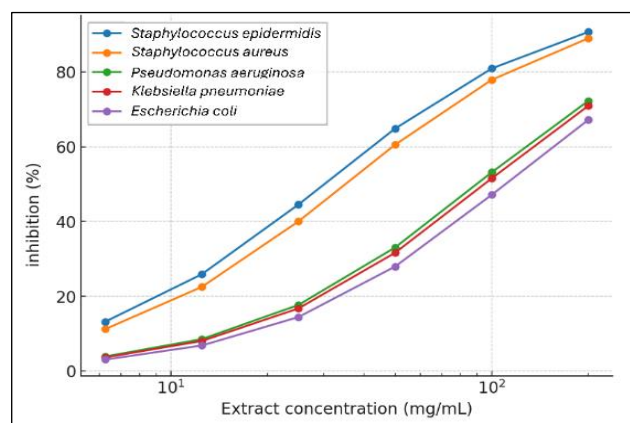


Figure 5. Dose–response curves of *Vernonia amygdalina* leaf extract against selected bacterial strains, showing inhibition zones (mm) across.

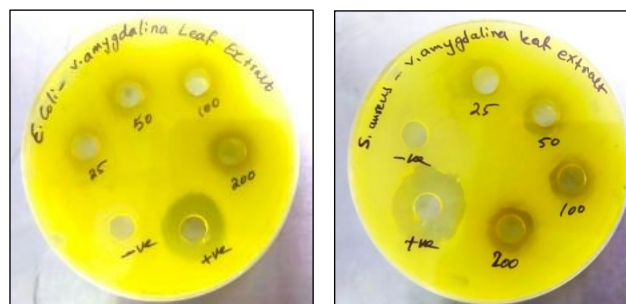


Figure 6. Antibacterial activity of *Vernonia amygdalina* leaf extract at different concentrations (25, 50, 100, and 200 mg/mL) against clinical isolates: (a) *Escherichia coli*; (b) *Staphylococcus aureus*. Ciprofloxacin was used as positive control (+ve) and 10% DMSO as negative control (–ve). different concentrations

The pursuit of novel antimicrobial agents from natural sources has become increasingly critical, particularly in low-income countries where the burden of infectious diseases remains disproportionately high. In fragile health systems, such as those of conflict-affected nations like Yemen, the lack of advanced diagnostic tools, the widespread reliance on conventional and often outdated treatment approaches, and the limited awareness of transmission routes among vulnerable populations exacerbates the spread of infections [39-41]. Therefore, the exploration of medicinal plants as alternative antimicrobial agents is not only scientifically relevant, but also a public health priority. Plant-based remedies are locally accessible, culturally accepted, and less costly than synthetic drugs, making them especially valuable in regions where economic hardship and warfare restrict access to modern health care. Within this context, validating the therapeutic potential of the *Vernonia amygdalina* contributes to bridging the gap between traditional knowledge and evidence-based medicine, offering a sustainable and locally adaptable solution to combat infectious diseases.

3.4. Limitations of the Study

Despite the promising findings, several limitations should be acknowledged. First, the study was restricted to in vitro assays, which, while informative, do not fully replicate complex interactions occurring within a living organism. Second, only a limited range of bacterial strains was tested; thus, the broader antimicrobial spectrum of the extract remains to be elucidated. Third, phytochemical characterization was based on standard screening methods and quantitative assays, which provide only preliminary insights into the active compounds. More advanced analytical techniques, such as HPLC, LC-MS, and NMR, are required to precisely identify and isolate the bioactive constituents. Finally, this study did not assess the potential cytotoxicity or safety profiles of the extracts, which are essential considerations for future therapeutic applications.

4. Conclusions

In summary, the methanolic extract of *Vernonia amygdalina* leaves collected from Socotra, Yemen, demonstrated notable phytochemical richness and specifically showed strong antioxidant activity and antibacterial effects against Gram-positive bacteria in vitro. These findings highlight the potential of plants as a valuable source of natural therapeutic agents. However, translating these results into practical medical applications requires further investigation including compound isolation, mechanistic studies, toxicity profiling, and in vivo validation. Considering the urgent global need for affordable and effective antimicrobial solutions, particularly in resource-limited and conflict-affected regions, this research highlights both the promise and necessity of continued exploration of *Vernonia amygdalina* as a complementary strategy in the fight against infectious diseases.

References

1. S. Degu, A. Meresa, Z. Animaw, M. Jegnie, A. Asfaw, and G. Tegegn, "Vernonia amygdalina: A comprehensive review of the nutritional makeup, traditional medicinal use, and pharmacology of isolated phytochemicals and compounds," *Front. Nat. Prod.*, vol. 3, p. 1347855, 2024, doi: 10.3389/fntpr.2024.1347855.
2. O. Kadiri and B. Olawoye, "Vernonia amygdalina: An underutilized vegetable with nutraceutical potentials – a review," *Turk. J. Agric. Food Sci. Technol.*, vol. 4, no. 9, pp. 763–768, 2016, doi: 10.24925/turjaf.v4i9.763-768.570.
3. O. Alara, A. Nour, S. Mudalip, and O. Olalere, "Phytochemical and pharmacological properties of Vernonia amygdalina: A review," *J. Chem. Eng. Ind. Biotechnol.*, vol. 2, no. 1, pp. 80–96, 2017, doi: 10.15282/jceib.v2i1.3871.
4. S. Al-Arnoot, Q. Y. Abdullah, M. A. Al-Maqtari, H. M. Ibrahim, H. A. Al-Shamahy, E. M. Salah, ... B. Al-Akhali, "Multitarget Antimicrobial Mechanisms of Plant Extracts: A Review of Harnessing Phytochemicals Against Drug-Resistant Pathogens," *Sana'a University Journal of Medicine and Health Sciences*, vol. 19, no. 4, pp. 292-309, 2025, doi: 10.59628/jchm.v19i4.1936
5. N. Uchegbu, K. Oni, and T. Olagunju, "Vernonia amygdalina processing as a functional ingredient: Oil-thermal influence on antioxidant, vitamin, mineral, and functional group retention," *J. Food Process. Preserv.*, vol. 46, no. 10, 2022, doi: 10.1111/jfpp.16846.
6. P. Hasibuan, U. Harahap, P. Sitorus, and D. Satria, "The anticancer activities of Vernonia amygdalina Delile leaves on 4T1 breast cancer cells through phosphoinositide 3-kinase (PI3K) pathway," *Heliyon*, vol. 6, no. 7, p. e04449, 2020, doi: 10.1016/j.heliyon.2020.e04449.
7. V. Rosalina, P. Hasibuan, D. Satria, E. Meiyanto, D. Putra, M. Chatri, and E. Septisetyani, "Antioxidant activity of flavonoid rich fraction of Vernonia amygdalina Delile leaves," *Int. J. Appl. Pharm.*, pp. 6–10, 2024, doi: 10.22159/ijap.2024v16s4.52249.
8. A. Prananda, A. Dalimunthe, U. Harahap, R. Syahputra, S. Nugraha, P. Situmorang, and M. Harahap, "Vernonia amygdalina protects against doxorubicin-induced hepatic and renal damage in rats: Mechanistic insights," *Pharmacia*, vol. 70, no. 3, pp. 825–835, 2023, doi: 10.3897/pharmacia.70.e112425.
9. M. N. Q. Al-Bana et al., "Seroprevalence and risk factors of herpes simplex virus type 2 among pregnant women in Dhamar city, Yemen," *MOJ Biol. Med.*, vol. 6, no. 3, pp. 108–114, 2021, doi: 10.15406/mojbm.2021.06.00141.
10. S. Al-Arnoot, S. M. Alghalibi, Q. Y. M. Abdullah, and A. Al-Thobhani, "Screening for susceptibility to Cytomegalovirus infection among pregnant women in Yemen," *J. Gynecol. Women's Health*, vol. 18,

- no. 3, p. 4, 2020, doi: 10.19080/JGWH.2020.18.5559890.
11. A. Al-Thobhani, Q. Y. Abdullah, S. M. Alghalibi, and S. Al-Arnoot, "Seroprevalence of Rubella virus antibodies among pregnant women in Hodeidah city, Western Yemen," *J. Hum. Virol. Retrovirol.*, vol. 10, no. 1, pp. 7–10, Jan. 2023, doi: 10.15406/jhvr.2023.10.00256.
12. Q. Y. M. Abdullah, Y. A. H. Al-Zuraei, M. F. Al-Helali, B. Al-Akhali, A. A. Abu Al-rejal, and S. Al-Arnoot, "Microbiological Quality Assessment of Ice Cream Produced in Yemen," *Sana'a Univ. J. Appl. Sci. Technol.*, vol. 3, no. 2, pp. 749–755, 2025, doi: 10.59628/jast.v3i2.1525.
13. M. N. Q. Al-Bana, S. M. S. Al-Ghalibi, Q. Y. M. Abdullah, S. Al-Arnoot, A. Al-Thobhani, and B. Al-Akhali, "A systematic review of the prevalence of *Campylobacter jejuni* in chicken populations within Middle East and North Africa (2014–2025)," *Sana'a Univ. J. Appl. Sci. Technol.*, vol. 3, no. 3, pp. 891–897, 2025, doi: 10.59628/jast.v3i3.1672.
14. Q. Y. M. Abdullah et al., "Seroprevalence of brucella infection among pregnant women in Sana'a city, Yemen," *Biom. Biostat. Int. J.*, vol. 7, no. 5, pp. 445–451, 2018, doi: 10.15406/bbij.2018.07.00245.
15. K. N. Q., A. A. M. Alaa, A. K. Amal, A. Q. M. Amira, F. A. A. Fatima, K. W. A. Kholood, M. N. M. Marwa, R. H. A. Rasha, and S. M. S. Shifa, "Isolation and Antimicrobial Susceptibility Profiles of Microorganisms Causing Urinary Tract Infection among Patients in Aden City, Yemen," *Electron. J. Univ. Aden Basic Appl. Sci.*, vol. 3, no. 3, pp. 163–175, 2022, doi: 10.47372/ejua-ba.2022.3.182.
16. E. Hussien and S. Endalew, "In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of *Vernonia amygdalina*," *BMC Complement. Med. Ther.*, vol. 23, no. 1, 2023, doi: 10.1186/s12906-023-03923-y.
17. S. I. Maryam, H. Abdulsalam, M. M. Namadina, A. Y. Yunusa, and S. A. Shehu, "Phytochemical, antioxidant and antibacterial activities of *Vernonia amygdalina* Del. leaf extract," *BEST J.*, vol. 21, no. 1, pp. 1–11, 2024, doi: 10.4314/bestj.v21i1.1.
18. P. Akinduti, V. Emoh-Robinson, H. Obamoh-Triumphant, Y. Obafemi, and T. Banjo, "Antibacterial activities of plant leaf extracts against multi-antibiotic resistant *Staphylococcus aureus* associated with skin and soft tissue infections," *BMC Complement. Med. Ther.*, vol. 22, no. 1, 2022, doi: 10.1186/s12906-022-03527-y.
19. M. Muhammad, E. Putra, H. Cintya, and D. Satria, "The effect of solvent towards antioxidant activity of *Vernonia amygdalina* Delile leaves," *Rasayan J. Chem.*, vol. 16, no. 2, pp. 760–765, 2023, doi: 10.31788/rjc.2023.1628059.
20. B. Fagbohunka, O. Nwolisah, K. Odufuwa, and P. Adetayo, "In vitro study of the inhibitory potentials of cold and hot aqueous extract of *Vernonia amygdalina*, *Calotropis procera*, and *Persea americana* on α -glucosidase," *Ann. Health Res.*, vol. 10, no. 2, pp. 152–162, 2024, doi: 10.30442/ahr.1002-07-235.
21. F. Tekou, D. Kuate, P. Nguekouo, C. Woumbo, and J. Oben, "Effect of cooking treatments on the phytochemical composition and antidiabetic potential of *Vernonia amygdalina*," *Food Sci. Nutr.*, vol. 6, no. 6, pp. 1684–1691, 2018, doi: 10.1002/fsn3.732.
22. A. M. Tura, M. Anbessa, E. D. Tulu, and B. Z. Tilinti, "Exploring *Vernonia amygdalina*'s leaf extracts for phytochemical screening and its antibacterial activities," *Int. J. Food Prop.*, vol. 27, no. 1, pp. 960–974, 2024, doi: 10.1080/10942912.2024.2377242.
23. E. Samuel et al., "Antimicrobial Activities of *Vernonia amygdalina* extract exhibit substantial antimicrobial activity against biofilm forming microbial pathogens," *bioRxiv*, preprint 2025.10.1101/2025.05.22.655479
24. U. J. Ugochi et al., "Therapeutic potential of *Chromolaena odorata*, *Vernonia amygdalina*, and *Cymbopogon citratus* against pathogenic Bacteria," *Scientific Reports*, vol. 15, article 217, 2025, doi: 10.1038/s41598-024-84696-3.
25. E. F. Al-Awadhi, S. Al-Arnoot, A. A. Ali, et al., "Antimicrobial efficacy of aqueous and ether extracts of clove (*Syzygium aromaticum*) against clinically significant gram-negative and gram-positive bacteria," *J. Bacteriol. Mycol. Open Access*, vol. 13, no. 2, pp. 129–133, 2025. [Online]. Available: <https://medcraveonline.com/JBMOA/JBMOA-13-00412.pdf>.
26. M. A. Edeoga, D. E. Okwu, and B. O. Mbaebie, "Phytochemical constituents of some Nigerian medicinal plants," *Afr. J. Biotechnol.*, vol. 4, no. 7, pp. 685–688, 2005. [Online]. Available: https://academicjournals.org/article/article1380041849_Edeoga%20et%20al.pdf
27. V. L. Singleton, R. Orthofer, and R. M. Lamuela-Raventós, "Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent," *Methods Enzymol.*, vol. 299, pp. 152–178, 1999, doi: 10.1016/S0076-6879(99)99017-1.
28. S. K. Algfri, K. S. Ali, G. A. Naser, and A. Bin Shuaib, "Physicochemical and phytochemical analysis, antioxidant and antimicrobial evaluation of aerial parts of *Jatropha spinosa*, *Jatropha variegata* and *Euphorbia milii*," *Electron. J. Univ. Aden Basic Appl. Sci.*, vol. 6, no. 1, pp. 61–74, 2025, doi: 10.47372/ejua-ba.2025.1.425.
29. I. F. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay," *Anal. Biochem.*, vol. 239, no. 1, pp. 70–76, 1996, doi: 10.1006/abio.1996.0292.

30. S. K. Algfri, G. A. Naser, R. S. Rageh, A. Shuaib, and A. H. Nasser, "Anatomical, physicochemical, phytochemical, antioxidant and antimicrobial investigations of *Tagetes minuta* L. aerial parts," *Electron. J. Univ. Aden Basic Appl. Sci.*, vol. 5, no. 4, pp. 507–520, 2024, doi: 10.47372/ejua-ba.2024.4.408.
31. K. M. Naji, M. A. Al-Maqtari, A. A. Al-Asbahi, et al., "Effect of daily chewing soft buds and leaves of *Catha edulis* (Khat) on the antioxidant defense system and oxidative stress markers in blood," *Arab J. Sci. Eng.*, vol. 40, pp. 1–6, 2015, doi: 10.1007/s13369-014-1492-x.
32. Clinical and Laboratory Standards Institute (CLSI), *Performance Standards for Antimicrobial Susceptibility Testing; 30th Informational Supplement (M100)*. Wayne, PA: CLSI, 2020. [Online]. Available: <https://clsi.org/standards/products/microbiology/documents/m100>
33. Q. A. A. Al Maqtari and M. A. Al Maqtari, "In vitro antibacterial activity of different Yemeni leaves extracts of *Lawsonia inermis* against some bacterial pathogens," *Int. J. Res. Stud. Biosci.*, vol. 2, pp. 52–57, 2014. [Online]. Available: <https://www.arcjournals.org/pdfs/ijrsb/v2-i10/8.pdf>
34. I. Wiegand, K. Hilpert, and R. E. W. Hancock, "Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances," *Nat. Protoc.*, vol. 3, no. 2, pp. 163–175, 2008, doi: 10.1038/nprot.2007.521.
35. Clinical and Laboratory Standards Institute (CLSI), *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—11th Edition (M07)*. Wayne, PA: CLSI, 2020. [Online]. Available: <https://clsi.org/standards/products/microbiology/documents/m07>
36. K. Tunasamy, N. Suryadevara, and T. Athimoolam, "Screening of *Vernonia amygdalina* leaf extracts for antioxidant and antimicrobial activity," *Mater. Today: Proc.*, vol. 16, pp. 1809–1818, 2019, doi: 10.1016/j.matpr.2019.06.055.
37. Q. Y. M. Abdullah et al., "Boswellia sacra in South Arabian Peninsula: A review," *Sana'a Univ. J. Appl. Sci. Technol.*, vol. 3, no. 1, pp. 604–620, 2025, doi: 10.59628/jast.v3i1.1475.
38. I. I. Ijeh and C. E. C. Ejike, "Current perspectives on the medicinal potentials of *Vernonia amygdalina* Del.," *J. Med. Plants Res.*, vol. 5, no. 7, pp. 1051–1061, Apr. 2011. [Online]. Available: https://academicjournals.org/article/article1380529017_Ijeh%20and%20Ejike.pdf.
39. S. Al-Arnoot, M. S. S. Alghalibi, Q. Abdullah, and A. Al-Thobhani, "Seroprevalence, risk factors and awareness of Cytomegalovirus infection among pregnant women in Hodeidah city, Western Yemen," in *Proc. Sci. Conf. Med. Labs, Sana'a, Yemen: Sana'a Univ.*, 2017.
40. M. Nowihi et al., "Comparison of standard agglutination test and enzyme-linked immunosorbent assay to detect brucella infection in Yemeni pregnant women," *J. Microbiol. Exp.*, vol. 5, no. 5, p. 00160, 2017, doi: 10.15406/jmen.2017.05.00160.
41. S. Al-Arnoot, Q. Y. Abdullah, A. Al-Thobhani, M. N. Al-Bana, B. Al-Akhali, S. M. Al-Ghalebi, et al., "Seroprevalence and research gaps in cytomegalovirus studies in Yemen: A comprehensive review," *Electron. J. Univ. Aden Basic Appl. Sci.*, vol. 6, no. 1, pp. 75–82, 2025, doi: 10.47372/ejua-ba.2025.1.426.



مجلة جامعة عدن للعلوم الطبيعية والتطبيقية

Journal homepage: <https://uajnas.adenuniv.com>

بحث علمي

الفحص الفيتوكيميائي والمضاد للأكسدة والميكروبات لأوراق المر (*Vernonia amygdalina*) من سقطرى، اليمن

خلود عبدالله احمد حسين المخزوم^{1*}، خليل منصور عبدة قاسم¹، حاتم احمد محمد صالح²

1. قسم الكيمياء، كلية التربية، جامعة عدن، اليمن.

2. قسم الكيمياء، كلية العلوم، جامعة عدن، اليمن.

<https://doi.org/10.47372/uajnas.2025.n1.a03>

المفاتيح البحث	الملخص
<p>التسليم: 2025 / 09 / 01</p> <p>القبول: 2025 / 10 / 20</p> <p>كلمات مفتاحية:</p> <p>النشاط المضاد للبكتيريا؛ المضادات للأكسدة؛ الفيتوكيميكال؛ سقطرى؛ <i>Vernonia amygdalina</i></p>	<p>تُعد نباتات <i>Vernonia amygdalina</i>، المعروفة محليًا باسم "الورق المر"، من النباتات الطبية واسعة الانتشار في إفريقيا، إلا أنها ما تزال غير مدروسة بشكل كافٍ في اليمن. هدفت هذه الدراسة إلى تقييم التركيب الكيميائي النباتي، والقدرة المضادة للأكسدة، والنشاط المضاد للبكتيريا لمستخلص الأوراق الميثانولي التي جُمعت من جزيرة سقطرى. أظهرت الفحوصات الكيميائية النباتية الأولية وجود مركبات ثانوية فعالة تشمل القلويدات، الفلافونويدات، التانينات، الصابونينات، التربينويدات، الجليكوسيدات القلبية، الفينولات، والستيرويدات. كما بينت التحاليل الكمية ارتفاع محتوى الفلافونويدات الكلي (4.2 ± 238.4 ملغم مكافئ كيرسيتين/غرام) ومحتوى فينولي ملحوظ (122.6 ± 3.1 ملغم مكافئ حمض الغاليك/غرام). وأظهرت الاختبارات المضادة للأكسدة باستخدام طريقة DPPH قدرة عالية على تثبيط الجذور الحرة بنسبة 84.7% عند تركيز 400 ميكروغرام/مل، مع قيمة IC_{50} بلغت 96.4 ميكروغرام/مل، فيما أظهر اختبار القدرة الاختزالية للحديد فعالية قوية في منح الإلكترونات ($OD_{700} = 0.732$). أما التقييم المضاد للبكتيريا بطريقة الانتشار في الآجار فقد أوضح فعالية مثبطة تعتمد على التركيز ضد البكتيريا موجبة وسالبة الغرام، مما يدل على الطيف الواسع للمستخلص. تؤكد هذه النتائج أن نبات <i>V. amygdalina</i> من سقطرى يُعد مصدرًا واعدًا للمركبات النشطة حيويًا ذات خصائص مضادة للأكسدة والبكتيريا، مما يدعم استخدامه التقليدي ويفتح المجال لتطويره كعامل علاجي تكميلي خصوصًا في البيئات محدودة الموارد. وتوصي الدراسة بإجراء أبحاث مستقبلية لعزل المركبات الفعالة، وتوضيح آليات عملها، واختبار فعاليتها وسلامتها في النماذج الحية.</p>