Interdisciplinary Research in Medical Sciences Specialty
ISSN: 3062-4401
2024, Volume 4, Issue 1, Page No: 144-153

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Evaluation of Methanolic Extracts from Six Ethnomedicinal Plants for Antibacterial Efficacy and Bioactive Constituents

Daniel O'Connor[1], Patrick Walsh[1], Brian Murphy[1]*

[1]Department of Translational Health Sciences, Faculty of Medicine, Trinity College Dublin, Dublin, Ireland.

***E-mail** ✉ brian.murphy.ths@outlook.com

Received: 01 February 2024; **Revised:** 06 April 2024; **Accepted:** 09 April 2024

ABSTRACT

This work aimed to investigate both the antibacterial potential and the phytochemical composition of six medicinal plants long utilized in local traditional remedies for various illnesses. Methanol-based extracts from these species were evaluated for antimicrobial performance through Agar well diffusion assays and Microtiter broth dilution techniques. Among the tested materials, the root extract of Andrachne aspera produced the largest inhibition zones against S. epidermidis, showing statistically significant values ($p < 0.05$) of 33 ± 0.17 at 100 mg/ml and 33.5 ± 0.84 at 200 mg/ml. The next strongest activity was observed from Dichrostachys cinerea leaf extract at 200 mg/ml, reaching 24.8 ± 0.41 against the same bacterium. Minimum inhibitory concentration results included 1.0 ± 0.0 for Andrachne aspera against E. faecalis, while Dichrostachys cinerea registered 2.0 ± 0.0 against S. aureus. Phytochemical screening indicated that both species were rich in Polyphenols and Flavonoids. These findings highlight the need for further in-depth research, including safety evaluations, structural analyses, and isolation of active secondary metabolites with potential application as new antimicrobial drugs.

Keywords: Agar well diffusion, Antibacterial activity, Gram-negative bacteria, Gram-positive bacteria, Minimum inhibitory concentration

How to Cite This Article: O'Connor D, Walsh P, Murphy B. Evaluation of Methanolic Extracts from Six Ethnomedicinal Plants for Antibacterial Efficacy and Bioactive Constituents. Interdiscip Res Med Sci Spec. 2024;4(1):144-53. https://doi.org/10.51847/zbJZnYvzjS

Introduction

Bacteria, which are single-celled prokaryotes, inhabit nearly every environment, and most play essential roles in maintaining ecological function. Beneficial species help recycle biological materials and support decomposition processes [1], and they also contribute substantially to agricultural systems and industrial production [2]. Only a limited subset of bacteria causes disease, yet these pathogenic species are capable of generating infections that threaten community health [3]. Without treatment, bacterial illnesses may progress to severe or life-endangering conditions, underscoring their public health significance.

The standard approach for managing bacterial infections involves the use of antibiotics chosen to target the specific pathogen involved [4]. Nevertheless, even with increased antibiotic availability, bacterial diseases remain a widespread concern. A major problem facing modern healthcare is the gradual erosion of antibiotic effectiveness, which compromises the capacity to treat infections reliably [5]. Escalating antimicrobial resistance raises the possibility that certain bacterial strains may eventually become untreatable with existing drugs [6]. Thus, resistance has emerged as a critical barrier in infectious disease management.

Discovering new antibacterial agents has therefore become a priority, and biologically derived compounds have gained attention as promising leads. Natural sources such as plants, fungi, microorganisms, algae, and animals provide chemically diverse molecules capable of combating resistant pathogens [7]. Growing scientific interest in these materials stems from their varied chemical structures and unique biological activities [8], positioning them as potential alternatives to conventional antibiotics.

In light of this, the aim of the present study was to assess the in vitro antibacterial effects of crude extracts prepared from six medicinal plants on both Gram-positive and Gram-negative bacteria, and to perform preliminary phytochemical examinations.

Materials and Methods

Collection and preparation of plant materials

Plant material was gathered from the Ensaro District in the North Shewa Zone of Ethiopia's Amhara Regional State, with the assistance of local knowledge holders. Species identification was performed using the Flora of Ethiopia and Eritrea [9] and later validated by trained botanists. Voucher samples were archived in the National Herbarium of Ethiopia at Addis Ababa University. Laboratory testing took place at the microbiology unit of the Traditional and Modern Medicine Research Directorate, Ethiopian Public Health Institute (EPHI). Six medicinal plants—Dichrostachys cinerea, Andrachne aspera, Psydrax shimperiana, Achyranthes aspera, Albizia anthelmintica, and Securidaca longipedunculata—were selected for antibacterial assays and phytochemical profiling **(Table 1).**

Table 1. Ethnobotanical notes for medicinal plants included in antibacterial assessment.

Scientific Name	Part Used	Family	Ethno-medicinal Uses Reported from Other Regions	Traditional Use in the Study Area
Andrachne aspera Spreng.	Root	Euphorbiaceae	Snakebite [10], Neisseria gonorrhoeae infection [11], malaria [12]	Treatment of snakebite wounds, tonsillitis
Achyranthes aspera L.	Leaf	Amaranthaceae	Wound healing [13], external injuries [14], gonorrhea [15], cough [16], stomach-ache [17]	Excessive bleeding, wounds
Dichrostachys cinerea (L.) Wight & Arn.	Leaf	Fabaceae	Stomach ache [18], scorpion sting [19]	Cellulitis (skin dermatitis)
Psydrax schimperiana (A. Rich.) Bridson	Leaf	Rubiaceae	No previous ethnobotanical reports documented	Diarrhoea, snakebite
Securidaca longipedunculata Fresen.	Root	Polygalaceae	Evil eye [18], malaria [16]	Headache, evil eye, tonsillitis
Albizia anthelmintica Brongn.	Stem bark	Fabaceae	Helminthiasis [20], taeniasis [19]	Taeniasis, abdominal pain

Preparation of methanolic extracts

Plant materials were first rinsed thoroughly—initially with clean water and then with distilled water—before being chopped into small fragments and left to dry naturally in shaded, room-temperature conditions. Once dry, the material was pulverized, screened through a sieve, and stored in polyethylene bags to prevent external contamination. Previous studies have indicated that organic solvents extract biologically active constituents more effectively than water-based systems [21]. For this reason, the current work employed 80 % methanol as the extraction solvent, as it consistently produces higher extraction yields and retrieves a wider array of secondary metabolites [22].

For each plant species, exactly 100 g of the prepared powder was transferred into an individual 2000 mL reagent bottle, followed by the addition of 1000 mL of methanol. The bottles were positioned on an orbital shaker and agitated for 24 h at room temperature. After shaking, the liquid portion was separated using Whatman No. 1 filter paper. This extraction sequence was repeated twice using fresh solvent. The combined filtrates were then concentrated under reduced pressure at 40 °C using a rotary evaporator. Remaining solvent was removed by transferring the concentrates to a water bath maintained at 40 °C. The resulting extracts were kept at 2–8 °C until further testing.

Tested bacterial species

Antimicrobial assessments were performed against nine reference strains obtained from the American Type Culture Collection (ATCC). The organisms included Staphylococcus aureus (ATCC 25923), Staphylococcus epidermidis (ATCC 12228), Enterococcus faecalis (ATCC 29212), Streptococcus agalactiae (ATCC 12386),

Streptococcus pyogenes (ATCC 19615), Salmonella typhimurium (ATCC 13311), Klebsiella pneumoniae (ATCC 700603), Proteus mirabilis (ATCC 35659), and Shigella flexneri (ATCC 12022).

All cultures were preserved in the microbiology facility of the Traditional and Modern Medicine Research Directorate (TMMRD), Ethiopian Public Health Institute (EPHI), using Triptosoya broth containing 20 % glycerol and stored at −78 °C.

Inoculum preparation

Except for S. pyogenes (ATCC 19615) and S. agalactiae (ATCC 12386)—which required growth on nutrient agar supplemented with 5 % sheep blood—the remaining organisms were revived by incubation on nutrient agar at 37 °C for 18–24 h. To prepare standardized inocula, 3–5 well-isolated colonies were transferred into nutrient broth. The resulting suspension was adjusted with a Thermo Scientific Evolution 60s UV–visible spectrophotometer (CAT 840210100) using a 1 cm cuvette until the optical density at 625 nm reached 0.08–0.1, corresponding to approximately 1×10^8 CFU/mL. A subsequent dilution (1:10) in appropriate broth produced a working concentration of 1×10^7 CFU/mL, which was used directly for antimicrobial testing alongside the appropriate control groups [23].

Antibacterial activity testing

The antimicrobial performance of crude extracts from the six medicinal plants was examined using both agar well diffusion and micro-titer dilution procedures, following the general outline provided by Degu *et al*. [21]. Standardized bacterial suspensions prepared in sterile saline were spread across Mueller–Hinton agar plates. Wells measuring 8 mm in diameter were created using a cork borer. Each well was filled with 100 µL of methanolic extract from Dichrostachys cinerea, Andrachne aspera, Achyranthes aspera, Albizia anthelmintica, Psydrax shimperiana, or Securidaca longipedunculata, tested at concentrations of 100 mg/mL and 200 mg/mL.

Erythromycin (15 µg) served as the benchmark for Gram-positive bacteria, while Ciprofloxacin (5 µg) was used for Gram-negative controls. Plates were incubated for 24 h at 37 °C, after which inhibition zones were measured. All assays were carried out in triplicate.

Minimum inhibitory concentration determination

All extracts prepared with 80 % methanol were subjected to a microtiter broth dilution assay. The Minimum Inhibitory Concentration (MIC) for each sample was established following the procedures recommended in the Clinical and Laboratory Standards Institute guidelines [24]. Stock solutions were produced by dissolving the crude extracts in distilled water to a final concentration of 64 mg/mL. Into every well of a 96-well plate, 100 µL of Mueller–Hinton broth was dispensed. Serial twofold dilutions were then generated directly on the plate, resulting in extract concentrations spanning 32 mg/mL down to 0.25 mg/mL.

A fresh bacterial culture was adjusted to approximately 5×10^5 CFU/mL, and 100 µL of this suspension was added to each well. Plates were incubated for 18–24 h at 37 °C. Afterward, 40 µL of a 0.2 mg/mL tetrazolium chloride solution (2,3,5-Triphenyltetrazolium chloride; TTC) was added to serve as a colorimetric indicator of cell viability, followed by a further 30 min incubation at 37 °C. MIC determinations were based on the absence of red coloration, representing the lowest concentration at which visible growth failed to occur. Each extract's MIC was evaluated in triplicate.

Every bacterial strain was tested alongside sterility and growth controls, and ciprofloxacin was employed as a positive reference drug beginning at 0.10 mg/mL. A negative control consisting solely of distilled water was also included.

Preliminary phytochemical analysis

An initial phytochemical assessment was undertaken to identify major groups of secondary metabolites, using standard classical procedures described in the literature [25].

Detection of alkaloids

Mayer's reagent was used to confirm alkaloids. A small amount of extract was gently mixed with two drops of the reagent along the inner surface of a test tube. Formation of a pale, creamy precipitate indicated a positive reaction [26].

Detection of polyphenols

Polyphenols were screened using the ferric chloride test described by Peter *et al*. [27]. Two milliliters of a 5 % $FeCl_3$ solution were added to 1 mL of crude extract. A blue-green coloration signified the presence of phenolic compounds.

Detection of saponins

To detect saponins, the froth test was applied. Roughly 3 mL of extract was shaken vigorously with 3 mL of distilled water. Stable, persistent foam formation was interpreted as evidence of saponins.

Detection of terpenoids

Terpenoids were screened by adding 2 mL of chloroform and 3 mL of sulfuric acid to 5 mL of plant extract. A reddish-brown coloration indicated a positive reaction.

Detection of steroids

The Salkowski method was used to identify steroids. Two milliliters of the sample were mixed with 5 mL of chloroform, after which 1 mL of 98 % sulfuric acid was carefully run down the tube wall. A reddish-brown ring at the interface confirmed the presence of steroids.

Detection of flavonoids

Flavonoids were tested using an alkaline reagent method. Three milliliters of extract were combined with 1 mL of a 10 % sodium hydroxide solution. The appearance of an intense yellow color suggested a positive reaction.

Detection of coumarins

To assess whether coumarins were present in the extracts, 2 mL of each sample was treated with 3 mL of a 10 % NaOH solution. The emergence of a yellow hue was taken as evidence that coumarins were contained in the material.

Detection of tannins

Tannins were examined using the gelatin precipitation method. The test sample was mixed with a 1 % (w/v) gelatin solution containing 10 % NaCl. The appearance of a white solid precipitate signified a positive reaction for tannins.

Data analysis

Antibacterial test results for the medicinal plants were reported as means ± standard deviations and visualized using bar and line charts with corresponding error bars. Comparisons between plant extracts and reference antibiotics were conducted using a t-test, with $p \leq 0.05$ indicating statistical significance. Minimum inhibitory concentration and phytochemical screening outcomes were likewise presented as mean ± standard deviation.

Results and Discussion

Antibacterial activity screening

Iniial antimicrobial responses of the extracts against gram-positive bacteria

Extracts from Dichrostachys cinerea, Andrachne aspera, Achyranthes aspera, Albizia anthelmintica, Psydrax shimperiana, and Securidaca longipedunculata were evaluated at 100 mg/mL and 200 mg/mL concentrations against S. aureus, S. epidermidis, E. faecalis, S. agalactiae, and S. pyogenes. Their activities were contrasted with the positive standard, erythromycin.

The methanolic root extract of Andrachne aspera produced the largest inhibition zones at both 100 mg/mL (33 ± 0.17) and 200 mg/mL (33.5 ± 0.84) against S. epidermidis ($p < 0.05$) **(Figure 1a)**. Similarly, the leaf extract of Dichrostachys cinerea generated a significantly elevated inhibition zone (29 ± 0.55) at 200 mg/mL **(Figure 1b)**. At 100 mg/mL, Dichrostachys cinerea produced an inhibition zone of 28 ± 0, statistically comparable to erythromycin ($p > 0.05$).

For S. aureus, the next strongest activities at 200 mg/mL were observed with Andrachne aspera (26 ± 0.89) and Dichrostachys cinerea (24.8 ± 0.41) **(Figures 1a and 1b)**. Achyranthes aspera leaves produced a notable inhibition zone (24.17 ± 0.75) against S. epidermidis at 200 mg/mL, although still below the reference antibiotic **(Figure 1c)**.

Psydrax schimperiana generated a mean inhibition diameter of 21.7 ± 0.82 toward S. pyogenes, slightly lower than the control drug (22.7 ± 0.52) **(Figure 1d)**. The weakest antimicrobial effects across most gram-positive targets were shown by Albizia anthelmintica and Securidaca longipedunculata **(Figures 1e–1f)**.

A clear dose-dependent pattern occurred in extracts of Andrachne aspera, Dichrostachys cinerea, and Psydrax schimperiana (**Figures 1a, 1b and 1d**).

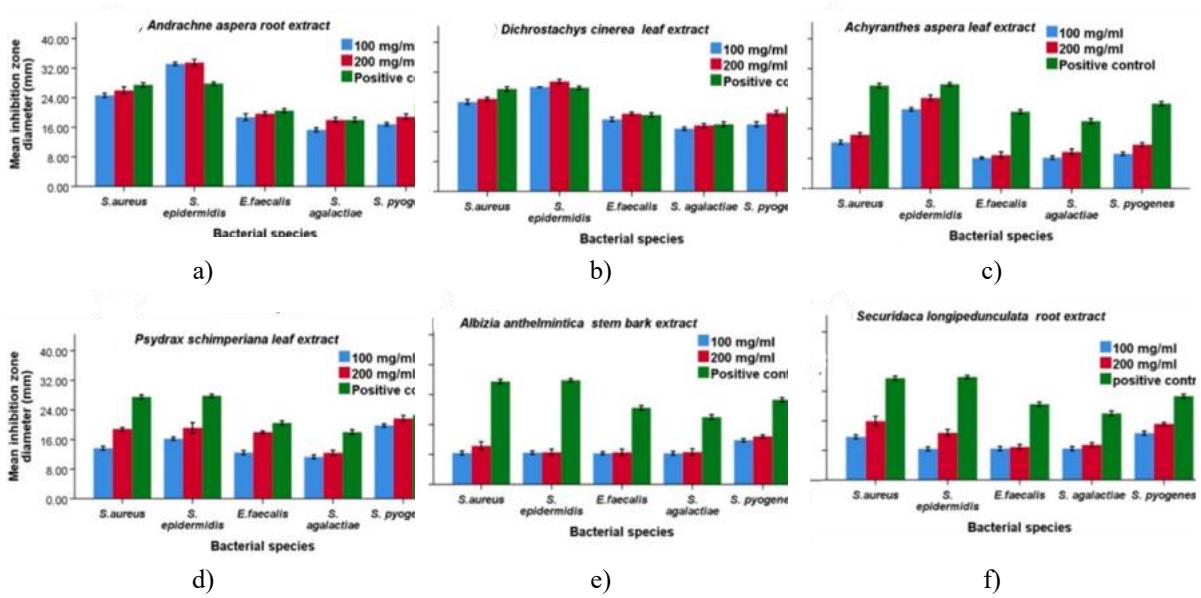

Figure 1. Antibacterial activity of crude methanolic extracts from selected medicinal plants against chosen Gram-positive bacterial strains.

Antimicrobial activity of crude extracts against Gram-negative bacteria

The study also examined the inhibitory effects of selected medicinal plant extracts on Gram-negative bacterial strains: *S. typhimurium*, *K. pneumoniae*, *P. mirabilis*, and *S. flexneri*. Relative to the reference antibiotic, Ciprofloxacin, all plant extracts showed markedly smaller inhibition zones ($p < 0.05$). Nonetheless, *Andrachne aspera* and *Dichrostachys cinerea* exhibited comparatively larger zones of inhibition against *P. mirabilis*, measuring 21 ± 0.67 and 21 ± 0 mm, respectively **(Figures 2a and 2b)**. These two plants also displayed comparatively higher activity against *S. typhimurium* and *S. flexneri* than the other tested extracts **(Figures 2a and 2b).**

A clear dose-dependent trend was observed: the inhibitory zones of *Dichrostachys cinerea* and *Andrachne aspera* against *P. mirabilis*, *S. typhimurium*, and *S. flexneri* increased when concentrations were raised from 100 mg/ml to 200 mg/ml. On the other hand, both extracts demonstrated the least effectiveness against *K. pneumoniae*. *Psydrax schimperiana* exhibited antibacterial activity against most of the tested bacteria, except for *P. mirabilis* **(Figure 2d)**. Similarly, *Achyranthes aspera* showed modest activity, producing inhibition zones of 13 ± 1.5 mm for *P. mirabilis* and 9.5 ± 0.55 mm for *S. flexneri* **(Figure 2c)**.

In contrast, *Albizia anthelmintica* and *Securidaca longipedunculata* exhibited negligible activity, showing inhibition solely against *S. typhimurium* at both 100 mg/ml and 200 mg/ml **(Figures 2e and 2f)**. The positive control, Ciprofloxacin (5 µg), produced significantly higher inhibition zones of 38.5 ± 0.55 mm against both *K. pneumoniae* and *S. flexneri*.

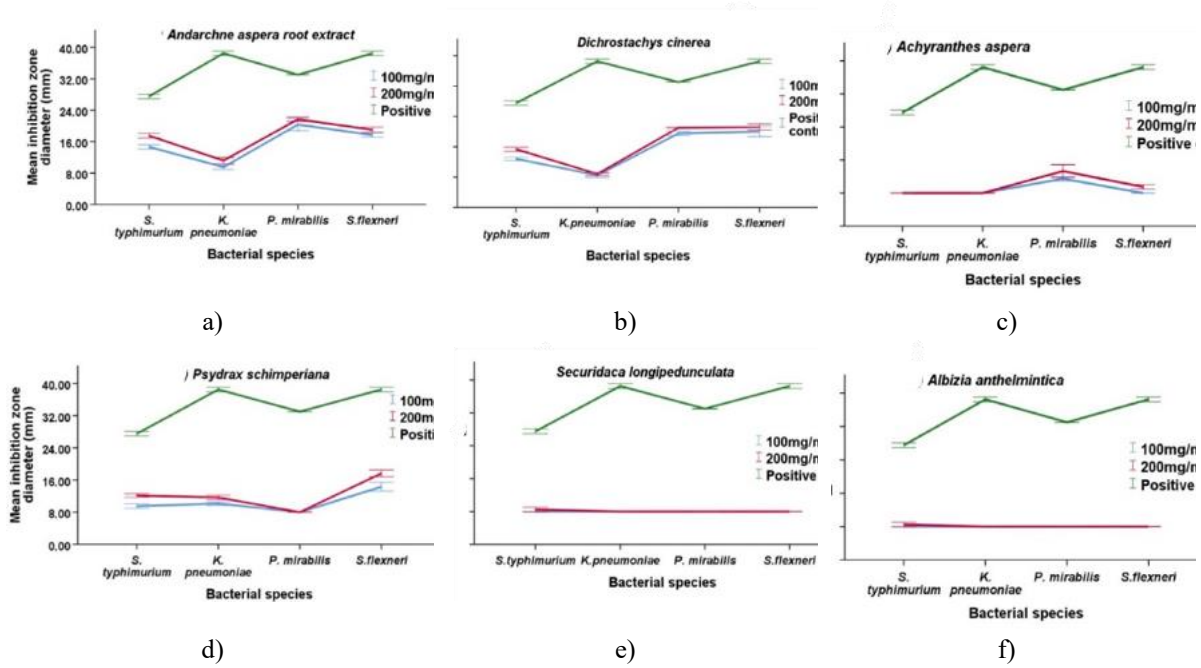

Figure 2. Antimicrobial activity of crude extracts from selected medicinal plants against Gram-negative bacteria.

Determination of minimum inhibitory concentration

The MIC procedure began at 64 mg/mL for each test microbe, followed by a two-fold dilution sequence down to 0.0625 mg/mL. Among the Gram-positive species, E. faecalis showed the greatest vulnerability to the methanolic extract of Andrachne aspera, requiring only 1.0 ± 0.0 mg/mL for growth inhibition. The next lowest MIC values—2.0 ± 0.0 mg/mL—were obtained for S. agalactiae and S. aureus when treated with Dichrostachys cinerea or Psydrax shimperiana extracts. Sensitivity of E. faecalis to Securidaca longipedunculata was also observed at 2.0 ± 0.0 mg/mL.

Within the Gram-negative group, S. flexneri responded to Andrachne aspera at 2.0 ± 0.0 mg/mL and to Dichrostachys cinerea at 3.3 ± 1.2 mg/mL. In contrast, Albizia anthelmintica generally showed weak inhibitory action; for nearly all organisms except S. flexneri and E. faecalis, MIC values exceeded 32.0 ± 0.0 mg/mL. Across all samples, the smallest MIC readings consistently appeared for E. faecalis.

Table 2. MIC values of ethanol extracts of the medicinal plants tested.

Plant species	**Microorganisms / Minimum Inhibitory Concentration (mg/ml)**							
	Gram-positive bacteria				**Gram-negative bacteria**			
	S. epidermidis	**S. agalactiae**	**E. faecalis**	**S. aureus**	**S. flexneri**	**P. mirabilis**	**S. typhimurium**	**K. pneumoniae**
Achyranthes aspera	10.7 ± 4.6	5.3 ± 2.3	4.0 ± 0.0	4.0 ± 0.0	13.3 ± 4.6	32.0 ± 0.0	32.0 ± 0.0	5.3 ± 2.3
Dichrostachys cinerea	8.0 ± 0.0	8.0 ± 0.0	4.0 ± 0.0	2.0 ± 0.0	3.3 ± 1.2	4.0 ± 0.0	5.3 ± 2.3	4.0 ± 0.0
Albizia anthelmintica	>32.0 ± 0.0	>32.0 ± 0.0	6.7 ± 2.3	>32.0 ± 0.0	16.0 ± 0.0	>32.0 ± 0.0	>32.0 ± 0.0	>32.0 ± 0.0
Andrachne aspera	8.0 ± 0.0	8.0 ± 0.0	1.0 ± 0.0	4.0 ± 0.0	2.0 ± 0.0	16.0 ± 0.0	10.7 ± 4.6	4.0 ± 0.0
Securidaca longipedunculata	13.3 ± 4.6	8.0 ± 0.0	2.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	32.0 ± 0.0	8.0 ± 0.0	4.0 ± 0.0
Psydrax shimperiana	8.0 ± 0.0	3.3 ± 1.2	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	16.0 ± 0.0	8.0 ± 0.0	5.3 ± 2.3

Note: erythromycin and ciprofloxacin were reference antimicrobials for Gram-positive and Gram-negative strains. Extract MICs are listed in mg/mL, while controls are reported in µg/mL.

Phytochemical analysis

To identify metabolic groups that may contribute to antimicrobial effects, all extracts were examined for secondary constituents. At least one extract from each of the six plants demonstrated the presence of alkaloids, polyphenols, saponins, terpenoids, steroids, flavonoids, coumarins, or tannins **(Table 3)**.

The root extract of Andrachne aspera tested positive for every class assessed and showed notably strong reactions for polyphenols, terpenoids, flavonoids, and tannins. Dichrostachys cinerea leaves contained especially high coumarin and polyphenol levels. Extracts from Achyranthes aspera and Psydrax shimperiana showed all metabolite groups except terpenoids, which did not appear in either one.

Table 3. Preliminary phytochemical profiles of the methanolic plant extracts.

Secondary metabolites	**Medicinal plants and plant parts used for phytochemical screening**					
	Dichrostachys cinerea	Albizia anthelmintica	Securidaca longipedunculata	Andrachne aspera	Achyranthes aspera	Psydrax shimperiana
Alkaloids	+	–	–	++	+++	+++
Polyphenols	+++	–	++	+++	+++	++
Saponins	+	–	–	++	+++	+
Terpenoids	+	++	+++	+++	–	–
Steroids	++	++	++	+	++	++
Flavonoids	+++	–	++	+++	++	++
Coumarins	+++	+	++	+	++	++
Tannins	+	–	++	+++	+++	+

Legend: +++ = very strong; ++ = strong; + = weak; — = negative.

Antibacterial activities and phytochemical analysis

Antibacterial evaluations were carried out to validate the healing potential of medicinal species with high informant consensus factor scores that are traditionally utilized in the Ensaro District. The experiments demonstrated that almost all selected plant extracts were capable of suppressing one or more Gram-positive and Gram-negative bacteria. Relative to the reference antibiotic (Erythromycin), the crude root extract of *Andrachne aspera* produced the largest average inhibition zones at 100 mg/ml and 200 mg/ml when tested against *S. epidermidis*. Likewise, the leaf extract of *Dichrostachys cinerea* generated its highest mean inhibition zone at 200 mg/ml for the same microorganism. These outcomes suggest that isolating and defining the active secondary metabolites in these plants could support the development of new, effective treatment options for infections caused by *S. epidermidis*.

Because this is the first study to analyze the antibacterial potential of *Andrachne aspera* root extract, comparison with earlier local or international work was not feasible. However, Banso and Adeyemo [28] documented a similar inhibition zone (24.0 ± 0.05) for *Dichrostachys cinerea* leaf extract against *Staphylococcus aureus*.

The leaf extract of *Achyranthes aspera* showed activity toward *S. pyogenes*, *S. aureus*, and *S. epidermidis*, although its performance was weaker than that of the positive control. Notably, no inhibitory effect was detected against *K. pneumoniae* or *E. faecalis*. This observation corresponds with the findings of Nigussie *et al*. [29], who also noted no activity against *K. pneumoniae* using methanolic leaf extract. Conversely, work by Mengie *et al*. [30] indicated that an extract from the same species was effective against *Klebsiella*. Such discrepancies may stem from differences in extraction approaches, plant maturity, or solvent selection.

Psydrax shimperiana produced inhibition zones against *S. aureus*, *S. epidermidis*, *S. pyogenes*, and *E. faecalis*, though these zones were smaller than those produced by the standard drug or by the more active extracts described earlier. This may reflect lower antibacterial potency or the presence of interfering non-active constituents that reduce direct interaction between active compounds and the microbes [31].

Although the inhibition zones were minimal, *Securidaca longipedunculata* demonstrated activity against *S. aureus* and *S. pyogenes* at 200 mg/ml. Previous investigations also report similar activity profiles for this species [32]. However, both in vivo and in vitro findings indicate that high doses of its root bark extract may be harmful [33].

In this study, *Albizia anthelmintica* showed inhibitory effects on *S. pyogenes*, aligning with results from Shatri [34]. Numerous investigations have confirmed that antimicrobial performance varies widely across medicinal plant species [35–37].

Overall, the data showed that higher concentrations of crude extracts produced larger inhibition zones in Gram-positive bacteria, suggesting a clear dose-dependent relationship between extract concentration and inhibitory activity. Variability in inhibition zone sizes among bacterial strains exposed to identical extract concentrations may be linked to differences in the nature and abundance of secondary bioactive compounds.

Compared to standard antibiotics, the antibacterial effects of the investigated medicinal plants were notably weaker against Gram-negative bacteria. The reduced susceptibility of these bacteria is likely due to their outer membrane, which provides a barrier and selectively restricts the entry of antimicrobial compounds [37]. This explains the smaller mean inhibition zones for Gram-negative strains relative to Gram-positive ones. These findings agree with Bobis *et al*. [38], who reported that Gram-negative bacteria are generally more resistant to antibiotics than Gram-positive bacteria.

Direct comparison of these results with previous studies on medicinal plants is difficult because of differences in extraction methods, crude extract concentrations, and the microbial strains tested. Nevertheless, MIC tests confirmed that both Gram-positive and Gram-negative bacteria were relatively more sensitive to *Andrachne aspera* and *Dichrostachys cinerea* extracts.

Phytochemical analysis

Phytochemical screening revealed that the tested medicinal plants contained various secondary metabolites such as alkaloids, polyphenols, saponins, steroids, terpenoids, flavonoids, coumarins, and tannins. The root extract of *Andrachne aspera* and the leaf extract of *Dichrostachys cinerea* had all these compounds in high amounts. *Psydrax schimperiana* lacked terpenoids, and the root of *Securidaca longipedunculata* did not contain alkaloids or saponins. Overall, *Dichrostachys cinerea*, *Andrachne aspera*, *Psydrax shimperiana*, and *Achyranthes aspera* contained a greater number of secondary metabolites, which may explain their relatively stronger antimicrobial activity. In contrast, *Albizia anthelmintica* stem bark had the fewest phytochemicals.

According to Hemeg *et al*. [39], the presence of secondary metabolites in medicinal plants contributes to their antimicrobial effects. Variations in the type and quantity of these compounds are likely responsible for differences in antibacterial activity among plants [40]. However, the mere presence of these metabolites does not guarantee strong antibacterial effects; activity depends on both their concentration and interactions [41]. A recent study by Efenberger-Szmechtyk *et al*. [42] also found that plants rich in polyphenols and flavonoids exhibited the most significant antibacterial activity, especially against Gram-positive bacteria.

Conclusion

The investigation into the antibacterial properties of selected medicinal plants produced promising results. Among the five Gram-positive bacteria tested, each exhibited sensitivity to at least one of the plant extracts. In particular, *Dichrostachys cinerea* and *Andrachne aspera* showed notably larger mean inhibition zones against *S. epidermidis* than the standard antibiotic, Erythromycin. These outcomes highlight the potential of these plants for developing potent antimicrobial agents.

Phytochemical screening revealed that the root extract of *Andrachne aspera* and the leaf extract of *Dichrostachys cinerea* contained all six major secondary metabolites analyzed, including alkaloids, polyphenols, saponins, terpenoids, steroids, flavonoids, coumarins, and tannins. The superior antibacterial activity observed for these two plants against both Gram-positive and Gram-negative bacteria is likely linked to the abundance and combination of these bioactive compounds.

It is therefore recommended that further studies focus on isolating and identifying the key secondary metabolites responsible for the antimicrobial effects of *Andrachne aspera* and *Dichrostachys cinerea*. Before undertaking isolation and characterization, in vivo assessments should be performed to evaluate their therapeutic efficacy and to monitor any potential toxicity. Such studies would provide a foundation for developing new, effective alternative antibiotics derived from these medicinal plants.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. R. Husain, N. Vikram, S. Pandey, G. Yadav, S.K. Bose, A. Ahamad, M. Shamim, K.G. Kumar, N. Khan, M. Zuhaib, Microorganisms: an eco-friendly tools for the waste management and environmental safety, in: Biotechnological Innovations for Environmental Bioremediation, Springer, 2022, pp. 949–981.
2. N. Akoijam, D. Kalita, S. Joshi, Bacteria and their industrial importance, in: Industrial Microbiology and Biotechnology, Springer, 2022, pp. 63–79.
3. M. Saha, A. Sarkar, Review on multiple facets of drug resistance: a rising challenge in the 21st century, Journal of xenobiotics 11 (4) (2021) 197–214.
4. M. Cavaco, M.A. Castanho, V. Neves, The use of antibody-antibiotic conjugates to fight bacterial infections, Front. Microbiol. 13 (2022), 835677.
5. D. Chinemerem Nwobodo, M.C. Ugwu, C. Oliseloke Anie, M.T. Al-Ouqaili, J. Chinedu Ikem, U. Victor Chigozie, M. Saki, Antibiotic resistance: the challenges and some emerging strategies for tackling a global menace, J. Clin. Lab. Anal. 36 (9) (2022), e24655.
6. M.H. Kollef, M. Bassetti, B. Francois, J. Burnham, G. Dimopoulos, J. Garnacho-Montero, J. Lipman, C.-E. Luyt, D.P. Nicolau, M.J. Postma, The intensive care medicine research agenda on multidrug-resistant bacteria, antibiotics, and stewardship, Intensive Care Med. 43 (2017) 1187–1197.
7. J. Li, S. Feng, X. Liu, X. Jia, F. Qiao, J. Guo, S. Deng, Effects of traditional Chinese medicine and its active ingredients on drug-resistant bacteria, Front. Pharmacol. 13 (2022), 837907.
8. F.J. A´lvarez-Martínez, E. Barrajo´n-Catal´an, V. Micol, Tackling antibiotic resistance with compounds of natural origin: a comprehensive review, Biomedicines 8(10) (2020) 405.
9. I. Hedberg, Flora of Ethiopia and Eritrea the biodiversity of african plants springer, Dordrecht 2 (2) (1996) 802–804.
10. A.A. Hinai, G. Al Issai, D.A. Lupton, Indigenous knowledge and folk use of medicinal plants in the eastern hajar mountains, Oman, Journal of Medicinal Plants Studies 8 (2020) 104–110.
11. A. Geyid, D. Abebe, A. Debella, Z. Makonnen, F. Aberra, F. Teka, T. Kebede, K. Urga, K. Yersaw, T. Biza, Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles, J. Ethnopharmacol. 97 (3) (2005) 421–427.
12. D. Hunde, Z. Asfaw, E. Kelbessa, Use of traditional medicinal plants by people of 'boosat'sub District, central eastern Ethiopia, Ethiopian Journal of Health Sciences 16 (2) (2006).
13. A. Fikru, E. Makonnen, T. Eguale, A. Debella, G.A. Mekonnen, Evaluation of in vivo wound healing activity of methanol extract of Achyranthes aspera L, J. Ethnopharmacol. 143 (2) (2012) 469–474.
14. T. Teklehaymanot, M. Giday, G. Medhin, Y. Mekonnen, Knowledge and use of medicinal plants by people around debre libanos monastery in Ethiopia, J. Ethnopharmacol. 111 (2) (2007) 271–283.
15. B. Bizuayehu, B. Garedew, A review on the ethnobotanical study of medicinal plants used for the treatment of gonorrhea disease in Ethiopia, Indian Journal of Natural Products and Resources (IJNPR)[Formerly Natural Product Radiance (NPR)] 9 (3) (2018) 183–193.
16. P. Nadembega, J.I. Boussim, J.B. Nikiema, F. Poli, F. Antognoni, Medicinal plants in baskoure, kourittenga province, Burkina Faso: an ethnobotanical study, J. Ethnopharmacol. 133 (2011) 378–395.
17. M.A. Rahman, J.S. Mossa, M.S. Al-Said, M.A. Al-Yahya, Medicinal plant diversity in the Flora of Saudi arabia 1: a report on seven plant families, Fitoterapia 75 (2004) 149–161.
18. Y.S. Birhan, S.L. Kitaw, Y.A. Alemayehu, N.M. Mengesha, Ethnobotanical study of medicinal plants used to treat human diseases in enarj enawga District, east gojjam zone, Amhara region, Ethiopia, SM J Med Plant Stud 1 (1) (2017) 1–9.
19. A. Enyew, Z. Asfaw, E. Kelbessa, R. Nagappan, Ethnobotanical study of traditional medicinal plants in and around fiche District, Central Ethiopia, Curr. Res. J. Biol. Sci. 6 (4) (2014) 154–167.
20. J.K. Muthee, D.W. Gakuya, J.M. Mbaria, P.G. Kareru, C.M. Mulei, F.K. Njonge, Ethnobotanical study of anthelmintic and other medicinal plants traditionally used in loitoktok District of Kenya, J. Ethnopharmacol. 135 (2011) 15–21.
21. S. Degu, A. Abebe, N. Gemeda, A. Bitew, Evaluation of antibacterial and acute oral toxicity of impatiens tinctoria A. Rich root extracts, PLoS One 16 (8) (2021), e0255932.

22. D.-H. Truong, D.H. Nguyen, N.T.A. Ta, A.V. Bui, T.H. Do, H.C. Nguyen, Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of severinia buxifolia, J. Food Qual. 2019 (2019).
23. J. Al-Salt, Antimicrobial activity of crude extracts of some plant leaves, Res. J. Microbiol. 7 (2012) 59–67.
24. CLSI, Global Laboratory Standards for a Healthier World, 2023. https://clsi.org/.
25. K.S. Banu, L. Cathrine, General techniques involved in phytochemical analysis, Int. J. Adv. Res. Comput. Sci. 2 (4) (2015) 25–32.
26. O. El-Guourrami, H. Elbouny, A.A. Benlabchir, S. Drioua, B. Ouahzizi, C. Alem, A. Doukkali, H. Benzeid, Phytochemical analysis, antioxidant, and antihyperlipidemic activities of teucriumtakoumitense, Journal of Taibah University Medical Sciences 18 (6) (2023) 1557.
27. E.L. Peter, P.B. Nagendrappa, C.O. Ajayi, C.D. Sesaazi, Total polyphenols and antihyperglycemic activity of aqueous fruits extract of abelmoschus esculentus: modeling and optimization of extraction conditions, PLoS One 16 (4) (2021), e0250405.
28. A. Banso, S. Adeyemo, Evaluation of antibacterial properties of tannins isolated from Dichrostachys cinerea, Afr. J. Biotechnol. 6 (15) (2007).
29. D. Nigussie, G. Davey, B.A. Legesse, A. Fekadu, E. Makonnen, Antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphoedema patients, BMC Complementary Medicine and Therapies 21 (2021) 1–10.
30. T. Mengie, S. Mequanente, D. Nigussie, B. Legesse, E. Makonnen, Investigation of wound healing and anti-inflammatory activities of solvent fractions of 80% methanol leaf extract of Achyranthes aspera L.(Amaranthaceae) in rats, J. Inflamm. Res. 14 (2021) 1775.
31. N. Vaou, E. Stavropoulou, C. Voidarou, Z. Tsakris, G. Rozos, C. Tsigalou, E. Bezirtzoglou, Interactions between medical plant-derived bioactive compounds: focus on antimicrobial combination effects, Antibiotics 11 (8) (2022) 1014.
32. A.A. Musa, A.O. Oyewale, I.G. Ndukwe, S.E. Yakubu, M.S. Abdullahi, Phytochemical screening and antimicrobial activity of solvent fractions of Securidaca longepedunculata (fresen) root bark methanol extract, J. Chem. Pharmaceut. Res. 5 (10) (2013) 28–33.
33. N.I. Mongalo, L. McGaw, J. Finnie, J. Van Staden, Securidaca longipedunculata fresen (polygalaceae): a review of its ethnomedicinal uses, phytochemistry, pharmacological properties and toxicology, J. Ethnopharmacol. 165 (2015) 215–226.
34. A.M. Shatri, Ethnomedicinal Uses, Phytochemical Characterization, and Antibacterial Activity of Grewia Tenax and Albizia Anthelmintica Extracts against Multidrug-Resistant Pneumonia-Causing Bacteria, 2021.
35. P. Arulmozhi, S. Vijayakumar, T. Kumar, Phytochemical analysis and antimicrobial activity of some medicinal plants against selected pathogenic microorganisms, Microb. Pathog. 123 (2018) 219–226.
36. A. Mehmood, S. Javid, M.F. Khan, K.S. Ahmad, A. Mustafa, total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems, BMC chemistry 16 (1) (2022) 1–10.
37. R. Chaudhari, K. Singh, P. Kodgire, Biochemical and molecular mechanisms of antibiotic resistance in Salmonella spp, Res. Microbiol. 174 (1–2) (2023), 103985.
38. O. Bobis, D. Dezmirean, L. Tomos, F. Chirila, L. Al Marghitas, Influence of phytochemical profile on antibacterial activity of different medicinal plants against gram-positive and gram-negative bacteria, Appl. Biochem. Microbiol. 51 (2015) 113–118.
39. H.A. Hemeg, I.M. Moussa, S. Ibrahim, T.M. Dawoud, J.H. Alhaji, A.S. Mubarak, S.A. Kabli, R.A. Alsubki, A.M. Tawfik, S.A. Marouf, Antimicrobial effect of different herbal plant extracts against different microbial population, Saudi J. Biol. Sci. 27 (12) (2020) 3221–3227.
40. A. Gupta, E. Jeyakumar, R. Lawrence, Journey of limonene as an antimicrobial agent, J. Pure Appl. Microbiol. 15 (3) (2021).
41. J.K. Dzotam, F.K. Touani, V. Kuete, Antibacterial and antibiotic-modifying activities of three food plants (xanthosoma mafaffa lam., moringa oleifera (L.) schott and passiflora edulis sims) against multidrug-resistant (mdr) gram-negative bacteria, BMC Compl. Alternative Med. 16 (2015) 1–8.
42. M. Efenberger-Szmechtyk, A. Nowak, A. Czyzowska, Plant extracts rich in polyphenols: antibacterial agents and natural preservatives for meat and meat products, Crit. Rev. Food Sci. Nutr. 61 (1) (2021) 149–178.