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Antibacterial and phytochemical analysis of traditional medicinal plants: An alternative therapeutic Approach to conventional antibiotics

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ABSTRACT

The purpose of this study was to carry out antibacterial and phytochemical analyses on six selected medicinal plants that have been traditionally used by the local people to treat and control different diseases. The antibacterial activities of methanolic extracts of these plants were assessed using the Agar well diffusion and Microtiter broth dilution methods. The root extract of *Andrachne aspera* showed significantly ($p < 0.05$) highest mean zone of inhibition at concentrations of 100 mg/ml (33 ± 0.17) and 200 mg/ml (33.5 ± 0.84) against *S. epidermidis*. The second highest mean zone of inhibition (24.8 ± 0.41) was recorded by *Dichrostachys cinerea* leaf extract against *S. epidermidis* at 200 mg/ml concentration. The minimum inhibitory concentrations 1.0 ± 0.0 was recorded by *Andrachne aspera* against *E. faecalis* and 2.0 ± 0.0 against *S. aureus* by *Dichrostachys cinerea*. The preliminary phytochemical analysis showed that *Andrachne aspera* and *Dichrostachys cinerea* contained strong concentration of Polyphenols and Flavonoids. Therefore, these two medicinal plant species have promising potential for further detailed investigations, including safety tests, characterization and isolation of bioactive secondary metabolites for the development of alternative drugs.

1. Introduction

Bacteria are single celled prokaryotic organisms that can be found everywhere and most of them have an important function in maintaining the ecological balance of our environment. Useful bacteria have a significant impact on the environment by successfully recycling the vital elements contained in decomposing organisms and other organic materials [1]. Additionally, they play vital roles in industries and agriculture [2]. However, only a small number of bacteria are harmful and can lead to infections that greatly affect public health [3]. Bacterial infections have been one of the most critical health problems, and if left untreated, they can cause serious and fatal illnesses. This implies that bacterial infections have a significant public health impact.

Administering antibiotics that specifically target the organisms responsible is a crucial part of treating bacterial infections [4]. Despite the substantial rise in antibiotic production, bacterial infections continue to be a major and escalating issue globally. The

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current challenge faced by modern medicine is the potential loss of effectiveness of antibiotics against bacteria, which would hinder the ability to treat bacterial infections [5]. The growing resistance of bacteria to antibacterial drugs indicates that there may come a time when antibiotics are no longer effective in treating bacterial infections caused by drug-resistant bacteria [6]. Antimicrobial resistance is therefore a serious public health issue and a major concern in the treatment of bacterial diseases. As a result, this resistance significantly increases the failure of antibiotic treatment.

The utilization of novel antibiotics including natural products is seen as a potential solution to combat bacterial resistance. Natural products can also be used as sources of novel antibacterial compounds that can combat antibiotic resistant bacterial infections [7]. Therefore, antimicrobial compounds derived from plants, animals, fungi, algae and microorganisms have recently received considerable attention as a new source of novel antimicrobial substances. In recent years, there has been a growing interest in studying natural antimicrobial products due to their wide range of chemical compositions and unique properties [8]. These compounds are being considered as potential alternative therapeutic substances to combat drug resistant bacteria.

Therefore, the purpose of this study was to evaluate the *in vitro* antimicrobial activities of crude extracts from six selected medicinal plants against Gram positive and Gram-negative bacterial strains and to carry out phytochemical screenings.

2. Material and methods

2.1. Collection and preparation of plant materials

Medicinal plant parts were collected from Ensaro District, located in the North Shewa Zone of the Amhara Regional State in Ethiopia. All plant parts were collected in consultation with residents and traditional healers. Identification was carried out using the Flora of Ethiopia and Eritrea [9] and confirmed by botanists. Voucher specimens were deposited in the National Herbarium of Ethiopia at Addis Ababa University. The *in vitro* experiments were conducted in the microbiology laboratory of the Traditional and Modern Medicine Research Directorate at the Ethiopian Public Health Institute (EPHI). Six medicinal plants, namely *Dichrostachys cinerea*, *Andrachne aspera*, *Psydrax shimperiana*, *Achyranthes aspera*, *Albizia anthelmintica*, and *Securidaca longipedunculata*, were selected to evaluate their antimicrobial activities and conduct phytochemical analysis (Table 1).

2.2. Preparation of methanolic extracts

Fresh parts of medicinal plants were washed in clean water and then rinsed with distilled water before being cut into pieces and dried at room temperature in the shade. The dried samples were ground, sieved, and the powder was packed in polyethylene bags to avoid contamination. According to various reports, organic solvents are more suitable for extracting bioactive ingredients than aqueous solvents [21]. The powder of medicinal plants in this study was extracted using 80 % methanol alcohol. This solvent was chosen because it provides higher yields and allows for the extraction of a greater number of secondary bioactive metabolites [22]. 100g of powdered sample from each plant extract was placed in separate 2000 mL reagent bottles. Then, 1000 mL of methanol was added to each bottle. The bottles were shaken on a shaking orbit machine for 24 h at room temperature. Then, the supernatant was filtered through a Whatman No. 1 filter paper. After filtration, the process was repeated for two more cycles with fresh solvent. The methanol filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator. It was then transferred to beakers and dried in a water bath set at 40 °C to remove any remaining organic solvents. Finally, the extracts were stored at 2–8 °C for further analysis.

2.3. Tested bacterial species

The antibacterial activities of selected medicinal plants were evaluated on nine standard strains of (American Type Culture Collection (ATCC)). The tested bacterial strains were *Staphylococcus aureus* (*S.aureus* (ATCC 25923)), *Staphylococcus epidermidis* (*S. epidermidis* (ATCC 12228)), *Enterococcus faecalis* (*E.faecalis* (ATCC 29212)), *Streptococcus agalactiae* (*S.agalactiae* (ATCC 12386)), and

Table 1
Ethnobotanical data on medicinal plants selected for antibacterial tests.

Scientific name	Family	Parts used	Locally used in the study area to treat	Ethno-medicinal use reports from elsewhere
Andrachne aspera Spreng.	Euphorbiaceae	Root	Snakebite wound healing, Tonsillitis,	Snakebite [10], Neisseria gonorrhoea [11], Malaria [12],.
Achyranthes aspera L.	Amaranthaceae	Leaf	Excessive bleeding, wound	The plant is reported to treat wounds [13], *e*xternal injury [14], Gonorrhea [15], Cough [16], Stomach-ache [17].
Dichrostachys cinerea (L.) Wight & Arn.	Fabaceae	Leaf	Cellulitis (skin dermatitis)	Stomach ache [18]; Scorpion bite [19].
Psydrax schimperiana (A. Rich.) Bridson	Rubiaceae	Leaf	Diarrhoea, snakebite	Has no ethnobotanical report yet.
Securidaca longipedunculata Fresen	Polygalaceae	Root	Headache, evil eye, tonsillitis	Evil eye [18], Malaria [16].
Albizia anthelmintica Brongn.	Fabaceae	Stem bark	Taeniasis, Abdominal pain	Helminthiasis [20], Taeniasis [19].

Streptococcus pyogenes (*S.pyogenes* (ATCC 19615)), *Salmonella typhimurium* (*S. typhimurium* (ATCC 13311)), *Klebsiella pneumoniae* (*K. pneumoniae* (ATCC 700603)), *Proteus mirabilis* (*P.mirabilis* (ATCC 35659)), and *Shigella flexneri* (*S. flexneri* (ATCC 12022)). These bacteria were kept in the microbiology laboratory of the Traditional and Modern Medicine Research Directorate (TMMRD) of the Ethiopian Public Health Institute (EPHI) on Triptosoya +20 % glycerol broth at −78 °C.

2.4. Inoculum preparation

With the exception of *S. pyogenes* (ATCC 19615) and *S. agalactiae* (ATCC 12386), which were cultured on 5 % sheep blood nutrient agar, all other strains were refreshed for the current examination by being incubated for 18–24 h at 37 °C in Petri dishes containing nutrient agar. To standardize, 3–5 colonies were taken from a pure culture of the test organisms and mixed with nutrient broth to create a suspension. The suspension was then measured for absorbance using a UV–visible spectrophotometer (Thermo Scientific Evolution 60s CAT 840210100) with a 1 cm light path until an absorbance reading of 0.08–0.1 at 625 nm was obtained. This reading is equivalent to 1×10^8 CFU/mL of bacteria. Thereafter, these suspensions were diluted with a suitable broth in a ratio of 1:10 to obtain a concentration of 1×10^7 CFU/mL. This concentration was used to evaluate the antimicrobial activity of the extract, comparing it to positive and negative controls [23].

2.5. Antibacterial activity testing

The antibacterial activity of crude extracts obtained from six medicinal plants was evaluated using agar well diffusion and microtiter dilution methods, as previously described by Degu et al. [21]. The selected test organisms, which had been prepared in a sterile saline solution, were introduced into Mueller Hinton agar plates through inoculation. A cork borer was used to create circular wells with a diameter of 8 mm. These wells were subsequently loaded with 100 μl of methanol extracts of *Dichrostachys cinerea, Andrachne aspera, Achyranthes aspera, Albizia anthelmintica, Psydrax shimperiana*, and *Securidaca longipedunculata*, individually, at concentrations of 100 mg/mL and 200 mg/mL. In the same way, Erythromycin (15 μg) and Ciprofloxacin (5 μg) were used as standard references for Gram-positive and Gram-negative bacterial strains, respectively. After that, the Petri dishes were incubated for 24 h at a temperature of 37 °C, and the zones of inhibition were assessed using a measuring instrument. These experiments were conducted in triplicate.

2.6. Minimum inhibitory concentration determination

The microtiter broth dilution method was performed on all crude extracts obtained using 80 % methanol. The determination of the Minimum Inhibitory Concentration (MIC) of the plant extracts was conducted in accordance with the protocols outlined by the Clinical and Laboratory Standards Institute guidelines [24]. Preparations of plant extracts were made by dissolving the extract in distilled water to create stock solutions with a concentration of 64 mg/mL. A volume of 100 μL of Muller Hinton broth was added to each well of the 96-well microplates. The serial dilutions were prepared in 96-well microplates using Mueller-Hinton broth. The concentrations ranged from 32 mg/mL to 0.25 mg/mL and were derived from a stock solution. A fresh culture was used to prepare a bacterial suspension containing approximately 5×10^5 colony-forming units/ml. One hundred microliters (100 μl) of the aforementioned suspension were added to each well and incubated for 18–24 h at a temperature of 37 °C. After incubation, 40 μl of a 0.2 mg/mL solution of 2,3,5-Triphenyltetrazolium chloride (TTC; Tetrazolium chloride) was added to each well as an indicator of microbial growth. The plates were then incubated at 37 °C for 30 min. Following incubation, the MIC values were determined by visually observing the presence or absence of a red color. The MIC was recorded as the lowest concentration of each extract that did not display any visible red color. The minimum concentration of every extract that did not show any visible red color was noted as the MIC. The MIC values were measured three times for each extract. A sterility control and a growth control well were also studied for each bacterial strain. To determine the sensitivity of the bacteria, positive control experiments were conducted in parallel using ciprofloxacin. The experiments started with a concentration of 0.10 mg/ml in sterile water. A negative control experiment was also conducted using only distilled water.

2.7. Preliminary phytochemical analysis

A preliminary phytochemical screening was carried out to detect secondary metabolites utilizing well-established conventional methodologies [25].

2.7.1. Detection of alkaloids

Alkaloids were detected through precipitation reactions utilizing Mayer's test reagent. A small volume of plant extract was combined with two drops of Mayer's reagent, which were carefully added along the inner walls of a test tube. The formation of a white, creamy precipitate signifies the presence of alkaloids [26].

2.7.2. Detection of polyphenols

Ferric chloride test was to detect the presence or absence of total polyphenols in the extracts as previously used by Peter et al. [27]. Two millilitres of a 5 % solution of ferric chloride were introduced into 1 mL of the crude extract. The observation of a blue-green colour signifies the existence of phenols.

2.7.3. Detection of saponins

The foaming test was employed as a means of detecting the presence of saponins. In this procedure, approximately 3 ml of plant extracts were combined with 3 ml of distilled water and vigorously agitated. The observation of a stable and persistent foam formation was regarded as an positive indication of the presence of saponins.

2.7.4. Detection of terpenoids

Terpenoids were detected through a chemical procedure involving the combination of 2 ml of chloroform with 3 ml of sulphuric acid, followed by the addition of 5 ml of plant extracts. The presence of a reddish-brown colour was regarded as an indication of the presence of terpenoids.

2.7.5. Detection of steroids

The Salkowski test was employed for the identification of steroids. A 2 mL of the sample solution was combined with approximately 5 mL of chloroform. Subsequently, 1 mL of 98 % concentrated sulphuric acid was cautiously introduced to the aforementioned mixture, ensuring it was added along the inner walls of the test tube. The appearance of a reddish-brown ring at the interface between the two layers signifies the existence of steroids.

2.7.6. Detection of flavonoids

In order to identify the presence of flavonoids in the plant extracts, an alkaline reagent test was utilized. This test involved treating 3 ml of the plant extract with 1 ml of a 10 % solution of Sodium hydroxide. The development of an intense yellow coloration served as an indicator for the existence of flavonoids.

2.7.7. Detection of coumarins

In order to determine the presence or absence of coumarins in the plant extracts, a solution consisting of 3 ml of 10 % sodium hydroxide was added to 2 ml of the plant extracts. The development of a yellow colour indicates for the presence of coumarins.

2.7.8. Detection of tannins

The gelatin test was employed to detect the presence of tannins. A solution of the sample was subjected to treatment with a 1 % w/v gelatin solution that also contained 10 % sodium chloride. The occurrence of a white precipitate indicated the presence of tannins.

2.8. Data analysis

All antibacterial activities of medicinal plants were computed as mean ± standard deviation and expressed in bar and line graphs with error bars, and the antibacterial activity of the samples was compared to standard antibiotics using the t-test. $P \leq 0.05$ values were used to denote a statistically significant difference. The minimum inhibitory concentration and phytochemical analysis data were presented as means ± standard deviations.

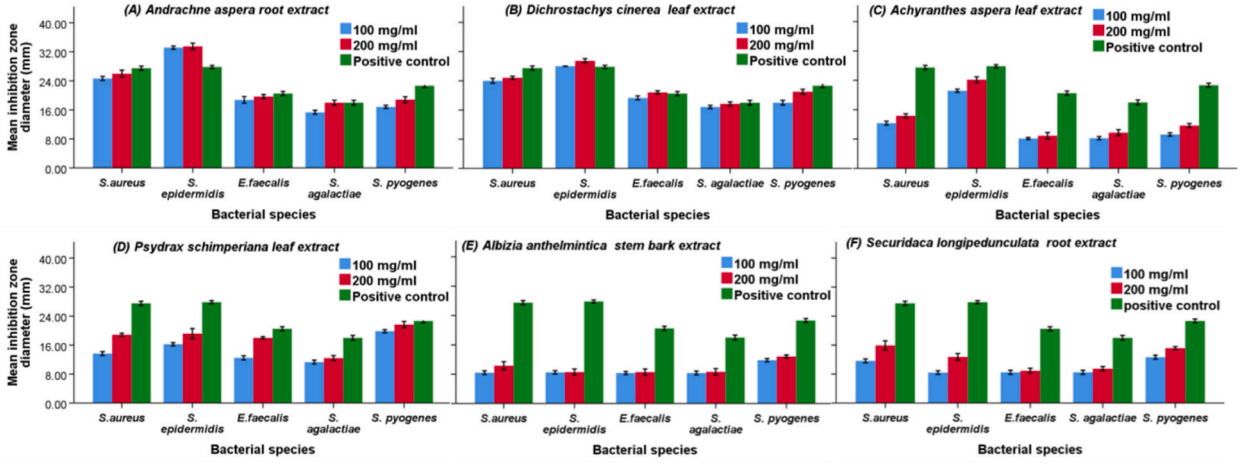

Fig. 1. Antimicrobial activities of selected medicinal plant crude extracts against some selected Gram-positive bacteria.

3. Results

3.1. Antibacterial activity screening

3.1.1. Preliminary antimicrobial activities of the extracts against different gram-positive bacterial species

The inhibitory effect of extracts from *Dichrostachys cinerea, Andrachne aspera, Achyranthes aspera, Albizia anthelmintica, Psydrax shimperiana,* and *Securidaca longipedunculata* were tested against *S. aureus*, *S. epidermidis*, *E. faecalis*, *S. agalactiae*, and *S. pyogenes* at concentrations of 100 mg/ml and 200 mg/ml. The result was compared to the inhibitory effect of the positive control, erythromycin. Based on this, the methanolic extracts of *Andrachne aspera* root showed significantly ($p < 0.05$) highest mean zone of inhibition at 100 mg/ml (33 ± 0.17) and 200 mg/ml (33.5 ± 0.84) concentrations against *S. epidermidis* (Fig. 1A). Similarly, *Dichrostachys cinerea* leaf extract showed a significantly ($p < 0.05$) higher mean zone of inhibition (29 ± 0.55) against *S. epidermidis* at a concentration of 200 mg/ml (Fig. 1B). *Dichrostachys cinerea* extracts exhibited similar antibacterial activity (28 ± 0) to the positive control (Erythromycin) against *S. epidermidis* at a concentration of 100 mg/ml ($p > 0.05$). The second higher mean zone of inhibition was recorded against *S. aureus* by the extracts of *Andrachne aspera* root (26 ± 0.89) and *Dichrostachys cinerea* (24.8 ± 0.41) at 200 mg/ml concentration (Fig. 1 A and B). The *Achyranthes aspera* leaf extract also showed a higher mean zone of inhibition (24.17 ± 0.75) against *S. epidermidis* at 200 mg/ml concentration, although it was lower than the control drug (Fig. 1C). The crude extract of *Psydrax schimperiana* showed a higher mean inhibition zone diameter (21.7 ± 0.82) against *S. pyogenes*, but lower than that of the control drug (22.7 ± 0.52) (Fig. 1D). *Albizia anthelmintica and Securidaca longipedunculata* showed the least potent antibacterial activity against almost all gram-positive bacteria that were tested (Fig. 1 E and F). A direct relationship between the concentration of plant extracts and the mean inhibitory zone was observed in *Andrachne aspera*, *Dichrostachys cinerea*, and *Psydrax schimperiana* (Fig. 1A, B, and D).

3.1.2. Antimicrobial activities against different gram-negative bacterial species

For this test, we also evaluated gram-negative bacterial species (*S. typhimurium*, *K. pneumoniae*, *P. mirabilis*, and *S. flexneri*) to assess the effectiveness of the crude extracts from the selected medicinal plants. Compared to the positive control (Ciprofloxacin), all plant extracts produced a significantly lower mean inhibition zone diameter ($p < 0.05$). However, *Andarchne aspera* and *Dichrostachys cinerea* showed relatively higher mean zone of inhibition diameter against *P. mirabilis*, with measurements of 21 ± 0.67 and 21 ± 0, respectively (Fig. 2 A and B). Likewise, the antibacterial activity of crude extracts from *Dichrostachys cinerea* and *Andarchne aspera* was found to be more potent against *S. typhimurium* and *S. flexneri* compared to other extracts (Fig. 2A and B).

Furthermore, the findings clearly indicated that the average zone of inhibition diameter caused by *Dichrostachys cinerea* and *Andarchne aspera* against *P. mirabilis*, *S. typhimurium*, and *S. flexneri* rose as the concentration escalated from 100 mg/ml to 200 mg/ml. However, the crude extracts of *Dichrostachys cinerea* and *Andarchne aspera* showed the least activity against *K. pneumoniae*. *Psydrax schimperiana* also had an antibacterial effect against the tested bacterial species, except for *P. mirabilis* (Fig. 2D). Similarly, *Achyranthes aspera* showed a slightly higher mean zone of inhibition against *P. mirabilis* (13 ± 1.5) and *S. flexneri* (9.5 ± 0.55) (Fig. 2C). In contrast to the other used medicinal plant extracts, *Albizia anthelmintica* and *Securidaca longipedunculata* did not exhibit antibacterial effects against any of the gram-negative bacteria tested except *S. typhimurium* at both concentration levels (Fig. 2E and F).The standard drug, Ciprofloxacin (5 μg), exhibited a higher average zone of inhibition against *K. pneumoniae* (38.5 ± 0.55) and *S. flexneri* (38.5 ± 0.55).

3.1.3. Determination of minimum inhibitory concentration

To determine the MIC value, each tested microorganism was evaluated starting from the higher concentration of 64 mg/ml and

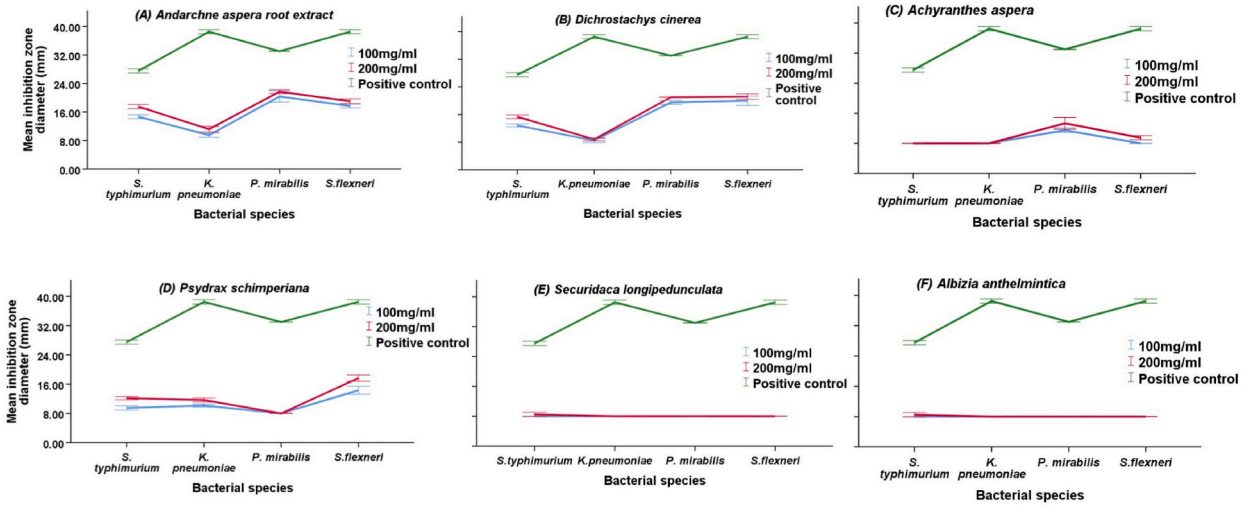

Fig. 2. Antimicrobial activities of selected medicinal plant crude extracts against some Gram-negative bacteria.

gradually diluted by a factor of two until reaching a lower concentration of 0.0625 mg/ml using serial bi-fold dilution. As a result, among Gram-positive bacteria, *E. faecalis* was found to be the most susceptible to methanol extracts of *Andrachne aspera*, with MIC values of 1.0 ± 0.0 mg/ml. The second lowest minimum inhibitory concentration was recorded by *S. agalactiae* and *S. aureus*, with minimum inhibitory concentration values of 2.0 ± 0.0 mg/ml for each of the methanol extracts of *Dichrostachys cinerea* and *Psydrax shimperiana*. *E. faecalis* was also sensitive to *Securidaca longipedunculata* extract, with a minimum inhibitory concentration of 2.0 ± 0.0 mg/ml. Among the Gram-negative bacteria, *S. flexneri* showed sensitivity to *Andrachne aspera* and *Dichrostachys cinerea*, with MIC values of 2.0 ± 0.0 mg/ml and 3.3 ± 1.2 mg/ml, respectively. The lowest antimicrobial activities were recorded by all tested bacterial species, except for *S. flexneri* and *E. faecalis*, when exposed to *Albizia anthelmintica* extract. The minimum inhibitory concentration of *Albizia anthelmintica* extract was found to be above 32.0 ± 0.0 mg/ml for most tested bacterial species (Table 2). The lowest inhibitory concentrations were recorded for *E. faecalis* by all of the plant extracts used.

3.2. Phytochemical analysis

Plant extracts under investigation were also subjected to phytochemical analysis in order to identify the presence of secondary metabolites that could potentially be responsible for their antimicrobial activities. The phytochemical examination revealed that at least one of the six selected medicinal plant extracts contained alkaloids, polyphenols, saponins, terpenoids, steroids, flavonoids, coumarins, and tannins (Table 3). The root extract of *Andrachne aspera* tested positive for all of the secondary metabolites examined. Furthermore, the root extract of *Andarchne aspera* exhibited significant levels of polyphenols, terpenoids, flavonoids, and tannins. *Dichrostachys cinerea* leaf extract also contains high concentrations of coumarins and polyphenols. *Achyranthes aspera* and *Psydrax shimperiana* both contained all the secondary metabolites that were evaluated, with the exception of the terpenoids.

4. Discussion

4.1. Antibacterial activities and phytochemical analysis

Antibacterial tests were conducted to verify the therapeutic properties of medicinal plants of high informant consensus factor values that are traditionally used for healing purposes in Ensaro District. The results confirmed that nearly all of the selected medicinal plant extracts exhibited antibacterial activities against one or more of the tested Gram-positive and Gram-negative bacterial species.

When compared to the positive control (Erythromycin), the crude extract of *Andrachne aspera* root showed significantly highest mean zone of inhibition at 100 mg/ml and 200 mg/ml against *S. epidrmidis*. Similarly, the leaf crude extract of *Dichrostachys cinerea* displayed significantly highest mean zone of inhibition at concentration of 200 mg/ml against *S. epidrmidis*. This indicates that isolation and characterization of the secondary bioactive compounds from these plants may help to develop alternative and potent drug for the treatment of infections caused by *S. epidrmidis*.

This study is the first of its kind to examine the effects of *Andrachne aspera* root extract. As a result, it was not possible to compare the findings of this study with other similar studies conducted locally or globally. However, an antimicrobial activity study on leaf extract of *Dichrostachys cinerea* by Banso and Adeyemo [28] showed the same mean zone of inhibition (24.0 ± 0.05) against *Staphylococcus aureus*.

The leaf extract from *Achyranthes aspera* has been found to have antibacterial properties, specifically against *S. pyogenes*, *S. aureus*, and *S. epidermidis*. However, the extract was not as effective as the positive control drug. It is important to note that *Achyranthes aspera* did not exhibit any antibacterial effects against *K. pneumoniae* and *E. faecalis*. This result aligns with the findings of Nigussie et al. [29], who also reported no antibacterial activity against *K. pneumonia* using a methanolic leaf extract of *Achyranthes aspera*. In contrast, a research conducted by Mengie et al. [30] demonstrated the efficacy of an *Achyranthes aspera* leaf extract against *Klebsiella* species. This observed variation could be attributed to several factors, including the specific extraction technique employed, the developmental stage of the plant, or the choice of extraction solvent.

Psydrax shimperiana exhibited wider mean zone of inhibition against *S. aureus, S. epidrmidis, S. pyogenes, and E. faecalis.* However,

Table 2
Minimum inhibitory concentration values of ethanol extracts of medicinal plant species against tested diseases causing microorganisms.

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

Keynotes: Erythromycin and ciprofloxacin are positive control drugs for Gram-positive bacteria and Gram-negative bacteria, respectively. The MIC values are expressed in mg/ml for extracts and in μg/ml for positive controls.

Table 3
Results of preliminary phytochemical screening of methanol extracts of selected medicinal plants.

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

+++ = very strong positive test, ++ = strong, + = Weak positive test, − = Negative test

the zone of inhibition formed by *Psydrax shimperiana* was narrower compared to the positive control and the other extracts mentioned above. This could be attributed to the plant's lower efficacy against these particular bacterial species or the presence of additional non-bioactive compounds that hinder direct contact between the bioactive compound and the bacterial species [31].

Even though the mean zone of inhibition was very small, *Securidaca longipedunculata* showed antibacterial activities against *S. aureus* and *S. pyogenes* at 200 mg/ml concentration. Other similar study has shown that *Securidaca longipedunculata* is effective against *S. aureus* and *S. pyogenes*, which is aligned with the result of this study [32]. Nevertheless, research conducted both in living organisms and in laboratory settings on *Securidaca longipedunculata* has shown that the administration of a larger amount of root bark extract can be harmful [33]. Based on the results of the current study, *Albizia anthelmintica* exhibited some activity against *S.pygones* bacterial strain, which is consistent with the findings of Shatri [34]. Studies on the antimicrobial properties of medicinal plant extracts have proved that different plants have different antibacterial activities [35–37].

The findings indicated that as the concentrations or dosages of crude extracts increased, the mean inhibition zone diameter in Gram-positive bacterial strains also increased. This indicates a strong and clear positive correlation between the diameter of the inhibitory zone and the concentrations of crude extracts derived from medicinal plants. Differences in the size of the mean inhibition zone diameter were observed among the tested bacterial strains, even when they were exposed to the same concentration of plant extracts. The types and concentrations of bioactive secondary metabolites might be the reason for these differences.

Compared to the positive control, the antibacterial activities of all selected medicinal plants were very low against the tested gram-negative bacteria. Gram-negative bacteria have a protective outer membrane that shields them from their environment, allowing them to selectively block the entry of antibiotics [37]. The lower mean zone of inhibition observed in gram-negative bacteria compared to gram-positive bacteria may be attributed to this factor. This finding is consistent with the result of Bobis et al. [38] which shows that Gram-negative bacteria are inherently more resistant to antibiotics compared to Gram-positive bacteria.

Overall, it was challenging to compare the findings of this research with other studies on the antibacterial properties of medicinal plants due to various factors. These factors include the use of different extraction solvents, concentrations of crude extract, and microbial strains in numerous studies conducted worldwide. The results of the minimum inhibitory concentration (MIC) test further validated the heightened susceptibility of both Gram-positive and Gram-negative bacterial species to both *Andrachne aspera* and *Dichrostachys cinerea*.

4.2. Phytochemical analysis

In the current study, the phytochemical analysis of medicinal plants revealed the presence of various secondary metabolites, including alkaloids, polyphenols, saponins, steroids, terpenoids, flavonoids, coumarins, and tannins. As a result, the root extract of *Andrachne aspera* and the leaf extract of *Dichrostachys cinerea* contained all types of these secondary metabolites in high concentration. On the other hand, terpenoids were absent in *Psydrax schimperiana*. Alkaloids and saponins were absent in *Securidaca longipedunculata* root extract. A greater number of phytochemicals have been detected in *Dichrostachys cinerea*, *Andrachne aspera, Psydrax shimperiana*, and *Achyranthes aspera*. This might be the reason why these plants, especially *Dichrostachys cinerea* and *Andrachne aspera*, exhibit relatively higher antimicrobial activity against tested bacterial strains. The stem bark extract of *Albizia anthelmintica* contains the lowest number of phytochemicals. According to a study by Hemeg et al. [39], the existence of secondary compounds in medicinal plants enables them to fight against and treat microbial illnesses. The differences in the types and amount of phytochemicals found in medicinal plants are responsible for the variations in their antimicrobial properties [40]. However, the presence of secondary metabolites in plant extracts does not guarantee their potent antibacterial properties in medicinal plants. Instead, the antimicrobial properties of plant extracts are determined by the concentration and interactions of secondary metabolites [41].

Recently published paper by Efenberger-Szmechtyk et al. [42] revealed that medicinal plants with higher concentrations of polyphenols and flavonoids showed the strongest and most significant antibacterial activity against Gram-positive bacterial strains.

5. Conclusion and RECOMMENDATIONS

Antibacterial activity tests were conducted on selected medicinal plants and the results were promising. From the five tested Gram-

positive bacterial strains, all of them were found to be highly sensitive to one or more medicinal plant extracts. The results of the study revealed that *Dichrostachys cinerea* and *Andrachne aspera* shown significantly higher mean zones of inhibition against *S. epidermidis* compared to the control drug, Erythromycin. Hence, it is imperative to conduct a thorough and comprehensive investigation to develop antimicrobial agents that exhibit superior efficacy against pathogenic microorganisms. The analysis of these two medicinal plants has revealed that most of them contain three or more secondary metabolites. Specifically, the root extract of *Andrachne aspera* and the leaf extract of *Dichrostachys cinerea* were found to contain all six primary metabolites that were tested, including alkaloids, polyphenols, saponins, terpenoids, steroids, flavonoids, coumarins, and tannins. Thus, the increased antibacterial effectiveness of these two plants against both gram-positive and gram-negative bacteria could be attributed to the presence of these secondary compounds. Therefore, it is essential to conduct additional detailed studies to isolate and identify the bioactive secondary compounds from *Andrachne aspera* and *Dichrostachys cinerea* in order to develop novel alternative antibiotics. However, prior to isolating and characterizing the bioactive compounds from these plants, researchers should thoroughly investigate the *in vivo* efficacy and potential toxicity of these plants.

Data availability statement

Data is available at https://doi.org/10.5061/dryad.ngf1vhj1n.

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CRediT authorship contribution statement

Asaye Asfaw: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ermias Lulekal:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition. **Tamrat Bekele:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Asfaw Debella:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Conceptualization. **Asfaw Meresa:** Supervision, Resources, Methodology, Investigation, Data curation. **Bihonegn Sisay:** Supervision, Resources, Methodology, Investigation, Data curation. **Sileshi Degu:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation. **Abiy Abebe:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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. Álvarez-Martínez, E. Barrajón-Catalán, 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.