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Antibacterial Potential of Selected Traditional Medicinal Plants for Wound Healing in Sekela District, Northwestern Ethiopia

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ABSTRACT

This study evaluated the antibacterial activity of medicinal plants (MPs) used to treat wounds. Methanol and ethyl acetate crude extracts of four medicinal plants were examined for antibacterial efficacy against gram-positive and gram-negative American Type Culture Collection (ATCC) strains. Plant crude extracts were produced using the maceration technique and analyzed using qualitative phytochemical tests. The antibacterial properties of plant products were evaluated using the agar disc diffusion assay. The broth microdilution method was used to determine the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations. The data were analyzed using Analysis of Variance (ANOVA). The methanol extract of *Rumex nervosus* had the highest yield (21%). The methanol extracts of all tested MPs tested positive for alkaloids, phenolics, and tannins. The methanol and ethyl acetate extracts exhibited a dose-dependent increase in the growth inhibition zone against all ATCC. The methanol extract of *Plantago lanceolata* demonstrated the highest antibacterial activity (16.67 ± 1.15) against *Staphylococcus epidermidis. S. aureus* and *S. epidermidis* recorded a relatively lower MIC (6.25 mg mL^{-1}) from methanol extracts of *P. lanceolata*, while *P. lanceolata* and *R. nervosus* recorded a lower MBC (25 mg mL^{-1}) against *S. aureus*.

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KEYWORDS
Antibacterial activity; inhibition zone; medicinal plants; MIC; phytochemical; wound

Introduction

Plants are a great source of medications, especially in traditional medicine, that can be utilized to treat a wide range of diseases.[1] Traditional medicine has not only played an essential role in healing, but it has also led to the identification of the majority of pharmaceutically active chemicals in plants that have been employed in drug synthesis.[2] Because of their natural nature, plant-derived antimicrobials are also thought to be safer than manufactured molecules.[3] It has been observed that the therapeutic impact of medicinal plants is related to the existence of secondary metabolites, such as alkaloids, terpenoids,

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glycosides, phenolic and other organic compounds, which are synthesized in all parts of the plant.[4]

Many types of synthetic antifungal and antibacterial medications are utilized in medicine to treat infections. However, the efficiency of these antifungal and antibacterial medicines is dwindling as drug-resistant strains emerge.[5] Folk medicine is a valuable and underutilized resource for the discovery and development of potential novel medications against microbial infections in order to reduce the formation of resistance and harmful drug effects.[6] Antibiotic resistance is the leading cause of illness in humans, resulting in the emergence and spread of antibiotic-resistant bacterial strains. Plants play a vital function in removing harmful bacteria's biofilms. Their extracts hinder the formation and development of biofilms.[7] Furthermore, the utilization of medicinal plants gives prospects to underdeveloped countries because they may be less expensive, more accessible, and more readily available.[8]

In underdeveloped countries, the majority of the population cannot afford contemporary pharmaceutical treatments and must rely on indigenous traditional medicinal plants.[8] Because of their variety and chemical diversity, tropical plants are the most valuable source of novel bioactives.[8] However, higher plants in general, and medicinal plants in our nation in particular, have not been tested from the standpoint of bioactive for phytochemical and pharmacological utilization from a broader perspective. As a result, proper study into the efficacy and safety of herbal therapies is required.

Wound infections are commonly associated with normal flora, environmental bacteria, or hospital-acquired infections.[9] When the skin surface is damaged in some manner, microorganisms infect soft tissue. People have been using plant products to cure different ailments since ancient times, even without knowing what causes them.[10] Medicinal plants are still utilized in traditional medicine to treat a variety of diseases in many nations, including Ethiopia.

According to a review[11] the flora of Ethiopia and Eritrea is exceptionally diverse and rich in endemic species; the country is estimated to have 6027 species of higher plants, 10% of which are endemic. However, only a small proportion of them have been studied for antimicrobial activity in the country.

The current study was motivated by prior finding.[,12] *Plantago lanceolata, Rumex nervosus, Rumex abyssinicus, and Zehneria scabra*, which is used to treat wounds, were chosen for this investigation. Antibacterial effect of selected medicinal plant extracts against pathogenic bacteria was reported.[13–16]

P. lanceolata has been used traditionally to cure a variety of ailments, including body cutting, wounds, and anthrax.[12,17] It is also useful for treating dysmenorrhea, stomach pain, laxatives, and astringents[18] and prevents bleeding and promotes repair of damaged tissue.[19] *P. lanceolata's* root, leaf, and

seed contain phytochemicals such as iridoid glycosides, polyphenols, polysaccharides, and flavonoids, all of which have medicinal potential.[20] Thus, the plant extracts have demonstrated antibacterial,[21] anti-inflammatory,[22] rheological, and viscoelastic[23] properties.

Rumex nervosus has been used ethnobotanically for a variety of purposes, including ascariasis, wound care, and snake bites.[12] Eritrean women used the stem and leaves of this herb to clean their bodies, believing that the steam and vapor would destroy and remove microorganisms from the body while also making them feel comfortable.[24] The results of phytochemical screening showed the presence of steroids, alkaloids, flavonoids, saponins, and terpenoids, and demonstrated promising antibiotic properties.[25,26] Traditionally, *R. abyssinicus* is used to heal wounds, ascariasis, and gastritis.[12] Phytochemical study found that *R. abyssinicus* contains anthraquinones, saponins, and tannins[28]. *R. abyssinicus* has been shown to have a number of pharmacological activities, including antibacterial activities,[27] antioxidant, anti-inflammatory, anticancer, anti-Alzheimer, anti-diabetes, and antibacterial effects both *in vitro* and in *vivo* .[28]

The leaves of *Zehnera scabra* have been used for wound healing,[29] **MICH**, and skin diseases.[29] The Amhara ethnic group utilizes the leaf juice to alleviate fevers and headaches.[30] Phytochemical tests revealed the presence of phenol, tannins, flavonoids, and steroids,[29] implying that plants have anti-inflammatory and antibacterial properties.[31] Furthermore, methanolic leaves of the extract have been shown to have anti-diarrheal, anti-secretary, and anti-malarial properties.[32] The current experimental plants are traditionally stated to have wound healing activity, which has been examined using different solvent polarity indexes.

Tested medicinal plants have a wide range of phytochemicals, including alkaloids, flavonoids, and terpenoids. These chemicals have different antibacterial activities and can limit the growth of pathogens often linked with wound infections, including *Staphylococcus aureus* and *Escherichia coli*. This work sheds light on how tested MPs have historically been employed for wound healing and infection prevention, directing scientific research into promising TMPs. Using locally accessible medicinal herbs can be a sustainable and cost-effective way to manage wound infections, especially in areas with limited access to synthetic antibiotics. This is especially important in developing nations such as Ethiopia.

Ethnobotanical investigations conducted in Ethiopia revealed that traditional healers employed medicinal plants to treat a variety of diseases, including wound infections.[33,34] People in Sekela District, like those throughout Ethiopia, have accumulated traditional knowledge on how to use medicinal plants to heal human and livestock illnesses, particularly wound infections.

However, there is a paucity of scientific evidence addressing the antibacterial action of traditionally used medicinal plants against bacterial pathogens involved in wound infections. Thus, the purpose of this study was to evaluate the antibacterial efficiency of selected medicinal plants utilized in wound healing against certain bacterial strains in the Sekela District of Northwestern Ethiopia.

Methods

Study Design

This was a laboratory-based experimental investigation that extracted plant leaf and root crude from the four (4) medicinal plants with their ICF and FL values using methanol and ethyl acetate solvents.[,12] Using the agar disc diffusion method, the extract was tested for antibacterial activity against specified bacterial pathogens at various test concentrations. The experimental data were compared to the reference data.

Medicinal Plant Parts Collection

The leaves of *Plantago lanceolata (*approximately 11°4'2"N, 37°17'12"E*), Rumex nervosus (*approximately 10°56'28"N,37°17'46"E*), Zehneria scabra (*approximately 10°49'39"N, 37°10'23"E) and roots of *Rumex abyssinicus (*approximately 11°3'56"N, 37°17'24"E) were collected from the district 463 km northwest of Addis Ababa on the way to regional capital (Bahir Dar), Ethiopia (Table 1). The plant components were packed in a paper bag and transported to Bahir Dar University's extraction laboratory. All plant material was collected after consulting the locals, traditional healers in the area, and a botanist. The plant was verified at the Department of Biology, Debre Markose University, Ethiopia.

Table 1. Details of the tested plant species used for wound healing in Sekela District, Ethiopia.

Plant name	Common name (Amharic)	Family	Part tested	Traditional uses	Collection number
Plantago lanceolata	Gorteb	Plantaginaceae	Leaf	Wound, body cutting	YD012
Rumex nervosus	Ambuacho	Polygonaceae	Leaf	Wound, ascariasis, snake bite, buginj	YD007
Rumex abyssinicus	Mekimeko	Polygonaceae	Root	Wound, ascariasis, gastritis	YD078
Zehneria scabra	Hareg resa	Cucurbitaceae	Leaf	Wound, sun strike, common cold	YD021

Preparation of Extracts

The extraction of each plant material was done following methods previously used with slight modifications.[35] The samples were washed thoroughly under running tap water and rinsed in distilled water, air-dried at room temperature under shade (23–27°C) and reduced to appropriate size by chopping.

Using a mechanical grinder, the dried plant parts were ground to a fine powder. The powder was sieved through 0.6 mm mesh and kept in polythene bags at 4°C until needed for extraction. The maceration technique was used for extraction. Powder and solvent 80% (methanol and ethyl acetate) were mixed in conical flasks in a 1:10 (w/v) ratio. The flasks were tightly closed, and the mix was shaken for 72 h at room temperature with an orbital shaker set to 200 rpm. After 72 h, the extract was filtered using cotton and then Whatman No.1 filter paper. A rotary evaporator (DW-RE -2000) was used to evaporate the solvent. The extract yield (%) was calculated gravimetrically using the extract's dry weight and the initial weight of the leaf powder as follows:

$$Extract\ yield(\%) = Dry\ weight\ of\ extract / Initial\ powder\ weight \times 100\%$$

Preliminary Phytochemical Screening of the Extracts

Phytochemical examination of methanol and ethyl acetate extracts of *Plantago lanceolata*, *Rumex nervosus, Rumex abyssinicus*, and *Zehneria scabra* plants was performed using established protocols to detect the active components present in the extracts. Alkaloids, saponins, phenols, tannins, anthraquinones, terpenoids, flavonoids, and steroids were tested.[36]

Test for Alkaloids (Wagner's Reagent Test)

Plant extracts were diluted in HCl and filtered. Wagner's reagent (which is an iodine solution in potassium iodide) was applied to filtrates. The presence of alkaloids in the extracts is confirmed by a reddish-brown precipitate.

Phenol Test (Ferric Chloride Test)

The extract (50 mg) was dissolved in 5 mL of purified water. A few drops of neutral 5% ferric chloride solution were added to these. The presence of phenolic compounds was indicated by bluish green or black coloration.

Test for Saponins (Foam Test)

The crude extract was mixed with 5 mL of distilled water in a test tube and was shake vigorously for 30 seconds. The formation of stable foam for 10 min indicates the presence of saponins.

Test for Terpenoids

The crude extract was dissolved in 2 mL of chloroform and dried by evaporation. To this, 2 mL of the concentrated sulfuric acid was added. Formation of a reddish-brown coloration at the interface indicates the presence of terpenoids.

Test for Tannins (Ferric Chloride Test)

Each plant extract was stirred with 1 mL of distilled water, after filtered, ferric chloride reagent was added to the filtrate. A blue-black, green, or blue-green precipitate indicates the presence of tannins.

Test for Steroids

To each of the four plant extracts, 10 mL of chloroform was added. 1 mL of acetic anhydride was added to these extracts, followed by 2 mL of concentrated sulfuric acid along the walls of the test tube. The appearance of blue green color at the junction indicates the presence of steroids.

Test for Flavonoids (Alkaline Reagent Test)

The extract was treated with 2–3 drops of Sodium hydroxide solution. Acute yellow color development shows the presence of flavonoids, which turn colorless when some drops of sulfuric acid were added.

Test for Anthraquinones

Chloroform was added to the extracts and mixed for 5 minutes. The extract was filtered and shaken with an equal volume of 100% ammonia solution. A pink, violet, or red hue in the ammoniacal layer (lower layer) showed the existence of free anthraquinones.

Bacterial Strains

Bacterial strains were selected as representative of both classes of two Gram-positive and two Gram-negative bacteria. The reference strains of *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC700603), *Staphylococcus aureus* (ATCC25923), and *S. epidermidis* (ATCC 12,228) were obtained from the Amhara Public Health Center. Pure cultures of the isolates were sub cultured and prepared for *in vitro* antimicrobial activity test using Muller Hinton agar.

Inoculum Preparation

The Kirby-Bauer technique was used to prepare inoculum with slight modification.[37] Active cultures were made by transferring a loop full of stock cultures to Mueller-Hinton agar and incubating them overnight at 37°C. A cotton swab was used to evenly distribute 0.5 mL of the bacterial

suspension onto the agar plate medium. To achieve the required standardized turbidity of the cell suspension, the inoculum suspension was prepared by picking colonies and diluting them with distilled water. The test tube's contents were thoroughly shaken by a vortex until a homogeneous suspension was formed. The inoculum's absorbance was measured with a spectrophotometer (LT-22313, India) at 600 nm and adjusted to 0.132. This value corresponds to the turbidity of a 0.5 McFarland standard. A 0.5 McFarland standard turbidity bacterial population is approximately 1.5×10^8 CFU/mL.

Antibacterial Activity Assay

Antibacterial activity of selected traditional medicinal plant extracts was examined by agar disc diffusion susceptibility method.[37] The extracts of selected medicinal plants were tested *in vitro* using the disc diffusion method against purposefully selected bacterial strains at serial test concentrations of 50, 25, 12.5, and 6.25 mg mL^{-1} from a stock concentration of 100 mg mL^{-1}. Discs of 5 mm diameter were made from Whatman No. 1 filter paper and soaked for 24 h in each test concentration. The discs were then taken out of the extract and placed on the inoculated Petri plates. Ciprofloxacin-impregnated discs served as standard controls, while those in 5% DMSO served as negative controls. The Petri plates were incubated at 37°C. Each test concentration was evaluated in triplicates. The diameter of the zone of inhibition was used to assess the antibacterial activity of extracts. Each test concentration's potency was compared to the positive and negative controls.

Minimum Inhibition Concentration and Minimum Bactericidal Concentration

Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts were conducted as described[38] with slight modification. One (1) mL of each test concentration of each extract was thoroughly mixed with 19 mL of Muller Hinton agar solution and poured to plates. The medium was allowed to solidify at room temperature and inoculated with 1 mL of *Escherichia coli, Klebsela pneumoniae, Staphylococcus aureus and Staphylococcus epidermidis* suspension adjusted to 0.5 McFarland turbidity standards. The plates were incubated at 37°C for 48 h. Petri plates without extracts were used as a control and the results were compared against these controls. MICs were based on the density of the growth control and expressed as the lowest extract concentrations that resulted in ≥ 80% reduction in bacterial growth compared to that of the extract-free growth control.[38] Test concentrations that didn't show bacterial growth were considered as MBCs.

Statistical Analysis

Antibacterial test results were analyzed using mean values of the inhibition zones. Values were expressed as mean ± SD and analyzed by one-way ANOVA ($p < .05$) using SPSS (ver. 26).

Results

Yield of Extraction

This study discovered that the yield of air-dried powder form of each medicinal plant varied in 80% methanol and ethyl acetate extraction. *R. nervosus* had the highest yield (21%) followed by *P. lanceolata* (17%). *Z. scabra* had the lowest yield (7%). Methanol extract had the highest percent (%) yield, while ethyl acetate extract had the lowest (Fig. 1).

Preliminary Phytochemical Screening of the Extracts

The methanol extracts of *P. lanceolata, R. nervosus, R. abyssinicus* and *Z. scabra* extracts tested positive for alkaloids, phenolic and tannins tests. Furthermore, *P. lanceolata and R. nervosus* contained flavonoides, phenols and steroids, whereas *R. abyssinicus* and *P. lanceolata* contained terpenoids and anthraquinones. However, *R. nervosus* and *Z. scabra* did not contain terpenoids or anthraquinones and saponins were absent from *Z. scabra*. *R. abyssinicus* was negative for the flavonoids and steroids test (Table 2).

The ethyl acetate extracts of *P. lanceolata, R. nervosus and R. abyssinicus* extracts tested positive for terpenoids flavonoids and tannins tests, whereas *Z. scabra* contained alkaloids, flavonoides and steroids. However, *R. nervosus*

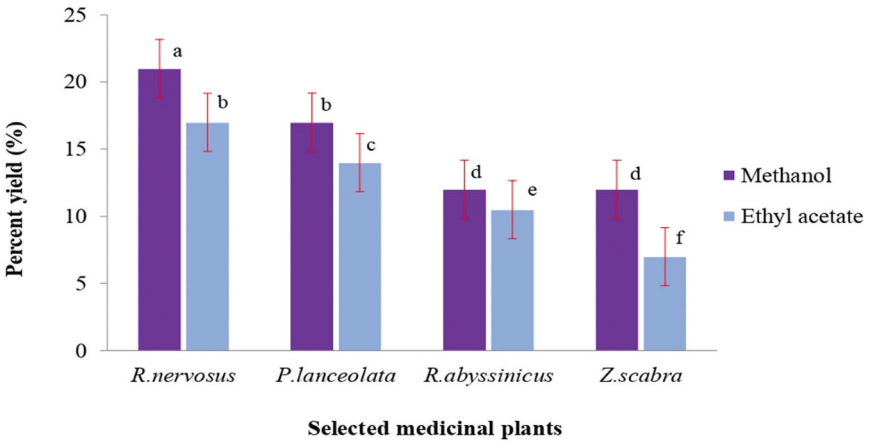

Figure 1. Yield percentage of crude extracts of rumex nervosus, plantago lanceolata, rumex abyssinicus, and Zehneria scabra in methanol and ethyl acetate.

Table 2. Preliminary phytochemical screening of rumex nervosus, plantago lanceolata, rumex abyssinicus, and Zehneria scabra extracts for secondary metabolites.

	P. lanceolata (Leaf)		*R. nervosus* (Leaf)		*R. abyssinicus* (Root)		*Z. scabra* (Leaf)	
Components	Methanol	Ethyl acetate	Methanol	Ethyl acetate	Methanol	Ethyl acetate	Methanol	Ethyl acetate
Alkaloids	+	+	+	_	+	+	+	+
Tannins	+	+	+	+	+	+	+	_
Saponins	+	_	+	_	+	_	_	_
Flavonoids	+	+	+	+	_	+	+	+
Terpenoids	+	+	_	+	+	+	_	_
Phenols	+	+	+	+	+	_	+	+
Steroids	+	_	+	+	_	_	+	+
Anthraquinones	+	+	_	_	+	+	_	_

+ = present, − = absent.

and *Z. scabra* did not contain saponins or anthraquinones and alkaloids were absent from *R. nervosus* (Table 2).

Antibacterial Activities of Selected Medicinal Plants

Medicinal plant extracts showed a dose-dependent progressive increase in the growth inhibition zone (Fig. 2). No difference was observed in terms of susceptibility among four medicinal plants. There was a difference in the

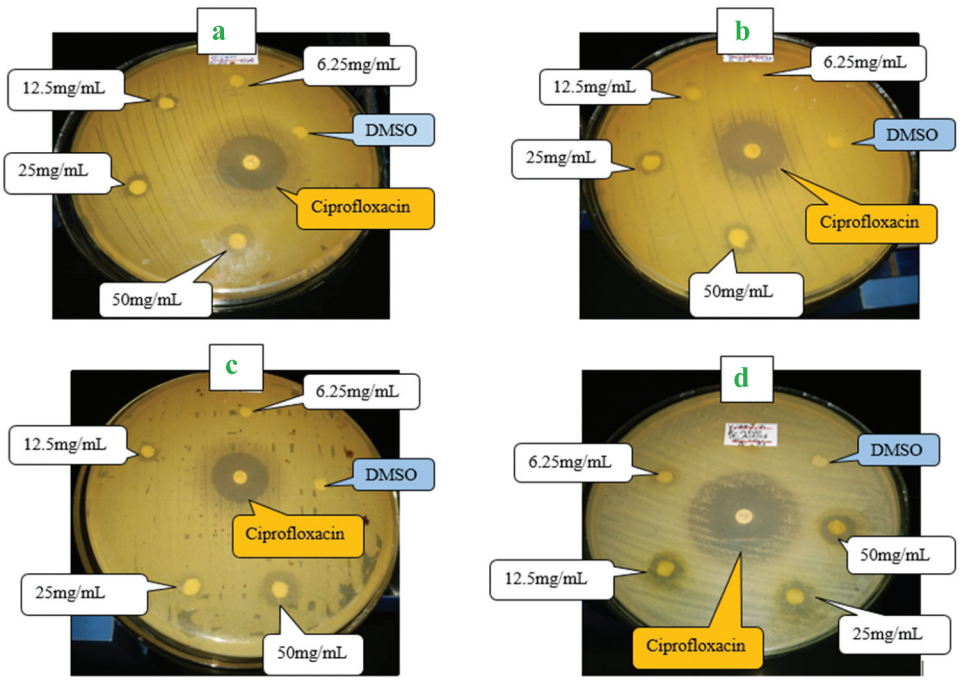

Figure 2. Antibacterial tests showing an inhibitory zone, (a) plantago lanceolata against Escherichia coli (b) rumex abyssinicus against Klebsiella pneumoniae, and (c) zehneria scabra against Staphylococcus aureus and (d) rumex nervosus against E. coli.

zone of inhibition among test concentration of plant extracts. However, no growth inhibition difference was detected among tested bacterial strains. The negative control (5% DMSO) showed no antibacterial activity (Table 3).

Antibacterial Activities of Plantago Lanceolata Against Tested Bacterial Strains

P. lanceolata crude leaf extract demonstrated effective bacterial growth inhibition against all tested bacteria. The highest antibacterial activity was observed from methanol extract against *S. epidermidis* isolates (16.67 ± 1.15 mm at 50 mg mL^{-1}) and the lowest growth inhibition zone (4 ± 1.7 mm at 6.25 mg mL^{-1}) was observed against *K. pneumoniae* strain. Similarly, the ethyl acetate extract inhibited the most *S. epidermidis* isolate (12.33 ± 1.53 mm at 50 mg mL^{-1}), while the least inhibition zone (1.67 ± 0.58 mm at 6.25 mg mL^{-1}) was observed against *E. coli* (Table 3).

Antibacterial Activities of Rumex Nervosus Against Tested Bacterial Strains

The antibacterial activity of *R. nervosus* leaf extracts in methanol and ethyl acetate showed that the zone of inhibition was between 2 and 14 mm. The highest zone of inhibition was recorded against *S. epidermidis* (14 ± 0.00) at 50 mg mL^{-1} in methanol extracts and the lowest zone of inhibition was observed against *E. coli* and *S. epidermidis* (2 ± 2) at 6.25 mg mL^{-1} in methanol and ethyl acetate extracts, respectively (Table 3).

Antibacterial Activities of Rumex Abyssinicus Against Tested Bacterial Strains

R. abyssinicus methanol and ethyl acetate root extract revealed zone of inhibition between 0.00 and 12.00 mm. The maximum zone of inhibition (12.00 ± 1) was observed from methanol extract against *S. aureus* at 50 mg mL^{-1}, and the lowest (0 ± 0) was recorded from *E. coli* and *S. epidermidis* at 6.25 mg mL^{-1} from both extracts (Table 3).

Antibacterial Activities of Zehneria Scabra Against Tested Bacterial Strains

The maximum zone of inhibition (13 ± 1) was recorded against *S. aureus* at 50 mg mL^{-1}, from methanol extract and the lowest inhibition zone (0 ± 0) was observed from *E. coli* at 6.25 mg mL^{-1} from ethyl acetate extracts (Table 3).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The extracts' effectiveness was also assessed using the MIC and MBC. Their MIC ranged from 6.25 mg mL^{-1} to 50 mg mL^{-1}, and their MBC ranged from 25 mg mL^{-1} to 100 mg mL^{-1}. *S. aureus* and *S. epidermidis* recorded a relatively lower MIC (6.25 mg mL^{-1}) from methanol extracts of *P. lanceolata*, while

Table 3. Mean inhibition zone of selected medicinal plants against tested bacterial strains.

			Mean inhibition zone (mm)			
			Gram-negative		Gram-positive	
Plant species	Solvent	Concentration (mg mL^{-1})	*Escherichia coli*	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Staphylococcus epidermidis*
Plantago ***lanceolata***	Methanol	50	13.67 ± 0.58a	14.67 ± 0.58b	15.33 ± 1.15c	16.67 ± 1.15d
		25	13 ± 1ab	9.67 ± 0.58c	14 ± 1 cd	11 ± 1e
		12.5	11 ± 1ab	9 ± 1c	7 ± 1de	9.67 ± 0.58e
		6.25	4.33 ± 0.58c	4 ± 1.70d	6.66 ± 0.58f	6.67 ± 0.58f
	Ethyl acetate	50	11 ± 1a	10.67 ± 0.58b	12.67 ± 1.15c	12.33 ± 1.53d
		25	10.67 ± 0.58a	10 ± 0b	10.67 ± 1.15c	10.33 ± 1.53 cd
		12.5	8.33 ± 0.58b	8 ± 1c	7.33 ± 1.15d	8.33 ± 0.58de
		6.25	1.66±.58c	3.67 ± 0.58d	2 ± 0e	3.33 ± 0.58f
Rumex ***nervosus***	Methanol	50	11.67 ± 1.15a	12.33 ± 0.58b	13 ± 1c	14 ± 0a
		25	9.33 ± 1.53ab	9 ± 1c	13.33 ± 1.15c	8 ± 1b
		12.5	5.67 ± 0.58bc	8 ± 0c	8 ± 1d	9 ± 0b
		6.25	2 ± 2 cd	2.33 ± 1.53d	5.33 ± 1.15d	4.67 ± 1.15c
	Ethyl acetate	50	9 ± 1a	9.67 ± 1.53b	13 ± 1c	12.33 ± 1.53d
		25	8.33 ± 1.15a	9.33 ± 1.15b	11 ± 1c	11.33 ± 1.15d
		12.5	2.33 ± 0.58b	6.67 ± 1.15b	6.67 ± 0.58d	7.33 ± 1.15e
		6.25	4.33 ± 0.58b	2 ± 2c	3.67 ± 0.58e	3.33 ± 0.58f
Rumex ***abyssinicus***	Methanol	50	10 ± 1a	8.67 ± 0.58b	12 ± 1c	10 ± 1d
		25	7.67 ± 1.53ab	8 ± 1.73b	10.33 ± 0.58c	8.33 ± 2.52d
		12.5	5.33 ± 1.15bc	4.33 ± 1.53c	6.33 ± 0.58d	6.67 ± 1.53de
		6.25	0 ± 0d	0.67 ± 1.15d	3.67 ± 0.58e	3.33 ± 1.15ef
	Ethyl acetate	50	9 ± 1.73a	10.33 ± 2.08b	10 ± 1c	11.33 ± 1.53d
		25	6.67 ± 2.31a	6.67 ± 1.15bc	9 ± 1 cd	7.67 ± 1.53c
		12.5	3.33 ± 1.15ab	3.67 ± 1.53 cd	7 ± 1de	5.33 ± 1.55c
		6.25	0 ± 0bc	0 ± 0de	0.67 ± 1.55f	1.67 ± 0.58e
Zehneria ***scabra***	Methanol	50	9.67 ± 1.53a	10 ± 1b	13 ± 1c	10.67 ± 0.58d
		25	8 ± 1ab	7.33 ± 1.55bc	8.67 ± 0.58d	8 ± 2d
		12.5	6 ± 1b	6 ± 2 cd	7.33 ± 1.55de	6.33 ± 2.52de
		6.25	1.33 ± 0.58d	1 ± 1e	4.33 ± 2.08ef	3.33 ± 1.55ef
	Ethyl acetate	50	8.67 ± 1.55b	9 ± 1b	11 ± 1c	9 ± 1d
		25	7 ± 1bc	7 ± 1b	9 ± 1 cd	8.33 ± 0.58d
		12.5	5.33 ± 1.55c	4.67 ± 0.58c	5.67 ± 2.52de	5.33 ± 1.55e
		6.25	0 ± 0d	0.33 ± 0.58d	2.67 ± 1.55ef	2.33 ± 1.53f
Positive	Ciprofloxacin (500 g)		**20.33 ± 1.15**	**21 ± 0.57**	**25.33 ± 1**	**24.67 ± 0.58**
Negative	DMSO (5%)		**NI**	**NI**	**NI**	**NI**

NI = no inhibition zone observed, values in a column followed by similar letter are not different ($p < .05$). This comparison was made between test concentrations of a species for each tested bacteria strain.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

			Tested strains			
			Gram negative		Gram positive	
Plants	Solvent		Escherichia coli	Klebsiella pneumoniae	Staphylococcus aureus	Staphylococcus epidermidis
Plantago lanceolata	Methanol	MIC	12.5	25	6.25	6.25
		MBC	50	80	25	50
	Ethyl acetate	MIC	50	50	25	50
		MBC	100	80	50	100
Rumex nervosus	Methanol	MIC	25	25	12.5	12.5
		MBC	80	80	25	80
	Ethyl acetate	MIC	50	50	12.5	25
		MBC	80	100	80	80
Rumex abyssinicus	Methanol	MIC	25	12.5	12.5	6.25
		MBC	80	80	50	50
	Ethyl acetate	MIC	50	25	25	50
		MBC	100	80	80	80
Zehneria scabra	Methanol	MIC	50	50	50	50
		MBC	80	80	80	80
	Ethyl acetate	MIC	50	50	50	50
		MBC	100	100	80	80

Values show concentration of plant extracts in mg mL^{-1}

P. lanceolata and *R. nervosus* recorded a lower MBC (25 mg mL^{-1}) against *S. aureus*. Ethyl acetate extracts of *R. nervosus*, on the other hand, had a lower MIC (12.5 mg mL^{-1}) against *S. aureus* (Table 4).

Discussion

The growing threat of drug-resistant bacterium strains against conventional antimicrobial medications prompted a global effort to identify remedies based on natural plant products as alternate means to combat these organisms.[39] The secondary metabolites present in plants may have different modes of antimicrobial action which help combat the emergence of resistance.[40] Bioactive plant products are used as drugs, lead compounds, biological or pharmacological tools, and as raw materials for the production of drugs.[41]

The antibacterial properties of *P. lanceolata, R. nervosus, R. abyssinicus*, and *Z. scabra* extracts were investigated. The highest extraction yield from methanol extracts was obtained from *R. nervosus*. Factors including plant bioactive constituents, extraction technique, plant parts used, concentration, and solvent type can all have a significant impact on extract yield.[42] The polarity difference in the solvent used in the study could explain the difference.

According to preliminary phytochemical analysis, methanol extracts contained more bioactive components than ethyl acetate extracts. It's because methanol is more polar than ethyl acetate. A previous study reported that methanol extracts contained more bioactive compounds than ethyl acetate extracts.[43] The presence of alkaloids, flavonoids, tannins and phenols in all four tested plant extracts accounted for the broad-spectrum antibacterial activities observed in this study, which is in agreement with the previous findings.[9]

Methanol and ethyl acetate extracts of chosen bacterial strains demonstrated a dose-dependent progressive rise in the growth inhibition zone, with a substantial difference in test concentrations inhibiting the development of tested pathogens. This suggests that the bioactive chemicals increase in concentration as the dose increases. The secondary metabolites derived from plants, such as alkaloids, anthraquinones, flavonoids, phenols, saponins, tannins, steroids, and terpenoids, have significant antibacterial activities,[44] which were present in the extracts tested in this experiment. Total phenolics, total flavonoids, total tannins, and total anthocyanins may be responsible for the highest biological activities.[45] Weak antibacterial activities of the studied plants may be attributed to the bacterial strains' resistance to the plant extracts.[46]

When methanol, ethyl acetate and DMSO were compared, methanol had a slightly higher inhibition zone which may be due to the polarity of the solvents.[7] Polar components cannot dissolve nonpolar components, reducing the antibacterial activity of the aqueous extract. Methanol has a great capacity to dissolve all polar and nonpolar molecules, hence the methanolic extract demonstrated higher inhibition against the pathogens.[47] Methanol was the most effective solvent that reduced the radial growth of the tested pathogens compared to other solvents.[48]

Staphylococcus aureus and S. epidermidis were the most susceptible strains compared to the other tested bacterial strains; however, *E. coli* was the least responsive pathogen. This could be because gram-negative bacteria have an outer phospholipid membrane structure that limits drug uptake.[49] Gram-positive bacteria, on the other hand, are more vulnerable because they only have an outer peptidoglycan layer, which is ineffective as a barrier.[49] Gram-negative bacteria have a cell envelope (lipopolysaccharide) that restricts access to the membrane more than Gram-positive bacteria do.[49] Similarly, others[50] reported *S. aureus* as the most sensitive to extracts obtained using methanol solvent. In contrast to the current study, others[51] reported that gram negative bacteria were the most susceptible strains to *Otostegia integrifolia* leaf extracts. This could be due to the microbial strain differences.

The extracts' efficacy was also evaluated using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The lowest concentration of the different extracts of the plant material that showed no observable growth of the tested bacteria was intended as the MIC.[52] The MBC was defined as the lowest concentration of the extracts at which there was no sign of bacterial growth.[53] *S. aureus* and *S. epidermidis* had a lower MIC from methanol extracts of *P. lanceolata*. This could indicate that *P. lanceolata* is effective against the tested bacterial strains. Other studies[54] explained that plants had varying degrees of MIC and MBC against the tested pathogens due to differences in plant bioactive constituents and the ability of the tested pathogen to respond to plant extracts.

The highest MIC and MBC values indicate that the plant extracts are less effective on some bacteria (gram-negative bacteria) or that the organism has the potential to develop antibiotic resistance, whereas low MIC and MBC values for other bacteria indicate that the plant extract is effective.[55] Other studies showed that chloroform extract of *Discopodium penninervium* had a lower MIC (12.5 mg mL^{-1}) against *staphylococcus aureus*.[51]

Conclusions

P. lanceolata, R. nervosus, R. abyssinicus, and *Z. scabra* methanol and ethyl acetate extracts displayed antibacterial efficacy against selected bacterial strains. The methanol extract of *P. lanceolata* exhibited significant antibacterial activity and the lowest MIC against *S. epidermidis*, the most sensitive organism; as a result, *P. lanceolata* may be identified as the most promising plant for wound healing as well as the development of novel drugs. *R. abyssinicus* and *Z. scabra* ethyl acetate extracts, on the other hand, showed no efficacy against *E. coli* at 6.25 mg mL^{-1}. These findings back up the long-held belief that the four medicinal herbs examined in this study may have antibacterial properties in wound infections.

Disclosure Statement

No potential conflict of interest was reported by the author(s).

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Data Availability Statement

All relevant data are within the manuscript and its supporting information files.

Author Contributions

YD conceived the study, carried out the experiments, analyzed and evaluated the experimental data, and wrote the draft and final manuscript. NA oversaw the work, engaged in the study design, and provided feedback on the write-up. AF helped collect plant samples, analyze data, and participate in manuscript preparation. ML oversaw the extraction and carried out the antimicrobial test. The final manuscript was read and approved by all of the authors.

Abbreviations

ATCC	American Type Culture Collection
ANOVA	Analysis of Variance

CFU	Colony Forming Unit
DMSO	Dimethylsulfoxide
MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibitory Concentration
WHO	World Health Organization

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