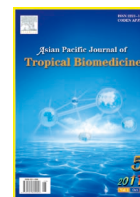




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Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia

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ABSTRACT

Objective: To evaluate the activity of selected Ethiopian medicinal plants traditionally used for wound treatment against wound-causing bacteria. **Methods:** Samples of medicinal plants (*Achyranthes aspera*, *Brucea antidysenterica*, *Datura stramonium*, *Croton macrostachyus*, *Acokanthera schimperi*, *Phytolacca dodecandra*, *Millettia ferruginea*, and *Solanum incanum*) were extracted using absolute methanol and water and tested for their antimicrobial activities against clinical isolates and standard strains of wound-causing bacteria using agar well diffusion and micro titer plate methods. **Results:** Most of the plant extracts had antibacterial activities, among which *Acokanthera schimperi* and *Brucea antidysenterica* inhibited growth of 100% and 35% of the test organisms, respectively. Methanolic extracts had higher activities compared with their corresponding aqueous extracts. The most susceptible organism to the extracts was *Streptococcus pyogenes* while the most resistant were *Escherichia coli* and *Proteus vulgaris*. **Conclusions:** This finding justifies the use of the plants in wound healing and their potential activity against wound-causing bacteria. Their toxicity level and antimicrobial activity with different extraction solvents should further be studied to use them as sources and templates for the synthesis of drugs to control wound and other disease-causing bacteria.

1. Introduction

Wounds, resulting from microbial infection, are the most common public health problems[1]. The common wound pathogens includes bacteria, fungi, protozoa and viruses among which the most common are beta-haemolytic *Streptococci* [*Streptococcus pyogenes* (*S. pyogenes*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*)] [2], *Proteus*, *Escherichia coli* (*E. coli*) and *Enterococcus* [3], *Acinetobacter* spp, *Klebsiella* spp [4], and *Coliforms* [5].

Although wounds may heal through the body's natural process of regenerating dermal and epidermal tissues, chronic forms cause significant impact on health and economic growth [6]. Current methods used to treat chronic wounds include debridement, irrigation, antibiotics, tissue grafts and proteolytic enzymes, which have

major drawbacks and unwanted side effects [7]. Topical antimicrobials may be indicated when the clinical signs and symptoms of an active infection are present. Complications of deep tissue infections such as bacteremia can be treated with systemic antibiotic [7]. However, the increase in antibiotic resistant strains together with the lack of and high cost of new generation antibiotics increased wound-related morbidity and mortality [8].

Ethnobotanical studies revealed that a wider range of Ethiopian plants are being used in the treatment of wounds and other diseases in the traditional health care system of the country [9–14]. Crude extracts of Ethiopian plants and others used elsewhere [15–19] revealed strong antibacterial activities indicating that these plants can serve as sources of effective drugs against wound-causing bacteria. The objective of this study was therefore to evaluate the activity of selected Ethiopian medicinal plants traditionally used for wound treatment against wound-causing bacteria.

2. Materials and methods

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2.1. Plant collection, identification and extraction

Selected Ethiopian plants that are used for traditional treatment of wounds^[9,10,15] were collected, identified, extracted and tested for their antibacterial activities against bacteria isolated from wounds. The plants were *Achyranthes aspera* (*A. aspera*) (leaf), *Brucea antidysenterica* (*B. antidysenterica*) (root), *Datura stramonium* (*D. stramonium*) (leaf), *Croton macrostachyus* (*C. macrostachyus*) (leaf), *Acokanthera schimperi* (*A. schimperi*) (leaf), *Phytolacca dodecandra* (*P. dodecandra*) (root), *Millettia ferruginea* (*M. ferruginea*) (leaf), and *Solanum incanum* (*S. incanum*) (leaf). Voucher specimens were stored at Aklilu Lemma Institute of Pathobiology, Addis Ababa University following identification. Plant materials were washed using tap water, air dried in shade and powdered using wooden-made pestle and mortar. The powdered plant materials were sieved and extracted in distilled water and absolute methanol.

2.2. Preparation of plant extracts and test organisms

Plant extracts were reconstituted with water to make a solution of 500 mg/mL and then filtered with a membrane of pore size of 0.2 μ m in a sterile nunc tubes. Sterility of the filtered extracts was checked by plating them on Muller Hinton agar^[8].

The test bacteria were *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, *Proteus vulgaris* (*P. vulgaris*), isolated from wound specimens, and standard strains [(American Type Culture Collection (ATCC)], *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 19615), *E. coli* (ATCC 25922), *P. aeruginosa* (27853) and *P. vulgaris* (PROVU-01). *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 19615), *E. coli* (ATCC 25922) and all the clinical isolates were obtained from stored samples at All Africa Leprosy, Tuberculosis and Rehabilitation Training Centre (ALERT)/Armauer Hansen Research Institute (AHRI) Research and Diagnostic Microbiology Laboratory. *P. aeruginosa* (27853) and *P. vulgaris* (PROVU-01) were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI) Microbiology Department.

The test organisms were grown in 5 mL Brain Heart Infusion (BHI) broth at 37 °C, authenticated and maintained in Muller Hinton agar medium. Twenty-four hour old pure cultured bacteria were used to prepare a density of 10⁸ cells mL⁻¹ of 0.5 McFarland standards during each test^[21]. Muller-Hinton agar was prepared according to the manufacturer's instruction, autoclaved and dispensed at sterile plate.

2.3. Antibacterial susceptibility tests

2.3.1. Agar well diffusion

Bacterial broth culture was prepared to a density of 10⁸ cells mL⁻¹ of 0.5 McFarland standard. The aliquot was spread evenly onto Muller Hinton agar or Muller Hinton agar supplemented with 5% sheep blood (for *S. pyogenes*) by

sterile cotton swab. Then, the plated medium was allowed to dry at room temperature for 30 minutes^[17]. On each plate, equidistant wells were made with a 6 mm diameter sterilized, cork borer, 2 mm from the edge of the plate. Fifty micro liter of each plant extract (500 mg/mL) was aseptically introduced into a respective agar well. Ciprofloxacin (5 μ g/mL) and amoxicillin (25 μ g/mL) were used as positive controls and the extraction solvents methanol and distilled water were included as negative controls. This was followed by allowing the agar plate on the bench for 40 minutes pre-diffusion followed by incubation at 37 °C for 24–48 h. The formation of clear inhibition zone of ≥ 7 mm diameters around the wells were regarded as significant susceptibility of the organisms to the extract^[22]. The experiment was performed in duplicate. Experiments that gave contradicting results were done for the third time for an easy decision.

2.3.2. Determination of minimum inhibitory concentration (MIC)

MIC was determined for extracts that showed ≥ 7 mm diameter growth inhibition zone. The test was performed using agar well diffusion and micro titer plate (Micro-tube dilution) methods. In agar well diffusion, the extract solution (500 mg/mL) was serially diluted as 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256 to bring 250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL, 15.63 mg/mL, 7.81 mg/mL, 3.95 mg/mL and 1.95 mg/mL concentrations, respectively. The extract was then aseptically introduced as described in section 4.3.1. The inhibition zone was measured after 24 h incubation at 37 °C and the minimum concentration that inhibited growth was considered as MIC value of the extract.

In micro titer plate (micro tube dilution), the 500 mg/mL extract was serially diluted as described above in nutrient broth and 20 μ L of a standard suspension of the test organism was then added to each concentration of the extract. The micro well plates were incubated at 37 °C for 24 h and presence of growth was evaluated by comparing the optical density (OD) of each well before and after incubation. The OD for micro tube dilution method was read by MULTISKAN PLUS VERSION 1.4 ELISA readers at 405 nm. When the difference of OD value (after incubation–before incubation) of the test (broth + extract + organism) was greater than that of the control (broth + extract) at each concentration, it was considered as presence of turbidity or growth of bacteria. The lowest concentration, at which there was no turbidity, was also regarded as MIC value of the extract.

2.3.3. Determination of the minimum bactericidal concentration (MBC)

MBC was determined by sub-culturing the samples having a value of lesser or equal to MIC value. The highest dilution (lesser concentration) that yielded no single bacterial colony was taken as MBC.

3. Results

Crude extracts of *A. schimperi*, *B. antidysenterica*, *P. dodecandra* and *S. incanum* showed bacterial growth inhibition of 100%, 35%, 25% and 25% against the test organisms, respectively. These plants inhibited growth of bacteria from 7 mm to 15 mm diameter (Table 1). Both aqueous and methanolic extracts of *A. schimperi* leaves caused 7 mm to 14 mm inhibition zones of bacterial growth. The bacterial strains, which were inhibited with a zone diameter of 14 mm, were *S. pyogenes* (ATCC) (with both methanol and aqueous extracts) and *S. aureus* (ATCC) with methanol extract. Its aqueous extract caused higher growth inhibition compared with its methanolic extract against clinical isolates of *S. aureus* (11 mm), *S. pyogenes* (12 mm) and *P. vulgaris* (10 mm). It showed low growth inhibition zones (7 mm diameter) against the clinical and standard strains of *E. coli*.

Methanolic extract of *B. antidysenterica* root was the second to inhibit the growth of 6 bacterial strains with inhibition zones ranging from 6 mm (against clinically isolated *P. aeruginosa*) to 15 mm [against standard *S. aureus* (ATCC)]. Its aqueous extract inhibited growth of *S. pyogenes* (ATCC) within 12 mm diameter.

P. dodecandra had activity on bacterial strains that were inhibited by *B. antidysenterica* except for clinical and standard strains of *S. aureus*. It inhibited growth of these organisms within 8 mm diameter except for *S. pyogenes* (ATCC), which extended to 10 mm diameter.

Methanolic extract of *S. incanum* inhibited growth of *S. aureus* (clinical isolate), *S. pyogenes* (ATCC) with growth

inhibition zone ranging from 8 mm to 9 mm. Aqueous extract of the plant showed better inhibition (10 mm diameter) on *S. pyogenes* (ATCC) than its methanolic extract (9 mm).

S. pyogenes (ATCC) was the most inhibited bacteria by most of the plant extracts. It was highly inhibited by the aqueous and methanolic extracts of *A. schimperi* and by methanolic extract of *B. antidysenterica*. The second most inhibited bacteria were the clinical isolates and standard strains of *P. aeruginosa*. Growth of these organisms was inhibited by aqueous extract of *A. schimperi* leaves and the methanolic extract of *B. antidysenterica*, *A. schimperi*, *P. dodecandra* and *S. incanum*. The most resistant bacterial strains against the plant extracts were both the clinical and standard strains of *E. coli* and *P. vulgaris*, which were susceptible only to the aqueous and methanolic extracts of *A. schimperi*.

The MIC value of plant extracts against the tested bacteria ranged from 1.95 mg/mL (methanolic extract of *P. dodecandra* on *S. pyogenes*) to 250.00 mg/mL (aqueous extract of *A. schimperi* on the same bacteria) (Table 2). The most frequent MIC value of the extracts was 62.50 mg/mL, followed by 15.63 mg/mL, 125.00 mg/mL, 31.50 mg/mL, 7.81 mg/mL, 3.90 mg/mL, 1.95 mg/mL and 250.00 mg/mL.

The MIC values of *A. schimperi* ranged from 3.91 mg/mL to 125.00 mg/mL. Its aqueous extract had higher MIC values than its methanolic extracts except for clinically isolated *P. aeruginosa* and standard strains of *P. vulgaris*, which had similar MIC values for both extracts. The MIC values of *B. antidysenterica* (methanol extract) ranged from 7.81 mg/mL to 125.00 mg/mL. Its aqueous extract has MIC value of 31.25 mg/mL against *S. pyogenes* (ATCC).

Methanolic extracts of *C. macrostachyus*, *D. stramonium* and *M. ferruginea* had MIC value of 7.81 mg/mL, 62.50 mg/

Table 1

Mean bacterial growth inhibition zones in agar well diffusion method treated with 500 mg/mL of plant extracts (mm).

Plant species		Bacteria									
		<i>S. aureus</i>		<i>S. pyogenes</i>		<i>E. coli</i>		<i>P. aeruginosa</i>		<i>P. vulgaris</i>	
		Clin	Stand	Clin	Stand	Clin	Stand	Clin	Stand	Clin	Stand
<i>A. aspera</i>	W	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	11	–	–	–	–	–	–
<i>A. schimperi</i>	W	11	13	12	14	7	7	9	10	10	12
	M	9	14	9	14	7	8	10	10	8	13
<i>B. antidysenterica</i>	W	–	–	–	12	–	–	–	–	–	–
	M	13	15	9	14	–	–	7	8	–	–
<i>C. macrostachyus</i>	W	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	10	–	–	–	–	–	–
<i>D. stramonium</i>	W	–	–	–	13	–	–	–	–	–	–
	M	–	–	–	9	–	–	–	–	–	–
<i>M. ferruginea</i>	W	–	–	–	8	–	–	–	–	–	–
	M	–	13	–	–	–	–	–	–	–	–
<i>P. dodecandra</i>	W	–	–	–	8	–	–	–	–	–	–
	M	–	–	8	10	–	–	8	8	–	–
<i>S. incanum</i>	W	–	–	–	10	–	–	9	8	–	–
	M	8	–	–	9	–	–	–	–	–	–
Amoxicillin		14	18	23	31	23	23	–	–	17	24
Ciprofloxacin		16	25	16	28	33	30	33	26	35	32

Clin = clinical isolate, Stand = standard (ATCC) strains, W = water extract, M = methanol extract.

Table 2

MIC values of selected plant extracts against the tested organisms using agar well diffusion and micro titration methods (mg/mL).

Plant species	Method		Bacteria									
			<i>S. aureus</i>		<i>S. pyogens</i>		<i>E. coli</i>		<i>P. aeuruginosa</i>		<i>P. vulgaris</i>	
			Clin	Stand	Clin	Stand	Clin	Stand	Clin	Stand	Clin	Stand
<i>A. schimperi</i>	Agar well diffusion	W	125.00	125.00	250.00	250.00	250.00	250.00	250.00	250.00	250.00	250.00
		M	125.00	125.00	500.00	250.00	250.00	500.00	125.00	125.00	500.00	500.00
	Micro titration	W	62.50	62.50	31.25	125.00	62.50	62.50	15.63	62.50	31.25	15.63
		M	3.91	15.63	15.63	7.81	15.63	15.63	15.63	15.63	15.63	15.63
<i>B. antidysenterica</i>	Agar well diffusion	M	125.00	62.50	250.00	62.50	–	–	500.00	125.00	–	–
	Micro titration	M	15.63	15.63	62.50	7.81	–	–	125.00	62.50	–	–
<i>P. dodecandra</i>	Agar well diffusion	M	–	–	250.00	250.00	–	–	250.00	250.00	–	–
	Micro titration	M	–	–	62.50	1.95	–	–	62.50	15.63	–	–
<i>S. incanum</i>	Agar well diffusion	M	–	250.00	–	250.00	–	–	250.00	250.00	–	–
	Micro titration	M	–	31.25	–	62.50	–	–	62.50	125.00	–	–

Clin = clinical isolate, Stand = standard (ATCC) strains, W = water extract, M = methanol extract.

Table 3

MBC of crude plant extracts (mg/mL).

Plant species		Bacteria									
		<i>S. aureus</i>		<i>S. pyogens</i>		<i>E. coli</i>		<i>P. aeuruginosa</i>		<i>P. vulgaris</i>	
		Clin	Stand	Clin	Stand	Clin	Stand	Clin	Stand	Clin	Stand
<i>A. aspera</i>	W	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	62.5	–	–	–	–	–	–
<i>A. schimperi</i>	W	62.50	62.50	31.25	125.00	62.50	62.50	15.63	125.00	31.25	15.63
	M	7.81	15.63	15.63	62.50	31.25	7.81	15.63	15.63	62.50	31.25
<i>B. antidysenterica</i>	W	–	–	–	125.00	–	–	–	–	–	–
	M	250.00	125.00	7.81	7.81	–	–	62.50	62.50	–	–
<i>C. macrostachyus</i>	W	–	–	–	–	–	–	–	–	–	–
	M	–	–	–	7.81	–	–	–	–	–	–
<i>D. stramonium</i>	W	–	–	–	250.00	–	–	–	–	–	–
	M	–	–	–	62.50	–	–	–	–	–	–
<i>M. ferruginea</i>	W	–	–	–	62.50	–	–	–	–	–	–
	M	–	62.50	–	–	–	–	–	–	–	–
<i>P. dodecandra</i>	W	–	–	–	125.00	–	–	–	–	–	–
	M	–	–	31.25	1.95	–	–	250.00	31.25	–	–
<i>S. incanum</i>	W	–	–	–	500.00	–	–	62.50	125.00	–	–
	M	62.50	–	–	62.50	–	–	–	–	–	–

Clin = clinical isolate, Stand = standard (ATCC) strains, W = water extract, M = methanol extract.

mL and 62.50 mg/mL against *S. pyogens* (ATCC), respectively. While the aqueous extract of *D. stramonium*, *P. dodecandra* and *M. ferruginea* had MIC value of 125.00 mg/mL, against this organism. The MIC value of *S. incanum* ranged from 31.25 mg/mL to 250.00 mg/mL. Its MIC value against *S. pyogens* (ATCC) was similar to that of *P. aeuruginosa* clinical isolate (62.50 mg/mL).

The MBC values, which were determined by sub-culturing the samples having dilution values of greater or equal to MIC values, were described in Table 3. The MBC values of the extracts ranged from 1.95 mg/mL (methanolic extract of *P. dodecandra*) to 500.00 mg/mL (aqueous extract of *S. incanum*) against the growth of *S. pyogens* (ATCC). The MBC and MIC values of most plant extracts were similar. Methanolic extract of *A. schimperi* leaves had MBC values which ranged from 7.81 mg/mL [against *S. aureus* clinical and *E. coli* (ATCC)] to 62.50 mg/mL [against *S. pyogens* (ATCC) and *P. vulgaris* (clinical)]. The corresponding result of its aqueous extract ranged from 15.63 mg/mL against *P. aeuruginosa*

(clinical) and *P. vulgaris* (ATCC) to 125.00 mg/mL against *S. pyogens* (ATCC).

B. antidysenterica, the second important plant in suppressing bacterial growth in this study, has MBC values of 7.81 mg/mL, 62.50 mg/mL, 125.00 mg/mL and 500.00 mg/mL against *S. pyogens*, *P. aeuruginosa*, *S. aureus* (ATCC), and *S. aureus* (clinical), respectively. Similarly, treatment with the aqueous extracts of *M. ferruginea* leaves, *P. dodecandra* root, *D. stramonium* leaves and *S. incanum* leaves showed MBC values of 62.50 mg/mL, 125.00 mg/mL, 250.00 mg/mL and 500.00 mg/mL, respectively against *S. pyogens* (ATCC). The methanolic extract of *S. incanum* caused the same MBC value (62.50 mg/mL) against clinical isolates of *S. aureus*, *P. aeuruginosa* and standard strain *S. pyogens*.

4. Discussion

Ethnobotanical investigations have been found to offer

important clues in the identification and development of traditionally used medicinal plants into modern drugs. Contribution of the field has also been reflected in the current study. Methanolic and aqueous extracts of *A. schimperi* leaves showed strong antibacterial activity against 10 bacterial strains, which strengthens the report by Tadege *et al*^[15]. Size of its growth inhibition zone (in diameter) was similar to that of *B. antidyenterica* and a little higher than the methanolic extract of *P. dodecandra* and *S. incanum*. Its methanolic extract was also reported to have activities against the viruses coxasackie (CVB3), influenza A and herpes simplex type 1 kupka (HSV-1)^[23]. This result, together with ethnobotanical studies, shows that the plant might have important compounds that can be used for the treatment of wound-causing bacteria and viruses.

Methanolic extract of *A. schimperi* showed minimum inhibition zones at a concentration of 125 mg/mL against *S. aureus* and *P. aeruginosa* in agar well diffusion method. This was similar to the methanolic extract of *M. ferruginea* on *S. aureus* (ATCC) and *B. antidyenterica* on clinically isolated *S. aureus* and standard strain of *P. aeruginosa*.

Most of the MBC and MIC value of *A. schimperi* as well as other plant extracts were almost similar, or MBC equal to one dilution factor less than MIC value of the extract. The similarity or closeness of the MBC and MIC values of the plant extracts could be due to the sensitivity of the micro titration method in detecting minimum amount of turbidity which was the indicator of the growth of the test organisms than visual inspection.

Methanolic extract of the root of *B. antidyenterica* was the second strong plant for its antibacterial activity. But its water extract had lower activity. This indicates that the active principle which inhibits the growth of susceptible bacteria may dissolve better in methanol than in water.

The root of *P. dodecandra* had strong activity against *S. pyogens* and *P. aeruginosa* comparable to the root of *B. antidyenterica*. Similarly, its petroleum ether extract was indicated to be active against gram-positive bacteria with inhibition diameter ranging from 12 to 20 mm^[24]. This is an added advantage since the berries of *P. dodecandra* are effective against the dermatophytes^[25] and equine histoplasmosis^[26].

Although the root and stem extracts of *D. stramonium* were reported to inhibit growth of *P. aeruginosa*, *Klebsiella pneumonia*, and *E. coli* with MIC of 25% W/V^[27] and *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*^[20], we found no activity in the present study. Our finding is in agreement with a study by Eftekhar *et al*^[28].

Methanolic extract of *S. incanum* was active against the clinical isolates of *S. aureus* and *P. aeruginosa* and standard strains of *S. pyogens* and *P. aeruginosa* (ATCC) which is in agreement with the study by Al-Fatimi *et al*^[29]. Methanolic extract of *A. aspera* has activity against *S. pyogens* (ATCC). Similar extract was reported to have activity against the pathogenic *Streptococcus mutants*^[15]. Except for

S. pyogens (ATCC), both the methanolic and aqueous extracts of *A. aspera* did not show antibacterial activity. In contrast, alcoholic and aqueous extracts of its leaves are indicated to have activities against *S. aureus* and *E. coli*^[16]. Lakshmi Naidu *et al*^[30] also indicated that methanol extract of *A. aspera* inhibited the growth of *S. aureus* with inhibition zone diameter of 9 mm. The inactivity of *A. aspera* on *S. aureus* or other tested organisms except for *S. pyogens* (ATCC) could be the difference in the concentration of the active principles that might vary due to climatic and geographical conditions.

The gram-positive *S. pyogens* (ATCC) was the most inhibited bacteria by most of the plant extracts while the most resistant were the gram-negative clinical and standard strains of *E. coli* and *P. vulgaris*, which were only inhibited by the aqueous and methanolic extract of *A. schimperi* leaf. Gram-negative bacteria are frequently reported to have developed multi-drug resistance to many of the antibiotics currently available in the market of which *E. coli* is the most prominent^[31–38].

In general, most of the methanolic and some of the aqueous extracts of the plants showed antibacterial activities. This indicates the potential of the plants to be used as antibacterial agents against wound causing pathogens. However, further studies should be conducted with different extraction solvents and toxicity and phytochemical analysis should also be performed on these plants to use them as sources and templates for the synthesis of drugs to control wound and other disease-causing bacteria.

Conflict of interest statement

We declare that we have no conflict of interest.

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