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Research Article
Phytochemical Screening and Evaluation of Antibacterial Activities of Root Bark Extracts of *Moringa stenopetala*

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Abstract

Background and Objective: Different parts of *Moringa stenopetala* are traditionally used to treat several human diseases and treatment of dirty water. We have revealed the bacterial activity of the root wood of *M. stenopetala* previously. This study was conducted to investigate the phytochemical constituents of root bark extracts of this plant and evaluating its antibacterial activity. **Materials and Methods:** The powdered plant material was subjected to extraction using solvent systems such as petroleum ether, chloroform and acetone involving and the maceration technique. After yield calculation, the crude extracts obtained were subjected to phytochemical screening and antibacterial activity tests. The antibacterial activity test was carried on four bacterial strains viz. *S. aureus*, *E. coli*, *P. aerugenosa* and *S. thyphimurium*. **Results:** The extraction gave 1.34 (0.54%), 2.26 (0.91%) and 3.80 g (1.54%) crude extracts from n-hexane, chloroform and acetone extracts, respectively. Phytochemical screening tests revealed the presence of alkaloids, saponin, terpenoids, anthraquinones, flavonoids, polyphenols and phytosterols in the various extracts. The extracts of *M. stenopetala* revealed antibacterial effects against the tested bacterial strains with zone of inhibition 15-25 mm and acetone extract was the most active. **Conclusion:** The findings of the study indicated that the plant has a great potential as a source of modern antibacterial agents against selected bacterial strains and potential role in disinfection of dirty water.

Key words: *Moringa stenopetala*, crude extract, antibacterial activity, phytochemical screening, disc diffusion method, secondary metabolites, natural products

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Moringa stenopetla belongs to the family of *Moringaceae.* The plant is nicknamed as the African *Moringa* as it is native to Africa. It is widely distributed in Eastern African countries such as Kenya and Ethiopia[1,2]. The plant is well known for its nutritional and medicinal uses in areas where it is native. For instance, it has been reported that the leaves are cooked and eaten as vegetables for humans in southern Ethiopia[2-5]. The presence of several essential amino acids, vitamins and minerals are the possible reasons for nutritional value/use of the plant's leaves[6-8]. Moreover, the plant has been given a due attention for its possible contribution in household food security in the areas where it is abundantly present[9-11].

In addition to its nutritional use, the plant has also been reported to have several medicinal uses. Some of its reported medicinal uses include that its leaves and roots (root barks) are used to treat malaria, wounds, diarrhea, induce vomiting, asthma, epilepsy, respiratory diseases, diabetes, hypertension[12], gastrointestinal problems[13] and to treat *Visceral leishmaniasis* or *Kala-azar*[2]. The stem bark is used to treat eye diseases, intestinal worms and to decrease or neutralize the venom power of snake, bee, scorpion and wasp[14]. This is a good indicator of the community's awareness and acceptance of the medicinal values of the tree as well as its huge potential for future drug discovery.

There are several scientific investigations that have been carried out on crude extracts obtained from different morphological parts of the plant aiming to validate its traditional medicinal uses and possibilities of identifying bioactive compounds. The reports of such investigations showed promising biological activities of crude extracts obtained from different morphological parts of the plant. For instance, crude extracts obtained from the leaves and roots of *M. stenopetala* reported to show antitrypansomal[13,15], diuretic[16] and antileishmanial[2,13,17,18], antiplasmodial[13,19], antidiabetic[20], antimicrobial[10,21-25] and oxytocic[13] activities. There are also reports that revealed crude extracts from this plant could lower glucose and cholesterol levels[26], blood pressure[27] and lipid levels[28,29] in experimental animals. *In vitro* antioxidant activity tests of methanol and aqueous extracts of leaves of *M. stenopetala* (growing in different parts of Ethiopia) showed the potential of the plant as sources of food additive or natural antioxidant to prevent stress related human diseases[30,31]. In line with such attempts by the scientific community, this study was initiated to carry out phytochemical screening and evaluation of antibacterial activities of root bark extracts of *M. stenopetala*.

MATERIALS AND METHODS

Study area: The study was carried out at The Department of Chemistry and Department of Microbiology, Jimma University, Ethiopia from November, 2012-June, 2013.

Plant material collection, preparation and extraction: The root bark of *M. stenopetala* was collected from Arba Minch College of Teacher Education, Arba Minch Town. The area is found 470 km South of Addis Ababa, Ethiopia. The sample was identified by a botanist Dr. Remesh Moochikkal, Department of Biology, College of Natural Sciences, Jimma University and was given a Voucher number of MTG/00190. The collected plant material was chopped into small pieces and air-dried under shade on a plastic material for a period of forty days. The dried plant material was powdered with the help of grinder at the College of Agriculture and Veterinary Medicine, Jimma University. The powder was stored in the refrigerator until it was used for extraction.

The plant material (250 g of powder) was sequentially extracted with petroleum ether, chloroform and acetone (Purchased from Sigma Aldrich Chemicals Co. Ltd agent, Addis Ababa) using the maceration technique and increasing order of the polarities of solvents[32,33]. Each extraction was done in 72 hrs with continuous shaking. The obtained extracts were filtered using Whatman No.1 filter paper (Purchased from Sigma Aldrich Chemicals Co. Ltd agent, Addis Ababa). The residual solvent in each gradient extract was removed under reduced pressure (using Heidolph, UK, Rotary evaporator) before extract was subjected to subsequent solvent extraction. The crude extract of each solvent was stored in the refrigerator at 4°C in sealed glass flasks until their use for further investigations. Each extract was weighed and the percent yields were calculated using the following formula:

$$\text{Extracts (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100\ (\%)$$

Phytochemical screening test: Phytochemical screening tests were carried out for all the extracts as per the standard methods reported in the literature to determine secondary metabolites (alkaloids, saponins, tannins, steroids, flavonoids, terpenoids, coumarins, anthraquinones, polyphenols and polysterols) present in the extracts[34-36].

Antibacterial activity test of the crude extracts (Disc diffusion method):

- **Microorganisms:** The microorganisms used for determination of antibacterial activities of the crude plant extracts and fractions were Gram-positive (*Staphylococcus aureus* ATCC 25903), Gram-negative (*Escherichia coli* ATCC 25722), Gram-negative (*Pseudomonas aeruginosa* DSMZ 1117) and Gram-negative (*Salmonella thyphimurium* ATCC 13311) were collected from Department of Biology, Jimma University, Ethiopia
- **Test solutions of crude extracts:** The test solutions were prepared by dissolving 50 mg of each crude extract in 1 mL of DMSO to achieve the final concentration of 50 mg mL^{-1}
- **Antimicrobial assay:** A standard disc-diffusion method was used to study the antimicrobial activities of the crude extracts[37]. A cell suspension of each bacteria strain equivalent to McFarland 0.5 turbidity standard was obtained by preparing 1% V/V of H_2SO_4 and 1% W/V $BaCl_2$ then 95.5 mL of 1% V/V of H_2SO_4 mixed with 0.5 mL of 1% $BaCl_2$ w/v for comparison of the turbidity to a cell suspension of each organism in order to have a suspension containing approximately $1\text{-}2\times10^8$ CFU mL^{-1}[37,38]. The bacterial suspensions spread over the 140 mm Petri dishes containing Mueller-Hinton agar that uses a sterile cotton swab. Then 6 mm diameter sterile discs (Whatman No 3 paper) were placed on the surface of the inoculated Agar in Petri dishes and 50 µL of each test solutions was applied onto the discs. After addition of test solutions on the discs, the extract was allowed to diffuse for 5 min and the plates were then kept in an incubator at 37°C for 24 hrs[39,40]. The standard disc of the antibiotic disc (ciprofloxacin, 50 µg) and DMSO (50 µL) were used as the positive and negative controls, respectively
- **Measuring antibacterial activity of crude extracts:** Antibacterial activity was determined by measuring the diameter of the inhibition zone around the disc. It was recorded only if the zone of inhibition was greater than 7 mm

RESULTS AND DISCUSSION

Yield of crude extracts obtained: The extraction yield is a measure of the solvent's efficiency to extract specific components from the original plant material and it was defined as the amount of extract recovered in mass compared with the initial amount of plant material. The yield is presented in percentage[35]. Based on the method indicated (Experimental Section), the gradient extraction of the root bark of the target plant, using three different solvents, gave different percentage yields. The percentage yield of the crude acetone extract was found to be higher than the other crude extracts (Table 1). The data is consistent with literature reports that reveal the percent yields extracts obtained from plants, using polar solvents are generally higher than those of non-polar solvents[32,41,42].

Table 1: Masses and percentage yields of crude extracts

Solvent system	Mass of extract (g)	Yield of extract (%)*
Petroleum ether	1.34	0.54
Chloroform	2.26	0.91
Acetone	3.80	1.54

*Mass of the plant material used was 250 g

Table 2: Phytochemical screening test results of the crude extracts from root barks of *M. stenopetala*

Metabolite	Petroleum ether extract	Chloroform extract	Acetone extract
Alkaloids	+	+	+
Saponins	+	+	+
Tannins	+	-	-
Steroids	+	+	+
Flavonoids	+	-	+
Terpenoids	+	+	+
Coumarins	-	-	+
Anthraquinones	-	+	-
Polyphenols	+	+	+
Phytosterols	+	+	+

+: Present, -: Absent

Phytochemical screening test results: All the crude extracts obtained from the root bark of *M. stenopetala* were subjected to phytochemical screening tests. The tests were performed following standardized procedures (tests) reported in the literature[34-36]. The results indicated the presence of secondary metabolites such as alkaloids, saponins, steroids, terpenoids, polyphenols and phytosterols in all the three extracts. Tannins and coumarins were detected only in petroleum ether extract and acetone extract, respectively. On the other hand, flavonoids were detected only in petroleum ether and acetone extracts but not in the chloroform extract. Anthraquinones were detected only in the chloroform extract (Table 2). The secondary metabolites found in this study are found to be consistent with reports of other researchers who reported the presence similar classes of secondary metabolites in extracts from *M. stenopetala* from Ethiopia[12,31] and Kenya[43].

Evaluation of antibacterial activities of the crude extracts: It has also been reported that the seed powder of

Table 3: Antibacterial activity profiles of the three extracts obtained from the root bark of *M. stenopetala*

	Zones of inhibition (mm) of extracts				
Bacterial strain	Petroleum ether extract	Chloroform extract	Acetone extract	Ciprofloxacin	DMSO
Escherichia coli	12	15	15	32	-
Pseudomonas aeruginosa	17	19	19	33	-
Salmonella thyphimurium	13	23	25	30	-
Staphylococcus aureus	15	23	24	30	-

(-): No activity (For DMSO)

M. stenopetala can be used to remove bacteria and heavy metals from waste or polluted water[44-46]. In the present study, the results indicated that all the crude extracts exhibited strong *in vitro* antibacterial activities (as the zones of inhibition are >7 mm) against the four bacterial strains that were used in the experiment with varying degrees (Table 3). When the zones of inhibitions of the three crude extracts were compared to each other, the antibacterial activity of the petroleum ether extract was found to be relatively lower than that of chloroform and acetone extracts. Its inhibition zones are in the range of 12-15 mm. On the other hand, the crude extracts of chloroform and acetone were found to show similar (or comparable) antibacterial activities with inhibition zones that are in the ranges of 15-23 and 15-25 mm, respectively (Table 3). It was also observed that the acetone extract showing slightly superior antibacterial activities on *S. thyphimurium* (25 mm) and *S. aureus* (24 mm) strains. This observation was found to be consistent with our previous report on the evaluation of antibacterial activities of the crude extracts of root wood of *M. stenopetala*[21,47]. Moreover, there other similar previous reports that state strong antibacterial activities crude extracts from the seed of *M. stenopetala*[22-24]. The observed antibacterial could be attributed to the presence of some of the secondary metabolites found in the extracts (Table 2). Though the activities of all the crude extracts were slightly lower than that of the reference drug (Ciprofloxacin), the activities of the chloroform and acetone extracts against *S. thyphimurium* and *S. aureus* were found to be comparable to that of the reference drug (Table 3)[21]. The finding of this study is also consistent with previous reports that reveal antimicrobial activities of secondary metabolites such as alkaloids, saponins, tannins, steroids, flavonoids, terpenoids, coumarins, anthraquinones, polyphenols and phytosterols[35,48-55]. The result of the present study also justifies the traditional medicinal use of the plant (discussed in the introduction section of this paper) and also the potential of *M. stenopetala* as future source of not only antimicrobial agents but also other agents for treatment of human diseases. However, further tests with large number of bacterial strains and plant extracts are recommended to reach at a comprehensive conclusion.

CONCLUSION

The result of the present study showed that the extracts of root bark extracts of *M. stenopetala* contain many phytochemical components. Their *in vitro* antibacterial activity tests revealed that the extracts are active against the bacterial strains (*S. aureus*, *E. coli*, *P. aeruginosa* and *S. thyphimurium*) used in the study. Moreover, the inhibitory activities of extracts of polar solvents (Chloroform and acetone) were slightly higher than that of the non-polar solvent (n-hexane) extract. As the tests were conducted in a limited number of bacterial strains, further investigations are recommended on multiple strains of bacterial species to draw a conclusion.

SIGNIFICANCE STATEMENT

This study discovers the secondary metabolites present in the root barks of *M. stenopetala* that can be helpful in substantiating the traditional medicinal use of the plant. The study would also help researchers working in discovery of antimicrobial agents to make focus on extracts obtained using polar solvents. Thus, based on the information, researchers may to isolate specific compounds for pharmacological activity tests.

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