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Original Research

Antibacterial Activity of *Moringa stenopetala* against Some Human Pathogenic Bacterial Strains

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Abstract

An emerging of antibiotic resistance brings most serious public health problems. It is therefore, important to look for more effective, safer and less toxic alternate options of treatment. The aim of the present study was to investigate antibacterial activity of Moringa stenopetala against some human pathogenic bacteria using disk diffusion method and agar dilution for minimum inhibitory concentration. The result revealed that, most of the plant extracts had antibacterial activity. *Staphylococcus aureus* was found to be the most susceptible bacteria to crude 80% methanol extract of seeds and ethyl acetate extract of root barks with inhibition zones of 18.66±0.88mm and 16.00±1.15mm and minimum inhibitory concentration of 1.25mg/ml and 2.5mg/ml respectively, whereas *Pseudomonas aeruginosa* was the most resistant bacteria to all of crude extracts. Similarly, *Staphylococcus aureus* was the most susceptible bacterial strain to chloroform fraction with inhibition diameter of 28.00±0.57mm and minimum inhibitory concentration of 0.31mg/ml, while *Pseudomonas aeruginosa* was the most resistant strain with inhibition zone of 9.66±0.33mm and minimum inhibitory concentration of10mg/ml respectively. In conclusion, this study is not only proves antibacterial activity of *Moringa stenopetala*, also provides a scientific basis for their traditional use. Pure chemical compounds and antimicrobial activity against many fungi and bacteria should be studied to use them as sources and templates for synthesis of drugs to control infectious diseases.

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INTRODUCTION

Medicinal plants are important sources of Traditional Medicine (TM) for millions of people and additional inputs to modern medicine in terms of exploring and producing new drugs to meet the need for the overgrowing population of the world (Abad *et al.*, 2007). More than 3.5 billion people rely on plants for the treatment of both human and livestock diseases (Newman *et al*., 2000). As elsewhere in Africa, indigenous people in Ethiopia, by large employed plant based TM to get cured from diseases arising from worms, fungi, bacteria, viruses and protozoa (Abebe, 2001). Although, herbal medicine represents one of the most important fields of TM all over the world, the search for the active ingredients for the synthesis of new drugs has not been extremely undertaken (Kumaraswamy *et al.*, 2008).

The wide use and misuse of antibiotics in the treatment of bacterial infections and using it in agriculture, livestock and poultry has led to the emergence and spread of resistant strains. As a result, society is facing one of the most serious public health problems over the emergence of infectious bacteria displaying resistance to even some effective antibiotics (Gibbons, 2005). In addition to increasing the cost of drug regimes, this situation has paved way for the re-emergency of the high frequency of opportunity and chronic infection cases in developing countries. It is therefore, important to look for more effective, safer and less toxic alternate options of treatment (Ako-nai *et al.*, 2003).

Medicinal plants have many biologically active compounds as secondary metabolites that have a capability of overcoming the problems of drug resistance (Douglas, 1987). In addition, they are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu *et al*., 1999). So, the examination of medical properties of crude medicinal plants is needed (Ranpal, 2009). Therefore, the studies of TM should have to continue which had different significance such as to develop local initiatives for pharmaceutical industry and to create possible motives for conserving biodiversity and to provide convenient access to modern medicines more easily that is available and acceptable by rural communities (Caceres *et al*., 1990).

About 65-85% of the populations in every country of the world rely on TM. In developed countries, this may be partly due to dissatisfaction with the conventional medicine while in the developing countries this is due to inaccessibility of modern medical system and TM are relatively safe. Easy accessibility, efficacy on treatment and affordable cost in getting health services are main reasons in preferring TM than costly synthetic drugs that have adverse effects (Sofowora, 1982). However, the majority of TM used in developing country has not been evaluated for their quality, safety and efficacy to some standards, while those in developed countries there are some remarkable claims made for their effectiveness (Yineger, 2005).

Ethiopia is rich of plant biodiversity. It is therefore, not surprising that some of these plants have chemical compounds of therapeutic value that may be used in the treatment of major diseases such as malaria, cancer and pathogenic microorganisms (Yirga, 2010). However, only few studies were geared towards indigenous medicine with an objective to improve their usage. Consequently, the overall use of these plants remained within the domain of local healers as they resort to them for the treatment of different health problems (Abbiw, 1996). However, in the recent years some studies have been undertaken regarding medicinal plants that have been screened for their antimicrobial activities (Mekonnen and Dräger, 2003; Bruck *et al.,* 2004; Tesfaye *et al.,* 2006; Asmamaw *et al*., 2007). So, Ethiopian flora offers great possibilities for the discovery of new compounds with antimicrobial activities (Fernandez *et al*., 2008).

Moringa stenopetala belongs to the family Moringaceae with one genus *Moringa* and with about fourteen species. It is a deciduous tropical plant widely distributed in the Southern parts of Ethiopia at an altitude range of about 1100-1600m. It is commonly used in folk medicines as antimalarial, antihypertensive, against stomach pain, antidiabetic, anticholesterol and to expel retained placentae during birth (Mekonnen and Gessesse, 1998; Mekonnen, 1999). Although, there were some studies made on the leaves and seeds of *Moringa stenopetala* (Mekonnen and Gessesse, 1998; Mekonnen *et al*., 1999; Mekonnen and Dräger, 2003; Sahilu, 2010), there was little information about different solvent extract antimicrobial property of its leaves, seeds, stem barks and root barks. Thus, the objective was to evaluate the antibacterial activity of a widely used medicinal plant *Moringa stenopetala* against four pathogenic bacteria namely *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Shigella boydii* by using different solvents.

MATERIALS AND METHODS

Plant Sample Collection and Identification

Fresh and matured seeds were separated from their pods, leaves, stem barks and root barks of *M. stenopetala* were collected from Southern parts of Ethiopia, the compound of Arbaminch University, Arbaminch in October 2011. The identity of plant specimen was confirmed and its voucher specimen was deposited at the National Herbarium, Department of Plant Biology and Biodiversity Management, College of Natural Science, Addis Ababa University (AAU).

Preparation of Crude Extract

The plant samples were dried under shade. The kernels of seeds after shelled from its husk, the leaves, stem barks and root barks were grounded by mortal and pistil, then by electronic mill (IKA).

Hundred gram powders of the leaves, stem barks and root barks were separately macerated in 600ml of chloroform, ethyl acetate and 80% methanol. Similarly, 100g powder of the seeds was dissolved only in 600ml of 80% methanol. These maceration processes was done in 1000ml Erlenmeyer flask on a rotary shaker (120rpm) at room temperature for 72 hrs. The extracts were filtered using filter paper (whatman No.1, Whatman Ltd., England) and the solvent was evaporated on the rotary evaporator (BÜCHI Rota-vapor R-205, Switzerland) under reduced pressure at 45^{0}C. The waxy residues of chloroform and ethyl acetate extracts were further dried at room temperature, where as 80% methanol extract was dried by lyophilizer (CHRiST). The resulting solidified plant extracts were kept in deep freeze (-20^{0}C) for future use.

Solvent Fractionation of *Moringa stenopitala* Seeds

Sixty gram of methanol extract (80%) was taken and packed in a thimble. Successive and exhaustive extractions were undertaken using petroleum ether (80-100^{o}C) (400ml), chloroform (400ml) and methanol (400ml) as fraction **I**, **II** and **III** respectively by using soxhlet apparatus (BÜCHI E-816 Sox, Switzerland). Each fraction was collected separately and concentrated at reduced pressure using rota-vapor at 45^{0}C. The semisolid mass was then dried at room temperature. The residue that was left in a thimble was dissolved in distilled water (200ml), then filtered by whatman No.1 filter paper and taken as fraction **IV**. Finally, it was dried by lyophilizer and weighted. These dried mass was powdered and kept in a deep freeze (-20^{0}C) for future use (Unasho, 2005).

Bacterial Strain

The bacterial strains used in this investigation were *S. aureus* ATCC25923*, E. coli* ATCC25922, *P. aeruginosa* ATCC 27053 and *S. boydii* ATCC 9289. They were obtained from Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa.

Preparation of Inocula and Culture Media

Purity of the culture was checked by them on differential or selective media. These are S-S Agar (OXOID) for *S. boydii*, Mannitol Salt Agar (OXOID) for *S. aureus*, MacConkey Agar (TECHNO PHARMCHEM) for *E. coli* and Pseudomonas Isolating Agar (OXOID) for *Pseudomonas aeruginosa*.

After purity of the cultures was checked, each bacterium was activated on Nutrient Agar (OXOID) for preparation of inocula. Inoculums were standardized by inoculating 18-24hr old culture bacteria in saline solution and compared with 0.5 McFarland turbidity standard that was prepared by adding a 0.5ml aliquot of 0.48 mol/L $BaCl_2$ (1.175% w/v $BaCl_2$. $2H_2O$) added to 99.5 ml of 0.18 mol/L H_2SO_4 (1%v/v) (McFarland, 1979).

Standard Antibiotics

Standard disks impregnated with amoxicillin (30µg), erythromycin (15µg), gentamicin (10µg) and kanamicin (30µg) were used as positive controls for the antibacterial susceptibility test; whereas 3% tween 80 served as a negative control.

Antibacterial Assay

The crude and semi-purified extracts were tested against the test organisms using the disc diffusion method as described by Bauer *et al*., (2006) and Lalitha (2008). Inoculums were prepared by mixing a few bacterial colonies from 18-24hr old culture in 5ml sterile saline solution and comparing the turbidity with that of the standard 0.5 McFarland solution which is equivalent to1.5 x 10^6-10^8cfu/ml. The sterile cotton swab was dipped into the properly adjusted inoculums and the excess was removed by gentle rotation of the cotton swab against the inner wall of the tube. The test bacteria were uniformly swabbed on the Mueller Hinton Agar (MHA) (OXOID) using the cotton swab. The inoculated plates were left at room temperature for 3-5 minutes to allow for any surface moisture to be absorbed before applying the extract. Each extract was dissolved in 3% of tween 80 with 300 mg/ml concentration. Ten micro litres of each plant extract was transferred onto a sterile filter paper disc (Whatman No.1; 6 mm in diameter) and allowed to dry for 15minute. Using aseptic conditions all the selected antibiotics and the plant extracts were applied on the MHA and left for 15 minutes to allow the extract to diffuse. The plates were then incubated at 37^0c for 18-24 hours in an incubator. All tests were performed in triplicate and zone of inhibition were measured after the incubation period by using ruler.

Determination of Minimum Inhibitory Concentrations

Minimum Inhibitory Concentrations (MIC) of the extracts that showed an inhibition zone $\geq$ 7 were carried out using the agar dilution method described by Aniel and Naidu (2007). The dried extracts were dissolved in 3% tween 80 at a concentration of 200mg/ml and then further serially diluted with 3% tween 80. Two milliliters of the extracts from each dilution was mixed with 18ml of molten MHA and poured into sterile petri-dishes allowing the agar to set. The final concentrations of the extracts in the culture medium ranged from 20 to 0.07mg/ml. The agar was streaked with fresh colonies of bacteria, of which its turbidity was adjusted as described before. One petri-dish with 2ml of 3% tween 80 and 18ml MHA was used as negative control, while other petri-dish with only 20ml MHA was used as positive control. Then, it was incubated at 37 ºC and examined after 18-24hr. The lowest concentration of each extract that did not allow any colony growth in the solid medium after the incubation period was regarded as the MIC. All tests were performed in triplicates

RESULTS

Antibacterial Assay of Crude Extracts

Crude extracts of the leaves, seeds, stem barks and root barks of *M. stenopetala* were tested for their antibacterial activity against *S. aureus, E. coli, P.aeruginosa* and *S. boydii*. Antibacterial activity was tested at 300gm/ml for both crude extracts and semi-purified fractions. Of 80% methanol crude extract of the seeds (18.66±0.88mm) showed highest antibacterial activity, while ethyl acetate extract of stem barks (11.00±0.57mm) showed lowest activity against *S. aureus*. All extracts of the leaves were inactive against tested bacterial strains. *S.aureus* was highly susceptible bacterial strain, while *P. aeruginosa* was the most resistant to all of the crude extracts (Table 1).

Table 1: Antibacterial activity of crude extracts of the plant parts at 300mg/ml concentration

		Zone of inhibition (mm)			
Plant parts	**Extracts**	***S. aureus***	***E. coli***	***P. aeruginosa***	***S. boydii***
Seeds	SM	18.66±0.88	11.66±0.88	-	14.66±0.33
Leaves	LM	-	-	-	-
	LE	-	-	-	-
	LC	-	-	-	-
Stem barks	BM	13.33±0.88	-	-	-
	BE	11.00±0.57	-	-	-
	BC	-	-	-	-
Root barks	RM	-	-	-	-
	RE	16.00±1.15	13.00±0.57	-	15.33±0.33
	RC	15.00±1.15	11.66±0.66	-	13.00±0.57

Note: SM: seeds 80% methanol extract, LM: Leaves 80% methanol extract, LE: Leaves ethyl acetate extract, LC: Leaves chloroform extract, BM: Stem barks 80% methanol extract, BE: Stem barks ethyl acetate extract, BC: Stem barks chloroform extract, RM: Root barks 80% methanol extract, RE: Root barks ethyl acetate extract, RC: Root barks chloroform extract, (-): No inhibitory activity.

Antibacterial Assay of Semi-purified Fractions

Table 2 indicated that, chloroform fraction of the seed showed best antibacterial activity against all tested bacterial strains, especially against *S. aureus* (28.00±0.57mm) followed by *S. boydii* (23.00±0.57mm). *P. aeruginosa* was the most resistant to this fraction, followed by *E. coli* with inhibition zone of 9.66±0.33mm and 14.66±0.33mm respectively. The second fraction that exhibited antibacterial activity was petroleum ether fraction. It was active only on *S. aureus* with inhibition zone of 14.33±0.88mm. Water fraction also showed antibacterial activity only on *S. aureus,* while methanol fraction was inactive against all selected bacterial strains.

Table 2: Antibacterial activity for semi-purified fractions of seeds at 300mg/ml concentration

		Zone of inhibition (mm)			
Plant part	**Solvents**	***S. aureus***	***E. coli***	***P. aeruginosa***	***S. boydii***
Seeds	Petroleum ether	14.33±0.88	-	-	-
	Chloroform	28.00±0.57	14.66±0.33	9.66±0.33	23.00±0.57
	Methanol	-	-	-	-
	Water	9.00±0.57	-	-	-

Note: - (-): no inhibitory activity

Comparisons with Controls

The activity of the standard antibiotics and the negative control are presented in Table 3. In this case, *S. aureus* was highly susceptible to all standard drugs, while *E. coli and S. boydii* were resistant to amoxicillin and erythromycin and susceptible to gentamicin and kanamicin. *P. aeruginosa* was also resistant to amoxicillin and erythromycin and intermediately succebtable to kanamicin, but susceptible to gentamicin.

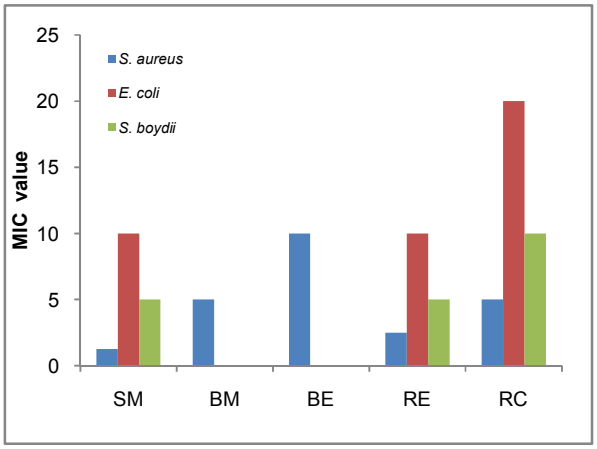
Table 3: Inhibition zone of standard antibiotics and tween 80 in mm

Controls	Concentration	Inhibition zone against tested bacteria (mm)			
		S. aureus	*E. coli*	*P. aeruginosa*	*S. boydii*
Amoxicillin	30µg	23.33±0.88	-	-	-
Erythromycin	15µg	28.00±1.15	9.66±0.88	8.66±0.33	11.33±0.88
Gentamicin	10µg	27.66±0.66	25.33±0.33	24.00±0.57	21.66±0.88
Kanamicin	30µg	26.33±0.33	25.66±0.88	13.66±0.33	21.33±0.88
Tween 80	3%v/v	-	-	-	-

Note: (-): no inhibitory activity

Minimum Inhibitory Concentrations of Crude Extracts

In agar dilution, MIC value was determined only for extracts that showed antibacterial activity against tested bacteria. The MIC value of crude extracts of plant parts against the tested bacteria ranged from 1.25 to 20 mg/ml. The most frequent MIC value of the extracts was 5 mg/ml and 10 mg/ml. Among crude extracts, 80% methanol extract of seeds had lowest MIC value of 1.25 mg/ml, followed by ethyl acetate extract of root barks with MIC of 2.5mg/ml against *S. aureus*. Both 80% methanol extract of seeds and ethyl acetate extract of root barks had same MIC value of 10 mg/ml against *E. coli* and same MIC value of 5mg/ml against *S. boydii*. Crude 80% methanol extract of stem barks showed lower MIC value of 5mg/ml than ethyl acetate extract of stem barks (10 mg/ml) against *S. aureus*. The crude extract which had highest MIC value was chloroform extract of root barks with MIC value of 20 mg/ml against *E. coli*. This extract also showed MIC value of 5mg/ml and 10mg/ml against *S. aureus* and *S. boydii* respectively (Figure 1).



Note: SM: seeds 80% methanol extract, BM: Stem barks 80% methanol extract, BE: Stem barks ethyl acetate extract, RE: Root barks ethyl acetate extract, RC: Root barks chloroform extract.

Figure 1: MIC value of crude extracts against selected bacterial strains

Minimum Inhibitory Concentrations of Semi-purified Fractions

Among solvent used, chloroform fraction showed best activity against tested bacteria which is followed by petroleum ether fraction. Chloroform fraction had lowest MIC value against *S. aureus* with MIC of 0.31 mg/ml and highest MIC against *P. aeruginosa* with MIC value of 10mg/ml. Chloroform fraction also showed an MIC of 0.62 and 2.5 mg/ml against *S. boydii* and *E. coli* respectively. Petroleum ether fraction was also inhibited *S. aureus* at 1.25mg/ml, while the MIC for water fraction against *S. aureus* was not within the concentration range tested (Figure 2).

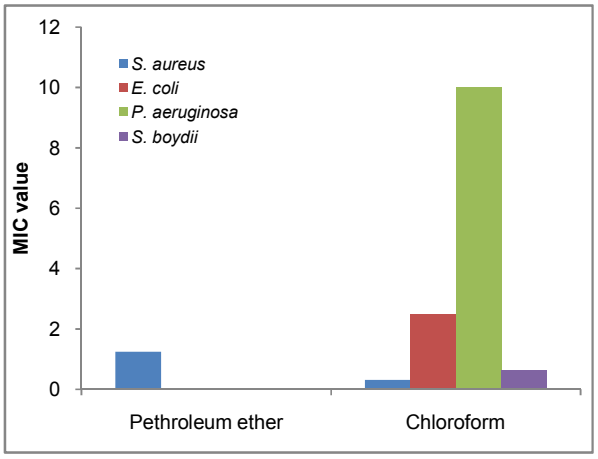


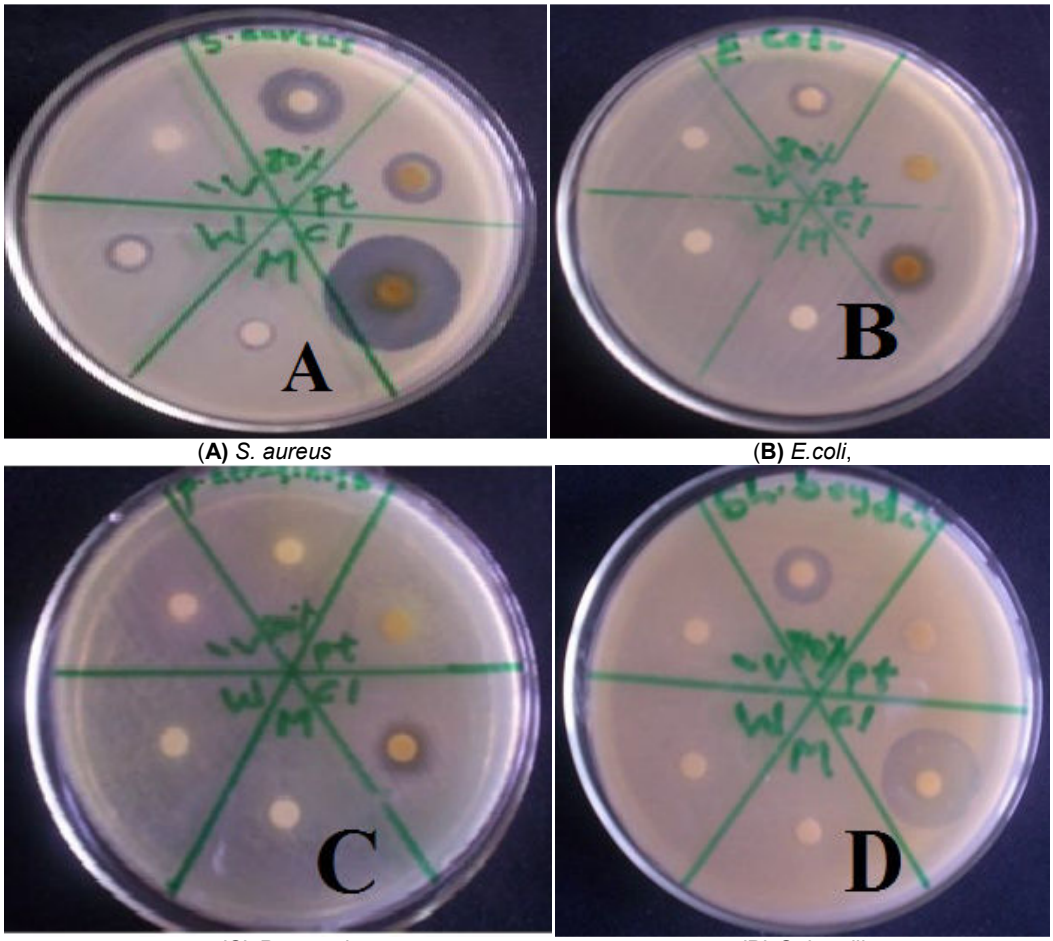
Figure 2: MIC value of semi-purified fractions against selected bacterial strains

DISCUSSION

Medicinal plants must be tested for their claimed activity and safety and efficacy profiles have to be assessed including the proposed dosage form of the extract before it is made available for use (Dahanukar *et al*., 2000). The traditional healers use primarily water as the solvent, but in the present study the plant extracts by petroleum ether, chloroform, ethyl acetate and methanol provided more consistent antibacterial activity compared to those extracted by water.

Antibacterial Assay of the Crude Extracts

The antimicrobial effect of many medicinal plants is well documented (Valero and Salmeron, 2003). The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains (Kone *et al*., 2004). In this study, using the disk diffusion method, it was observed that extracts of seeds, stem barks and root barks of *M. stenopetala* produce antibacterial activity against both Gram negative and Gram positive pathogens.



(**A)** *S. aureus* (**B)** *E.coli*,

(C) *P. aeruginosa* **(D)** *S. boydii*

Figure 3: Antibacterial activity of semi-purified fractions of seed against tested bacteria.

The result from Table 1 showed that, 80% methanol extract of the seeds showed maximum antibacterial activity against *S. aureus* (18.66±0.88mm) followed by *S. boydii* (14.66±0.33mm). But, it exhibit less activity against *E. coli* (11.66±0.88mm) and inactive against *P. aeruginosa*. This activity strengthens the report of Mekonnen and Dräger (2003), in which crude water extract of the seeds of *M. stenopetala* showed strong antibacterial effect against *S. aureus, Salmonella typhi* and *Shigella* species.

These results are also in agreement with the study conducted by Sahilu (2010), in which antibacterial activity for crude water extract of the seeds of *M. stenoptala* was analyzed against five bacteria strains. The maximum antibacterial activities were observed against *S. aureus* (28.2mm) and *S. boydii* (26.4mm) at a concentration of 200mg/ml, which closely agreed with this study. Among the five bacterial organisms the growth of *E. coli* was not inhibited by the crude water extract of the seed. But, in this study crude 80% methanol extract showed inhibitory activity against *E. coli* (11.66±0.88mm). This indicates that methanol extract was better than water extract against *E. coli*. In other word, methanol had the potential to extract active compounds than water which inhibit the growth of *E. coli*. The antibacterial activity of methanol extracts of *M. oleifera* and *M. stenopetala* seeds was also conducted by Walter *et al.* (2011), on three bacterial strains, which closely agreed with this study, that both methanol extracts of *M. oleifera* and *M. stenopetala* seeds showed antibacterial activity against *E. coli*.

Saadabi and Abu-Zaid (2011) evaluated aqueous and methanol extracts of *M. oleifera* seeds for their antibacterial activity against four types of bacteria namely, *S. aureus, B. subtilis, E. coli and P. aeruginosa*. Its aqueous extract showed superior antibacterial activity against all bacterial strains than its methanol. Both of the seeds extracts strongly inhibited the growth of Gram positive bacteria (*S. aureus* and *B. subtilis*) than Gram negative bacteria (*E. coli* and *P. aeruginosa*). It is common observation that Gram-negative bacteria are more resistant to many compounds than Gram-positive ones. This is might be because of morphological differences that Gram-negative bacteria have an outer membrane (Nikaido and Varra, 1985). Particularly, *E. coli* and *P. aeroginosa* are incriminated in several infections for their insensitivity to antibiotics (Morse *et al*., 1986). Hence, this is in congruency with the observation in this experiment that most of the extracts have shown lesser activity against *P. aeruginosa* and *E. coli* than that of *S. aureus*.

The Gram-positive bacteria are more susceptible due to the fact that their outer peptidoglycan layer which is not an effective permeability barrier (Nikaido and Varra, 1985). In spite of the permeability differences, some of the tested extracts demonstrated reasonable activities against

Gram-negative bacteria; especially ethyl acetate extract of root barks and chloroform fraction which showed better activity on *E. coli* and *S. boydii* strains than the other plant extracts.

The extracts of the leaves of *M. stenopetala* did not shown any antibacterial activity against all tested bacteria. This may indicate that, the active compounds are not extracted by these solvents or this plant part may not contain bioactive compounds against these bacteria. Similar to this study, Mekonnen and Dräger (2003) reported that aqueous extract of *M. stenopetala* leaves did not shown any antibacterial activity against *S. aureus*, *Salmonella typhi* and *Shigella* species.

In this study, *S. aureus* was the only bacterium which was inhibited by extracts of stem barks. The antibacterial activity assay of these three solvent extracts of stem barks revealed that methanol extract showed higher antibacterial activity (13.33±0.88mm) than ethyl acetate extract (11.00±0.57mm), suggesting that the bioactive compounds are better extracted with methanol than ethyl acetate and immiscible with chloroform (Table 1).

In the study of Chetia and Gogoi (2011), the methanol extract of *M. oleifera* stem barks was studied for their antibacterial activities against *E. coli*, *P. aeruginosa* and *S. aureus* using disc diffusion method. The results suggest that, this extract had significant antibacterial activities against *E coli,* but inactive against *P. aeruginosa* and *S. aureus.* However, Sarin *et al*. (2010) reported that methanol extract of *M. oleifera* stem barks inhibited the growth of both *S. aureus* and *E. coli* with inhibition zone of 15.0±0.0 and 7.0± 0.0 mm respectively. This report was confirmed by the present work, in which methanol extract of stem barks showed best activity against *S. aureus*.

The results from Table 1 also show that, all extracts of root barks were active, except methanol extract against all tested bacteria except *P. aeruginosa.* Among these strains, *S. aureus* was highly sensitive to ethyl acetate extract (16.00±1.15mm) and chloroform extract (15.00±1.15mm), followed by *S. boydii* with antibacterial activity of (15.33±0.33mm) and (13.00±0.57mm) respectively. Chloroform extract (11.66±0.66mm) of this plant part showed less activity, followed by ethyl acetate extract (13.00±0.57mm) against *E. coli.*

In the study conducted by Raj *et al*. (2011), the antibacterial activity of root barks of *M. oleifera* was analyzed against four bacterial strains. Its ethyl acetate extracts showed higher antibacterial activity than its chloroform extract against *P. aeruginosa* with inhibition zone of 18.2 ± 0.2 mm and 12.2±0.2mm respectively. Ethyl acetate extract also showed higher antibacterial activity than chloroform extract against *S. aureus* with inhibition zone of 11.10±0.1 and 8.2±0.2mm respectively and lower activity against *E. coli* (9.9±0.1mm), but chloroform extract was inactive against same bacteria. Dewangan *et al*. (2010) also reported that, ethyl acetate and chloroform extracts of root barks of *M. oleifera* inhibited the growth of four bacteria. The ethyl acetate and chloroform extracts of *M. oleifera* root barks were found to have strong antibacterial activity against *S. aureus* with inhibition zones of 19.66±0.88mm and 16.66±0.33mm, followed by *P. aeruginosa* with inhibition zone of 19±0.57mm and 15±0.57mm respectively. But, *E. coli* was the least susceptible test organism to ethyl acetate and chloroform extracts with inhibition zone of 13.66±0.33mm and 13.00±0.57mm respectively. In agreement with this study, *S. aureus* was highly susceptible than *E. coli* and ethyl acetate extract showed maximum antibacterial activity than chloroform extract. This indicates that the active ingredient which inhibits the growth of these bacteria might be better extracted by ethyl acetate than in chloroform and immiscible in methanol.

The results from Table 3 also showed that, the four tested *M. stenopetala* parts have varied antibacterial activity against the tested organisms. Among these, seeds and root barks were most effective against the selected strains. Stem barks extracts were slightly active against these strains, while the leaves extracts were inactive totally. When comparing tested bacteria, *S. aureus* was highly susceptible bacterial strain, while *E. coli* was less sensitive and *P. aeruginosa* was totally resistant bacterial strains. The difference in efficacy of these different extracts might be due to the distribution of the active ingredient in plant parts, potency of extracting solvent and inherent resistance of the tested bacteria species (Nayan *et al*., 2011). The present phyotochemical study of *M. stenopetala* are similar to those plant extracts that have been reported by possessing strong antibacterial activity against both Gram positive and Gram negative bacteria.

Antibacterial Assay of the Semi-purified Fractions

Isolating active compounds from crude plant extracts needs extracting this component from the complex matrix of the crude extracts to get the pure form. One of such attempt is solvent fractionation that involves extracting components depending on their polarities (Nostro *et al*., 2000).

Table 2 indicated that, the entire fractions showed antibacterial activity against *S. aureus*, except methanol fraction, but chloroform fraction (28.00±0.57mm) showed highly significant activity. Methanol fraction was inactive against all selected bacteria. *P. aeruginosa* was the most resistant to this fraction, followed by *E. coli* with inhibition zone of 9.66±0.33mm and 14.66±0.33mm respectively. The results from Table 2 also illustrated that the non-polar fractions (petroleum ether and chloroform) were stronger in their activity compared to polar fractions (methanol and water). Antibacterial activities were found to be decreased with increasing polarity, indicating that the active compounds responsible for antibacterial activity of the extract reside in the non-polar fractions in relatively higher concentrations. This result strongly supported the report of Taddese (2004), who analyzed antibacterial activity of selected medicinal plants topically applied in Ethiopia by using petroleum ether, chloroform, methanol and acetone. Among these, non polar fractions (petroleum ether, chloroform) showed maximum antibacterial activity than polar fractions (methanol and water).

The antibacterial effect was noticeably higher by chloroform fraction than activity in other fractions. This difference might be due to a) the active compounds are concentrated by using chloroform as a solvent; b) the concentration of inhibiting components are decreased by selecting the solvent (Taddese, 2004). Hence, the active ingredient is most likely non-polar and further activity-guided fractionation work on the non-polar fraction seems to be beneficial.

The results in Table 1 and Table 2 indicated that, semi-purified (chloroform fraction) showed significantly higher antibacterial activity than all crude extracts against all selected bacterial strains. The reason behind is that the active compounds in semi-purified fractions are relatively concentrated to particular solvent (chloroform in this case) than in crude extracts against tested bacteria.

Comparison with Controls

According to WHO (2012) the relationship between susceptibility of bacterial strains against standard antibiotics applied and the nearest inhibition zone diameter can be classified as follows: For amoxicillin (30µg), erythromycin (15µg) and kanamicin (30µg), if the diameter is ≤13mm: resistant; between 14 to 17mm: intermediate and ≥ 18mm: the bacteria is susceptible and for gentimicin (10µg) ≤12mm: resistant, between13-14mm: intermediate and ≥15mm: the bacteria is susceptible. From this it can be deduced that, *S. aureus* was susceptible to all standard antibiotics, while *P. aeruginosa* was susceptible to gentamicin, intermediate to kanamicin and resistant to amoxicillin and erythromycin. *E. coli* and *S. boydii* were resistant to amoxicillin and erythromycin and susceptible to genthamicin and kanamicin (Table 3).

When the activities of *M. stenopetala* were compared with these standard antibiotics, all crude extracts showed lower antibacterial activity than all standard antibiotics against *S. aureus*, but better activity than amoxicillin and erythromycin and lower activity than gentamicin and kanamicin against *E. coli*, *P. aeruginosa* and *S. boydii* (Table 1 and 3). Among semi-purified fractions, chloroform fraction was as potent as erythromycin and slightly more potent than amoxicillin, gentamicin and kanamicin against *S. aureus.* Similarly, it showed more antibacterial activity than amoxicillin and erythromycin and lower activity than gentamicin and kanamicin against *E. coli* and *P. aeruginosa.* It is also more active than all selected standard antibiotics against *S. boydii* (Table 2 and 3). Generally, chloroform fraction and some crude extracts showed relatively better antibacterial activity, when compared with standard antibiotics. This might be due to the appearance of drug resistant bacterial strains to these standard antibiotics. Therefore, if active ingredients of these extracts are purified, the extracts of this plant parts will show best antibacterial activity at low concentration.

Minimum Inhibitory Concentration of Crude Extracts

The MIC assay was also employed to evaluate the effectiveness of the extracts to inhibit the growth of test bacteria. Among crude extracts, 80% methanol extract of seeds had lowest MIC value of 1.25 mg/ml, followed by ethyl acetate extract of root barks with MIC of 2.5mg/ml against *S. aureus*. This indicated that 80% methanol extract of seeds was more effective against this bacterium. The crude extract which had highest MIC value was chloroform extract of root barks with MIC value of 20 mg/ml against *E. coli* (Figure 1).

In Ethiopia, Sahilu (2010) reported that crude water extract of *M. stenopetala* seeds inhibited *S. aureus* and *E. coli* at MIC of 1.25 and 10 mg/ml respectively. This report agreed with the present work in which, water extract of *M. stenopetala* seeds was equivalent in effectiveness to crude 80% methanol extract of the seeds against *S. aureus* with MIC value of 1.25mg/ml and it is also equivalent in effectiveness with crude 80% methanol extract of the seeds and ethyl acetate extract of root barks against *E. coli,* ethyl acetate extract of stem barks against *S. aureus* and chloroform extract of root barks against *S. boydii* at MIC value of 10mg/ml.

Lalas *et al*. (2012) analyzed antibacterial activity of n-hexane extract of *Moringa peregrina* seeds with MIC value of 4.5mg/ml against *S. aureus*. Busani *et al*. (2012) also reported that, acetone extract of *M. oleifera* leaves inhibited the growth of *S. aureus* at MIC value of 5mg/ml. According to Bukar *et al*. (2010), chloroform extract of *M. oleifera* seeds and chloroform extract of *M. oleifera* leaves inhibited the growth of *S. aureus* at MIC value of >4mg/ml, while methanol extract of *M. oleifera* seeds and chloroform extract of *M. oleifera* leaves inhibited *S. boydii* at same concentration. When these reports were compared with this study, they were relatively equivalent in effectiveness to 80% methanol extract of stem barks and chloroform extract of root barks of against *S. aureus* and crude 80% methanol extract of the seeds and ethyl acetate extract of root barks against *S. boydii* at 5mg/ml MIC value. Ethyl acetate extract of root barks was showed MIC value at 2.5mg/ml against *S. aureus*, which relatively agreed with the report of Bukar *et al*. (2010) in which methanol extract of *M oleifera* leaves inhibited *S. aureus* with MIC value of 2mg/ml.

Although the MIC result showed that *E. coli* was the highest resistant bacterial strain to have been inhibited at 20 mg/ml concentration of the chloroform extract of root barks, Sarin *et al*. (2010) reported that, this test organism can be inhibited at a concentration as low as 0.62mg/ml of the hexane and methanol extracts of *M. oleifera* stem barks. Sarin *et al*. (2010) also reported that, hexane and methanol extracts of *M. oleifera* stem barks gave MIC values of 0.312 and 0.078 mg/ml on *S. aurues* respectively. Sahilu (2010) reported, crude water extract of *M. stenopetala* seeds inhibited *S. boydii* with MIC of 0.63mg/ml. Comparing these results with the present study, solvent extracts of *M. stenopetala* parts were less effective in inhibiting the tested bacteria than the extracts of *M. oleifera* stem barks and water extract of *M. stenopetala.* Generally, among crude extracts 80% methanol extract of the seed highly potent against *S. aureus*, while chloroform extract of root bark was less efficient against *E. coli*.

Minimum Inhibitory Concentration of Semi-Purified Fractions

Minimum inhibitory concentration for semi-purified fraction of the seeds was done for petroleum ether, chloroform and water fractions. Chloroform fraction had lowest (0.31mg/ml) and highest (10mg/ml) MIC value against *S. aureus* and *P. aeruginosa* respectively. Petroleum ether fraction exhibited MIC value of 1.25mg/ml against *S. aureus*. Water fraction was inactive at these concentrations range (Figure 2).

Nikkon *et al*. (2003) conducted a research work to investigate antibacterial activity of the compound N-benzyl, S-ethly thioformate isolated from *M. oleifera* against fourteen bacterial strains. This compound-1 isolated from chloroform fraction of ethanol extract showed lowest MIC value of 0.032mg/ml against *S. aureus* and *S. boydii*. Although fraction of *M. oleifera* was more potent than *M. stenopetala* fraction, this report agreed with the present study in which chloroform fraction

had lowest MIC value against *S. aureus* and *S. boydii*. Generally, semi-purified fraction (chloroform fraction) had lowest MIC value than all crude extracts, which indicated that semi-purified fractions are more effective against tested bacteria than crude extracts, because bioactive compounds in semi-purified fractions were concentrated to specific solvent (chloroform) than in crude extracts.

CONCLUSIONS

From the above results, it can be concluded that traditional medicinal plant, *M. stenopetala* possess diverse antibacterial activity in its different parts against the selected bacterial strains. The chance to find active compounds was more apparent in crude 80% methanol extract of the seeds, followed by ethyl acetate extracts and chloroform extracts of root barks. The pattern of inhibition also showed that, *S. auseus* was the most susceptible bacterial strain followed by *S. boydii* to both crude and semi-purified fractions, while *P. aeruginosa* was the most resistant bacteria followed by *E. coli.* Much of the antibacterial activities of semi-purified fractions were because of the non-polar fractions than the polar one. Among these, chloroform fraction had highest inhibition zone and lowest MIC value. This means, chloroform fraction is more effective against test bacteria than both crude extracts and other fractions. This indicated that, active compounds in semi-purified fraction were concentrated to specific solvent (chloroform). Thus, *M. stenopetala* could become promising natural antibacterial agents with potential applications in pharmaceutical industry as sources and templates for the synthesis of new drugs to control infectious diseases. However, if plant extracts are to be used for medicinal purposes, issues of safety and toxicity will need to be considered and determined in near future for further application. Based on these findings the following are recommended. The antibacterial testing was conducted on limited number of bacteria. So, the same work should be carried out on large variety of fungal and bacterial strains so as to have a clear picture of the spectrum of antimicrobial activity of the herbal drugs. Further work is needed to know and characterize the pure chemical compounds of the plant that are responsible for antimicrobial properties and to increase the efficiency of these extracts.

Conflict of Interest

Conflict of interest none declated

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