

Full Length Research Paper

Antimicrobial properties of *Moringa Stenopetala* seed oil

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***Moringa stenopetala* is a multipurpose tree with considerable economic and social potential as it has vital nutritional, industrial, and medicinal applications. The study was aimed to investigate the antimicrobial activities of *M. stenopetala* seed oil against pathogenic microorganisms. *M. Stenopetala* seeds were collected from three locations (Damba Gofa, Shelle, and Konso) and extracted using two different solvents (hexane and petroleum ether). Pathogenic microorganisms: bacteria (gram-positive, *Staphylococcus aureus*, and gram-negative *Escherichia coli*) and the fungal strains (*Trichophyton mentagrophytes* and *Candida albicans*) were used in this study. Standard procedures were followed to determine antimicrobial activities of *M. stenopetala* extract against pathogenic microorganisms. The result revealed that *M. stenopetala* seed extract has shown inhibitory activity against *T. mentagrophytes* fungi at the concentration $\geq 12.5\%$ at all locations and both extraction solvents used. However, the extract did not show any inhibitory activity against tested bacteria and *C. albicans* fungi. The finding indicated that *M. stenopetala* seed could be used as an alternative to chemical fungicide to control *T. mentagrophytes* fungi. Further investigation is needed on the identification of compounds that inhibits the pathogenic microorganism.**

Key words: Antimicrobial activity, bacteria and fungi, *Moringa stenopetala* seed, extract.

INTRODUCTION

World Health Organization (WHO) reported that 80% of the population in developing countries relies on medicinal plants to acquire primary health care needs (WHO, 2002). This is likely in Ethiopia where 80% of the human

population and 90% of livestock depend on traditional medicines (Abebe, 2001). The majority of these come from plant sources, which are the main sources of antimicrobial molecules (Adnan et al., 2015). These

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include secondary metabolites synthesized by the plants, more likely phenolic compounds (Hu et al., 2021). In addition, they have an advantage over synthetic products due to fewer side effects (Adnan et al., 2015).

Furthermore, they are the source of new antimicrobial drugs due to the increment of microorganisms resistant to conventional antimicrobials (Silva and Fernandes Júnior, 2010).

Moringa stenopetala belongs to the Moringaceae family and it is one of the species of the thirteenth *Moringa* geniuses (NRC, 2001). It is an underutilized, fast-growing vegetable food crop indigenous to East African lowlands and southern Ethiopia (Abuye et al., 2003). In Ethiopia, *M. Stenopetala* is commonly known as Shiferaw (Amharic), Aleko, Aluko, Halako (Gamo Gofa), Kallanki (Benishangul), Telahu (Tsemay), Haleko, Shelchada (Konso) and Haleko (Burji) (UNIDO, 2015). In English, it is named as Africa Moringa tree, Ben oil tree, Cabbage tree, and Horse-radish tree (Demeulenaere, 2001). Various parts of Moringa are used for human food, fuelwood, livestock forage, medicine, dye, water purification, soil and water conservation, quality of cooking oil, green manure, and as a source of income for Moringa cultivators (Demeulenaere, 2001; Abay et al., 2015).

M. stenopetala is used traditionally as food and to treat malaria, hypertension, asthma, diabetes, common cold, wounds, retained placenta, and stomach problems (Mekonnen and Gessesse, 1998). The seeds show a flocculating property, important in purifying turbid water (Abuye et al., 2003; Prashith et al., 2016). It is a major source of oil which could be important for cooking, salad (Raghavendra et al., 2016), and for different industrial applications (Seifu, 2015).

Furthermore, the seed possesses coagulant activity is useful for clarifying water and possesses antimicrobial activity (Rani et al., 2018). *M. stenopetala* seed extraction using different extraction solvents like hexane and methanol exhibits inhibition against waterborne disease, caused by *Salmonella typhi*, *Vibrio cholera*, and *Escherichia coli* (Walter et al., 2011). This is mainly due to biologically active compounds of a plant relying on the type of solvent used in the extraction procedure (Seleshe and Kang, 2019).

Even though *M. stenopetala* has a remarkable role in the lives of a large population of Southern Ethiopian, there is a lack of research conducted on the antimicrobial activities of *M. stenopetala* seed extract in the study area. Furthermore, the growing pressure on food manufacturers to avoid the use of chemical preservatives needs to search for alternative preservatives. Therefore, the present study aimed to evaluate the antimicrobial activity of *M. stenopetala* seed solvent extract collected from different locations against four pathogenic microorganisms, namely *Staphylococcus aureus*, *Escherichia coli*, *Trichophyton mentagrophytes*, and yeast *Candida*

albicans.

MATERIALS AND METHODS

Samples collection

The identification of *M. stenopetala* used in this study was done with the help of a botanist from Arbaminch university and the dominance of *M. stenopetala* in the sites considered by the study (Abuye et al., 2003; Gebregiorgis et al., 2012; Seifu, 2015). Matured pods of *M. stenopetala* with similar color were collected from three locations in Southern Ethiopia; Gofa Zone (Demba Gofa district), Gamo Zone (Shelle district), and Segen Area Zone (Konso district) from January to February 2022. The locations were selected purposely based on the availability and abundance of *M. stenopetala* trees in the area.

Shelle district is located about 27 km from Arba Minch town and 532 km from Addis Ababa. Demba Gofa district is located 526 km from Addis Ababa. Konso district is located about 600 km southwest of Addis Ababa capital city of Ethiopia.

M. stenopetala seed powder preparation

The powder preparation was performed following the procedure indicated by Haile et al. (2019). Briefly, the matured seeds were separated from their pods and cleaned by removing the bark. The seeds with even appearance in size and shape were selected. The seeds were sun-dried to separate the husk from the seed kernel and the seed powder was prepared using a mechanical grinder. The powders obtained were sieved and then stored in polythene bags until extraction at Arba Minch University Chemistry laboratory.

Oil extracts preparation

The oil was extracted using a semi-continuous process; soxhlet procedure, through repeated washing (percolation) with n-Hexane and petroleum ether. Seed powders of 40 g were placed in a porous cellulose thimble. Then the thimble was placed in an extraction chamber in between flask containing solvents of 150 ml and condenser. Heat was applied into the flask where the solvent evaporates into a condenser and converted to liquid that flows into the extraction chamber containing the sample. At the end of extraction, the remaining solvent in a flask is evaporated in an oven and the oil was collected (Adejumo et al., 2013).

Test organisms

The pathogenic microorganisms used in this study were gram-positive bacteria *S. aureus* and gram-negative bacteria *E. coli*; the fungal strains *T. mentagrophytes* and *C. albicans* (Yeast). The strains were clinical isolates obtained from Bacteriology and Mycotic disease reference laboratory of Ethiopian Public Health Institute, Addis Ababa, Ethiopia.

Inoculum preparation

The inoculum for bacteria was prepared from the stock cultures and sub cultured onto nutrient agar using a sterilized wire loop and incubated at 37°C for 24 h. Whereas the yeast and fungi were inoculated with Sabouraud Dextrose Agar (SDA) media and

incubated at 25°C for 72 h. The required working suspension of the inoculum was prepared by transferring morphologically similar colonies of each organism from a young culture in 5 ml nutrient broth (for bacteria) and Sabouraud Dextrose Broth (SDB) for fungi. Then the turbidity of the inoculum was standardized to 0.5 McFarland turbidity standards by measuring with OD 600 nm spectrophotometer to have inoculum size which is equivalent to 1×10^8 CFU/ml. Then the suspension was diluted to 1:100 and used as a starting inoculum for the test (Cheesbrough, 2002).

Controls used in the study

Chloramphenicol for *S. aureus* and *E. coli* and Ketoconazole for *T. mentagrophytes* and *C. albicans* was used as a positive control but 5 % Tween 80 was utilized as a negative control.

Antimicrobial assay

Antibacterial activity of n-Hexane and petroleum ether extracts of *M. stenopetala* seed oil were evaluated by the modified agar well diffusion technique (Bauer et al., 1996). Standardized inoculum of bacterial and fungal culture suspension was uniformly swabbed on the Mueller Hinton Agar (MHA) (OXOID) and SDA (PARK) media respectively by using a sterile cotton swab. The inoculated plates were left at room temperature for 10 minutes to absorb any surface moisture before applying the extract. Thus, wells were aseptically punched on both MHA and SDA plates equidistant of 6 mm in diameter by using a sterile stainless still borer and labeled at the backside of the plates. Each well was filled with 100 μ l of n-hexane and petroleum ether extracts at concentrations of 3.13, 6.25, 12.5, 25 and 50%. Accordingly, all plates were kept to settle down on a working bench for 1hr to allow proper diffusion of the extract into the media. The bacteria cultures were incubated at 37°C for 24 h while the fungal culture was incubated at 25°C for 72 h. The solvents that were used to reconstitute the extract were set up in parallel. Antimicrobial activity was determined by measuring the zone of inhibition around each well. For each extract duplicate trials were conducted against each organism.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the seed oil was determined against the test organisms by using the agar dilution technique (Griffin et al., 2000). This was conducted by mixing the sterile cooled at 45°C MHA and SDA media with different concentrations (4 and 2%) of n-Hexane and petroleum ether extract and poured into Petri dishes (90 mm) and left to solidify then the plates were left upside down at room temperature for 10 to 15 minutes to avoid moisture. In the same fashion controls without the extract were set up in parallel using 5% Tween 80 for negative control and Chloramphenicol and Ketoconazole for positive control. Mueller Hinton Agar and SDA were inoculated with the strains to confirm the viability of the culture. Followed by these 10 μ l from each standardized bacterial and fungal suspension was taken and inoculated on the media that were incorporated with plant extracts. The plates were allowed to stand for 5 min and incubated at 37°C for 24 h for bacteria and 25°C for 72 h for fungi. The procedure was performed in duplicate at different concentrations of the extract.

Statistical analysis

The zone of inhibition around each disc was measured in mm and

the results were presented as means \pm SD using IBM SPSS Statistics software (version 25).

RESULTS

Antibacterial activity of *M. stenopetala* seed oil

The results have shown that n-Hexane and petroleum ether extracts of *M. stenopetala* seed from the different locations used at different concentrations has shown no zones of inhibition of bacterial growth (Table 1). The inhibition zone for the standard drug chloramphenicol was 11.8 mm for *E. coli* and 14.0 mm for *S. aureus*.

Antifungal activity of *M. stenopetala* seed oil

Minimum inhibitory concentration of *M. stenopetala* seed oil

The finding of this research has indicated no inhibition for all tested microorganisms at the concentrations of 2 and 4%. Furthermore, no inhibitory effect was observed in the presence of 5% Tween 80 which was used as a negative control (Table 1).

DISCUSSION

The study did not show any inhibition activity of *M. stenopetala* extracts against bacteria. Previous study reported controversial results from the present study (Chekesa and Mekonnen, 2015); methanol crude extract and ethyl acetate extract of the *M. stenopetala* seeds showed the highest antibacterial activity, against *S. aureus* and *E. coli* but petroleum ether extract of the seeds only showed inhibition on *S. aureus* but not in *E. coli*. The resistance of *E. coli* to the extract matches findings from a study on the antibacterial activity of *Moringa* leaf extract to be ineffective against *E. coli* (Bhawasari et al., 1965; Peixoto et al., 2011). In line with the current study petroleum ether leaf extract of *M. oleifera* didn't show inhibition against *S. aureus* and *E. coli* isolated from urinary tract-infected patients (Abdalla et al., 2016).

The organisms that are included in this study are clinical isolates that are obtained from symptomatic patients. Hence, they may have a high chance of exposure to anti-bacterial agents that may bring change to the molecular and other factors. Therefore, the microorganisms are expected to be less sensitive compared to standard organisms with no chance of exposure to any antimicrobial agents. Moreover, a previous study (Rahman et al., 2008) reported that petroleum ether extract from the stem bark of *M. oleifera* did not show antibacterial activity in both *E. coli* and *S.*

Table 1. Antifungal effect of different solvent extracts of *M. stenopetala* seed oil.

Zone of inhibition (mm)				
Test organisms				
Location	Extraction solvent	Concentration (%)	<i>T. mentagrophytes</i> (mean±SD)	<i>C. albicans</i>
Shelle	Pet ether	50	14±0.28	-
		25	10±0.28	-
		12.5	7±0.25	-
		6.25	-	-
		3.13	-	-
Shelle	n-Hexane	50	17.75±0.73	-
		25	17±0.32	-
		12.5	12.38±0.01	-
		6.25	11.2±0.01	-
		3.13	11±0.63	-
Goffa	Pet ether	50	12±0.91	-
		25	7±0.21	-
		12.5	-	-
		6.25	-	-
		3.13	-	-
Goffa	n-Hexane	50	10±0.28	-
		25	7.5±0.19	-
		12.5	5±0.28	-
		6.25	-	-
		3.13	-	-
Konso	Pet ether	50	10±0.14	-
		25	8±0.50	-
		12.5	7±0.77	-
		6.25	-	-
		3.13	-	-
Konso	n-Hexane	50	9±0.35	-
		25	7±0.14	-
		12.5	-	-
		6.25	-	-
		3.13	-	-
Ketokonazo Dist. H ₂ O		3.13	-	-
Negative control Tween80		0.1 mg/ml	21.0±0.05	15.4±0.00
		5%	-	-

Mean±SD- mean±standard deviation, _ No inhibition zone (no activity), Pet ether-Petroleum ether.

Source: Authors

aureus. Furthermore, a study made by Shailemo et al. (2016) showed antimicrobial activity *M. oleifera* n-Hexane seeds and bark extracts against pathogens of water-borne diseases was lower than other solvents used for extraction. The inactivity of both extracts against bacteria

might be because of the presence of polar compounds in the plant that can bind to the cytoplasmic membrane of the organism but since both the extracts are non-polar the activity of the compound becomes inactive against the tested organism (Boyd and Beveridge, 1981).

Both n-Hexane and petroleum ether extract of *M. stenopetala* seed showed antifungal activity against *T. mentagraphytes* at the concentration $\geq 12.5\%$ except n-Hexane extract collected from Shelle which has shown antifungal activity at the concentration of ≥ 3.06 . In line with the current finding, Dinesha et al. (2018) reported that Moringa seed kernel oil presented excellent antifungal activities. Furthermore, Anthonia (2012) reported that *T. mentagraphyte* growth was inhibited by inhibition zone of 22 mm using ethanolic extract *M. oleifera* leave. In other study, *M. stenopetala* methanolic leaf extract results in concentration dependent inhibition of mycelial growth of *Aspergillus flavus* (Kekuda et al., 2016).

The result has demonstrated an increase in the extraction concentration resulted in gradual increases in the inhibition zone. Similar result has been reported by Prabakaran et al. (2018) for *M. oleifera* extract. Both n-Hexane and petroleum ether extract of *M. stenopetalla* seed has shown no antifungal activity against *C. albicans* (Table 1). This result was in line with a study conducted by Rahman et al. (2008) where petroleum ether extract from the stem bark of *M. oleifera* did not show antifungal activity against *C. albicans*. The inhibition zone for the standard drug Ketoconazole was 21.0mm for *T. mentagraphyte* and 15.4 mm for *C. albican*. In a study done by Lalas et al. (2012) *Moringa peregrina* seed oil extracted by n-Hexane a low activity to *C. albicans* was found compared to other microorganisms *C. albicans* was also found to be the most resistant compared to the tested organism for cold pressed and n-Hexane extracted *Moringa peregrina* seed oil (Osman et al., 2022). In our study both n-Hexane and petroleum ether extract did not show any activity against *C. albicans* this might be due to different species of Moringa.

Generally, the variations in the antimicrobial activities of different study reports could be due to differences in Moringa species, environment conditions, extraction methods, extraction solvent used, age and parts of Moringa used.

Conclusion

The results of the study revealed that *M. stenopetala* seed extract has shown the potential to inhibit the activities of *T. mentagraphyte* fungi even at a lower concentration. The result of the present study is promising as the *M. stenopetala* seed extract exhibited marked antifungal potential which could be used as an alternative to the fungicide chemical. Further studies need to be conducted with various pathogenic microorganisms and extraction with more polar extraction solvents such as Carbon tetrachloride, chloroform, ethyl acetate, etc. Identification of compounds that are responsible to inhibit pathogenic microorganisms also

needs further investigation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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