

# *In Vitro* Antimicrobial Activity of Different Solvent Extracts from *Moringa stenopetala* Leaves

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**ABSTRACT:** This study was conducted to investigate the antimicrobial activities of different solvent extracts of *Moringa stenopetala* (MS) leaves against nine selected pathogenic microorganisms. The disc diffusion method was used to assess antimicrobial activity and determine minimum inhibitory concentrations (MIC). Methanol, and chloroform extracts showed significant inhibitory activity against *Klebsiella pneumoniae* and *Bacillus cereus*. The greatest antimicrobial activity, determined by lowest MIC values (62.5 µg/mL), was observed for inhibition of *C. albicans* by the ethanol extract and *Streptococcus pneumoniae* by the methanol extract. The MICs of chloroform extract were 125 µg/mL against *Escherichia coli*, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Listeria monocytogenes*, and *B. cereus*. Water extract showed the lowest inhibition against these microorganisms, with MIC values of 250 µg/mL. The data presented in this study suggest that MS leaves have great potential in the development of food preservatives and antibiotic drugs.

**Keywords:** extracts, disc diffusion, minimum inhibitory concentrations, inhibition

## INTRODUCTION

*Moringa oleifera* (MO) and *Moringa stenopetala* (MS) are underutilized species of tropical trees that may play an important role in dietary diversification and contribute to alleviation of hidden hunger in less developed tropical and subtropical countries (1). In countries where it is grown, *Moringa* is considered a multipurpose species of tree (2). Various *Moringa* tissues are used as food, herbal medicine, fodder, hedges, firewood, gum, and for water purification (1). *Moringa* leaves contain more vitamins than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges, and more potassium than bananas; the protein quality of *Moringa* leaves even rivals that of milk and eggs (3). *Moringa* is a rich source of certain micronutrients that are commonly deficient in cereal-based diets, such as selenium (4).

MS is indigenous to Kenya and Ethiopia. MS (commonly known as cabbage tree in English, Haleko in Walayita, and Gamo languages in southern Ethiopia) produces edible foliage, immature pods, and seeds that are rich in essential amino acids, vitamins, and minerals (5,6). MS is particularly important as a human food source since the leaves, which have high nutritional value, grow towards

the end of the dry season when few other sources of green vegetables are available (7). MS is often known as 'shiferaw' among local communities for its many medicinal uses, and the leaves, roots, and seeds have long been used in folk medicine. Various parts of MS trees are thought to contain disease-preventing chemicals (8). People with high blood pressure boil the leaves and drink the extract for relief from ailments (8) and leaf extracts are used to lower blood glucose and cholesterol levels. Ghebreselassie et al. (9) reported that aqueous MS leaf extract increases body weight and reduces serum glucose and cholesterol levels in mice. Similarly, experiments conducted on animal models showed that the butanol fraction of the ethanol extract from MS leaves has anti-hyperglycemic and anti-hyperlipidemic effects and, therefore, may be used to treat diabetes (10).

Due to the cost of efficient antimicrobials, a large proportion of the population in developing countries utilize medicinal plants for treatment of infectious diseases. The World Health Organization's estimates that traditional healing is the source of primary health care for a large majority (80%) of the population in Africa (3). Moreover, antimicrobial resistance has become a global problem, and ongoing strategies include researching new and in-

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novative antimicrobials. Traditional medicines from plant extracts, such as *Moringa*, may represent clinically effective, cheap, and relatively less toxic treatment than existing drugs (11). Previous studies indicate that successful determining biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (12). Many solvents are used to extract active materials from plants, including alcohols (ethanol or methanol), diethyl ether, chloroform, ethyl acetate, *n*-butanol, and water (13). Despite its significant economic contributions to the livelihood of millions of people in the eastern Africa, MS has not been given much attention for research and development. There remains a research gap and lack of fundamental information, in particular regarding antimicrobial activity of different parts of MS. There is therefore a need to research the activity of this important but neglected indigenous vegetable tree, and promote and commercialize the findings. The aim of the present study was to evaluate the antimicrobial activities of different solvent extracts of MS leaves. We assessed the antimicrobial activities using the disc diffusion method and determined the minimum inhibitory concentrations against nine selected strains of pathogenic microorganisms.

## MATERIALS AND METHODS

### MS leaves powder preparation

Leaves from healthy and uninfected MS trees were collected from Hosaena, southern Ethiopia. Leaves were washed using running tap water to eliminate dust and other foreign particles, and to thoroughly clean the leaves. The leaves were air dried under the shed and ground into powder using a pestle and mortar. The powders obtained were sieved and then stored in polythene bags for transport to the experimental site: Life and Natural Science College of Daegu University, Korea.

### Extracts preparation

Extractions from MS leaves powder were carried out using four different solvents: 100% chloroform, 100% methanol, 70% ethanol, and water. Samples (15 g) of the powdered leaves were poured into four 500 mL conical flasks (flask A, B, C, and D). 150 mL of chloroform, methanol, ethanol, and distilled water were added to samples in flasks A, B, C, and D, respectively. Flask D (sample and distilled water) was boiled at 80°C for 30 min in a water bath (VS-8480, Vision Science Co., Ltd., Gyeongsan, Korea). The four mixtures were periodically shaken every 6 h to mix and were kept for two days. The extracts were filtered using filter paper (Whatman No. 1, GE Healthcare, Buckinghamshire, UK). Extracts (filtrate) were concentrated at 45°C under reduced pressure using an evap-

orator (EYELA CCA-1110, Tokyo Rikakikai Co., Tokyo, Japan). The extracts were lyophilized and stored in a desiccator until further use.

### Antimicrobial activities of extracts using the disc diffusion method

The test organisms were purchased as lyophilized samples from the Microbial Resource Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea. Organisms included: the Gram-negative bacteria *Escherichia coli* (KCTC 1682, serotype, serogroup O6), *Klebsiella pneumoniae* subsp. *pneumoniae* (KCTC 2208), and *Salmonella* Typhimurium (KCTC 1926); the Gram-positive bacteria *Staphylococcus aureus* (KCTC 1929), *Streptococcus pneumoniae* (KCTC 5412), *Listeria monocytogenes* (KCTC 3569), and *Bacillus cereus* (KCTC 3624); the yeast: *Candida albicans* (KCTC 7007); and the fungi: *Aspergillus niger* (KCTC 6971).

The bacteria were inoculated with tryptic soy broth (Difco Laboratories Inc., Detroit, MI, USA) and incubated at 37°C for 24 h (NU-5810, NuAire Laboratory Equipment, Plymouth, MN, USA). The yeast and fungi were inoculated with potato dextrose broth (Difco Laboratories Inc.) and incubated at 25°C for 72 h. For *in vitro* assessment of antimicrobial activity, we followed a modified protocol for the disc diffusion method (14). Extracts at concentrations of 20 mg/mL were dissolved in dimethyl sulfoxide and filtered through a 0.45 µm microporous filter. 15 mL nutrient agar was added to each petri dish. After solidification, 100 µL of each bacterial suspension contained 10<sup>6</sup> colony-forming unit (CFU)/mL; each yeast and fungal culture was standardized to 10<sup>5</sup> CFU/mL and spread onto petri plates. Sterile filter paper discs (8.0-mm diameter, 1.0-mm thickness) (Advantec, Tokyo, Japan) containing 20 µL of each prepared extracts were placed on the surface of seeded petri plates. Plates were incubated at 35°C for 24 h for the bacterial test and at 25°C for 72 h for the yeast and the fungal test. The growth inhibition zones around each disk impregnated with differing concentrations of extracts were measured to the nearest millimeter. The diameters of the inhibition zones were expressed by comparing the inhibition zones for test organisms used for this study; the average readings from each duplicate was used for comparison.

### Determination of the minimum inhibitory concentration (MIC)

The MIC values were defined as the lowest concentration of extracts that inhibited growth. Extract MIC values was evaluated using a resazurin-based microtiter dilution assay according to Elshikh et al. (15), with a slight modification: assays were carried out under aseptic conditions in 96 well microtiter plates (Nunc, Roskilde, Denmark). The first column of each microtiter plate was filled with

100 µL of test materials (from 1 mg/mL extract stock solution), and the 2nd to 10th wells were filled with 50 µL of sterile water. A two-fold serial dilution (throughout 2nd to 10th wells) was achieved by transferring 50 µL of test material wells in the first column to subsequent wells of each row, so that each well had 50 µL of test material in serially descending concentrations. From wells in the 10th columns, 50 µL solution was removed. The working solution of extracts was diluted across the 96-wells using a two-fold serial dilution to give final testing concentrations of 1,000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.9, and 1.95 µg/mL. Each microtiter plate had a set of 2 controls: (a) test organisms without test extract as a (positive control; 11th wells), and (b) solutions without test organisms (12th wells), as a control for contamination during plate preparation. Aliquots (20 µL) of bacterial, yeast, and fungal suspensions (test organisms) were added to each well. The plates were incubated in a temperature-controlled incubator at 37°C for 24 h for bacteria, and at 48 h for yeast and fungi. After the incubation period, 80 µL resazurin dyes were added and re-incubated for 2 h for color development, and the color changes in each well were observed. Following addition of resazurin, blue coloration indicated pathogen inhibition, and a change from blue to red/pink indicated the presence of live micro-organisms. All experiments were performed in triplicate. The average values were calculated for the MIC of the test material.

### Statistical analysis

The data from the study assessed by analysis of variance (ANOVA) using SAS version 8.1 (SAS Institute, Cary, NC, USA). Statistically significant differences concentrations of solvent extracts needed for antimicrobial activity, and the MIC for each solvent extract were determined. Duncan's multiple range test ( $P < 0.05$ ) was used to com-

pare variations between samples.

## RESULTS AND DISCUSSION

MS is a multipurpose tree, which can be used in food, medicines and in a host of other commodities. The MS leaves are a major food crop in Ethiopia where they are eaten every day, especially during the dry season (16). The leaves are eaten like spinach together with cereal balls. It has been suggested that about 50% of the people in the Konso district of southern Ethiopia get their food from MS (8). In recent decades, there has been an increasing interest in the use of plants as potential antimicrobial agents (17). The antimicrobial activities of various parts of MS have not previously been thoroughly studied, and there is a lack of the fundamental information needed to commercialize its contribution. The present study was conducted to address this research gap through examining the antimicrobial activity of MS leaves. Different solvent extracts of MS leaves were examined for their antimicrobial activities against nine selected pathogenic microorganisms. Evaluation of antimicrobial activity was performed using a disc diffusion method (conc. 1,000 µg/disc) and MICs were determined for different solvent extracts against the test organisms; these results are presented in Table 1 and 2, respectively. The inhibitory effect of the ethanol extract significantly varied ( $P < 0.05$ ) between test organisms. The inhibitory activity was more potent against the Gram-negative bacterium *K. pneumoniae*, the Gram-positive bacteria *B. cereus* and *S. pneumoniae*, and the fungi strains *A. niger* and *C. albicans*. Based on the sensitivity of the test organisms to the MS ethanol extract, as the organisms can be ordered as *B. cereus* > *A. niger* > *C. albicans* > *K. pneumoniae*, *S. pneumoniae* > *S. Typhimurium*, *S. aureus*, and *L. monocytogenes* > *E. coli*. *E.*

**Table 1.** The antimicrobial activities of different solvent extracts against nine selected pathogens (zone of inhibition to the nearest mm)

Microorganisms <sup>1)</sup>	Ethanol	Methanol	Chloroform	Water
<i>Escherichia coli</i>	8.00±0.00 <sup>dB</sup>	12.00±1.41 <sup>cdA</sup>	11.50±1.00 <sup>cdeA</sup>	8.00±0.00 <sup>CB</sup>
<i>Salmonella</i> Typhimurium	8.50±0.58 <sup>cdNS</sup>	9.00±1.15 <sup>e</sup>	9.50±0.58 <sup>f</sup>	9.50±0.58 <sup>ab</sup>
<i>Klebsiella pneumoniae</i>	9.00±0.58 <sup>abcC</sup>	18.25±1.26 <sup>aA</sup>	16.75±1.26 <sup>aB</sup>	8.00±0.00 <sup>CD</sup>
<i>Staphylococcus aureus</i>	8.50±0.58 <sup>cdB</sup>	11.00±1.41 <sup>deA</sup>	10.50±1.73 <sup>defA</sup>	8.00±0.00 <sup>CB</sup>
<i>Streptococcus pneumoniae</i>	9.00±0.00 <sup>bcdB</sup>	13.75±1.26 <sup>bcA</sup>	12.75±0.96 <sup>CA</sup>	8.50±0.58 <sup>bcB</sup>
<i>Listeria monocytogenes</i>	8.50±0.58 <sup>cdB</sup>	11.25±2.63 <sup>dA</sup>	10.00±1.10 <sup>efAB</sup>	8.50±0.58 <sup>bcB</sup>
<i>Bacillus cereus</i>	10.50±1.73 <sup>abB</sup>	15.50±1.29 <sup>bA</sup>	15.00±0.82 <sup>bA</sup>	9.00±1.15 <sup>bcB</sup>
<i>Candida albicans</i>	9.50±0.58 <sup>abcB</sup>	10.25±0.50 <sup>deB</sup>	11.75±0.50 <sup>cdA</sup>	10.50±1.00 <sup>abB</sup>
<i>Aspergillus niger</i>	10.00±1.15 <sup>abNS</sup>	11.25±0.50 <sup>d</sup>	10.50±0.58 <sup>def</sup>	10.25±1.50 <sup>a</sup>

Data represent mean±standard deviation.

Means with different letters (a-f) in column are significant different within the same solvent extract against different test microorganism.

Means with different letters (A-D) in row are significant different with the different solvent extract within the same microorganism.

<sup>1)</sup>Microorganisms, nine different strain of human pathogens used for the test.

<sup>NS</sup>Not significant.

*coli* were comparatively tolerant to the ethanol extract. Methanol and chloroform extracts showed a similar, significant ( $P < 0.05$ ) trend of inhibitory activity against all the test organisms. Methanol and chloroform extracts showed significantly pronounced inhibitory activity against the Gram-negative bacterium *K. pneumoniae*, with the zones of inhibition measuring 18.25 and 16.75 mm, respectively. With methanol and chloroform extracts, the Gram-positive bacterium *B. cereus* showed the second greatest amount of bacterial inhibition; the zone of inhibition measured 15.50 and 15.00 mm, respectively. The Gram-negative bacterium *S. Typhimurium* showed the lowest inhibition of the tested bacteria; inhibition with methanol and chloroform extracts induced zones of inhibition measuring 9.00 and 9.50 mm, respectively. For the methanol and chloroform extracts, inhibitory activity against the test organisms can be ordered as follow: *K. pneumoniae* > *B. cereus* > *S. pneumoniae* > *E. coli* > *C. albicans* > *A. niger*, *S. aureus* > *L. monocytogenes* > *S. Typhimurium*. In this study, we showed that water extract (boiling distilled water extract) showed a comparatively moderate inhibitory activity against the bacterium *S. Typhimurium*, the fungi *A. niger* and the yeast *C. albicans*, inducing inhibition zones of 9.50, 10.25, and 10.50 mm, respectively. Water extract did not show inhibitory activity against the rest of the six pathogenic bacterial strains tested.

Lalitha and Jayanthi (12) indicated that successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The results of our disc diffusion antimicrobial activity assay revealed differing responses of same test organisms to four different MS leaf solvents extracts (ethanol, chloroform, methanol, and water). Chloroform and methanol solvent extracts showed greater inhibition of *E. coli*, *K. pneumoniae*, *S. aureus*, *S. pneumoniae*, *L. monocytogenes*, *B. cereus*, and *C. albicans* compared with ethanol and water (boiling distilled water) extracts. There was no significant difference in inhibitory activity of the four solvent extracts against *S. Typhimurium* or *A. Niger*. Moreover, water extract didn't show inhibitory activity against four of the test organisms, and only showed minimal inhibition of the rest (Table 1). Earlier studies investigating aqueous extracts of Australian native herb extracts have typically shown poor antimicrobial activity compared with other solvent extracts (18). Wigmore et al. (19) suggested that aqueous extracts consist of a mixture of non-active components including carbohydrates, organic acids, proteins, and minerals. In general, we showed that MS leaf different solvent extracts had differing inhibitory activities against the various microorganisms studied. This may be due to differences in the solvents' abilities to successfully extract biologically active compounds from MS leaves, which is in agreement with the previous study by Lalitha and Jayanthi (12).

**Table 2.** Minimum inhibitory concentration values of different solvent extracts against the test microorganisms (unit:  $\mu\text{g/mL}$ )

Microorganisms <sup>1)</sup>	Ethanol	Methanol	Chloroform	Water
<i>Escherichia coli</i>	125	125	125	250
<i>Salmonella</i> <i>Typhimurium</i>	125	125	125	250
<i>Klebsiella pneumoniae</i>	250	125	500	250
<i>Staphylococcus</i> <i>aureus</i>	250	250	125	250
<i>Streptococcus</i> <i>pneumoniae</i>	500	62.5	250	500
<i>Listeria</i> <i>monocytogenes</i>	500	250	125	250
<i>Bacillus cereus</i>	250	125	125	500
<i>Candida albicans</i>	62.5	250	500	250
<i>Aspergillus niger</i>	250	500	1,000	250

<sup>1)</sup>Microorganisms are the same as described in Table 1.

Therefore, selection of appropriate solvent extracts for inhibition of target organisms is very important in the development of food preservative and antibiotics from plant origins.

In microbiology, MIC is the lowest concentration of an antimicrobial (such as an antifungal, antibiotic, or bacteriostatic) drug that will inhibit the viable growth of a microorganism after an overnight incubation (20). To quantitatively evaluate the antimicrobial activities of different solvent extracts of MS leaves for inhibiting nine strains of pathogenic organisms, we used the disc diffusion method; these results are presented in Table 2. All four solvent extracts (ethanol, chloroform, methanol, and water) exhibited marked antimicrobial potential after MIC determination. The MIC for the different solvent extracts ranged from 62.5  $\mu\text{g/mL}$  (ethanol extract against *C. albicans*, and methanol extract against *S. pneumoniae*) to 1,000  $\mu\text{g/mL}$  (chloroform extract against *A. niger*). Mann et al. (21) showed that antimicrobial agents exhibiting low activity against an organism have high MIC, whereas agents with high antimicrobial activities show low MIC. The highest antimicrobial activity for ethanol extract was 62.5  $\mu\text{g/mL}$  (against the fungi *C. albicans*) and the lowest activity was observed for the Gram-positive bacteria *S. pneumoniae* and *L. monocytogenes* at a MIC of 500  $\mu\text{g/mL}$ . In contrast, the methanol extract showed lowest MICs, and therefore highest antimicrobial activities, against *S. pneumoniae* (MIC of 62.5  $\mu\text{g/mL}$ ) and a comparatively highest MIC value was observed for *A. niger* (MIC of 500  $\mu\text{g/mL}$ ). Moreover, the chloroform extract showed a MIC of 125  $\mu\text{g/mL}$  against *E. coli*, *S. Typhimurium*, *S. aureus*, *L. monocytogenes*, and *B. cereus*, and the water extract showed a MIC of 250  $\mu\text{g/mL}$  against all strains of microorganisms except *S. pneumoniae* and *B. cereus* (both MIC of 500  $\mu\text{g/mL}$ ). Test organisms inhibited by water extracts at a MIC of 250  $\mu\text{g/mL}$  were inhibited at lower concentrations by extracts of ethanol, methanol, and chloroform (MICs of

62.5, 62.5, and 125 µg/mL, respectively). These results demonstrate that the lowest antimicrobial activity (highest MIC) occurs when water is used as the solvent for extraction. In general, the different microorganisms varied widely in their susceptibility to antibacterial agents from solvent extracts of MS leaves. In conclusion, our disc diffusion assay and MIC antimicrobial activity results imply that the esteemed *Moringa* plants have promising potential for use in development of food preservative and antibiotic drugs.

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## AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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