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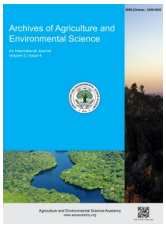

ORIGINAL RESEARCH ARTICLE

Phytochemical screening and *in vitro* antibacterial activity of *Moringa oleifera* (Lam.) leaf extract

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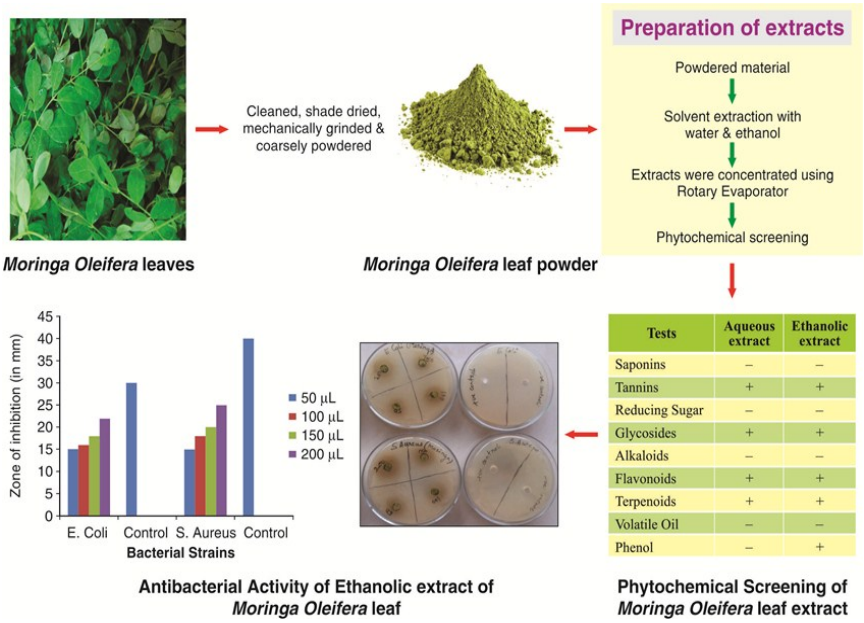
GRAPHICAL ABSTRACT



Antibacterial Activity of Ethanolic extract of *Moringa Oleifera* leaf

Phytochemical Screening of *Moringa Oleifera* leaf extract

Keywords

Agar well diffusion method
Antibacterial activity
Moringa oleifera Lam.
Phytochemical Screening

ABSTRACT

The aim of the study was to investigate the phytochemical constituents and antibacterial activity of ethanolic extract of *Moringa oleifera* Lam. belonging to family Moringaceae. Distilled water and ethanol was used to extract the bioactive compounds from the leaves of *M. oleifera* to detect the phytochemical constituents and to screen its antibacterial activity. The phytochemical constituents were screened by qualitative analysis method. The phytochemical screening indicated the presence of tannins, flavonoids, glycosides, terpenoids, phenols, etc., in leaf extract of *M. oleifera*. The antibacterial activity of ethanolic leaf extract of *M. oleifera* was examined against gram positive bacteria (*Staphylococcus aureus)* and gram negative bacteria (*Escherichia coli)*. Antibacterial assay were done with ethanolic extract of *M. oleifera* in volumes 50, 100, 150 and 200 µL/well, using agar well diffusion method. The study showed that ethanolic extract of *M. oleifera* showed potent antibacterial activity against *S. Aureus* and *E. coli.*

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INTRODUCTION

Moringa (*Moringa oleifera* Lam.) is a type of local medicinal Indian herb belonging to the family of Moringaceae. The tree is often referred to as a "wonder-tree" for its multipurpose usability and also known as "Drumstick-tree", "Horseradish-tree" and "Ben-oil tree". *M. oleifera* is found in many tropical and sub-tropical regions (Berkovich *et al*., 2013; Rockwood *et al*., 2013; Daba, 2016). Moringa can be grown in the even the harshest and driest of soils, where scarcely anything else will grow. Moringa is nick-named "never die" because of its staggering capacity to endure harsh climate and even dry season. Traditionally, besides being a daily used vegetable among people of these regions, Moringa is also widely known and used for its health benefits. *M. oleifera* is considered as "miracle tree" due to its amazing healing abilities for various ailments and even some chronic diseases because all its parts are used, especially for their pharmacological and nutritional properties (Rockwood *et al*., 2013).

Moringa is wealthy in nutrition attributable to the presence of a spread of essential phytochemicals gift in its leaves, pods and seeds. In fact, Moringa provides 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 more potassium than bananas and 25 times more iron than spinach (Rockwood *et al*., 2013). The leaves of *Moringa oleifera* are rich in minerals like calcium, potassium, zinc, magnesium, iron and copper (Kasolo *et al*., 2010).Vitamins like beta-carotene of vitamin A, vitamin B such as folic acid, pyridoxine and nicotinic acid, vitamin C, D and E also present in *M. oleifera* (Vinoth *et al*., 2012; Mbikay, 2012). Phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugar present along with anti-cancerous agents like glucosinolates, isothiocyanates, glycoside compounds and glycerol-1-9-octadecanoate (Berkovich *et al*., 2013). *M. oleifera* leaves show around 25.5–31.03 mg of zinc per kg, which is the daily requirement of zinc in the diet (Barminas *et al*., 1998).

Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess anti-tumour, antipyretic, anti-epileptic, anti-inflammatory, anti-ulcer (Pal *et al*., 1995). Other important medicinal properties of the plant include anti-spasmodic (Cáceres *et al*., 1992), diuretic (Morton, 1991), anti-hypertensive (Dahot, 1988), cholesterol lowering (Mehta *et al*., 2003), antioxidant, anti-diabetic, hepatoprotective (Ruckmani *et al*.,1998), anti -bacterial and anti-fungal activities (Nikkon *et al*., 2003). The aqueous extracts of roots and barks were found to be effective in preventing implantation, aqueous extracts of fruits have shown significant anti-inflammatory activity, methanolic extracts of leaves have shown anti-ulcer activity and ethanolic extracts of seeds exhibited anti-tumour activity (Patel Rameshwar *et al*., 2010). The leaves of Moringa contains bioactive compounds called β-sitosterol which are highly involved in the stabilization of the cholesterol level in the serum of the high fat diet fed rats (Ghasi *et al*., 2000). Moringa leaves are highly rich in β–carotene and leutin which supplies the vitamin A that is highly responsible to prevent the night blindness and also the eye problems in the children. The juices of the Moringa leaves were also involved in the treatment of the conjunctivitis. *M. oleifera* can be used as an anti-cancer agent as it is natural, reliable and safe, at established concentrations. Studies have shown that Moringa can be used as an anti-neoproliferative agent, thereby, inhibiting the growth of cancer cells. Soluble and solvent extracts of leaves have been proven effective as anti-cancer agents due to its ability to induce reactive oxygen species in the cancer cells which lead to apoptosis. This is further proved by the up regulation of caspase 3 and caspase 9, which are part of the apoptotic pathway (Liou and Storz, 2010; Jung, 2014; Leelawat and Leelawat, 2014).

Moreover, the ROS production by Moringa is specific and targets only cancer cells, making it an ideal anticancer agent. The present study was undertaken to identify phytochemical constituents present in the leaves of *M. oleifera* and to explore its antibacterial activity.

MATERIALS AND METHODS

Collection of plant materials

Plant collection

Plants leaves were collected from Visakhapatnam, A.P, India. Plant leaves were initially dried in an air conditioned, dehumidified room, then further dried in an oven at ca. 40°C for a total of seven days, and then finally ground to a fine powder.

Chemicals used

Ethanol and distilled water were used in the preparation of extracts. Mueller-Hinton agar, Nutrient broth, disposable sterile petri dishes, cotton swabs, sterile saline, test tubes was purchased from HiMedia Laboratories Pvt. Ltd., Mumbai. Ampicillin was used to check susceptibility and resistance pattern of the bacterial strains. The antibiotic was obtained from local pharmacy store and working solution having 10 mg/ml concentration of the antibiotic was used for the study.

Bacterial strains

Bacterial cultures of Gram positive bacteria *Staphylococcus aureus* (ATCC25923) and Gram negative bacteria *Escherichia coli* (ATCC25922), were obtained from Microbial Type Culture Collection Center (MTCC), Chandigarh, India. Bacteria were grown in nutrient broth and maintained on nutrient agar slants at 4°C. They were cultured on nutrient broth (Himedia) at 37°C for 24 h.

Preparation of *M. oleifera* leaf extracts

Aqueous *M. oleifera* leaf extract

Immerse 30 g of Moringa Oleifera leaf powder in 300 ml of boiled de-ionized water (DI-H_2O) under magnetic stirrer for about 1h 45 at 50°C. The mixture was cooled to room tempera-

ture and filtered through nylon mesh, followed by Whatmann Filter paper no.1. The filtered *M. olefeira* leaf extract was stored in refrigerator at 4°C for further studies.

Ethanolic *M. oleifera* leaf extract

Immerse 50 g of *M. oleifera* leaf powder in 100 ml of 100% ethanol for overnight. The ethanol fraction was separated using sterile muslin cloth and filter through sterile Whatmann filter paper no. 01. The aqueous and ethanolic extract of *M. oleifera* leaves was protected from light by aluminum foil and stored in refrigerator until used.

Sterility test of the plant extracts

The aqueous and ethanolic extracts were tested for growth or contamination. This was carried out by inoculating 1ml each of them on nutrient agar and incubated at 37°C for 24 hours. The plates were observed for growth. No growth in the extracts after incubation indicates that the extracts were sterile. The extracts were then accessed for antimicrobial activity.

Phytochemical screening

The different qualitative phytochemical tests were carried out as per the standard tests for the phyto-constituents such as alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, glycosides, reducing sugars, fats and oils, etc., present in the leaf extracts. The positive tests were noted as present (+) and absent (-).

Saponins

Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Tannins

To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue colour is observed for gallic tannins and green colour indicates for catecholic tannins.

Reducing sugars

To 0.5 ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.

Glycosides

25 ml of dilute sulphuric acid was added to 5 ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, then 5 ml of Fehling's solution added. Glycosides are indicated by a brick red precipitate.

Alkaloids

2 ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.

Flavonoids

4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange colour for flavones.

Terpenoids

Four milligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoids.

Volatile oil

2 ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is formed if volatile oils are present.

Phenols

To 2 ml of extract, a few drops of ferric chloride solution was added. The appearance of a greenish yellow colour, confirms the presence of phenol.

Standardization of inoculum media

The inocula were prepared from the stock cultures, which were maintained on nutrient agar slant at 4^0C and subcultured into nutrient broth using a sterilized wire loop. Cultures were then adjusted to a concentration of 10^8CFU/mL by making a suspension in 0.85% saline solution match the 0.5 McFarland turbidity standards (Hindler and Jorgensen, 1995). Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated at 24 hrs at 37° C.

Agar well diffusion method

The antibacterial activity of ethanolic leaf extract of *M. oleifera* (1.0 g/ml) against each strain was studied by agar well diffusion method using 50,100,150 and 200 µL/well. The Muller Hinton agar plates was prepared and kept for sterilization. After sterilization the media was poured in to sterile petriplates and were allowed to solidify for thirty minutes. After the medium was solidified, it was inoculated with 18 hours old cultures (100µl) of the given test organisms microorganisms by spreading the bacterial inoculums on the over the Muller-Hinton agar medium using sterile cotton swab horizontally and vertically in order to get a uniform microbial growth. Wells of 6 mm. were punched in the agar and filled with plant extracts. The control plates were made by using Ampicillin (10 mg/ml) as positive control and ethanol as a negative control to determine the sensitivity of bacterial strains. The plates were incubated at 37°C for 18-24 hours. Antimicrobial activity was evaluated by measuring the zones of inhibition against the tested bacteria. Each assay was carried out in triplicate.

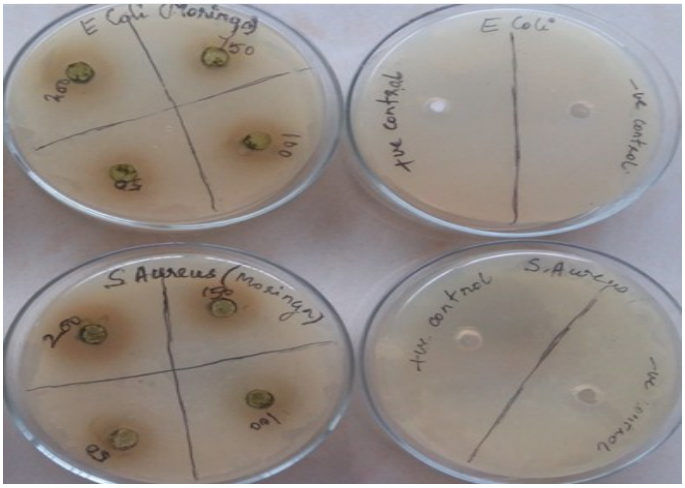

Figure 1. Antibacterial disc diffusion assay plates.

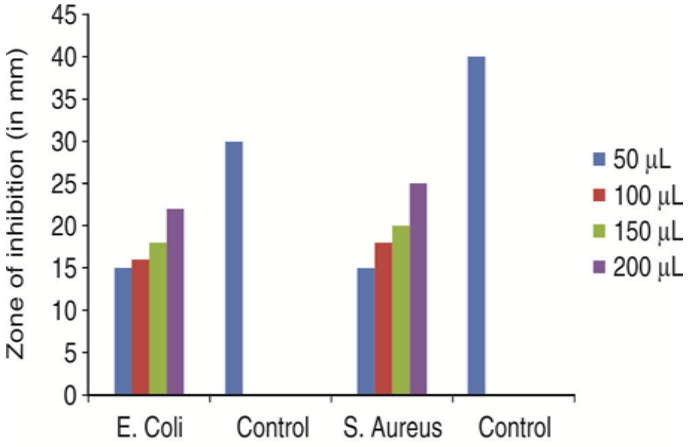

Figure 2. *Anti-bacterial of ethanolic extract of M. oleifera.*

RESULTS AND DISCUSSION

The qualitative phytochemical analysis of *M. oleifera* leaf extracts was done to test for presence of various phytochemicals. The results of the phytochemical analysis of *M. oleifera* leaf extracts using water and ethanol are shown in Table 1. The phytochemical screening indicated the presence of tannins, flavonoids, glycosides, terpenoids, phenols, etc., in leaf extracts of Moringa *oleifera* that are responsible for its antibacterial activity. The antibacterial activity of ethanolic extract was investigated using agar well diffusion method, against gram-positive species (*S. aureus*) and gram-negative strain (*E. coli*). The results of antibacterial activity of ethanolic leaf extract of *M. oleifera* are shown in Figure 2 and Table 2. The results show that ethanolic extract of Moringa *oleifera* had activity against both the test bacteria. The maximum zone of inhibition against *S. aureus* was 25 mm *and E. coli was* 22 mm.

The antibacterial activity of the ethanolic extract was greater against gram-positive species (*S. aureus*) than against gram-negative strain (*E. coli*). Similar studies conducted by Bukar *et al.* (2010) and Nepolean *et al.* (2009) reported that ethanol leaf extracts were sensitive to *S. aureus* and *E. coli* at concentration of 200 mg/ml. However, a contrary finding was reported by Arzai (2008) where no antimicrobial activity was observed at 125 mg/ml concentration on *S. aureus* but activity was obtained at higher concentration of 250 mg/ml. Findings by Maroyi (2006) also found that leaf extract has antibacterial activity against *S. aureus.* The antibacterial properties of the leaf and seed of *M. oleifera* as shown in the present study was in conformity with earlier findings by Anwar *et al.* (2007); Akhtar *et al.* (2007) and Foidl *et al.* (2001) who reported antibacterial properties of seed and leaves extracts of *M. oleifera*. The antimicrobial activity of *M. oleifera* is due to the presence of a significant phytochemical of a short polypeptide called 4 (ά–L–rhamnosyloxy) benzyl-isothiocyanate (Eilert *et al.*, 1981; Guevara *et al.*, 1999). This peptide act directly on bacteria and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes (Silvestre *et al.*, 2000). Flavonoids and tannins have been reported to possess antimicrobial activity, the antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelope proteins.

Table1. Phytochemical Screening of Moringa *oleifera* Lam Leaf extracts.

Tests	Aqueous extract	Ethanolic extract
Saponins	–	–
Tannins	+	+
Reducing Sugar	–	–
Glycosides	+	+
Alkaloids	–	–
Flavonoids	+	+
Terpenoids	+	+
Volatile Oil	–	–
Phenol	–	+

Table 2. Antimicrobial activity of ethanolic leaf extract of Moringa *oleifera* against *S. Aureus* and *E.Coli.*

Bacterial strains	Zone of inhibition (mm)				
	Ampicillin	50 µL/ Well	100 µL/ Well	150 µL/ Well	200 µL/ Well
S. aureus	40	15	18	20	25
E. coli	30	15	16	18	22

Conclusion

The ethanolic leaf extract of *M. oleifera* used in this experiment showed significant antibacterial activity against test pathogens, this thus supports the fact that *M. oleifera* contain contains active phytochemicals with wide-spectrum antibacterial activity, capable of inhibiting the growth of gram-positive and negative bacteria. The antibacterial activity of ethanolic leaf extract of *M. oleifera* could be due to the better solubility of its components in organic solvent, which indicates that the active components responsible for the bactericidal activity are more soluble in organic solvents. Detailed study is needed to investigate the active compounds present in these plant parts having antibacterial activity that may help us to design more effective chemotherapeutic agent to heal bacterial infections.

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