



Original Research Article

Antimicrobial activity of different extracts of leaf of *Moringa oleifera* (Lam) against gram positive and gram negative bacteria

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A B S T R A C T

Keywords

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The antimicrobial activity of petroleum ether, chloroform, ethanol and aqueous extracts of *Moringa oleifera* leaf against four microorganisms viz., *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*. The antimicrobial activity was performed by Kirby-Bauer disc diffusion method. Ethanolic extract showed maximum zone of inhibition against *Staphylococcus aureus*.

Introduction

Air is important as it provides oxygen and other gases that are essential for the survival of human beings and animals. Humans breathe in oxygen from the air in order to live as it facilitates many processes in the body (Sahagun 2008). Certain respiratory tract infections are transmitted through air. Airborne pathogens or allergens often cause inflammation in the nose, throat, sinuses and the lungs. This is caused by the inhalation of these pathogens that affect a person's respiratory system or even the rest of the body (Mitchall 1994). Food plays very vital role in maintaining proper health and also helps in prevention and cure of diseases. Good nutritive food makes health, but at the same time bad or unhealthy food gives rise to several diseases.

Bacteria and viruses are the most common cause of food poisoning. (De Giusti 2007). Plants have been used as valuable sources of natural products for maintaining animal and human health. Plants have been reported to contain large varieties of chemical substances that possess important preventative and curative therapies. *Moringa Oleifera (lam)* is considered a complete food it has an impressive range of medicinal uses with high nutritional value. Its multiple pharmaceutical effects are capitalized as therapeutic remedy for various diseases in traditional medicinal system (Anwar, 2007). The extracts of the leaves are known to have biological properties and these are usually found to vary with the type of solvent used to extract the active components. The plant has been reported

to possess antimicrobial properties and this explains the reason for its wide use in the treatment of human respiratory tract diseases (Lockett *et al.*, 2000; Anwar *et al.*, 2007).

Bukar *et al.*, (2010) reported that *Moringa oleifera* leaf ethanolic extract had the broadest spectrum of activity on the test bacteria. Doughari *et al.*, (2007) reported antibacterial activity from the aqueous, acetone and ethanolic extracts of the leaves of *Moringa oleifera*. Of the three solvents used, ethanolic extract of the plant demonstrated the highest activity, while the aqueous extract showed the least activity at 100 mg/ml. Renitta *et al.*, (2009) reported antimicrobial activity from the ethanolic extract of leaves, seeds and flowers of *Moringa oleifera* against microorganisms like *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter spp*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* A, *Staphylococcus aureus*, *Streptococcus* and *Candida albicans*.

Materials and Methods

Collection of sample

The plant material of *Moringa oleifera* Lam) or Drumstick leaves is used in this study was collected from Tindivanam in month of October 2012 and authenticated by Mrs. Prema sambath vice principal of Plant Biology and Plant Biotechnology Department from Ethiraj college for Women, Egmore, Chennai. Following identification voucher specimen of the plant was deposited in herbarium (PB &PB.Tech./2013/Flora of the Presidency of Madras, J.S Gamble, vol. I, II, III).

Fresh material of the plant sample was collected, cleaned, washed, shade dried

and homogenised to a fine powdered and stored in airtight bottle.

Preparation of plant extracts:

30g of collected powdered form of leaves weighed and extracted with 90% of chloroform, 80% of petroleum ether, 70% of ethanol and aqueous (distilled water), made upto 250ml and purified by soxhlet apparatus with their respective boiling temperature, allow to evaporate and collected in small labelled vials (Balajee *et al*, 2004).

Test organisms

Pathogens tested for antimicrobial activity

Gram negative bacteria (Food borne pathogens)

Escherichia coli
Klebsiella pneumoniae

Gram positive bacteria (Respiratory pathogens)

Staphylococcus aureus
Streptococcus pneumoniae

Screening of antimicrobial activity

Media for test organisms

27g of Muller Hinton Agar was added to 100ml of sterile distilled water and autoclaved at 121°C for 15 minutes at 15lbs. 1.0g of dextrose was added to 10ml of sterile distilled water and steam sterilized for 15 minutes. After cooling both the content was mixed and poured into sterile petriplates approximately 4mm and allowed to set at ambient temperature and used.

Antimicrobial activity by agar disc diffusion method

Antimicrobial activity of each plant extracts determined by using a modified Kirby –Bauer (Bauer *et al.* , 1966) disc diffusion method. Briefly, broth culture of test bacteria were spreaded on the Muller Hinton Agar media in petriplates and microbes broth culture were applied on media by swabbing, under lab condition. The extracts were tested using 5mm sterilized filter paper discs which impregnated with 200mg to 800mg concentration of test samples (Aqueous, petroleum ether, chloroform and ethanol) allowed to dry for few minutes at room temperature, plates were incubated at 37°C about 24 hours. Then, the diameters of the inhibition zones were measured in millimeters. Standard antibiotics, tetracycline (10mcg/disc) served as positive control. A filter disc impregnated with 10µl of DMSO (Dimethylsulphoxide) were used as vehicle control. Each extracts of different concentrations 200, 400, 600, 800mg/ml was impregnated on to the disc and all assays performed in triplicates to consider mean values as a standard one.

Result and Discusion

The antimicrobial activity of petroleum ether, chloroform, ethanol and aqueous extracts of leaves of *Moringa oleifera* (Lam) with 200-800mg/ml concentration against selected gram positive bacteria and gram negative bacteria. All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. The results were expressed as mean \pm standard deviation. Ethanolic extract showed maximum zone of inhibition against *Staphylococcus aureus* and aqueous extract showed lowest activity against *Streptococcus pneumoniae*.

Petroleum ether extract of leaves of *Moringa oleifera* against *Escherichia coli* ranges from 5mm to 9mm and *Klebsiella pneumoniae* ranges from 4mm to 7mm, for *Staphylococcus aureus* ranges from 7mm to 11mm whereas against *Streptococcus pneumoniae* shows 3mm to 6mm at 200, 400, 600, 800 mg concentrations respectively. There was no inhibition of growth with vehicle control. Antimicrobial activity of petroleum ether extract of leaves of *Moringa oleifera* was significantly effective against the gram positive and gram negative bacteria. The antimicrobial activity increased significantly with the increasing concentration, 800 milligram being the maximum concentration tried.

Chloroform extract of leaves of *Moringa oleifera* against *Escherichia coli* ranges from 8mm to 11mm and *Klebsiella pneumoniae* ranges from 6mm to 9mm, for *Staphylococcus aureus* ranges from 7mm to 11mm at 200, 400, 600, 800 mg concentrations respectively, whereas against *Streptococcus pneumoniae* shows 5mm to 7mm which is lesser than other microbes at same concentration of chloroform extracts. Antimicrobial activity of chloroform extract of leaves of *Moringa oleifera* was significantly effective against the gram positive and gram negative bacteria. The antimicrobial activity increased significantly with the increasing concentration, 600 milligram being the maximum concentration tried.

Ethanolic extract of leaves of *Moringa oleifera* against *Escherichia coli* and *Klebsiella pneumoniae* organisms showed from 7mm to 12mm for zone of inhibition at 200mg to 800mg concentration respectively.

Table.1 Effect of ethanolic extract of leaves of *Moringa oleifera* (Lam) against gram positive and gram negative bacteria

S.No	Name of test organisms	Zone of inhibition(mm) Mean \pm standard deviation				Positive control	Vehicle Control
		200mg	400mg	600mg	800mg	Tetracycline (10mcg)	
1.	<i>Escherichia coli</i> (Gram negative)	8.3 \pm 0.47	8.0 \pm 0.47	9.3 \pm 0.46	11.0 \pm 0.00	12.3 \pm 6.8	-
2.	<i>Klebsiella pneumoniae</i> (Gram-ve)	5.6 \pm 1.24	6.3 \pm 0.94	8.3 \pm 0.91	9.3 \pm 0.46	6.6 \pm 2.3	-
3.	<i>Staphylococcus aureus</i> (Gram positive)	10.0 \pm 0.7	15.0 \pm 0.9 4	12.6 \pm 0.94	13.0 \pm 0.00	15.0 \pm 0.0	-
4.	<i>Streptococcus pneumoniae</i> (Gram +ve)	4.3 \pm 0.71	6.3 \pm 0.46	5.3 \pm 1.00	7.0 \pm 0.81	6.0 \pm 2.5	-

For *Staphylococcus aureus* have 10mm, 14mm and 12mm diameter of zone of inhibition at 200, 400, 600, and effect of chloroform extract against *Streptococcus pneumoniae* shows 6mm to 8mm zone of inhibition which is slightly lesser than other microbes.

The aqueous extract of *Moringa oleifera* (lam) against *Staphylococcus aureus* showed the highest inhibition 6mm with the concentration 800 milligram. Whereas *Streptococcus pneumoniae* ranged from 2mm to 3mm showing the highest inhibition at 800 milligram of plant extract. Antimicrobial activity of aqueous extract of *Moringa oleifera* against *Escherichia coli* ranged from 3 to 5 mm and *Klebsiella pneumoniae* shows 2mm to 4mm. There was no inhibition of growth with vehicle control.

From the result the ethanolic and chloroform leaf extracts had strong inhibitory effect against all the gram positive and gram negative bacteria. The

study reported strong antimicrobial activity for all the four extracts in general ethanol and chloroform extracts as comparatively strong and slightly less inhibitory effects in petroleum ether and aqueous extracts against gram positive and gram negative bacteria. Comparison with tetracycline showed 15mm which is same inhibition zone of ethanolic extract against *Staphylococcus aureus*. No inhibition of growth with vehicle control.

Previous study reported the *Moringa oleifera* leaf extract the non activity of the water extract against microbes investigated study is in agreement with previous works which showed that aqueous extracts of plants generally exhibited little or no anti microbial activity (Aiyegoro *et al.*, 2008).

Ethanolic extract of leaf of *Moringa oleifera* showed maximum diameter of zone of inhibition against *Staphylococcus aureus* i.e (15mm). The chance to find antimicrobial activity was more apparent

in ethanol and chloroform than petroleum ether and aqueous extracts of the same plant. This study that the extract of *Moringa oleifera* is active against the tested gram positive and gram negative microorganisms and the results confirm the use of the plant in traditional medicine.

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