

Original Research Article

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Phytochemical Screening and Antibacterial Assay of extracts of different parts of *Moringa oleifera* Lam

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ABSTRACT

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Antibiotic resistance remains the serious concern to the global health. Antibiotic are becoming less effective against various pathogenic organism. The present study was aimed to evaluate antibacterial efficacy of Leaf, Flower and bark of *Moringa oleifera* (Drumstick). This result shows methanolic extracts of leaf had best zone of inhibition against *E.coli* and *Streptococcus pneumonia* and the most susceptible bacteria was found to be *S. aureus* pseudomonas Ethyl acetate extract of bark against *Pseudomonas* showed better result than other solvent (12mm). Zone of inhibition of petroleum ether and ethyl acetate extract of flower shows better zone against *Streptococcus pneumoniae*. Hence it proves that selected part of *Moringa oleifera* contains bioactive components which are antibacterial in nature.

Introduction

The increasingly large numbers of bacteria that are developing resistance against orthodox antibiotics pulls attention of researchers toward herbal antimicrobial molecules in hope that they may provide useful leads into anti-infective drug with least side effects. Several secondary metabolites were isolated from plant which includes terpenoids, steroids, alkaloids, tannins,

benzophenones, coumarins and flavonoids. These new herbal phytochemical substances can also serve as templates for producing more effective drugs through semi-synthetic and total synthetic procedure.

Moringa oleifera (Lam) is the most cultivated species of a single genus found in Moringaceae family, commonly known as Drumstick. Other common names of this plant are horse radish tree, benzolive tree, mother's

best friend, marango, Mulangay, Sajna or Ben oil tree. *Moringa* species have been utilized by Traditional medicine practitioners in curing various diseases as it has medicinal values (Rathi *et al.*, 2004). It is considered as Miracle tree as all the parts of the plant are useful for human. It is also known as multipurpose plant due to its various functions almost every parts of the plant are exploring by many in different industries. Some of the valuable uses of *Moringa oleifera* are medicine, nutrition, water management, livestock feed, biodiesel and landscaping among others (Bennet, *et al.*, 2003).

Many part of the plant such as leaves, fruits, roots were used as vegetables (Siddhuraju, *et al.*, 2003). This plant has plethora of Minerals and vitamins (Latida, *et al.*, 2013). Different studies have showed that various parts of *Moringa oleifera* such as stem, flowers, bark, roots as well as seeds have antimicrobial activities (Lockett, *et al.*, 2000 and Fahey, *et al.*, 2005). *Moringa oleifera* have been considered as antimicrobial agent after the discovery of several Antimicrobial bioactive components with inhibitory activity against many pathogenic microorganisms (Fozia, *et al.*, 2012). leaves are known to have biological properties due to presences of useful phytochemicals compounds such as saponins, flavonoids, tannins and other phenolic compounds that have antimicrobial properties (Bako, *et al.*, 2010). The seed pods (fruits) of the *Moringa oleifera* tree are one of the most nutritive and useful parts of this versatile plant.

The root and bark of young trees are considered rubefacient, stomachic carminative, vesicant and abortifacient. The flowers and roots contain an antibiotic that is highly effective in the treatment of cholera. Torres-Castillo, *et al.*, 2013 stated that the antioxidant activities of *Moringa* plant extracts is due to the presence of polyphenolic

compounds, which can act as great antimicrobial agents in a quantum of crucial phytoconstituents such as tannins, saponins, alkaloids, steroidal aglycones, reducing sugars, terpenoids, and so on, that act as a cardiac and circulatory stimulants, possess anti-tumour, antipyretic, anticonvulsant, anti-inflammatory (Lockett *et al.*, 2000), antiulcer, antispasmodic, antidiabetic, diuretic, antihypertensive, cholesterol-lowering, antioxidant, antifungal, abortifacient, antibacterial (Anwar and Rashid, 2007).

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* (Renitta, *et al.*, 2009). *Moringa* leaves have been reported to contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges, and more potassium than bananas, and that the protein quality of *Moringa* leaves rivals that of milk and eggs.

The solo aim of the present study is an attempt to explore the potentiality of *Moringa oleifera* leaves, flower and bark (Methanol, Petroleum ether, Ethyl acetate & Aqueous) extracts Phytochemical & Antibacterial angle. The Antibacterial activity of above extracts was evaluated by using gram positive & gram negative strain of human pathogenic bacteria.

Materials and Methods

Collection of plant material

The plant material of *Moringa oleifera* (Fresh leaves, flower and bark) used in study were collected from botanical garden of A.N.College, Patna, Bihar, India.

Preparation of plant extracts

Fresh material of the plant samples were collected, cleaned, washed, air dried and homogenized to a fine powdered using mechanical stirrer and stored in airtight bottle. 25gm of collected powdered form of leaves, flower, bark weighed and extracted with Ethanol, Methanol, Petroleum ether, ethyl acetate and aqueous extracts by soaking the powdered materials in 200 ml of solvents & run in Soxhlet. The process of extraction continues until solvent becomes transparent. Extract was dried by rotary evaporator at 40°C for 36 hours. Extract yield were weighed and mentioned in table 1. The extracts were kept in sterile bottles under refrigerated condition until use.

Identification, selection and maintenance of test bacteria

Four bacterial cultures of *Escherichia coli*, *Streptococcus pneumonia* (Gram negative Bacteria) *Staphylococcus aureus* and *pseudomonas aeruginosa* (Gram positive Bacteria) were selected for antibacterial assay. These clinical bacteria were brought from laboratory of Microbiology Department of Patna Medical Hospital. The isolates were cultured and identified by using microscopy (by Gram staining procedures), colony examination and biochemical tests (BPC, 1994). The subculture was maintained on nutrient broth at 37°C and were cultured on nutrient agar to perform Antibacterial activity.

Phytochemical screening

The qualitative Phytochemical screening of the extracts was done to assess the presence of the following active ingredient: Alkaloid, Flavonoid, Glycosides, Steroids, Polyphenol, Terpenoids, Saponins and Tannins, (Sofowora, *et al*, 1989; Trease, *et al*, 1989).

Antibacterial activity

Antibacterial activity of selected plant parts extracts was carried out by using agar disc diffusion method in nutrient agar media with little modifications. About 25 ml of media was poured into each Petri plate. After solidification of media, the bacteria were inoculated by spreader on the surface of the plates. 6 mm disc of Whatman No. 1 filter paper were loaded with 25 µl of plant different solvent extract along with positive control and negative control and air dried. The discs were placed in the medium and after incubation at 37°C for 24 hrs, zone of inhibitions was observed and measured in mm. (Murray, 2009).

Results and Discussion

Phytochemical screening

The selected parts (Leaves, Flower, Bark) of different extracts of *Moringa oleifera* Lam were dried, finely powdered and extracted, used for this study (Table 1). The results of the phytochemical analysis revealed varying constituents of these extracts. Alkaloids, glycosides, terpenoids, saponins and tannins were detected in all extracts of leaf but aqueous extract only contain flavonoid and steroids. (Table 2). Flower extract is devoid of steroids, terpenoids and tannins (Table 3) while bark extract does contain steroids (Table 4).

Antibacterial testing

The antimicrobial assay results revealed that *M. oleifera* extracts exhibited antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Pseudomonas aeruginosa*. The *Moringa oleifera*, leaf methanolic extract was active against *Escherichia coli* and *Streptococcus pneumonia* but shows less

active against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Standard Amikacin (controls) inhibited the test organisms by 100% at 10 mg/ml concentrations respectively (Tables 5, 6 and 7).

Fig. shows that in *Moringa oleifera* leaves, all extracts had higher antibacterial activity against *E.coli* compared to *S.aureus*. In *Moringa oleifera* flower, all extract had higher antibacterial activity against *S. pneumoniae*. Ethyl acetate showed highest

antibacterial activity against four microorganisms tested. In *Moringa oleifera* bark, methanol and petroleum ether extract showed better antibacterial property in comparison to others in *E. coli* (12mm).

In case of *pseudomonas*, ethyl acetate showed better result than other solvent (12mm). Petroleum ether and ethyl acetate extract was most active against *Streptococcus pneumoniae*. *Moringa* leaf was active against pathogenic bacteria as compared to flower and bark.

Table.1 Dry weight of extract after drying in Rota Vapour (gm/100gm)

Sl.No.	Sample (100g)	Ethanol Extract (gm/100gm)	Methanol Extract (gm/100gm)	Ethyl Acetate Extract	Aqueous Extract (gm/100gm)	Petroleum's Ether (gm/100gm)
1.	Leaf	14.26	17.72	11.82	7.27	10.84
2.	Bark	9.4	10.24	8.22	5.42	9.4
3.	Flower	13.2	8.7	8.5	7.34	12.2

Table.2 Phytochemical screening of selected solvent of leaf extract

Sl. No.	Phytochemicals	Methanol	Ethanol	Petroleum ether	Ethyl acetate	Aqueous
1	Alkaloids	+	+	+	+	-
2	Flavonoids	+	+	+	+	+
3	Glycosides	+	+	+	+	-
4	Steroids	+	+	+	+	+
5	Polyphenol	+	+	+	+	-
6	Terpenoids	+	+	+	+	-
7	Saponins	+	+	+	+	-
8	Tannin	+	+	+	+	-

(+) indicates presences (-) indicates absences

Table.3 Phytochemical screening of selected solvent of flower extract

Sl. No.	Phytochemicals	Methanol	Ethanol	Petroleum ether	Ethyl acetate	Aqueous
1	Alkaloids	-	-	-	-	+
2	Flavonoids	-	-	+	+	+
3	Glycosides	+	-	+	+	-
4	Steroids	-	-	-	-	-
5	Polyphenol	+	+	-	+	-
6	Terpenoids	-	-	-	-	-
7	Saponins	+	+	-	-	+
8	Tannins	-	-	-	-	-

(+) indicates presences (-) indicates absences

Table.4 Phytochemical screening of selected solvent of bark extract

Sl. No.	Phytochemicals	Methanol	Ethanol	Petroleum ether	Ethyl acetate	Aqueous
1	Alkaloids	-	-	-	+	-
2	Flavonoids	+	+	+	+	+
3	Glycosides	+	-	+	-	+
4	Steroids	-	-	-	-	-
5	Polyphenol	-	+	-	+	-
6	Terpenoides	+	-	-	-	-
7	Saponins	+	-	-	-	+
8	Tannins	-	+	-	-	-

(+) indicates presences (-) indicates absences

Table.5 Leaf extract antibacterial analysis on selected bacterial strain (10mg/ml)

Sl. No	Bacterial Strain	P+ Control	N- Control	Methanol	Ethanol	Petroleum ether	Ethyl acetate	Aqueous
1	<i>E.Coli</i>	32 mm	0 mm	12 mm	10 mm	8 mm	10 mm	6 mm
2	<i>Streptococcus pneumonia</i>	28 mm	0 mm	12 mm	9 mm	6 mm	8 mm	3 mm
3	<i>Pseudomonas aeruginosa</i>	24 mm	0 mm	10 mm	7mm	10 mm	8 mm	2 mm
4	<i>Staphylococcus aureus</i>	25 mm	0 mm	12 mm	10 mm	8 mm	10 mm	6 mm

Note: - P+ Positive Control = Amikacin (10µl) and N – Negative Control = Sterilized of distilled Water

Table.6 Flower extracts antibacterial analysis on selected bacterial strain (mm)

Sl. No.	Bacterial Strain	P+ Control	N- Control	Methanol	Petroleum Ether	Ethanol	Aqueous	Ethyl acetate
1	<i>E.coli</i>	30 mm	7 mm	10 mm	11 mm	10 mm	2 mm	12 mm
2	<i>Streptococcus pneumonia</i>	30 mm	7 mm	8 mm	12 mm	12 mm	10 mm	12 mm
3	<i>Pseudomonas aeruginosa</i>	30 mm	7 mm	10 mm	8 mm	4 mm	2 mm	10 mm
4	<i>Staphylococcus aureus</i>	30 mm	7 mm	10 mm	12 mm	11 mm	6 mm	12 mm

Table.7 Bark extracts antibacterial analysis on selected bacterial strain (mm)

Sl. no.	Bacterial Strain	P+ Control	N- Control	Methanol	Petroleum Ether	Ethanol	Aqueous	Ethyl acetate
1	<i>E.coli</i>	30 mm	7 mm	12 mm	12 mm	7 mm	8 mm	10 mm
2	<i>Streptococcus pneumoniae</i>	30 mm	7 mm	8 mm	12 mm	10 mm	2 mm	12 mm
3	<i>Pseudomonas aeruginosa</i>	30 mm	7 mm	6 mm	8 mm	3mm	6 mm	12 mm
4	<i>Staphylococcus aureus</i>	30 mm	7 mm	11 mm	10 mm	10 mm	10 mm	11 mm

Many researchers have proven that *Moringa oleifera* parts such as bark, stem, flowers, fruit, roots as well as seeds have antimicrobial activities (Fahey, *et al.*,2005: locket, *et al.*,2000). *Moringa oleifera* have been considered as bactericidal agent after the discovery of several antibacterial constituents with inhibitory activity against pathogenic bacteria. Finding of new antimicrobials that would be effective against drug resistant strains, phytochemical screening and antibacterial activity, investigation of the *M. oleifera* selected parts extract were carried out. It is suggested that this plant drug would have enormous health benefits with little side effects that is common with the synthetic drugs. During the course of this research, investigations have founded many phytochemicals bioactive compound with promising antibacterial activity.

Various researchers reported antimicrobial activity of *Moringa oleifera* against variety of pathogens including *S. aureus*, *S. albus*, *S. pyogenes* *P. aeruginosa*, *Salmonella gallinarum*, *B.subtilis* and *E. coli* (Thilza,*et al.*,2010 and Kumar, *et al.*,2012).Antimicrobial activity from the seeds of *Moringa oleifera* were assayed for the evaluation of antimicrobial activity against bacterial strains (*Pasturella multocida*, *Escherichia coli*, *Bacillus subtilis* and *Staphlococcus aureus*), which was evaluated by Amer Jamil *et al.*, (2008).

Phytochemicals are present in virtually all plant tissues of *Moringa oleifera* e.g. leaves, roots, stem and fruits (Siddiqui, *et al.*,2009). Presence of phytoconstituents like alkaloids, flavonoids, tannins, saponins are responsible for antibacterial activity (Patil, *et al.*,2013). Alkaloids, phenols, flavonoids and glycosides

have a number of biological activities and strong antibacterial potentials (Robbers *et al.*, 1996). Alkaloids have exhibited promising activity against *H. pylori* (Hadi and Bremner, 2001) and a number of other bacterial strains (Sinha *et al.*, 2001; Saeed and Sabir, 2001; Khan *et al.*, 2001; Kren and Martinkova, 2001) Similarly, a few glycosides have presented with antibacterial activities The antibacterial potential of terpenoids have been documented.

Terpenoids are bioactive molecules which are a part of plants' defence mechanisms as phyto protectants (Morrissey and Osbourn, 1999). Studies have shown that the antimicrobial potential of *M. oleifera* leaves extract may be attributable to the presence of an array of phytochemicals.

This study revealed that *Moringa oleifera* is highly potential medicinal plant of multiple use. It has high antibacterial property and effective secondary metabolite make them more suitable for newer and safer nutraceutical and pharmaceutical products. *Moringa oleifera* parts could be good source of drugs that may be used against bacterial infection if it is found effective and non-toxic in animal trial. 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 chemo therapeutic agent to heal bacterial infections. The result strengthens the scientific data base.

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