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Research Article

ANTIMICROBIAL EVALUATION OF LEAF EXTRACT OF *MORINGA OLEIFERA* LAM

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ABSTRACT
Moringa oleifera tree is known as a Miracle tree as almost every part of this tree possesses product useful for humans. The leaves and pods are eaten. The plant is also reported to be medicinally important and almost all parts of the *Moringa oleifera* tree are considered to possess medicinal properties and are used in the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulant
Leaves are also known to have anti-oxidant properties and are known to cures hallucinations, dry tumors, hiccups and asthma. The root and bark are useful in treatment of heart complaints, eye diseases, inflammation, dyspepsia and enlargement of spleen.
In Present study the antimicrobial activity was investigated by employing main model Kirby-bauer disc diffusion method. The results showed that 50% ethanolic extract of *Moringa oleifera* Lam leaf have very little antibacterial activity and it shows mild inhibitory activity at high concentration of extract of *Moringa oleifera*.
Keyword: *Moringa oleifera*, Kirby-bauer, Antimicrobial activity

INTRODUCTION

Herbal medicines are the finished, labeled medicinal products that contain active ingredients from aerial or underground parts or other plant materials, or combination thereof, whether in the crude state or as plant preparations. Demand for herbal medicine sector is growing fast, increasing by 12-15% value per year. As per WHO reports about 4 billion people of the world population presently use herbal medicines for their primary health care as alternative system of medicine i.e. Ayurvedic, Homeopathic, Naturopathic, oriental and native American Indian medicine[1]

Habitat

Moringa oleifera Lam. is a small or medium-sized tree, about 10m high, found wild in the sub-Himalayan tract, from Chenab east wards to Sarda and cultivated all over the plains of the India[2]

Chemical Constituents

Leaves of *Moringa oleifera* have been reported to contain flavonoid pigments such as kaempferol, rhamnatin, isoquercitrin, and kaempferitrin[3]. The flowers contain traces of alkaloids; they also contain a wax, quercetin and kaempferol; the ash is rich in potassium and calcium. The roots contain an active antibiotic principle, pterygospermin ($C_{22}H_{18}O_2N_2S_2$). The root bark contains two alkaloids, viz. moringine and moringinine. The nutritious tender pods are rich in vitamins and are a good source of α– linolenic acid in human diets

Table- 1 : Phytochemical constituents isolated from *Moringa oleifera* Lam.

Parts	Phytochemical constituents
Roots	4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate and benzylglucosinolate 10
Stem	4-hydroxymellein, vanillin, β-sitosterone, octacosanic acid and β-sitosterol 11
Bark	4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate 10
Whole gum exudates	L-arabinose, D-galactose, D-glucuronic acid, L-rhamnose, D-mannose, D-xylose and leucoanthocyanin 12-13
Leaves	Glycoside niazirin, niazirinin and three mustard oil glycosides, 4-[4'-O-acetyl- α -L-rhamnosyloxy) benzyl] isothiocyanate, niaziminin A and B 14-15
Mature flowers	D-mannose, D-glucose, protein, ascorbic acid, polysaccharide 16
Whole pods	Nitriles, isothiocyanate, thiocarbanates, 0-[2'-hydroxy-3'-(2''-heptenyloxy)]-propylundecanoate, 0-ethyl-4-[(α -1-rhamnosyloxy)-benzyl] carbamate, methyl-p-hydroxybenzoate and β-sitosterol 14-15
Mature seeds	Crude protein, Crude fat, carbohydrate, methionine, cysteine, 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate, benzylglucosinolate, moringyne, mono-palmitic and di-oleic triglyceride 10
Seed oil	Vitamin A, beta carotene, precursor of Vitamin A 17-18

Traditional Uses

Moringa oleifera tree is known as a Miracle tree as almost every part of this tree possesses product useful for humans. The leaves and pods are eaten. The plant is also reported to be medicinally important and almost all parts of the *Moringa oleifera* tree are considered to possess medicinal properties and are used in the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulant
Leaves are also known to have anti-oxidant properties and are known to cures hallucinations, dry tumors, hiccups and asthma[4]. The root and bark are useful in treatment of heart complaints, eye diseases, inflammation, dyspepsia and enlargement of spleen. The flowers are known to cure inflammations and muscle diseases. Seed oil is to be useful in treatment of leprous ulcers

MATERIAL AND METHODS

Collection of the plant materials

Moringa oleifera Lam. leaves (Family - Moringaceae) were collected from Botanical Garden of N.B.R.I. (National Botanical Research Institute), Lucknow, India in month of October 2010. The plant materials were authenticated by Dr. Tariq Husain chemo taxonomist at National Botanical Research Institute, Lucknow and the voucher specimens (97816) were deposited in the departmental herbarium of

National Botanical Research Institute, Lucknow, India for future reference.

Preparation of 50% Ethanolic extract of *Moringa oleifera* Lam. leaves

The freshly collected leaves (4kg) of *Moringa oleifera* were first washed with distilled water and air-dried at 30 ± 2^0C. Then dry in tray drier under controlled conditions and powdered. The powdered plant materials (1000g) were macerated with petroleum ether to remove fatty substances and the marc was further exhaustively extracted with of 50% ethanol for 3 days (3 X 5L). The extract was separated by filtration and concentrated on rotavapour (Buchi, USA) and then dried in lyophilizer (Labconco, USA) under reduced pressure the yield of dried extract obtained was 95.0g (9.5% w/w). The 50% ethanolic extracts obtained was further subjected to toxicological and pharmacological investigations.

Antimicrobial Studies

Disc diffusion test / kirby-bauer disc diffusion method[5]

Preparation of testing solution of extract:

1.0gm of dried 50% ethanolic extract of leaves was dissolved in 5.0ml. of DMSO (Dimethyl sulfoxide) to get 200mg/ml. solution. Different dilutions of extract were prepared in DMSO to get following concentration of extract:

1. Undiluted solution (200mg/ml)
2. 1.5ml solution + 0.5ml DMSO (150mg/ml.)
3. 1.0ml solution + 1.0ml DMSO (100mg/ml.)
4. 0.75ml extract + 1.25ml DMSO (75mg/ml.)
5. 0.5ml extract + 1.5ml DMSO (50mg/ml.)

Preparation of Disc

Five vials each containing one hundred discs (6mm diameter) of whatman paper no. 1 were sterilized. One milliliter each of different dilutions of extract was added on discs in separate vials. The Impregnated discs contain 2.0mg, 1.5mg 1.0mg, 0.75mg and 0.5mg of extract.

Preparation of Media

Sensitivity testing of test solution was done on Muller Hinton Agar Media. This media is ideal for disc diffusion method of antimicrobial drug testing.

Composition of Media (MHA)

Ingredients	
Beef infusion	300grams /L
Casein acid hydrolysate	17.5 grams/L
Starch	1.5 grams/L
Agar	17.0 grams /L
Final pH:	7.3

The ingredients were suspended in 1 liter of infusion. It is boiled to dissolve completely. It is sterilized by autoclave. Cooled down and poured in Petri dishes and allowed to jellify.

Microorganisms

Standard strains of *Staphylococcus aureus (ATCC 25923)*, *Klebsiella pneumoniae (NCTC, 13368), Escherichia coli (ATCC 25922)*, *Pseudomonas aeruginosa (ATCC 27853*) were inoculated in infusion broth and incubated for 24 hours. Then turbidity of each organism was adjusted with sterile infusion broth. (MacFarlane tube no. 4).

Procedure

A small amount of diluted bacterial suspensions were poured over Muller Hinton media to spread uniformly on the surface. After a few seconds excess of broth was discarded. Later when the surface is little dried disc impregnated various concentration of extract and discs impregnated with two standard antibiotics (Amoxicillin 10mcg and Ciprofloxacin 5mcg: Himedia) were placed on the surface at equal distance. Plates were incubated overnight at 37^{o}C.

Observation

Next day zone of inhibition of bacterial growth was measured with scale .

Interpretation:	**Zone Size**	**Result**
	No inhibition	Resistant (-)
	Less than 10mm	Mildly Sensitive (+)
	10 to 20mm	Moderately Sensitive (++)
	20 or more	Highly Sensitive (++)

Table 2 -: Antimicrobial effect of 50% ethanolic extract of *Moringa oleifera* Lam. leaf

S. No		*Staphylococcus Aureus ATCC 25923*		*Klebsiella Pneumoniae NCTC 13368*		*Escherichia Coli ATCC 25922*		*Pseudomoas Aeruginosa ATCC 27853*	
	Concentration of Extract/disc	**Inhibition zone in (m.m)**	**Interpretation**	**Inhibition zone in (m.m)**	**Interpretation**	**Inhibition zone in (m.m)**	**Interpretation**	**Inhibition zone in (mm)**	**Interpretation**
1	2mg/ml	10	Sensitive	10	sensitive	8	sensitive	zero	resistant
2	1.5mg/ml	zero	Resistant	zero	resistant	zero	resistant	zero	resistant
3	1mg/ml	zero	Resistant	zero	resistant	zero	resistant	zero	resistant
4	0.75mg/ml	zero	Resistant	zero	resistant	zero	resistant	zero	resistant
5	0.50mg/ml	zero	Resistant	zero	resistant	zero	resistant	zero	resistant
Antibiotics									
6	Amoxycillin (10mcg/ml)	15	Sensitive	15	sensitive	12	sensitive	15	sensitive
7	Ciprofloxacin (5mcg/ml)	17	sensitive	13	sensitive	14	sensitive	17	sensitive

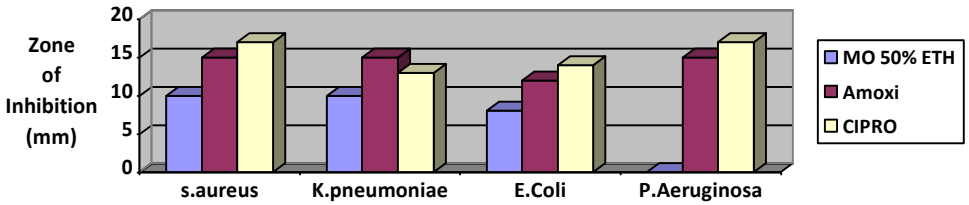

Fig- 1 : Antimicrobial effect of 50% ethanolic extract of *Moringa oleifera* Lam. leaf (Graphical representation)

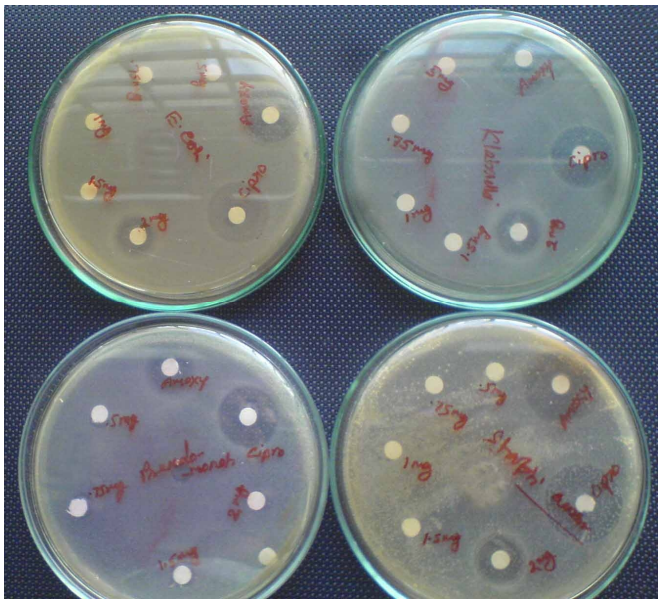

Fig 2 -: Antimicrobial effects of 50% ethanolic extract of *Moringa oleifera* Lam. leaf

DISCUSSION

Antibacterial studies

In antibacterial studies, 50% ethanolic extract was tested against Standard strains of *Staphylococcus aureus (ATCC 25923), Klebsiella pneumoniae (NCTC, 13368) Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853)* and inoculated in infusion broth and incubation for 24 hours. Then turbidity of each organism was adjusted with sterile infusion broth. (MacFarlane tube no. 4).

A small amount of diluted bacterial suspensions were poured over Muller Hinton media to spread uniformly on the surface. After a few seconds excess of broth was discarded. Later when the surface is little dried disc impregnated with various concentration of extract and discs impregnated with two standard antibiotics (Amoxicillin 10mcg/ml and Ciprofloxacin 5mcg/ml: Himedia*)* were placed on the surface at equal distance. Plates were incubated overnight at 37^{o}C.

Next day zone of inhibition of bacterial growth around was measured with scale for determination of minimum inhibitory concentration.

The results revealed that 50% ethanolic extract of *Moringa oleifera lam* leaves showed little antibacterial activity. In future the antimicrobial activity may be performed on the various fractions and isolated products of *Moringa oleifera*, so the Plant may be developed as an antimicrobial drug.

CONCLUSION

From the above table it is clearly noted that 50% ethanolic extract of *Moringa oleifera* Lam. leaf have very little antibacterial activity and it shows mild inhibitory activity at high concentration of extract of *Moringa oleifera* but no activity against pseudomonas.

In conclusion the action of extract upon the antimicrobial models justified its usefulness in herbal formulation further isolation and identification of the compound responsible for biological activity need to be explored.

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