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A STUDY ON PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *MORINGAOLEIFERA*

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ABSTRACT

Moringaoleifera is commonly known as "Drumstick". It is the most popular tropical crop. All its parts were used especially for their pharmacological, nutritional and water purification properties. The seeds of *Moringa* tree is used as a natural flocculant which is considered to be a natural alternative in purification of water. The pure cultures of bacterial strains were isolated from various sources. The isolated organisms were characterized by biochemical tests. The organisms were sub cultured in nutrient broth for further use. *Moringaoleifera* leaves were collected fresh and air dried. The leaves were grounded into fine powder. The leaves were soaked in three different solvents such as water, ethanol and chloroform, kept overnight and filtered out using whatmann filter paper no.2. The phytochemical analysis were carried out. Antimicrobial assay were done with aqueous, ethanol, chloroform extract at different concentrations. The zone of inhibition were observed and tabulated.

KEYWORDS: *Moringaoleifera*, Bioflocculation, Phytochemical Studies, Antimicrobial Studies

INTRODUCTION

Moringaoleifera is a growing fast, drought-tolerant, and, must be cut back several times to make it branch out more. It will readily sprout again and all the valuable products will remain within safe. It seems to thrive in impossible places and never dies. It can be developed easily from seeds or cuttings, compost or manure are not necessary. It can be densely seeded with high yielding. The light shade of the tree is a considerable help to most vegetables. Flowering can be induced through small watering to have a nearly continuous yield. Different parts of this plant contain a sketch of important minerals, and are a good source of protein, various phenolics, vitamins, β – carotene and amino acids. The Moringa plant offers a rich and exceptional combination of zeatin, kaempferom, quercetin and many other phytochemicals. It is very significant for its medicinal value. Numerous parts of the plant such as the roots, seed, bark, leaves, fruit, and immature pods, flowers act as cardiac and circulatory drugs, antipyretic, anti ulcer, anti-inflammatory, antiepileptic. Other chief medicinal properties of the plant include antispasmodic, diuretic, antihypertensive, cholesterol lowering, hepatoprotective, antioxidant, antidiabetic, antibacterial and antifungal activities. *M. oleifera* parts are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia. In addition, *M. oleifera* seeds possess water purifying powers by flocculating Gram positive and Gram negative bacterial cells. *M. oleifera* seeds can also be used as a less expensive bio absorbent for the removal of heavy metals.

In traditional Indian medicine various parts of the tree are used therapeutically for treatment of venomous bites, ascites and rheumatism and helps in lowering blood pressure. 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. As the global scenario is now changing towards the use of non- toxic and eco-friendly products, development of modern drugs from traditional medicinal plants should be emphasized for the control of various human and animal diseases. *Moringaoleifera* (Drumstick) is one such plant which is reported to possess several medicinal properties. The different parts of this plant viz. leaves, stem bark, root bark, flowers, fruits and seeds are used in the indigenous systems of medicine for the treatment of variety of human ailments and some parts are also eaten as vegetable. During recent years considerable work has been done to investigate the pharmacological actions of the leaves and seeds of *Moringaoleifera* on scientific lines but only limited work has been reported so far on antibacterial activity of *Moringaoleifera* root bark though it is reported to possess varied medicinal properties. Therefore, it was considered worthy to investigate the antibacterial activity of *Moringaoleifera* root bark.

MATERIALS AND METHODS

Collection of Plant Materials

Leaves were collected from the *Moringaoleifera* tree at the college campus. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and rinsed with distilled water. The samples were dried under shade paper towel in laboratory and then homogenized into fine powder using a mortar and pestle and stored in air tight bottles and were used for further studies.

Preparation of Leaf Extract

- **Aqueous Extract**

Plant material (100 g) was crushed in sterile water (250 ml) for preparation of aqueous extract. The extract was separated using sterile muslin cloth and filter through sterile Whatmann filter paper (no. 02).

- **Ethanol Extraction**

Moringaoleifera leaves (100 g) were ground into fine powder using a stainless-steel grinder, deep in100% ethanol (200 mL) for overnight. The ethanol fraction was separated using sterile muslin cloth and filter through sterile Whatmann filter paper (no. 02). The filtered extract was concentrated by a rotary film evaporator.

- **Chloroform Extraction**

For preparation of chloroform extract ground plant sample (100 g) was added in chloroform respectively (200ml each case) and left for overnight at room temperature. The extracts were separated using sterile muslin cloth and filter through sterile Whatmann filter paper (no. 02).

Phytochemical Analysis

- **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 10ml 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 0.5ml 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**

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

- **Alkaloids**

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

- **Flavonoids**

4ml 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 color 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 color was observed for terpenoid.

- **Volatile Oil**

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

Isolation and Characterization of Microbes from Sewage Sample

For the isolation of organisms, samples (Sputum, Urine etc.,) were collected randomly from the infectious patients from the hospital. The samples collected were then plated on to Mac Conkey's agar, Mannitol salt agar and Nutrient Agar plates for bacterial isolation using sterilized loop. The plates were then incubated at 37°C for 24 hrs. The plates were observed for bacterial growth after 24 hours. In some plates, there were mixed cultures of organisms. These plates were subsequently sub cultured to isolate the pure strain. Morphological identification done by using Gram staining technique. Further, characterization of organisms was carried out by various biochemical test and the results were tabulated.

Determination of Anti-bacterial Activity

The antibacterial activity of the leaf extracts was determined using agar well diffusion method. Muller Hinton agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells were punched in the agar and filled with plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 37°C for 18 hours and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The antibacterial potential of the different extracts was evaluated by comparing their zones of inhibition.

RESULTS AND DISCUSSIONS

Phytochemical Analysis

The table 1 shows the following Phytochemicals are present in the Moringa leaf

Table 1: Qualitative Phytochemical Analysis of the Extracts of *Moringa* Leaf

Tests	Aqueous	Ethanol	Chloroform
Saponins	-	-	+
Tannins	+	+	+
Reducing Sugar	-	-	-
Glycosides	+	+	-
Alkaloids	-	-	+
Flavonoids	+	+	-
Terpenoids	+	+	-
Volatile Oil	-	-	-
Phenol	-	+	+

Isolation of Organisms

Microbial strains like *Escherichia coli, Salmonella* sp, *Proteus vulgaris, Klebsiella species* were isolated from the Infectious subjects

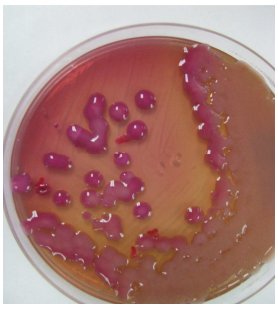

Figure 1: *Klebsiella Species*

Figure 2: *Salmonella* Sp

Figure 3: *Proteus Vulgaris*

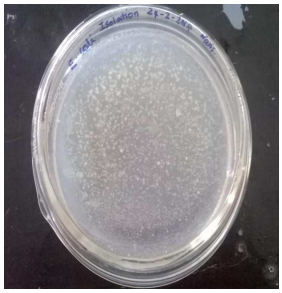

Figure 4: *E. coli*

Characterization of Organisms by Biochemicaltests

The isolated microorganisms were subjected to Various biochemical test such as Indole, MR VP, Citrate utilization test, Triple sugar iron agar test, Catalase, Oxidase test etc., and the results are tabulated in the table 2

Table 2: Biochemical Analysis of Isolated Organisms

Test	*E. Coli*	*Salmonella* Sp	*Klebsiella Species*	*Proteus Vulgaris*
Indole	+	-	-	+
Methyl red	+	-	-	-

Table 2: Contd.,

Vogesproskauer	-	-	+	-
Citrate test	-	-	+	-
Catalase test	-	-	-	-
Oxidase test	-	-	-	-
Triple sugar ion	+	+	-	-

Antimicrobial Studies of Isolated Organisms

Antimicrobial Activity of Aqueous Extract

The aqueous extract of Moringa leaf showed varied zone of inhibitions (Figure 5) against different organisms which is tabulated in table 3. It is found that the organism *Proteus* sppossessed maximum inhibition of 1mm at 100µL dilution.

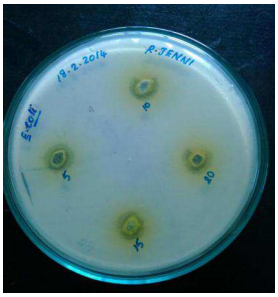

Figure 5a*: E. coli*

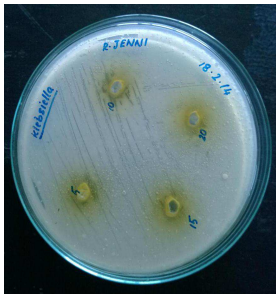

Figure 5b: *Klebsiella* sp

Figure 5c: *Salmonella* sp

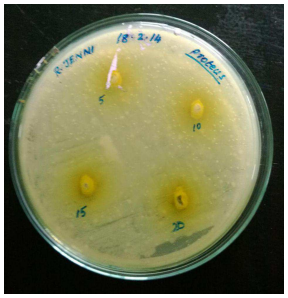

Figure 5d: *Proteus* sp

Figure 5: Antimicrobial Activity of Aqueous Extract against Different Organisms

Table 3: Antimicrobial Activity of the Aqueous Extract against Different Organisms

Organism	25µl	50µl	75µl	100µl
E. coli	0.4mm	0.5mm	0.5mm	0.6mm
Klebsiella species	0.5mm	0.6mm	0.7mm	0.8mm
Salmonella sp	0.4mm	-	-	-
Proteus sp	0.6mm	0.8mm	0.9mm	1mm

Antimicrobial Activity of Ethanol Extract

The Ethanolic extract of Moringa leaf showed varied zone of inhibitions (Figure 6) against different organisms which is tabulated in table 4. *Proteus species* and *Salmonella* sp showed the maximum zone of inhibition of 1.4mm and 1mm at 100µL dilution. Hence *Proteus* is more sensitive to ethanol extract.

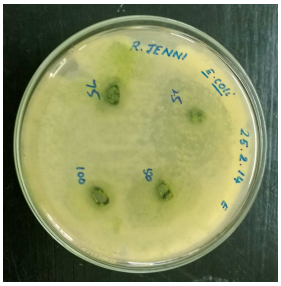

Figure 6a*: E. coli*

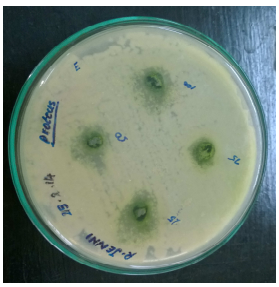

Figure 6b: *Klebsiella* sp

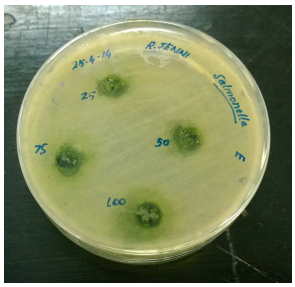

Figure 6c: *Salmonella* sp

Figure 6d: *Proteus* sp

Figure 6: Antimicrobial Activity of Ethanol Extract against Different Organism

Table 4: Antimicrobial Activity of the Ethanol Extract against Different Organisms

Organism	25µl	50µl	75µl	100µl
E. coli	-	-	-	0.4mm
Klebsiella species	0.6mm	0.6mm	0.7mm	0.8mm
Salmonella sp	-	0.4mm	0.7mm	1mm
Proteus species	0.6mm	1mm	1.2mm	1.4mm

Antimicrobial Activity of Chloroform Extract

The Chlroform extract of Moringa leaf showed varied zone of inhibitions (Figure 7) against different organisms which is tabulated in table 5. *Proteus species* and showed the maximum zone of inhibition of 1.8mm at 75µL dilution. Hence *Proteus* spis more sensitive to Chloroform extract.

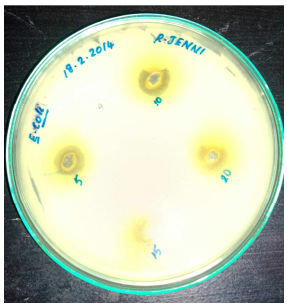

Figure 7a*: E. coli*

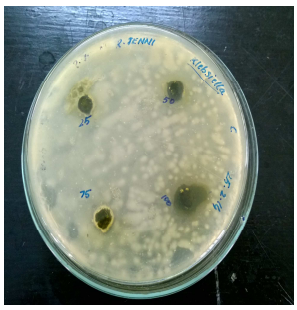

Figure 7b: *Klebsiella* sp

Figure 7c: *Salmonella* sp

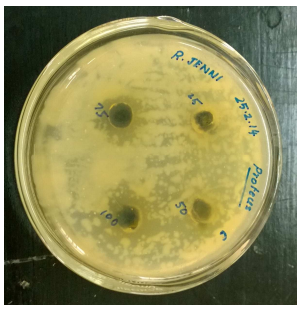

Figure 7d: *Proteus* sp

Figure 7: Antimicrobial Activity of Chloroform Extract against Different Organisms

Table 5: Antimicrobial Activity of the Chloroform Extract against Different Organisms

Organism	25µl	50µl	75µl	100µl
E. coli	-	0.2mm	0.3mm	0.6mm
Klebsiella species	0.1mm	0.3mm	0.4mm	0.8mm
Salmonella sp	0.4mm	-	-	-
Proteus sp	-	-	1.8mm	1mm

CONCLUSIONS

Moringa is the perfect example of the third world producing what it does not consume and increasingly consuming what it does not produce. Moringa is a gift of the nature, a pure magic natural agro-biodiversity able to save billion of thirsty people in the world with a better health and sanitation. This study exposed that traditional medicines are still used by tribal peoples and it is established the value of a great number of plants used in tribal medicine especially for wound healing. Thus, this tree offers very interesting opportunities as food supplement, nutrition, vegetable, oil, water treatment, green manure, foliar spray, natural fertilizer, livestock feed, fodder, medicine, cosmetic and care products.

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