Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2130

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **3 (5):** 95-100 (2015)

International Journal of Pure & Applied Bioscience

Research Article

OPEN ACCESS

Pharmacognostical Evaluation and Antimicrobial activity of *Moringa oleifera* Lamk. Leaf

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ABSTRACT

Moringa oleifera Lamk. (Moringaceae) is a very useful tree in tropical countries. In folklore and ayurvedic all parts of the tree used in different healing procedures for different diseases. The plant leaves are very good nutrient supplement for malnutrition and also used as an antimicrobial agent. The crude aqueous and alcoholic leaf extract of Moringa oleifera was subjected to phyto-chemical analysis to determine the constituents of the leaf extracts. Results of phyto-chemical analysis showed that the crude extracts contained Carbohydrates, Proteins, Resins, Tannin and Alkaloids. The chemical compounds found in these extracts of Moringa oleifera leaf have good pharmacological properties. The Solubility of Moringa leaf with nine solvents, results highest in water (32%) and lowest in Hexane (5.0 %) solvent. The antimicrobial activity of the Moringa leaf was assayed against one Gram- positive strains (Staphylococcus aureus), two Gram-negative strains (Escherichia coli, Pseudomonas aeruginosa), and five fungal strains of agro-food interest (Penicillium aurantiogriseum, Penicillium expansum, Penicillium citrinum, Penicillium digitatum, and Aspergillus niger spp.). Staphylococcus aureus, E.coli as well as the fungal strains were sensitive to the Moringa leaf extract . The traditional application of the leaf of Moringa oleifera as poultice and decoction may have a scientific or pharmacological basis. These studies provide an evidence to support traditional medicinal uses of the plant.

Key words: *Phyto-chemical, Moringa oleifera, Gram- positive and Gram-negative bacteria, Pharmacognostical.*

INTRODUCTION

The history of plants being used for medicinal purpose is probably as old as the history of mankind. Herbal medicine is an achievement of popular therapeutic diversity since they may possess hundreds of medicinal materials and produce their curative effects. Awareness of the herbal plant's chemical constituent is helpful in the discovery of effective therapeutic agents. Extraction and characterization of several active phytocompounds from the medicinal plants is the foundation for the formation of some high activity profile drugs[1].

Moringa, native to Asia and spread in most parts of Africa, is the sole genus in the flowering plant family *Moringaceae*. This genus is made of 12 species[2] .

Cite this article: Shukla, P. and Tripathi, M., Pharmacognostical Evaluation and Antimicrobial activity of *Moringa oleifera* Lamk. Leaf , *Int. J. Pure App. Biosci.* **3 (5):** 95-100 (2015). http://dx.doi.org/10.18782/2320-7051.2130

Moringa oleifera Lam. is one of the most economically important species indigenous to dry tropical areas in the Northwestern India, at the Southwestern foot of the Himalayas[3]. Moreover, it is widely cultivated in different countries[4]. An extensive variety of nutritional and medicinal uses have been attributed to its roots, bark, leaves, flowers, fruits and seeds[5]. Almost all parts of this plant have been used for various diseases in the folk medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, haematological and hepatic and kidney disorders[6]. Leaves of *Moringa oleifera* are traditionally used as purgatives and in the treatment of headaches, haemorrhoids, fevers, inflammation of noise and throat, bronchitis, eye and ear infections, and to combat vitamin C deficiency. The leaf juice is believed to control glycaemia and is applied for swollen glands[7]. Leaves of *Moringa. oleifera* are cooked and eaten like spinach or used to prepare soups and salads. Fresh leaves have been reported to contain vitamin C and vitamin A, more than those reported in carrots and oranges[8]. Leaves are also used to contrast hypertension and cholesterol, indeed, anticancer, antitumor, anti-inflammatory, diuretic properties as well as antihepatotoxic, antifertility, antiurolithiatic and analgesic[9]. *Moringa oleifera* is also known for its antioxidant activity, essentially due to the presence of high amounts of polyphenols[10]. The work was undertaken in the trust as a part of a program of testing and validation of traditional practices of using the Ayurvedic medicine. The current work deals with detailed standardization guidelines involving Good Manufacturing (GMP) prescribed for preparation of Ayurvedic medicine. Pharmacognostical evaluation guidelines to be followed[11] for herbals products provided by World Health Organization (WHO), and Ayurvedic pharmacopoeia of India have been considered.

MATERIALS AND METHODS

Collection of plant materials

Leaf samples of *Moringa. oleifera* was collected at Sirsavan , Chitrakoot, district Satna, (M.P.) on month of the march. The leaves sample was collected from *Moringa* tree about 15 years old. After removing the petioles, leaf samples were dried at 35^0C for 48 hours and grounded by mixer grinder and powdered to obtained fine powder. The sample were labeled and kept in air-tight plastic container until analysis[12].

Macroscopy , microscopy & powder microscopy

Macroscopic characters like appearance, taste, colour, and odour were evaluated. Leaf sections were cut by free hand sectioning and numerous sections were examined microscopically. For powder microscopic analysis about 2 gm of powder in a small beaker and wash thoroughly with water, pour out the water without loss of material, mount a small portion in glycerine; warm a few mg with chloral hydrate solution, wash and mount in glycerine; treat a few mg with iodine in potassium iodide solution and mount in glycerine. Observed the characteristics in the various mounts[13-14].

Physico-chemical tests

Physico-chemical tests were carried out such as loss on drying at 105^0C, extractive value **(**distilled water soluble, Ethyl alcohol, Toluene, Ethyl acetate, Chloroform, Acetone, Diethyl ether, Methanol, and Hexane soluble extractive values)[15].

Fluorescence analysis

The powdered sample was treated with different chemical reagents to observe various colour reactions which may help to confirm the purity of the Drug[16].

Quantitative Biochemical analysis

The amount of Biochemical constituents like Carbohydrate, Protein, Crude fibres, Ascorbic acid present in powdered drug are analyze by specified methods[17].

Determination of antimicrobial activity (Preparation of Methanolic Extract)

The Methanolic extract of the drug by soaking 75 g of drug powder in 150 ml of 95% methanol. The mixture is allowed to stay for 72 hours in dark away from direct sunlight . it was stirred at 12 hours. The resulting solution was filtered using Whatman Filter paper 1. Than the filtrate was evaporated in a shallow dish to dryness. The dried powdered of extract was scratched off the dish and dissolved in small amount of methanol. This solution was used as antimicrobial agent in the test .

Preparation of Hot Water Extract

Hot water extract of the drug sample was prepared by dissolving 75 g of powdered drug in 200 ml of distilled water for 4 hours. It was then further extracted using the Soxhlet apparatus for further 2 hours. The resulting infusion was filtered using Whatman Filter paper 1. The filtrate was than subjected to evaporation till dryness. The dried powdered of extract was scratched off the dish and dissolved in small amount of distill water. This solution was used as antimicrobial agent in the test .

Procedure of Antimicrobial Activity

The antimicrobial activity of the leaf extracts was determined using agar well diffusion method by following the known procedure. Nutrient agar was inoculated with the given microorganisms by spreading the bacterial and fungal 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 of bacterial microorganisms were incubated at 37°C for 24 hours and Fungal plates keep 25^0C for 72 hours in room temperature. The antimicrobial activity was assessed by measuring the diameter of the zone of inhibition. The antimicrobial potential of the different extracts was evaluated by comparing their zones of inhibition[18].

RESULT AND DISCUSSION

Macroscopy - Fresh leaves compound, alternate, 30 to 45 cm long, 5 to 10 mm in width, pale green, dried leaves are shrivelled, broken, never attached with main rachis. Odour characteristics, taste bitter.

Microscopy - TS passing through the midrib of a leaflet is broadly convex on the lower side and almost straight or slightly depressed on the upper side, shows a layer of upper and lower epidermii traversed with stomata, they being very few on upper side, covered with cuticle and bear few thick-walled simple trichomes of various sizes, some being very short , straight or bent, embedded with brownish yellow pigment, some very long, thick-walled and warty ; unlike the upper epidermis the cells of lower one are highly papillose ; 2 to 3 layers of palisade cells run underneath the upper epidermis, they are bit smaller in height or occasionally obscure when extended to a small distance in the midrib region, the remaining tissue of the mesophyll being occupied by 5 to 6 rows of spongy parenchyma traversed with obliquely cut vascular strands, an arc of well developed meristele lies in centre of midrib encircled by collenchymatous ground tissue, reaching up to the epidermis and embedded with rosette crystals of calcium oxalate Figure-A-B.

Powder microscopy - Shows fragments of upper and lower epidermii in surface view embedded with anomocytic stomata, rosette crystals and simple starch grains scattered as such or embedded in parenchymatous cells of the ground tissue of the midrib and rachis, thick walled, warty, short and long trichomes or their broken fragments scattered as such, or attached with the cells of epidermis, longitudinally cut fragments of annular and spiral vessels, tears of gums, pigment and mucilage cells scattered as such from the rachis Figure-C,D, E, G,H & I .

Physico - chemical analysis

The LOD shows moisture content in powdered drug. Extractive value with different solvents are primarily useful for the determination of exhausted or adulterated drug. The results of Physico-chemical analysis of the leaf of *Moringa oleifera* is given in table- 1.

Fluorescence analysis

Powdered drug was treated with different reagents and was examined under UV light (254 & 366 nm) the results are given in table -2.

Quantitative Biochemical analysis

Leaves of *the Moringa oleifera Lamk.* tree can be an extremely valuable source of nutrition for people of all ages. Higher amount of carbohydrates, protein, fibre and ascorbic acid obtain after analysis. Results are given in Table -3.

Determination of Antimicrobial Activity

The antimicrobial activity of the *Moringa* leaf was assayed against one Gram- positive strains (*Staphylococcus aureus*), two Gram-negative strains (*Escherichia coli, Pseudomonas aeruginosa*), and five fungal strains of agro-food interest (*Penicillium aurantiogriseum, Penicillium expansum, Penicillium citrinum, Penicillium digitatum, and Aspergillus niger* spp.).

Staphylococcus aureus, E.coli as well as the fungal strains were sensitive to the *Moringa* leaf extract. The methanolic and hot water extract of leaf because of its strong microbicidal property and superiority over commercial microbicides, may prove to be an effective herbal protectant against a wide spectrum of pathogenic bacteria and fungi, since herbal microbicides are non-toxic and ecofriendly. Results are given in Table- 4.

Table- 1: Physico-chemical tests of *Moringa oleifera* leaf

Parameter	Percentage (%)
Loss on Drying (LOD)	7.42%
Water soluble extractive	32.18%
Alcohol soluble extractive	14.45%
Toluene soluble extractive	7.71%
Ethyl acetate soluble extractive	6.2%
Acetone soluble extractive	7.7%
Chloroform soluble extractive	7.86%
Diethyl ether soluble extractive	10.03%
Methanol soluble extractive	31.08%
Hexane soluble extractive	5.06%

Table- 2: Fluorescence analysis of powdered leaf of *Moringa oleifera*

Name of the Reagents	At 254 nm	At 366 nm
Powder as such	Dark green	Green
Powder + 1N HCL	Brown	Black
Powder + 1N NaOH in Water	Dark brown	Light yellow
Powder + 1N NaOH in Methanol	Light yellow	Reddish pink
Powder + 50% KOH	Light yellow	Light pink
Powder + 50% H_2SO_4	Light yellow	Light pink
Powder + Conc. H_2SO_4	Dark brown	Light green
Powder + 50% HNO_3	Light yellow	Dark blue
Powder + Acetic acid	Brownish	Pink
Powder + Iodine water	Light yellow	Blue

Table- 3 : Quantitative biochemical screening of *M. oleifera* Lamk. leaf extract

Name of Biochemicals	Quantity of Biochemicals
Crude fibre	153.1455 %
Protein	0.572 mg/l
Carbohydrate	0.931 mg/l
Ascorbic acid (Vitamin C)	888.2364 mg/100g

Table- 4 : Screening of Antimicrobial activity of *Moringa oleifera* leaf extract

Name of the pathogen	Name of antibiotics	Diameter of Zone of inhibition in mm		
		Antibiotics	Sample extract	
			Methanolic	Aqueous
Staphylococcus aureus	Ofloxacin	25	15	12
Escherichia coli	Streptomycin	16	8	12
Pseudomonas aeruginosa	Gentamicin	30	10	10
Penicillium aurantiogriseum	Clotrimazole	11	11	10
Penicillium expansum	Clotrimazole	15	10	10
Penicillium citrinum	Clotrimazole	11	12	13
Penicillium digitatum	Clotrimazole	10	10	12
Aspergillus niger spp.	Clotrimazole	11	21	14

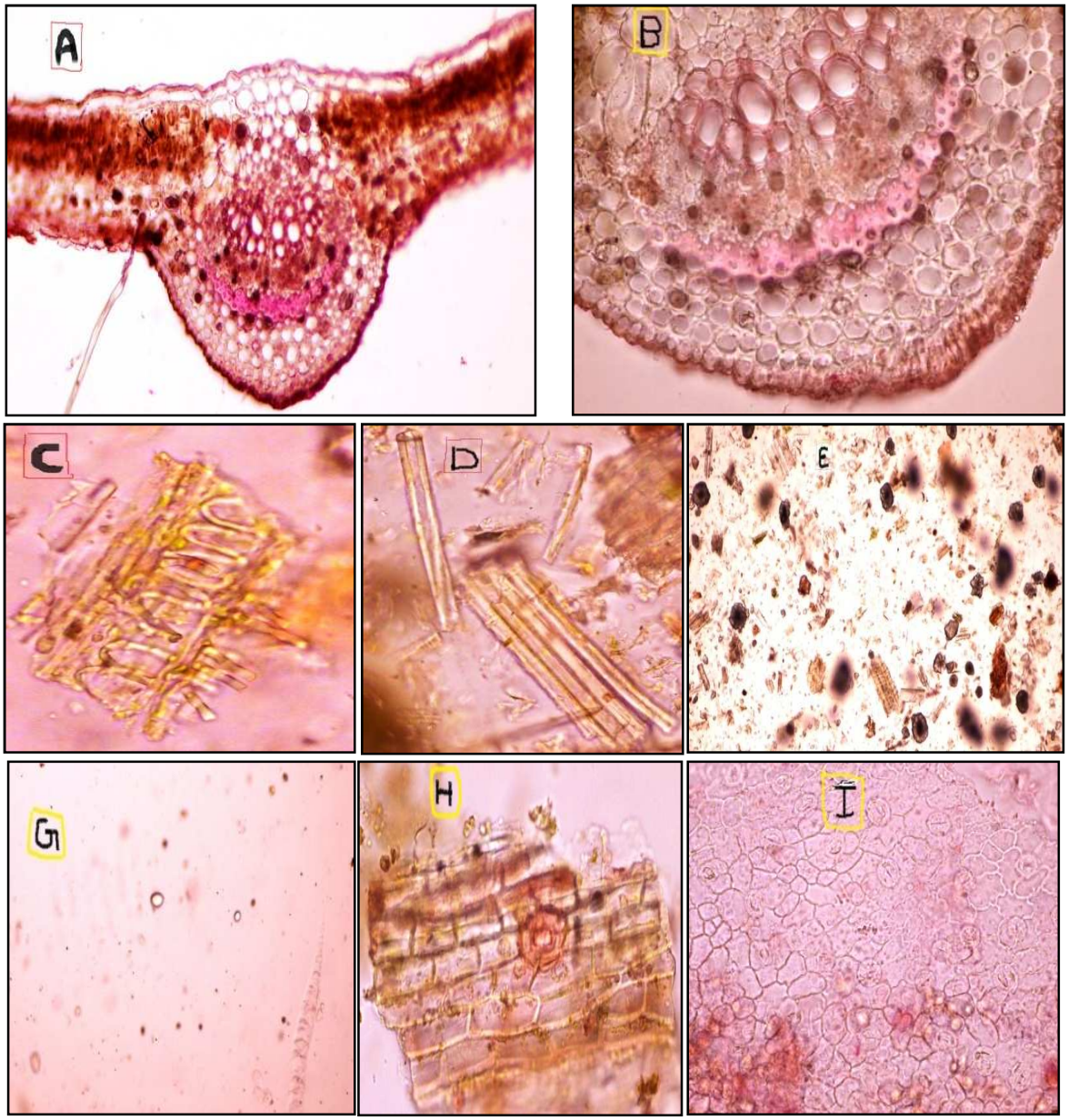

Fig. Anatomical approach of *M. oleifera*. Lamk. leaf (A) Transverse section of a leaflet; (B) Visualization at central vein of a leaflet; (C) Spiral and annular vessels ; (D) Fibres ; (E) Rosette crystals of calcium oxalate ; (G) simple starch grains ; (H, I) cells of lower epidermis showing anomocytic stomata.

CONCLUSION

Morinna oleifera is indeed a very useful breakthrough in the demand of alternative natural medicine for the treatment of various disease activities by pathogenic organisms. This is proved by the good antimicrobial activity and the presence of secondary metabolities showed by the leaf extract. Therefore the plant could be used in the treatment of typhoid fever, diarrohea, stomach ulcer, tumors, post menopausal syndrome, arteriosclerosis, control of blood sugar level, as anti inflammatory drugs, gastrointestinal disorder, anti oxidant, cancer, diabetes etc. These findings support the fact that *moringa oleifera* appears in the hierarchy of the medicinal plants used for the treatment of microbial infections. Because of the antimicrobial activity and the phytochemical content of *moringa oleifera*, it is recommended that the plant should be used in the manufacture of antimicrobial drugs. *Moringa oleifera* is ranked among many tribes in India and the world at large for the treatment of infections caused by micro organisms. The Pharmacognostic and Physico-chemical parameters can be used for judging the adulteration and purity of this drug. These findings could be helpful in identification and authentication of herbal drug.

Acknowledgment

The Authors are thankful to Shri Abhay Mahajan, Organizing Secretary, Arogyadham, Deendayal Research Institute, Chitrakoot, Satna (MP) and Vice Chancellor, APS university, Rewa for providing the facilities to Carry out this work.

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