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Phytochemical screening and antimicrobial activity of Moringa *oleifera* and Mass spectra

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ABSTRACT

The present study deals with phytochemical screening of various solvent of Moringa oleifera and antimicrobial activity of methanolic extract of Moringa *oleifera*. The second part of study deals with Mass spectra of Moringa plant.The Moringa Plant is known as Miracle Plant in Plant Industry. The widespread pan-tropic Moringaceae family comprises of about 13 Species. Moringa *oleifera* the most known genera of medicinal plants, used for traditional medicinal purposes**.**

Key Words: Moringa *oleifera*, phytochemical, antimicrobial, mass spectra methanolic.

INTRODUCTION

Medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care (Farnsworth et al. 1985; Akinyemi et al. 2005). Over the years, the World Health Organization advocated that countries should encourage traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins (WHO 1978). In recent years, Pharmaceutical companies have spent a lot of time and money in developing Natural products extracted from plants, to produce more cost effective remedies that are affordable to the population (Doughari 2006). Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century (Zaika 1975). It is estimated that today, plant materials are present in, or have provided the models for 50% Western drugs (Robbers et al. 1996).

Moringa *oleifera* is the most widely cultivated species of a monogeneric family, the Moringaceae; it is a fast-growing, drought-resistant tree that is native to the sub-Himalayan tracts of India, Pakistan, Bangaladesh and Afghanistan (R.K. Gupta, 2010).The “Moringa” tree is grown mainly in semi- arid, tropical and sub- tropical areas. While it grows best in dry sandy soil, it tolerates poor soil, including coastal areas.

In aurveda the medicinal properties of Moringa *oleifera* are very well explained, this plant is light to digest and cause dryness of tissue. Due to these properties the usage of plant vitiates pitta and alleviates vata and kapha. It is also used to treat headache, hiccough, paralysis, appetite, reduce stomach cramps, intestinal worms, menstrual cramps, ulcer, mental illness, tumors , infections and its reduce blood cholesterol, piles (K.M. Nadkarni 2009 and C.P. Khare, 2007).

The main consitutents of Moringa plant are: deic, palmtic and stearic acid, saponins, glycosides, gum, protein vitamins: A (8855 IU per 100g), B1, B2, B3, C Minerals: calcium, iron, phosphorus, magnesium. The leaves, flowers and pods are used as significant sources of vitamins A, B and C, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta-carotene, calcium, iron, and alpha-tocopherol (Dahot, 1988). The root bark contains two alkaloids: moringine and moringinine. Other isolateted compounds from plants are 4-(4'-O-acetyl-α-L-rhamnopyranosyloxy)benzyl,4-(-Lrhamnopyranosyloxy)benzylisothiocyanate , niazimicin ,

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pterygospermin, benzyl isothiocyanate, and 4-(α-L-rhamnopyranosyloxy)benzylglucosinolate4. (Garima mishra et,al 2011).

Based on the previous reports, the study was designed to evaluate the phytochemicals present in the various solvent extract of Moringa *oleifera* plant and subsequently, identified a variety of phytocomponents in the methanolic extract of Moringa *oleifera* by mass spectrometry. And antimicrobial activity tested by disc diffusion method.

MATERIAL AND METHODS:

Chemicals and regents

Methanol, Ethnol, Chloroform, DMSO, Sulphuric Acid, Diluted ammonia, Ferric chloride, hydrogen chloride (HCl), Distilled water, Folin-Ciocalteau reagent, Draggendroff's reagent, sodium carbonate and gallic acid were used for phytochemicals screening, Nutrient agar Media, Potato dextrose agar Media were used for the antibacterial assay. The entire reagents used were of merck and Himedia, India.

Instruments

Grinder, water bath, glassware(conical flask, beakers, test tubes, petri plates), micropipettes, gel puncher, digital balance, Rotary vacuum evaporator, Autoclave, Electronic, Vortex shaker, Refrigerator 4^0C, -20°C fridge, UV visible spectrophotometer, Mixer grinder, Heating mental, Rectangular water bath, Bacteriological Incubater 37^0C, Hot air oven 70^0C, Ultra-centrifuge , Silica gel sheets (silufol 60 F254, aluminum support; Merck). IR, NMR and Mass were performed using sophisticated instrument facilities of SIRT Bhopal, and IIT Indore.

Microogranism

Bacterial Strain – E. Coli (MTCC No. 739), S. aureus (MTCC No. 87), B. Subtilis (MTCC No. 441) and P. aeruginosa. **Fungal Strain –** C. albicans (MTCC No. 183), A. niger (MTCC No. 872)

Plant Material

The whole plants of Moringa *oleifera* were collected from the local surroundings at Bhopal city of M.P, during the month of November to December. The plant was acknowledged by a senior Botanist Head of the Department of Botany M.L.B. Autonomous College Bhopal. The whole plant washed thoroughly with sterilize distill water in order to remove dirt and dust present on the surface. The plan dried in shed for 15 days. The branch of plants separately made into a fine powder in the grinder, which pw12ass through 4C micron mesh. The powder was kept in air tight bottles till used.

Preparation of Plant Extract: The plant extract were prepared with different solvent like ethanol, petether , hydroalcoholic by Soxhlet assembly.

Phytochemical screening Qualitative analysis:

The Premilanry phytochemicals (Carbohydrates, fatty acids, proteins, saponins, tannins, flavonids, alkalodis, terpenoids, glycosides and aminoacids). Screenings of various solvent extract were carried using the standard procedure to identify the various photo constituents.

Quantitative estimation of Tannins: The quantitative determination of Tannins in moringa oleifera plant sample was performed as per protocol. Briefly, 500 mg of dried extract was dissolved in 50 ml of distill water in a screw capped conical flask and allowed to shake for 1-hr. Approximately 5ml of filtrated was mixed with 2 ml of 0.1 MFecl3 in 0.1 N0Hcl and 0.008 M Pottasium ferrocynide. The absorbance was measured at 12NM with in 10 min by UV-Visible spectrophotometer. All the experiment was carried out in triplicate.

Quantitative determination of total phenolic content: The total phenol content in Moringa *oleifera* determined by the spectrophotometric method as per protocol. The extract was boiled with 50 ml of ether for the extraction of phenolic component for 15 min. 5 ml of extract was mixed with 10 ml of distilled water, 2ml

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of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol into a 50 ml flask and incubated at room temp for 30 min for color development. Absorbance was measured at 505 nm by spectrophotometer. All the experiments were performed in triplicate and assay of the total phenolic content was expressed in mg gallic acid equivalent per g of the extract by comparing with gallic acid as standard.

Antimicrobial susceptibility test (Parija, 2007)

Screening for antimicrobial activity of methnolic extract of moringa *oleifera* was carried out by commonly known as disc diffusion method. Approximately 4 to 5 well isolated colonies of the bacterial strain are inoculated into 5 ml of nutrient broth and incubated at 37°C. Standard Drug Used Ofloxacin (100µg/ml). After standardization of bacterial suspension, sterile cotton swap was immerged in it and the swap was rotated several times, with firm pressure on the inside wall of the tube to remove excess fluid. Nutrient agar media plate was prepared with a depth of 4mm. Dried surface of nutrient agar plate was inoculated the by streaking the swab 3 times over the entire agar surface. A sterile 5mm cork borer was used to punch holes after solidification of media. The wells formed were filled with different concentrations of the sample which were labeled accordingly. The plates were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37°C for 48hours in upright condition. After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimeters along two axis i.e. 90° to each other and the mean of the four reading were then calculated included 5mm well.

Antifungal Activity : Standard Drug Used Amphoterecin-B (100µg/ml)

Petri plates containing 20ml PDA medium were seeded with 24hr culture of fungal strains. Wells were cut and 20µl of the given sample (of different concentrations) were added. The plates were then incubated at 37°C for 24 hours. The antifungal activity was assayed by measuring the diameter of the inhibition zone formed around the well. Amphoterecin-B was used as a positive control. After 16 to 18 hours of incubation, each plate was examined.

Biostatical Interpretation

All data are presented in mean ± SD. Data were analyzed by One Way ANNOVA followed by Benferronni's test. $P<0.05$ was considered as level of significance.

Result & Discussion: Phytochemicals of Moringa *oleifera* in Table 1 represents the qualitative analysis of phytochemicals present in various solvent extract of Moringa *oleifera*. Total phenolic content and total flavonoid content was estimated using standard Gallic acid and Rutin. This showed that extracts were having significant amount of phenolic and flavonoid content. The qualitative analysis of methanolic extract is showed the table 2 and table 3. Mass spectrometry is an analytical technique that measures the mass-to-charge ratio of charged particle. It is used for determining masses of particles, for determining the elemental composition of a sample or molecule, and for elucidating the chemical structures of molecules. **(Sparkman *et. al*, 2000).** Fig.1 showed various peaks, in mass spectra of Moringa *oleifera*, these are showed many bioactive components are present in methanolic extract of Moringa *oleifera* the components details are showed in table 4. the antimicrobial activity of the extract of Moringa *oleifera* were studied in different concentrations (100,200,300 and 400mg/ml) against four pathogenic bacterial strains, two Gram-positive and two Gram - negative E. Coli (MTCC No. 739) S. aureus (MTCC No. 87), B. Subtilis (MTCC No. 441) and P. aeruginosa ant two fungal strain C. albicans (MTCC No. 183) A. niger (MTCC No. 872). Zone of inhibition of extracts were compared that standard drugs. The result showed that the remarkable inhibition of the bacterial growth was shown of against the tested organism.

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Table 1: Qualitative analysis of phytochemicals present in moringa oleifera with various solvents extract

S.No.	Name of Phytochemical	Pet ether Extract	Hydroalcoholic Extract	Methanol Extract
1	Carbohydrate	-	+	+
2	Alkaloids	+	+	+
3	Terpenoids	-	+	+
4	Flavonoids	+	+	+
5	Tannins & Phenolic Compounds	-	+	+
6	Saponins	-	+	-
7	Protein and amino acid	-	+	+
8	Glycosides	-	+	+
9	Fats & Oils	-	+	+

Table 2: Total Flavonoid content in Extract of Moringa oleifera

S. No.	Absorbance	Concentration	TFC content in mg/g equivalent of Rutin
1	0.236	1mg/ml	118
2	0.234	1mg/ml	116
3	0.236	1mg/ml	118
MEAN±SD			**117.0±1.154**

Table 3: Total Phenolic content in Extract of *Moringa olifera*

S.No.	Absorbance	Concentration	TPC content in mg/g Equvilent of Gallic acid
1	**1.011**	**1mg/ml**	**189.2**
2	1.003	1mg/ml	187.6
3	0.997	1mg/ml	186.4
MEAN±SD			**187.7±1.404**

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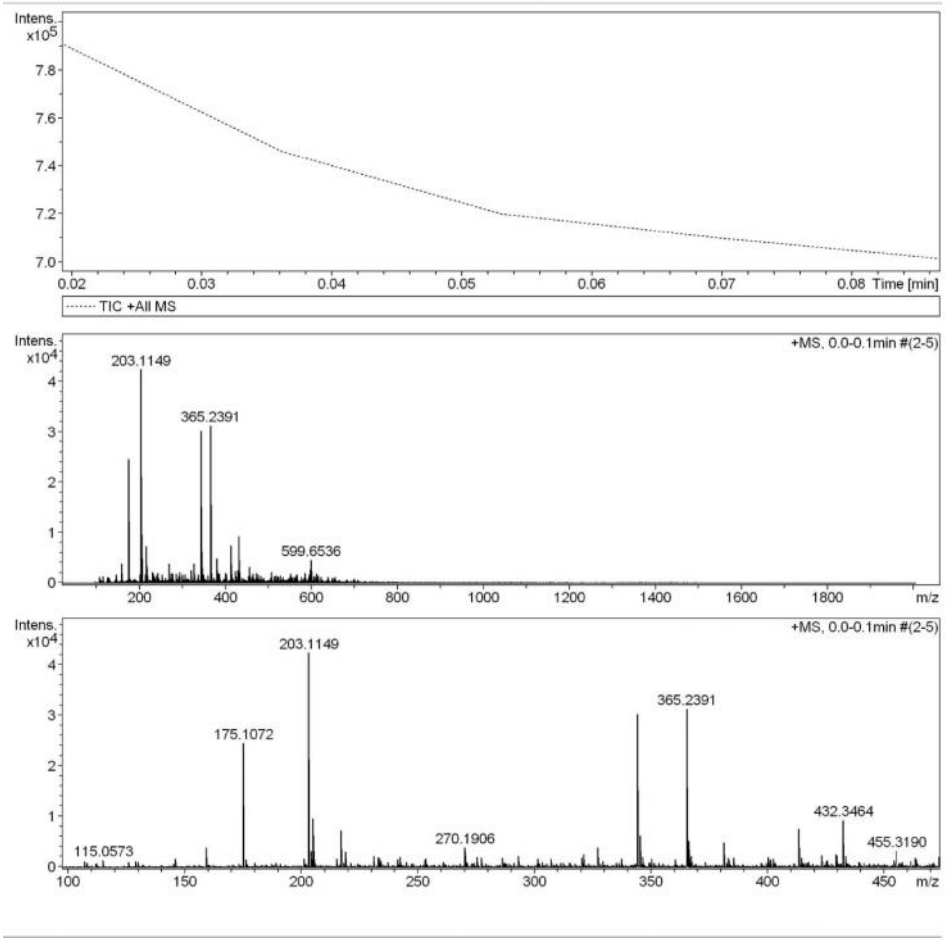

Fig. 1: Mass Spectra of Moringa *oleifera* .

Phytocomponents identified in the methanolic extract of Moringa *oleifera*

203	Prove presence of sugar in the compound
566	Presence sugar with aromatic ring
434,364, and 328	Indicate the polyphenol compound

Table 5: Antibacterial Activity methanolic extract of *Moringa oleifera*:

S. No.	Organism	Zone of Inhibition				Standard (Ofloxacin) **100 µg/ml**
		100 mg/ml	**200 mg/ml**	**300 mg/ml**	**400 mg/ml**	
1	*Staphylococcus Aureous*	21.25±0.957	23.50±0.577	26.00±0.816	30.75±0.957	39.75±0.645
2	*Bacillus Subtilis*	21.75±0.957	25.50±1.291	28.50±1.291	32.25±0.957	38.75±0.645
3	*Escherichia Coli*	20.00±0.816	22.75±0.500	26.75±1.258	31.50±1.291	36.88±0.854
4	*Pseudomonas Aeruginosa*	18.00±0.816	22.50±1.291	26.75±0.957	31.25±0.957	37.63±0.750

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Table 6: Antifungal Activity methanolic extract of *Moringa oleifera*

S. No.	Organism	Zone of Inhibition				Standard (Amphotericin B)**100 µg/ml**
		100 mg/ml	**200 mg/ml**	**300 mg/ml**	**400 mg/ml**	
1	*Aspergillus Niger*	20.25±0.957	22.75±0.500	25.00±0.816	29.75±0.957	37.75±0.645
2	*Candida lbicans*	20.25±1.258	24.75±0.957	28.75±1.708	34.75±1.500	36.25±0.866

Conclusion: The Phytochemical analysis revealed the present of various secondary metabolites. . Antimicrobial potential of extract was also investigated against *StaphylococcusAureous, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger and Candida albicans.* Extract exhibited good antimicrobial effect against both gram negative and gram positive bacteria, and also against fungus. Mass spectra of moringa oleifera shows sugar, polyphenols , aeromatic rings of carbohydrate are present in extract.

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