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Phytochemical analysis and characterization of *Moringa oleifera* for its antimicrobial and antioxidantactivity

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Abstract
Ayurveda is one of the traditional medicinal systems of Indian culture. The philosophy behind Ayurveda is preventing unnecessary suffering and living a long healthy life. Ayurveda involves the use of natural elements to eliminate the root cause of a disease by restoring balance and at the same time creating a healthy life-style to prevent the recurrence of imbalance. Herbal medicines have existed world-wide with long recorded history. World Health Organization (WHO) have estimated that 80% of the world's inhabitants still rely on traditional medicines for their health care. India is well-known to be one of the major biodiversity centre with about 45,000 plant species, including 15,000 medicinal plants. The concept of polyherbalism is to achieve greater therapeutic efficacy. The active phytochemical constituents of individual plants are insufficient to achieve the desirable therapeutic effects.
When combining this research mainly focuses on the importance of polyherbalism and its clinical significance. For this study medicinal plant *Moringa Oleifera* have been taken and extracted for their study of anti-bacterial and anti- oxidant activity. The phytochemical compounds were screened by qualitative analysis method and the detected phytochemicals are tannins, saponins, alkaloids, phenols, terpenoids, flavonoids. The different solvents such as methanol, petroleumether, chloro formandaqueouswereusedto extract the bioactive compounds from various parts of the selected medicinal plant. The anti-bacterial activity were demonstrated against the bacterial strains like *Staphylococcus aureus* and *Escherichia coli* by disc-diffusion method. The anti-oxidant activity was evaluated by DPPH radical scavenging method.

Keywords: phytochemical screening, anti-microbial activity, anti-oxidant activity, DPPH method, phytotherapy, traditional medicine, polyherbal formulation

Introduction

Moringa oleifera an aboriginal of Indian subcontinent, is a member of the Moringaceae family of perennial angiosperm plants, which includes 13 other species (Nadkarni, 1976 and Farooq *et al*., 2012). The plant is grown best in dry sandy or loamy soil that is slightly alkaline (Abdul, 2007 and Asante *et al*, 2014). Although it is adaptable to various soil conditions from varying pH of 4.5 to 8.0, it is not able to tolerate water logging, freezing or frosts conditions (Asante *et al*, 2014 and Radovich, 2011). Moringa is a fast growing tree with about 10m in height and a diameter of 2.04m at chest heightIt has a soft trunk, white corky; and a gummy bark bearing branches. Each twice or thrice pinnate compound leaf bears small leaf leg. The flowers are pleasantly fragrant,white in colour; and the three wings seeds are scattered by the winds. MO flowers, tenders leaves and pods are eaten as vegetables. India being the largest producer of Moringa, has an annual production of between 1.1 to 1.3 million tonnes of tender fruits from an area of 380 km² (Rajangam *et al*., 2001). leaf leg. The flowers are pleasantly fragrant, white in colour; and the three wings seeds are scattered by the winds. MO flowers, tenders leaves and pods are eaten as vegetables. India being the largest producer of Moringa, has an annual production of between 1.1 to 1.3 million tonnes of tender fruits from an area of 380 km² (Rajangam *et al*., 2001)

Fig 1: *Moringa oleifera*

Characteristics

Moring aoleifera is a small and fast growing evergreen tree. The bark is thick, soft, corky and deeply fissured, the leaves are usually tripinnate, the leaflets are elliptic, the flowers are generally white and fragrant in large panicles, the pods are pendulous green in colour triangular and ribbed within trigonous winged seeds. 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, vescicant and abortifacient. The flowers and roots contain an antibiotic that is highly effective in the treatment of cholera. The leaves, rich in vitamin A and C, are considered useful in respiratory ailments. The juice extracted from the leaves has strong antibacterial and anti-malarial properties.

Taxonomical Classification of *Moringa oleifera*
Kingdom: Plantae (Plants)
Subkingdom: Tracheaobionta (Vascular Plant)
Superdivision: Spermatophyta (Seed Plants)
Division : Magnoliophyta (Flowering Plants)
Class: Magnoliopsida (Dicotyledone)
Subclass : Dilleniidae
Order: Capparales
Family: Moringaceae
Genus: Moringa
Species: oleifera

Materials and methods
Microorganisms: *Staphylococcus aureus and Escherichia coli* MTCC (Microbial type culture collection)

Glasswares: Petri plates, Pipettes (1ml & 2ml), Measuring cylinder, Flask, Beaker, Jam bottles, Glass rod, Volumetric Flask, Test tubes, Conical Flask,Funnel.

Miscellaneous: Cotton, Inoculation loop, Whattmann filter paper, Centrifuge tubes, Micropipettes, Disk, Tips, Forceps, Hi Media antibiotic Zone Scale (for Zone measurement), Dropper, Aluminum foil, Rubber band, Glossy papers, Pipette bulbs, test tube stand, Wash Water, Glass slide, Icepack.

Chemicals Required: 95% ethanol, Distilled water, Nutrient Broth, Agar, Nutrient Agar Media, Culture, Herbal Drug powder (Moringa oleifera), Chloroform, Methanol, Petroleum ether, Fehling solution A & B, Ferric chloride, Mayer's reagent (Mercuric Chloride, Potassium Iodide), Ninhydrin solution, DPPH **(**Diphenylpicryl Hydrazine), Sodium Hydroxide, Biuret Reagent, Conc. Sulphuric Acid, Acetic Acid, Dilute Hydrochloric Acid, Diclofenac Sodium.

Instruments
- Soxhlet Assembly (J-Sil, 50/42, Borosil glass) - For extracting the phytochemicals of powdered drug with the help ofsolvents.
- Vacuum Rotary Evaporator (Scientech) - For evaporating the phytochemicals present in theextraction.
- Digital Balance (Denver, Germany) - For weighing chemicals in microquantities.
- Hot Air Oven (Scientech, 325 L) - For sterilizing the glass wares afterwashing.
- Laminar Air Flow Chamber Horizontal - For maintenance of asepticcondition.
- Incubator (Scientech) - For the growth of themicroorganism.
- Cyclo Mixer (REMI) - For mixing thesuspensions.
- Antibiotic Zone Scale Laboratories Ltd - For the measurement of zone ofinhibition

Sample Collection
The plant was collected from Govt. Nursery of Ujjain, M.P. India.

Fig 2

Preparation of Plant Extracts
200 ml of solvent (Chloroform, Methanol, Petroleum ether, aqueous) was taken in a round bottom flask. Then 20 gm of drug powder was weighed in a digital weighing machine was wrapped in a filter paper to make a thimble. It was then placed in the central compartment & it was heate data temperature range between 50^0C-60^0 Cinaheatingmantle. After heating the vapour passes through the side arm up into the reflux condenser. Here the vapour condenses, liquefies & drips into the thimble containing the material to be extracted. The warm solvent percolates through the material & the wall of the thimble & the extract gradually collects in the central compartment. Once the height of the extract reaches the top of the siphon, the entire liquid in the central compartment flows through this & back into the lower round bottomed flask. Then the process is further repeated as required. In this method the extract gets collected in the lower vessel and gradually becomes more & more concentrated. When the drug powder was completely extracted, the solvent collected in the middle compartment displayed transparent colour. Assuming that there are novolatile substances present, the vapourisation from the heated extract is pure solvent in the vapour form & so the liquid dripped into the material from the condenser is essentially pure solvent, though derived from the extract, thus although a relatively small volume of solvent is needed. The effective volume of solvent used for the extraction is proportional to the time for which the process is allowed to continue. The extraction process was repeated for Chloroform, Methanol and Petroleumether.

Table 1: Phytochemical Analysis Test Chart of *Moringa oleifera*

Phytochemical Test	*Moringa oleifera*			
	Chloroform	**Methanol**	**Petroleum Ether**	**Aqueous**
Alkaliods	+	-	-	-
Flavonoids	+	+	-	+
Tannins	+	+	-	+
Phenols	-	-	-	+
Terpenoids	+	+	-	+
Saponins	-	+	+	+

(+) --- Positive; (-) --- Negative

Anti-Bacterial Activity by Disc Diffusion Method

Preparation of Inoculum

Staphylococcus aureus and *E. coli* strains were used. 60 ml of Nutrient broth was prepared in 100 ml conical flask. It was sterilized & then inoculated with inoculum with the help of sterile loop in laminar air flow from preserved slants. They were then kept in incubator at 37^0C for sufficient period of time for organism to grow.

Disc Diffusion Method

After solidification the disc of whatmann filter paper imbibed with 20 µl plant extracts were carefully placed with the help of forceps at the centre of the petri dish and then kept in incubator for 24hrs.

Measurement of Zones

With the help of antibiotic zone scale the zone of inhibition (ZOI) were measured.

Antioxidant activity

Preparation of Reagent

DPPH Reagent 2 mg of DPPH was taken & dissolves in 100 ml of Methanol.

Ascorbic Acid-- 0.2 gm of Ascorbic acid in 100 ml of distilled water.

Method

11 clean test tubes were taken and ascorbic acid solution was added to each of the test tubes in an increasing amount from 0.2, 0.4, Theeleventh test tube was kept blank with noascorbic acid. Then methanol was added to make the final volume to 2 ml. Then 0.5 ml of DPPH solution was added to each of the test tubes. The test tubes were allowed to stand for the reaction to occur for 10 min in dark conditions. Finally the readings were noted down by the help of UV VIS SHIMADZU 1800 Spectrophotometer at 517nm. In case of extracts obtained from herbal sample same procedure was used. 20 µl of the samples were taken & volume was made to 2 ml with methanol. 0.5 ml of DPPH solution was added to each of the test tubes and it was allowed to stand for reaction for 10 min in dark conditions. Reading was noted down on UV VIS SHIMADZU 1800 Spectrophotometer at 517nm. Determination of percentage inhibition of DPPH Activity by using following formula:

% Inhibition of DPPH Activity = A-B/A *100

Where, A = Optical Density (O.D.) of the blank B = Optical Density (O.D.) of the sample.

Results and discussion

The powdered drug was subjected to successive extraction protocol soxhalation. The extract so obtained was tested for the presence of phytochemical like alkaloid, flavonoids and Tannins. The anti-bacterial activity of the powder extract was done with Chloroform, methanol and petroleum ether and aqueous. The results indicate that the anti- microbial activity of the methanolic extract of *Moringa oleifera* was comparable with standard antibiotic. This shows the *Moringa oleifera*has an anti-bacterial activity and this may be due to the extracted phytochemicals in methanolic extract. But further chemical characterization is needed to confirm the molecule responsible for the activity. The anti-bacterial activity of this herbal formulation was comparable with standard antibiotics like Penicillin G and Ofloxacin.

Table 2: Colour of Successive Extracts

Sl. No.	Name of Reagent	Name of Drug	Colour of Extract
01.	Chloroform	*MoringaOleifera*	Reddish brown
02.	Petroleum Ether	*MoringaOleifera*	Reddish brown
03.	Methanol	*MoringaOleifera*	Yellowish
04.	Aqueous	*MoringaOleifera*	Blue green

Table 3: Anti-Bacterial Activity of various Extract of *Moringa oleifera*

Sl. No.	Name of the Drug	Microorganism	Zone of Inhibition (in mm)					
			Chloroform Extract	Petroleum Ether Extract	Methanol Extract	Aqueous Extract	Standard Drug	
							Penicillin G	Ofloxacin
01.	*MoringaOleifera*	*E. coli*	11.4 mm	5.1 mm	10 mm	No ZOI	17 mm	19.5 mm
02.	*MoringaOleifera*	*S. aureus*	12.8 mm	5.6 mm	6 mm	10 mm	12 mm	15 mm

Anti-Oxidant Activity of *Moringa oleifera*

Phytochemical screening reveals that the major constituents of *Moringa oleifera* extract are phenolic compound, alkaloid and flavanoid. Among these phenolic compounds which may be responsible for the activities ofanti-oxidant.

DPPH Radical Scavenging Activity

Moringa oleifera had significant scavenging effect on the DPPH free radical which increased with increasing concentration. The scavenging effect of sample was lower than that of Ascorbic acid.

Table 3: Observation Table of DPPH Method for Determining the Percentage of Inhibition

Sl. No.	Volume of Sample (200µl)	Volume of Methanol (in ml)	Volume of DPPH (inml)	Absorbance (at 517 nm)	Percentage (%) of Inhibition
01.	Petroleum Ether	3 ml	0.7	0.159	47.4
02.	Chloroform	3 ml	0.7	0.165	49.2
03.	Methanol	3 ml	0.7	0.206	66.5

Conclusion and Future Prospects

The results of this study clearly indicate that *Moringa oleifera* have high anti-oxidant activity and radical scavenging activity against various anti-oxidant systems in vitro. These assays have important applications for the food and pharmaceutical industry. Moreover, *Moringa oleifera* can be used as an easily accessible source of natural antioxidants and as a possible food supplement. In our present study we conclude that *Moringa oleifera* has good anti-oxidant property and could be attributed to the presence of flavonoids, alkaloids, tannins, saponin and phenolic compounds. It was already reported that naturally occurring phenolic compounds have free radical scavenging property.

The Herbal formulations have its own importance and advantages as compare to any other forms of medicines. As discussed in the present research the herbal formulations are free from any undesirable side effects and more or less they are non habit forming. The Indian climate favours the growth of many rare varieties of medicinal Plants. But the need of the hour is, these plants should be identified and much extensive research should be done on it so that new drug discovery can be made to cure many threatful diseases. Many research organizations and Industries are pursuing research on exploring the flora like CIMAP, Himalayan Drugs etc. and many success stories are daily published. But the research should be carried out in a large scale and should be region specific so that new formulations can be prepared. Much work is also going on Polyherbal Formulation, in which many herbal drugs are scientifically mixed to get the synergistic effect.

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