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Moringa oleifera: A STUDY ON ITS PRELIMINARY PHYTOCHEMISTRY AND ANTI OXIDANT ACTIVITY

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Abstract

The herbal medicine has been in use by diverse civilizations in different parts of the world for centuries. The present study investigated the phytochemical analysis of crude extract of *Moringa oleifera* leaf and pod in petroleum ether and methanol respectively. The phytochemical screening of these extracts showed the presence of alkaloids, terpenoids, flavonoids, saponins and glycosides. The extracts were also screened for their free radical scavenging properties using ascorbic acid as standard antioxidant. The results thus obtained showed that the methanolic extract of *Moringa oleifera* leaf exhibit significant anti-oxidant activity than petroleum ether extracts.

Keywords: *Moringa oleifera*, herbal medicine, antioxidant activity.

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INTRODUCTION

In India and other countries of the world, phytomedicines have been used since time immemorial to treat various ailments long before the introduction of modern medicine. Herbal medicines are still widely used in many parts of the world especially in areas where people do not have access to modern medicines [1,2]. Moreover in most Asian countries where herbal medicines are still heavily relied upon because of high cost of chemotherapeutic drugs, there is a need for scientific research to determine the biological activities of medicinal plants. The findings obtained from such research may lead to the validation of traditionally used medicinally important plants and enable full usage of the properties of these plants.

Natural products or phytochemicals are still recognized as one of the most important resources of bioactive compounds [4]. Phytochemicals are chemical compounds that are naturally found in plant. They are responsible for the colour and organoleptic properties of the plant [5]**.** It is also referred to as those chemicals that may have biological significance but are not established as an essential nutrient in plant [6]**.** Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures. Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity [7]. The extract thus obtained, after standardization, may be used as medicinal agent and contains complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans [8].

Antioxidant compounds are playing an important role in health protecting factor. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and

flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [9]. Thus interest in natural antioxidant has increased considerably. Nowadays, it is well known that natural antioxidants extracted from herbs and spices having high antioxidant properties and are used in many food applications [10]. Natural antioxidants from plant sources are potent and safe due to their harmless nature; wild herbs have their antioxidant properties [11]**.** One of such plants of medicinal value is *Moringa olifera,* belonging to the family Moringaceae, commonly known as 'sahajan' in Hindi, Horse radish in English. It is a small, fast, growing, evergreen, or deciduous tree that usually grows upto 10 or 12 m in height. It is distributed among Sub Himalayan Tracts, Assam, Bengal and Peninsular India[12]. Various properties are attributed to it like antispasmodic, diuretic, anti-diabetic, anti-inflammatory, anti-arthritic etc. [13]

MATERIALS AND METHODS

Collection of plant material

The leaves and pods of *Moringa oleifera* were collected in the month of October November from the surroundings of Bhopal and were identified by Dr Rakesh Mehta H.O.D Botany Govt M. G. M. College Itarsi Bhopal. The plant parts were shade dried and turned into powdered form by means of grinder.

Preparation of Extracts

The dried powder of pods and leaves were continuously refluxed with petroleum ether and methanol respectively using Soxhlet apparatus. The extracts were dried under reduced pressure using rotary evaporator to get the crude. It was stored below 4°C until further used,when needed the extract was suspended/dissolved in the desired solvent and was then further subjected to phytochemical studies.

Phytochemical Screening

The phytochemical screening was performed using standard procedures [14].

Determination of anti oxidant activity

DPPH Radical Scavenging Assay

The DPPH assay of *Moringa oleifera* extract was determined by the method as reported by *Floegel et. al., 2011* [15]. The procedure involved UV-spectrophotometric determination. Three solutions i.e. Standard, Test and Control were Prepared.

Preparation of Standard Ascorbic acid solutions

Different solutions (1 - 10µg/ml) of the ascorbic acid were Prepared in methanol. 1.5 ml of each solution of ascorbic acid were mixed with 1.5 ml of 200µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of each solution was taken after 30 min against methanol (as blank) at 517 nm.

Preparation of Test solutions

Different solutions of the extract were Preparedd in methanol to give concentrations (10 - 100µg/ml). 1.5 ml of each solution of *Moringa olifera* extract was mixed with 200 µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of each solution of *Moringa olifera* extract was taken after 30 min against methanol (as blank) at 517 nm.

Preparation of Control solution

For control, 1.5 ml of methanol was mixed with 200 µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of the control was taken after 30 min against methanol (as blank) at 517 nm.

Percentage antioxidant activity of plant extract and Ascorbic acid was calculated by using formula:

$$I\% = \frac{Ac - (At - Ab)}{Ac} \times 100$$

Where,

I% = Percentage inhibition

Ac = Absorbance of control (methanol and 200 µM DPPH solution)

At = Absorbance of ascorbic acid/plant extract with 200 µM DPPH solution after 30 min.

Ab = Absorbance of ascorbic acid/plant extract without 200 µM DPP solution

RESULTS AND DISCUSSIONS

Phytochemical Screening

The phytochemical screening of the extracts revealed the presence of following bioactive compounds as given in Table 1. These are the phytochemicals which are essential in many medicinal plants responsible for the antioxidant property either by scavenging free radicals or by preventing their formation. The reported medicinal property of the plant might be due to the presence of these bioactive components [16].

Table 1: The phytochemical screening of the extracts revealed the presence of following bioactive compounds

Phytochemicals	Tests	Pet ether extract		Methanolic extract	
		POD	LEAVES	POD	LEAVES
Alkaloids	Mayer's Test	−	−	−	+
	Wagner's Test	` −	−	−	+
	Hager's Test	−	−	−	+
Terpenoids	Salkowski test	−	+	+	+
	Libermann Burchards Test	−	+	+	+
Flavonoids	Lead Acetate Test	−	+	+	+
	Alkaline reagent test	−	+	+	+
Carbohydrates	Molish test	+	−	−	+
	Fehling's Test:	+	−	−	+
	Breford's Test	+	−	−	+
Glycosides	Killer Killians test	−	+	+	+
	Legal's Test	−	−	+	+
Tannins and phenolic compounds	$FeCl_3$ test	+	+	+	+
	Lead Acetate Test	+	+	+	+
	Gelatin Test	+	+	+	+
Saponins	Froth test	+	+	+	+
Amino acid Proteins	Biuret's Test	−	−	+	−
	Ninhydrin test	−	−	+	−

+ = Presence, − = Absence

Anti oxidant Activity

Free radical scavenging assay (DPPH assay)

The DPPH radical reacts with suitable reducing agents losing color stoichometrically with the number of electrons consumed, which is measured spectrophotometrically at 517 nm. The obtained results of the different extracts of *Moringa oleifera* plant are shown below. The order of the scavenging activity of different extracts of *Moringa oleifera* was found to be methanolic leaf extract (IC_{50} 27.42 µg/ml) > methanolic pod extract (IC_{50} 60.41 µg/ml) > petroleum ether leaf

extract (IC_{50} 143.35 µg/ml) > petroleum ether pod extract(IC_{50} 214.61 µg/ml). The scavenging effect was compared to that of the standard ascorbic acid with IC_{50} value18.53 µg/ml. The results thus obtained suggest that methanolic extracts have the proton donating ability and can serve as free radical inhibitors or scavenger or exhibit significant DPPH radical inhibition than the petroleum ether extracts.

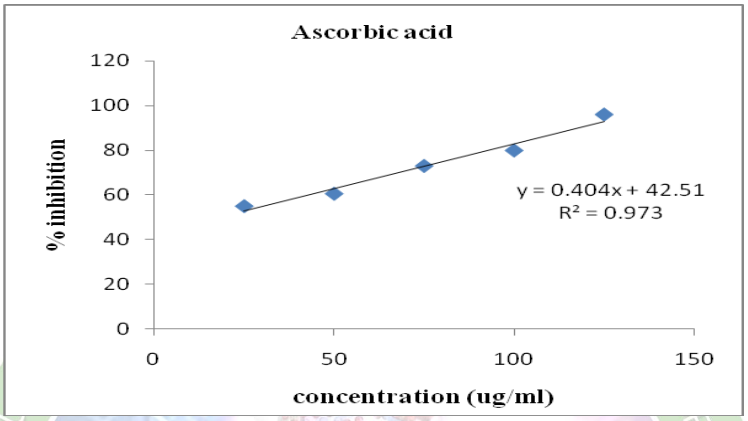

Fig.1: Standard curve of ascorbic acid. Graph represent regression curve of Ascorbic acid by DPPH assay method.

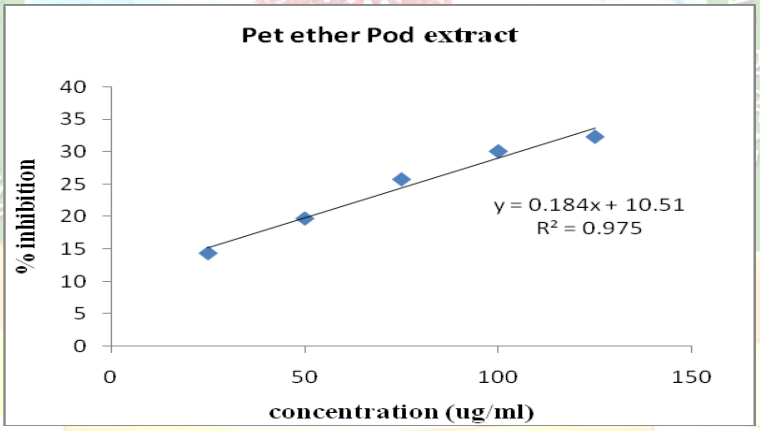

Fig.2: Graph represent regression curve of Pet ether Pod Extract by DPPH assay method

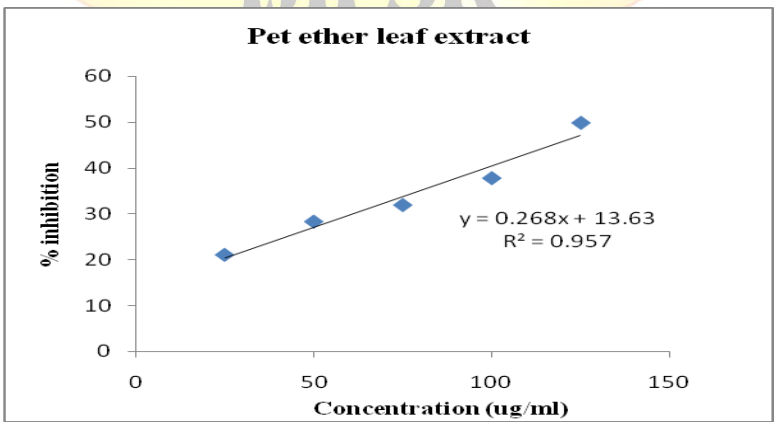

Fig.3: Graph represent regression curve of Pet ether leaf Extract by DPPH assay method

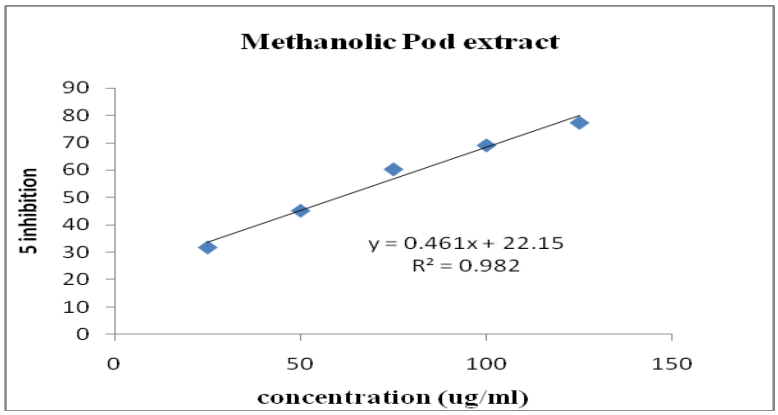

Fig.4: Graph represent regression curve of Methanolic Pod Extract by DPPH assay method

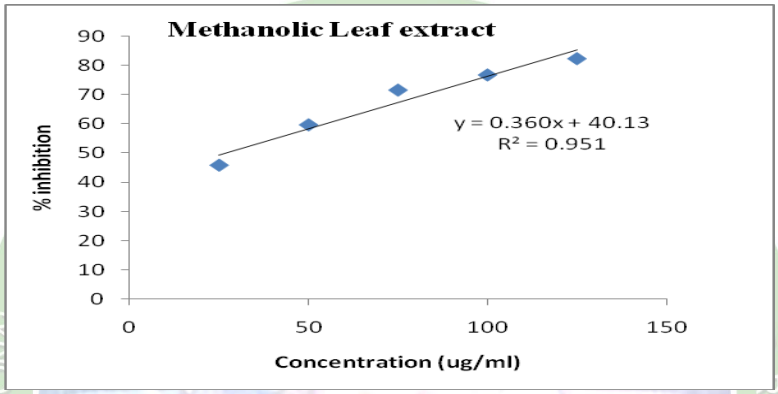

Fig.5: Graph represent regression curve of Methanolic leaf Extract by DPPH assay method

CONCLUSION

The present study indicated that all the essential bioactive compounds such as flavonoids, terpenoids, alkaloids, polyphenols etc are present in the *Moringa oleifera* leaf extracts. It was also evident that the methanolic extract of leaf exhibited potent antioxidant activity than other extracts of the plant. Thus the free radical scavenging ability of *Moringa oleifera* leaf will provide good health benefits to the humans. The antioxidants act as defense mechanism that protects against oxidative damage, and include compounds to remove or repair damaged molecules and sufficient intake of antioxidants is supposed to protect against diseases. The phytochemical antioxidants have potent potential to neutralize free radicals or oxidants responsible for the cell damage. Thus the present study scientifically validates the use of *Moringa oleifera* leaf for making different medicinal preparations for treating different ailments of our body.

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