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Original Research Article

Pharmacognostical, phytochemical and antioxidant potential of hydroalcoholic extract of *Moringa Oliefera* lam leaves

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ABSTRACT

Background: Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Secondary constituents contain alkaloids, flavonoids, phenol, saponin, steroids and tannins. Medicinal plants have anticancer, antimicrobial, antidiabetic, antidiuretic and anti- inflammation activities ect. The increasing interest in powerful biological activity of secondary metabolites outlined the necessity of determining their contents in medicinal plants. *Moringa oleifera* Lam (*M. oleifera*) is a fast-growing and drought-resistant tree of the *Moringaceae* family. The tree is known with some common names: miracle, ben oil, drumstick, horseradish or simply moringa. Phytochemical studies of plant organs showed the plant as a rich source of primary and secondary metabolites belonging to different classes of organic compounds. Pharmacological studies confirmed the use of the plant to cure several diseases and to possess nutraceutical properties.
Objective: The objective of this study was to investigate pharmacognostical, phytochemical features and antioxidant activity of hydroalcoholic extracts of *M. oleifera* leaves by using DPPH assay method.
Material and Methods: The different pharmacognostical parameters were evaluated as per standard protocols with some modifications. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. The *in vitro* antioxidant activity of hydroalcoholic extract of the leaves was assessed against DPPH method using standard protocols.
Results: Phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, tannins, amino acid and carbohydrate. The total phenolic and flavonoids content of *M. oleifera* leaves of hydroalcoholic extract was 0.864and 1.014 mg/100mg respectively. The activities of hydroalcoholic leaves extract against DPPH assay method were concentration dependent with IC_{50} values of ascorbic acid and extracts 17.68 and 79.10μg/ml respectively.
Conclusion: The present study concluded that the crude extract of *M. oleifera* is a rich source of secondary phytoconstituents which impart significant antioxidant potential. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

1. Introduction

India is a rich source of medicinal plants and a number of plant derived oils and extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Only a few of them have been

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scientifically explored. Plant derived natural products such as phenols, flavonoids, terpenes and alkaloids[1,2] have received considerable attention in recent years due to their diverse pharmacological properties. The qualitative analysis of phytochemicals of a medicinal plant is reported as vital step in any kind of medicinal plant research. Screening of plants constituents accurately can be done by employing chromatographic techniques.[3] Quantification usually employs the use of gravimetric and spectroscopic methods with several advanced approaches now available.[4] Reactive Oxygen Species (ROS), such as hydrogen peroxide, super oxide anion and hydroxyl radical, capable of causing damage to DNA, have been associated with carcinogenesis, coronary heart disease and many other health problems related to advancing age.[5] Antioxidant that scavenges these free radicals proves to be beneficial for these disorders as they prevent damage against cell proteins, lipids and carbohydrates.[6] Erythrocytes, which are the most abundant cells in the human body, possessing desirable physiological and morphological characteristics are exploited extensively in drug delivery.[7] Oxidative damage to the erythrocyte membrane (lipid/ protein) may be implicated in haemolysis associated with some haemoglobinopathies, oxidative drugs, transition metal excess, radiation, and deficiencies in some erythrocyte antioxidant systems.[8] This assay is useful either for screening studies on various molecules and their metabolites, especially on one hand, molecule having an oxidizing or antioxidating activity or on the other hand, molecule having a long term action.[9] Several herbal secondary metabolites such as flavonoid have been found to protect cells from oxidative damage.[10] These compounds have been evidenced to stabilize RBC membrane by scavenging free radicals and reducing lipid peroxidation.[11,12]*M*. oleifera is a fast-growing and drought-resistant tree of the *Moringaceae* family. It is commonly known with several names including moringa, drumstick tree (for the long and slender seedpods), horseradish tree (for the roots taste resembling horseradish), ben oil tree (being rich in behenic acid) and miracle tree (for the medicinal properties).[13]*M. oleifera* is among the food plants richest in nutrients.[14] It has a high content in essential amino acids, proteins, minerals, vitamins and polyphenols. It is a rich source of phytochemicals including flavonoids, anthocyanins, isothiocyanates, anthraquinone, alkaloids, essential oils, tannic acid, saponins, steroids, terpenoids, and cardiac glycosides.[15] In addition, it is used to treat individuals with extreme malnutrition as well as for its pharmacological (hepatoprotective, antihypertensive, cholesterol-lowering, anti-urolithiasis, antifertility, antidiabetic, and antioxidant activity, nutraceutical properties, and antimicrobial).[15] Moreover, *M. oleifera* is being used to help to breastfeed mothers improving postpartum milk production.[16] It is also used in Ayurvedic tradition specifically for cancer treatment.[17] *M. oleifera* leaves and buds were used against headache by rubbing them on the temples. Roots and root barks were used as anti-scorbutic.[15] The eye diseases were treated with the juice of the leaves added with honey. Dried seeds of *M. oleifera* were used in ophthalmic preparation, venereal affection anti-inflammatory and purgative and as tonic.[15] The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds and *in vitro* antioxidant activity of leaves of *M. oleifera* in Sagar region of Madhya Pradesh.

2. Materials and Methods

2.1. Plant material

The leaves of plant *M. oleifera* were collected in the month of August 2019 from the local area of Sagar, MP. Herbarium file of plant part was prepared and authenticated by Dr. Pradeep Tiwari (Professor), Department of Botany, Dr. HS Gour University Sagar, (M.P.) and the specimen voucher no. assigned was BOT/H/09/23/318. After that Herbarium file was submitted in Department. Plant material (leaves) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

2.2. Chemical reagents

All the chemicals used in this study were obtained from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

2.3. Defatting of plant material

M. oleifera leaf powder was produced after shade drying at room temperature. The shade-dried plant material was coarsely ground up and put through a petroleum ether extraction process utilizing soxhlet equipment. The extraction process was continued until the material had been sufficiently defatted.

2.4. Extraction by soxhletion method

Using the soxhletion method, 300gm of dried plant material were thoroughly extracted with a hydroalcoholic combination (500 ml, 70:30 v/v methanol: water) at 60°-

70°C for 24 hours. Over their boiling temperatures, the extract was evaporated. To determine the extractive yield, the dried crude concentrated extract was weighed. It was then transferred to glass vials (6×2 cm) and kept in a refrigerator (4°C) until it was needed for analysis.[18]

2.5. Macroscopical evaluation

The taste, size, colour, and odour of the leaves of M. oleifera were determined by doing a macroscopical study, which is the morphological description of the leaves that can be seen with the unaided eye.[19]

2.6. Physicochemical parameters

2.6.1. Loss on drying

A tarred petridish containing 10 gm of the powdered medication was precisely weighed. It was 105°C dried in a hot air oven for an hour before being reweighed. Calculating the initial and final weights allowed for the determination of loss on drying.

2.7. Total ash value

A silica dish was used to burn 5 gm of powdered medication at a temperature no higher than 450°C until it was carbon-free in a muffle furnace. After cooling, it was weighed. It was determined what percentage of the drug's weight was ash.

2.8. Acid insoluble ash value

By placing the crucible on a water bath and covering it with a watch glass, 1gm of ash was cooked for 5 minutes with 25°C of hydrochloric acid, and then cooled. Five ml of hydrochloric acid were used to rinse the watch glass before being placed to the crucible. The material was then filtered on a filter paper that had already been weighed, and the filtrate was dried and weighed. Calculating the percentage of content left over after subtracting the weight of the filter paper allowed for the determination of the acid insoluble ash value.

2.9. Water soluble ash value

By placing the crucible on a water bath and covering it with a watch glass, 1gm of ash was cooked for 5 minutes with 25 ml of distilled water, and then cooled. 5 ml of distilled water were used to rinse the watch glass before being placed to the crucible. To calculate the water soluble ash value, the original percentage of ash collected (i.e. 100%) was subtracted from the percentage of remaining content.

2.10. Alcohol soluble extractive value

In a closed flask, 5 gm of coarsely powdered, air-dried medication was macerated for 24 hours with 100 ml of alcohol, shaking frequently for the first six hours, and then left to stand for the last 18 hours. After that, it was quickly filtered to prevent alcohol loss. A 25 ml sample of the filtrate was dried to dryness in a shallow dish with a flat bottom, dried at 105°C, and weighed. The percentage of extractive that is soluble in alcohol was estimated using the air-dried medication as a base.

2.11. Water soluble extractive value

A closed flask was used to macerate 5gm of coarsely powdered, air-dried medication with 100 ml of chloroform water for 24 hours, stirring frequently for six hours, then allowing to stand for eighteen hours. Following that, it was quickly filtered while taking measures to prevent chloroform water loss. A 25ml sample of the filtrate was dried to dryness in a flat-bottomed dish that had been dried at 105^0C before being weighed.

2.12. Foaming index

Weighting 1 gm of coarse powder, 100 ml of water was added to a 500 ml conical flask. On a water bath, it was kept at a moderate boil for 30 minutes. It was filtered into a 100 ml volumetric flask while still cold. Water was added in a enough amount to dilute the volume. The decoction was placed into a test tube and shaken for 15 seconds lengthwise. After 15 minutes of standing, the height of the foam was measured to determine the foaming index.[20,21]

2.13. Phytochemical screening of the extract

Various phytoconstituents, including alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids were analysed qualitatively in the *M. oleifera* extract.[22,23]

2.14. Total phenol determination

The Olufunmiso et al. technique[24] was used to calculate the total phenolic content. Two ml of each extract or standard were combined with one ml of the Folin Ciocalteau reagent, which had previously been diluted 1:10 v/v in distilled water, and one ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15 seconds before being left to stand for 10 minutes to develop the colour. A UV/visible spectrophotometer were used to detect the absorbance at 765 nm. Gallic acid's standard graph was used to compute the total phenol concentration, and the findings were represented as mg per 100 mg of gallic acid.

2.15. Total flavonoids determination

The Olufunmiso et al.[24] technique was used to determine the total flavonoid content. A UV/visible spectrophotometer was used to measure the reaction mixture's absorbance at

420 nm after adding 1 ml of 2% AlCl3 solution to 3 ml of extract or standard and letting the mixture sit for 15 minutes at room temperature. Using the quercetin standard graph, the amount of flavonoids was estimated, and the results were reported as quercetin equivalent (mg/100mg).

2.16. Antioxidant activity

2.16.1. DPPH free radical scavenging assay

A modified approach was used to test the DPPH scavenging activity.[24] The spectrophotometer measured the DPPH scavenging activity. 1.5 ml of the stock solution (6 mg in 100 ml methanol) was made so that it produced an initial absorbance when combined with 1.5 ml of methanol. After 15 minutes, a decrease in absorbance was seen when the sample extract was present at various concentrations (10-100μg/ml). After diluting 1.5 ml of the DPPH solution with methanol to make 3 ml, the absorbance was measured right away at 517 nm for the control reading. In a series of volumetric flasks, 1.5 ml of DPPH and 1.5 ml of the test sample at various concentrations were added. The final volume was then adjusted to 3 ml with methanol. Three test samples were collected, and they were all handled similarly. The mean was finally taken. Each concentration was measured using absorbance at zero time. After 15 minutes, at 517 nm, DPPH absorbance finally decreased with the sample at a varied concentration. The following equation was used to compute the percentage of free radical DPPH inhibition: Inhibition percentage is equal to [(control absorbance - sample absorbance)/control absorbance] 100%. The 50% inhibitory concentration (IC50), which is how the activity is expressed, was determined based on the proportion of DPPH radicals that were scavenged. The level of antioxidant activity increases with decreasing IC50 values.

3. Results and Discussions

The so-obtained crude extracts were concentrated on a water bath in order to thoroughly evaporate the solvents and obtain the real yield of extraction. The hydroalcoholic extract yield from *M. oleifera* was 6.4%w/w. Table 1 summarizes the morphological properties of M. oleifera leaves. A fast-growing perennial tree, *M. oleifera* can grow to a height of 7 to 12 metres and a diameter of 20 to 40 cm. Their leaves are a dark green colour, have distinct flavours and odours, are smooth in appearance, and are typically 20–70 cm long. *M. oleifera* leaves were shade dried and ground into powder to measure a number of physiochemical parameters, including loss on drying, total ash value, extractive soluble in alcohol, extractive soluble in water, and foaming index (Table 2). Table 3 displays the findings of a qualitative phytochemical examination of the unprocessed leaves of *M. oleifera.* hydroalcoholic extracts contained alkaloids, glycosides, flavonoids, tannins, amino acids, and carbohydrates. Using the calibration curve equation: y = 0.015x - 0.001, R2= 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance, total phenolic compounds (TPC) were expressed as mg/100mg of gallic acid equivalent of dry extract sample. The equation based on the calibration curve: y = 0.035x + 0.009, R2=0.999, where X is the quercetin equivalent (QE) and Y is the absorbance, was used to determine the total flavonoids content as quercetin equivalent (mg/100mg). The hydroalcoholic extracts of M. oleifera leaves yielded content values of 0.864 and 1.014 for total phenolic and flavonoids, respectively, as shown in Table 4. The hydrogen-donating character of extracts was evaluated by the DPPH radical scavenging test.[25] The inhibitory concentration 50% (IC50) value of M. oleifera hydroalcoholic extract was determined to be 79.10 g/ml as compared to that of ascorbic acid (17.68 g/ml) under DPPH radical scavenging activity. Table 5 shows a dose-dependent action with regard to concentration.

Table 1: Morphological characteristic of *M. oleifera* leaves

Plant name	Colour	Odour	Appearance
M. oleifera	Dark green	Characteristic	Smooth

Table 2: Physiochemical analysis of powder of *M. oleifera* leaves

S. No.	Parameters	Observations
1	Loss on drying	3.8 % w/w
2	Total Ash value	5.6 % w/w
	Acid insoluble ash	1.9% w/w
	Water soluble ash	3.07% w/w
3	Alcohol soluble extractive	26.25%
4	Water soluble extractive	23.59%
5	Foaming index	24 (ml)

Table 3: Phytochemical screening of *M. oleifera* leaves extracts

S. no.	Phytochemical	Test	Result
1	Alkaloids	a) Dragendroffs reagents	+
		b) Mayer's reagents	+
		c) Hager's reagents	+
2	Glycosides	Bontrager test	+
3	Tannins	Ferric chloride test	+
4	Carbohydrates	Molisch test	+
5	Flavonoids	Shinoda test	+
6	Protein	Heat test	-
		Biuret test	-
7	Amino acid	Millon's test	+
		Ninhydrin test	+
8	Tannins	Ferric chloride	+

Table 4: Results of total phenol and flavonoids content

S. No.	Extract	Total phenol content	Total flavonoids content
		mg/100mg	
1	Hydroalcoholic	0.864	1.014

Table 5: % Inhibition of ascorbic acid and hydroalcoholic extract using DPPH method

S. No.	Concentration (μg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	44.65	20.06
2	20	48.62	26.25
3	40	65.34	37.85
4	60	69.65	42.48
5	80	77.41	50.57
6	100	84.13	57.03
	IC 50	17.68	79.10

4. Conclusion

The plant under study can be considered a possible source of beneficial medications due to the presence of significant amounts of secondary metabolites in its leaves, including flavonoids, alkaloids, and phenolics. The fact that phytoconstituents are present in significant amounts may help us to understand the potential pharmacological significance of this plant in the prevention and treatment of disease. The chemical components in plants that have distinct physiological effects on the human body are what give them their therapeutic worth. Additionally, it supports the statements made regarding the therapeutic benefits of this plant as a treatment method and its folkloric medical usage. Therefore, in order to produce viable chemotherapeutic drugs, we advise additional separation, purification, and characterization of the bioactive components from *M. oleifera* leaf, stem, flower, and seed.

5. Abbreviations

1. QE: Quercetin Equivalent
2. GAE: Gallic Acid Equivalent
3. TPC: Total Phenolic Compounds
4. DPPH: 2,2-diphenylpicrylhydrazyl

6. Source of Funding

None.

7. Conflict of Interest

None.

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