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Physicochemical And Phytochemical Standardization of Moringa Oleifera Leaves & Pods: An Indian Traditional Recipes

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Authors' contributions

This work was carried out in collaboration among all authors. Author AKT designed the study, performed the statistical analysis, wrote the protocol. Author SB performed the experimental work and wrote the first draft of the manuscript. Authors MK and SS managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Aim of the study was to analyse the physicochemical and phytochemical analysis of Moringa oleifera extracts.
Methodology: The physicochemical & phytochemical analysis of Moringa oleifera includes ash values, extractive value, LOD, pH etc.
Results: The physicochemical parameters of the methanol extract were analysed as per standard method. The aqueous & methanolic extract of Moringa oleifera showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, phytosterol saponins and tannins. The amounts of

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total flavonoid and phenolic were found to be 71.63 mg and 10.31 mg/g of the methanol extract, respectively. It was found that the phytochemical constituents are very much enriched in the Moringa oleifera extract and can the used for the development of new formulations.

Keywords: Moringa oleifera; physicochemical; phytochemical; total phenolics; total flavonoids.

1. INTRODUCTION

Nature is constantly a prime illustration of the age-old symbiotic phenomenon. Each biotic and abiotic element interacts with the others. The existence of plants is important for man. To treat any sickness that affects people, nature has provided a wide range of treatments. Man's natural curiosity has led to the accumulation of pharmacological knowledge through thousands of years, and as a result, we now have a wide range of efficient methods for ensuring healthcare. Since the beginning of human have been used extensively in medicine.

“Moringa oleifera is most well-known and widely cultivated medicinal plants belonging to family Moringaceae. The leaves, fruit, flowers, and immature pods of this tree are used as a highly nutritive vegetable in many countries. It is widely cultivated for the diversified use of its young seed pods and green leaves as vegetables and for medicine. It is considered as a very good supplement because of its high protein value. Without that, it is known as the miracle tree because of its diversified beneficial features, e.g., 10 times more vitamins than carrots, 7 times more vitamin C than oranges, 17 times more calcium than milk, and 15 times more potassium than bananas. World Health Organization has promoted Moringa as an alternative to imported food supplies to treat malnutrition. The nutritional and therapeutic values of moringa are used to prevent or treat protein-energy, malnutrition, and other nutritional related diseases. The plant is frequently used in the traditional medicine for treating tumours, glandular swelling and headache promoting more digestion, and lower cholesterol levels. It is still commonly used in folk medicine to treat tumours, headaches, and diabetes” [1].

“Several bioactive compounds present in *Moringa oleifera* plant, such as flavonoids, saponins, tannins, anthraquinones and alkaloids. They are synthesized by plants to combat environmental and physiological stresses such as ultraviolet radiation and microbial attack” [2,3]. Therefore, moringa leaves has beneficial for nutritional and therapeutic applications. Phytoconstituents present in fruits and vegetables such as tocopherols, carotenoids, and ascorbic acid can scavenge the reactive oxygen species (ROS) to a considerable extent when combined with a sensible eating routine .Similarly, carotenoids are linked with reducing the risk of cardiovascular disease (CVD), cancer, and age-associated macular degeneration. These various bioactive compounds, often referred to as phytochemicals (dietary antioxidants and polyphenols, etc.), are massive in *M. oleifera* leaves.

Plant Description [3]

Classification:

- **Kingdom** - Plantae
- **Sub kingdom** - Tracheobionta
- **Super Division –** Spermatophyta
- **Division** – Magnoliophyta
- **Class –** Magnoliopsida
- **Sub class –** Dilleniidae
- **Order** - Capparales
- **Family –** Moringceae
- **Genus** – Moringa
- **Species** – oleifera
- **Binomial name:** *Moringa oleifera Lam*

Botanical Background of *Moringa oleifera* [4-7]

The height of Moringa tree is ranging from 5-12 m with an open umbrella-shaped crown, straight trunk (10-30 cm thick), and a corky, whitish bark. The plant leaflets diameter is 1-2 cm and length 1.5-2.5 cm . Tuberous tap root of the tree which explains its tolerance to drought conditions. It is the tree of hot semi-arid regions (annual rainfall 250-1500 mm). Moringa is adaptable to a wide range of environmental conditions from hot and dry to hot, humid, and wet conditions. The tree is tolerant to light frosts but does not survive as a perennial under freezing conditions. Cultivation led to the collection of seeds from the tree. The development of plantlets in the greenhouse for 2

-3 month and the transplantation of mature stems (1-1.5 m long) to the main fields. The leaves, seeds, flowers, pods (fruit), bark, and roots are all seen as a vegetable and each part is uniquely harvested and utilized. Fresh leaves are picked, shade dried, ground to a powder, and then stored for later as a food flavouring or additives. Dried or fresh leaves are also used in foods such as soups and porridges [2], curry gravy and noodles, rice, or, wheat. Farmers have added the leaves to animal feed to maintain healthy livestock and vegetable compost for crop growth [5]. The seeds can be eaten green, roasted, or powdered, and steamed in tea and curries.The pods and seed often referred to as Moringa kernels, have a taste that ranges from sweet to bitter and is most popularly consumed after frying to get a peanut-like taste.

Hence this study was undertaken to develop comparative quality standards of moringa. This may be useful to pharmaceutical industries for authentication of commercial sample and to explore the possibility of using other species as complementary to each other.

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Materials

The fully matured pods and leaves of Moringa oleifera samples were collected from farmer land of Shirpur 425405 Dist: Dhule, Maharashtra, India. The plant was identified and authenticated by Dr. S.R. Kshirsagar, plant Taxonomist, Dept. of Botany SSVPS College, Dhule, Maharashtra.

2.2 Preparation of Leaves and Pods Powder

The fresh plant leaves were washed thoroughly with distilled water and air dried for 7 days. Leaves was crushed and blended for size reduction to mesh size #40 and used for further analysis. The powdered was preserved in closed container for further extraction purposes.

Approximately 5 kg of moringa pods were cooked in a pressure cooker for 2 hours, after which the pulp was scraped off and collected in a stainless-steel plate to be dried for 2 days at room temperature [8].

2.3 Physicochemical Evaluation of Crude Drug

The dried powder of *moringa oleifera leaves & pods* were standardized for physicochemical parameters as per standard methods [9]. The detailed procedures are given below.

2.3.1 Determination of ash values [10,11].

This parameter is used to detect adulterated or low-grade crude drugs that are exhausted and sandy or earthy particles. It is also used to detect minerals present in water-soluble and acid-insoluble ash.

2.3.1.1 Total ash [10,11]

2 g crude drug powder was incinerated in silica crucible at 450°C until the crude drugs get carbon free. The ash was allowed to cool before being weighed. The ash value was calculated using following Formula 9.

Total ash (%) = Weight of ash X 100 / *W*

2.3.1.2 Acid insoluble ash

The ash value was to determine whether the raw drug had any undesirable, harmful or earthy compounds. To determine the acid insoluble value, total ash obtained previously was placed in 25 ml of diluted hydrochloric acid and kept on the heating mantle. The mixture was then cleaned, burnt, and weighed after being filtered through ash-less filter paper.

Acid insoluble ash (%) = Weight of ash X 100 / *W*

2.3.1.3 Water soluble ash

To determine the water-soluble ash value, 25 ml of water were mixed with the previously obtained ash. After filtering, gathering, and weighing the mixture on the filter paper. The amount of insoluble materials was subtracted from the weighed amount of ash to determine the amount of ash that was water-soluble. This weighted amount was used to calculate the percentage of water-soluble ash value 9.

2.3.2 Determination of extractive values [10,11]

2.3.2.1 Alcohol soluble extractive value

5 g of coarsely air-dried crude drug powder was macerated in 100 ml of ethanol for twenty-four hours in a closed flask, stirring frequently for the first six hours and allowed it to stand for eighteen

hours. It was then filtered to prevent solvent loss, and 25 ml of the filtrate was evaporated to dryness in a flat-bottomed shallow dish with a tared bottom and dried at 105°C to a constant weight. Alcohol soluble extractives value was calculated by following

Alcohol soluble extractive (%) = Weight of extract in 25 ml X 4 X 100 / W

2.3.2.2 Water soluble extractive value [10,11]

5 g of coarsely air-dried crude drug powder was macerated with 100 ml of chloroform water for 24 hours in a closed flask, with frequent shaking and then allowed to stand for twenty-four hours. To prevent the loss of chloroform water, it was then immediately filtered. 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed plate dried at 105^0C, then weighed.

Water-soluble extractive (%) = Weight of extract in 25 ml X 4 X 100 / W

2.3.3 Loss on drying [10,11]

The loss on drying was calculated using the method of API. Weighed amount of crude drugs was added to a petri plate and was put in the oven and weighed at various time intervals at 105^0C.The percentage loss during drying was calculated according to following formula

LOD (%) = (Initial weight of porcelain dish with the drug – final weight of porcelain dish after 6 h) / (Initial weight of porcelain dish with the drug -Weight of empty porcelain dish)

Loss on drying (%) = Loss in weight X 100 / W

2.3.4 pH determination [10,11]

For pH determine the extract was dissolved in 10 ml of water. A digital pH meter was used to determine the pH. The pH is measured thrice.

2.4 Phytochemical Screening of *Moringa oleifera*

“Qualitative phytochemical screenings of plant extract were performed to investigate the presence of major chemical classes of components e.g. alkaloids, flavonoids, glycosides, tannins, phenols and steroids using following qualitative chemical tests” [9-11].

2.4.1 Test for alkaloids

“Small quantity of extract was mixed with a few drops of dil.hydrochloric acid. The acidic filtrate was used to detect the presence of alkaloids with various reagents like Mayer’s reagent (cream ppt), Hager’s reagent (yellow ppt), Wagner’s reagent (reddish brown ppt), and Dragendroff’s reagent (reddish brown ppt)” [12].

2.4.2 Tests for carbohydrates

Molisch’s test: “Few drops of Molisch’s reagent was added to small amount of extract followed by the addition of conc. H2SO4 along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 mL of distilled water. The formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates” [12].

Fehling’s test: The small amount of moringa extract was treated with 5 ml of Fehling’s solution (A and B) and kept in boiling water bath. The formation of yellow or red color precipitate indicates the presence of reducing sugar.

2.4.3 Test for saponins

“To a small amount of the moringa extract few drops of distilled water was added and shaken vigorously until persistent foam was observed for 15 min and the height of foam develop upto 1 cm” [12].

2.4.4 Tests for flavonoids

With sodium hydroxide: “Plant extract was treated with Sodium hydroxide solution. Blue to violet colors show the presence of anthocyanins while yellow to orange colors indicates flavanones and yellow for flavones” [12].

Concentrated sulphuric acid: when conc. sulphuric acid was added to plant extract. Yellow-orange color indicates the presence of anthocyanins and orange to red color for flavones.

Shinoda test: Magnesium turnings and dil.HCL were added to plant extract, change in blue from magenta to purple indicates the presence of flavonoids.

2.4.5 Test for protein [12]

Mix and warm plant extract and Millon's reagent it will either become brick red or disintegrate and release a reddish-colored fluid.

2.4.6 Test for phytosterol [12]

The plant extract was treated with a solution of alcoholic KOH until it was completely saponified. Saponified portion was extracted with ether and the ether layer was evaporated to obtain the residue for further analysis.

Liebermann-Burchard test: The residue obtained was dissolved in a few drops of diluted acetic acid, followed by addition of acetic anhydride and a few drops of conc. sulphuric acid. Bluish green color obtained for the presence of phytosterol.

Salkowski Reaction: The residue was dissolved in chloroform & add of conc. sulphuric acid. The chloroform layer shows red color and acid layer shows green fluorescence for presence of phytosterol.

2.4.7 Test for phenolic compounds and tannins [12-14]

Lead Sub Acetate Test: To plant extract add few drops of lead sub acetate solution. A cream gelatinous precipitation indicates positive test for Tannins.

Ferric Chloride Test: A small amount of plant extract mixed with ferric chloride solution, greenish to black color indicates the presence of phenolic & Tannins.

2.5 Estimation of Phytochemical Constituents

2.5.1 Estimation of total phenol

"Total phenolic contents (TPC) in the leaves & pods were determined by Folin–Ciocalteu colorimetric method as described by Singleton et. al. with some modification. Gallic acid was used as standard. 10 mg/10mL solution in methanol was prepared in methanol. Different dilutions of gallic acid like 20, 40, 60, 80, and 100 µg/mL were prepared from the standard solution. To each concentration, 5 mL of 10% Folin–Ciocalteu reagent (FCR) and 4 mL of 7% Na_2CO_3 were added making a final volume of 10 mL. Thus, the obtained, blue-colored mixture.it was shaken well and incubated for 30 min at 40°C in a water bath. The absorbance of colored solution was determined at 760 nm against blank. All the experiments were carried out in triplicates, and the average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve" [15-16]

2.5.2 Determination of total flavonoids

The aluminum chloride method was used to determine total flavonoid contents of plant extracts by colorimetric assay. Quercetin was used as standard solution. Stock solution was prepared by dissolving 10 mg of quercetin in 10 mL of methanol. Out of this stock solution, serial dilutions to make various concentrations of 0.25,0.5, 0.75 and 1 mg/mL solutions. One mL of standard quercetin from each concentration was added to the test tube containing 4 mL of distilled water, 0.3 mL of 5% $NaNO_2$ and 0.3 mL of 10% $AlCl_3$. Then, 2 mL of 1 M NaOH was added to the mixture. The volume of the mixture was made to 10 mL by immediately adding distilled water. The total flavonoid content was expressed as a quercetin equivalent.

"Sample prepared was also prepared and make different dilutions concentrations 0.25,0.5, 0.75 and 1 mg/mL solutions. A similar procedure was followed for the extracts also, and the absorbance was measured by spectrophotometer at 510 nm. The flavonoid content was expressed as quercetin equivalent (mg QE/g) using the linear equation based on the standard calibration curve" [15-16].

3. RESULTS AND DISCUSSION

3.1 Organoleptic Characters of *Moringa oleifera*

Moringa was subjected to physicochemical analysis using relevant parameters. The analysis was carried out aiming to gain information about the qualitative and quantitative composition of the test drug. The observations like colour,taste and consistency obtained are presented in Table 1. The ash value of the test drug was found to be 1.74% w/w, and it is one of the salient parameters for the standardization of herbal drugs.

Table 1. Organoleptic characters of *Moringa oleifera Plant*

Parameters	*Moringa oleifera*	
	Leaves	**Pod pulp**
Color	Greenish	Pale brown
Taste	salty	Sweet
Consistency	Course Powder	Powder

3.2 Extraction of *Moringa oleifera* Powder

According to the protocol, the crude drugs *Moringa oleifera* was extracted using aqueous and methanol solvents. When compared to other solvent, the aqueous solvent was shown to have a higher percentage yield of crude extract. The yield of the aqueous extract is determined to be 43.2% and 44%. The yield of the methanol extract is determined to be 27.6% and 12% respectively. This demonstrates that when ethanol is used instead of distillation, more phytochemical components diffuse and become soluble.

Fig. 1. *Moringa oleifera* leaves crude drug

3.3 Physicochemical Parameters

These standardization parameters were performed as per the guidelines of Ayurvedic Pharmacopoeia of India [9]. The physicochemical characteristics of the aqueous solutions were examined. Parameters are total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, loss on drying, foreign, and pH (Table 3).

3.4 Phytochemical Screening

Preliminary phytochemical investigation of moringa plant extracts revealed the presence of alkaloids, glycosides, sterols, carbohydrates, flavonoids, saponins [10-13] The results of phytochemical screening are depicted in Table 4.

3.5 Estimation of Phytochemical Constituents

The major phytochemical constituents present in this moringa are believed to be total flavonoid & total phenol [14-16]. The total phenol was estimated by the Folin-ciocalteu method. Total flavonoids & total phenolics were found to be 52 mg, 30.25 mg per 1 gm of aqueous extract, and 10.31 mg & 71.63 mg per 1 gm of leaf methanolic extract respectively whereas 10.1 mg, 36.83 mg per 1 gm of aqueous extract, and 23 mg & 21.61 mg per 1 gm of methanolic extract of pods. When methanol extract is compared to aqueous extract, the number of phytochemical constituents was found to be higher in methanol extracts (Table 5)

Fig. 2. *Moringa oleifera* seed crude drug

Table 2. Nature and percentage yield of extracts

Content	Solvent	Color	Type of extract	Consistency	%Yield (w/w)
MOL	Aqueous	Slight reddish Brown	Crude	Solid	43.2
	Methanol	Greenish Black	Crude	Solid	27.6
	Ether	Green	Crude	Solid	20.0
MOP	Aqueous	Brown	Crude	Solid	44.0
	Methanol	Yellowish	Crude	Solid	12.0
	Ether	Dark Brown	Crude	Solid	0.80

MOL: Moringa Oleifera Leaves, MOP: Moringa Oleifera Pods Pulp

Table 3. Physicochemical evaluation of *Moringa oleifera*

S. No	Parameters	Results % w/w	
		Leaves	Pods
1	Total ash	10.5	6.0
2	Acid insoluble ash	4.5	3.0
3	Water soluble ash	8.0	1.2
4	Water soluble extractives	23.2	64.0
5	Alcohol soluble extractives	17.6	12.0
6	Loss on drying	77 (for fresh leaves) 6.5 (for Dry Leaves)	86.6
7	pH (aqueous solution)	6.8	5.8

Table 4. Phytochemical screening of *Moringa oleifera*

S. No	Name of tests	Leaves		Pods	
		Aqueous extract	Methanol extract	Aqueous extract	Methanol extract
Test for alkaloids					
1	Dragendroff's	+	+	+	+
2	Mayer's	-	+	-	+
3	Hagers	+	+	+	+
Test for flavonoids					
4	With sodium hydroxide	+	+	+	+
5	With conc. sulphuric acid	+	+	+	+
6	Shinoda	+	+	+	+
Test for tannins					
7	Ferric Chloride	-	-	-	-
8	Lead acetate	+	+	+	+
9	Gelatin	-	+	+	+
Test for phenols					
10	Lead acetate	+	+	+	+
11	Gelatin test	-	+	+	+
Test for saponins					
12	Foam test	++	++	++	+
Test for carbohydrates					
13	Molisch's test	-	-	++	-
14	Fehling's test	-	-	++	-
Test for phytosterol					
15	Libermann burchard	-	+	+	+
16	Salkowski reaction	+	+	+	+
17	Proteins	+	+	+	+
18	Amino Acids	+	+	+	+

Table 5. Estimation of phytochemical constituents In *Moringa oleifera* extract in methanol

Herbal drug	Solvent used	Total Flavonoid (mg QE/g)	Total phenolic (mg GAE/g)
MOL	Aqueous	17-52	30.25-38.42
	Methanol	10.31	71.63-72.00
MOP	Aqueous	10.1	11.62-36.83
	Methanol	23.0	21.61±1.035

QE: Quercetin Equivalent; GAE: Gallic Acid Equivalent

4. CONCLUSION

The results obtained from proximate analysis reveal that dried moringa leaves are a potent source of essential nutrients. Consequently, these plants hold promise as valuable additions to both human and animal diets. Notably, Moringa oleifera exhibits a higher extractive value in methanol compared to water, suggesting a greater concentration of

phytoconstituents in a methanolic extract. The methanolic extract of *Moringa oleifera* contains proteins, alkaloids, flavonoids, tannins, phenols, and saponins. The presence of these phytochemicals in Moringa oleifera extract opens avenues for creating novel recipes.

The chemical composition of moringa plants deserves further investigation in future studies. Exploring the phytochemical components of *Moringa oleifera* leaves could uncover additional applications for these plants in herbal treatments. As we investigate into this research, documenting, substantiating, and maintaining the quality standards of moringa plants become crucial aspects in the context of the globalization of Ayurveda.

This study serves as a significant initial step towards establishing the Pharmacognostical, analytical, and nutraceutical profile of Moringa—a vital plant in the Ayurvedic system of medicine. It puts the groundwork for future endeavours aimed at understanding and utilizing the diverse potential of Moringa in the field of healthcare.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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