

Proximate Analysis of Nutritional and Antioxidant Properties of Moringa Oleifera Leaves and Pods

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Abstract: *Moringa oleifera*, a plant widely recognized for its nutritional and medicinal value, has been the subject of increasing interest due to its potent antioxidant properties. This study focuses on the physicochemical evaluation and in-vitro antioxidant activity of *Moringa oleifera* leaves and pods. Standardized procedures were used to determine ash content, moisture levels, pH, and various extractive values (water, alcohol, and ether-soluble), providing a detailed phytochemical profile of both plant parts. The antioxidant potential was assessed using DPPH radical scavenging and total antioxidant capacity assays. Results indicated that the leaves exhibited higher antioxidant activity and contained greater amounts of polyphenols compared to the pods, suggesting their superior free radical scavenging ability. The study confirms that *Moringa oleifera* is a rich source of natural antioxidants and essential nutrients, supporting its traditional use in promoting health and preventing disease. These findings contribute to the validation of *Moringa* as a valuable nutraceutical with potential applications in food and pharmaceutical industries

Keywords: *Moringa oleifera*, pods and leaves antioxidants, physicochemical evaluation, DPPH radical, nutraceutical

I. INTRODUCTION

Nutraceuticals

Nutraceutical word was first time coined (nutrition & pharmaceutical) by Stephen L De Felice in 1989. The term is applied to products that range from isolated nutrients, dietary supplements & herbal products as well as products of animal & marine origin. The Indians, Chinese, Egyptians & Samaritans are just few civilizations that use food as medicine. Hippocrates considered by some to be the father of medicine said that people should “take food as medicine”. “A nutraceutical is any non-toxic food extract of marine or animal part that has scientifically confirmed health benefit for the prevention & treatment of diseases”.

Nutraceutical Benefits

- May increases the health value of our diet.
- May help us live longer.
- May help us to avoid particular conditions. May have a psychological benefit from doing something for oneself.
- May have a perceived to be more “Nature” than traditional medicine & less likely to produced unpleasant side effect.
- May present food for population with special needs. General perception of “Natural is always good & safe”.

PLANT PROFILE

Moringa oleifera

Biological Source: Drug consists of fresh Leaves & Pods of *Moringa oleifera* belonging to Family: Moringaceae





Fig 1. Moringa oleifera Leaves



Fig 2. Moringa oleifera Pod

Kingdom	Plantae
Subkingdom	Tracheobionta
Super Division	Spermatophyta
Division	Magnoliophyta
Class	Eudicots
Subclass	Rosids
Order	Brassicales
Family	Moringaceae
Genus	Moringa

Table 1- Taxonomic Classification of Moringa Oleifera

Morphology

Leaves:

Colour : Green

Shape : Tripinnate, rachis slender thickened & articulated at base

Length : leaflets 1-2cm long

Pods:

Colour : Green

Shape : Pendulous, greenish, triangular

Length : 30-120cm long 1.8cm wide.

Phytochemical Constituent

Moringa oleifera is a good source of various phytochemicals like alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, terpenoids. Leaves of Moringa Oleifera have been reported to contain flavonoid, pigment such as kaempferol, rhamnetin, isoquercitrin, kaempferitrin.

MATERIAL AND METHODS

Collection and Authentication of Plant Material

The fully matured pods and leaves of Moringa oleifera samples were collected from farmer land of Shahada 425409 Dist: Nandurbar, Maharashtra, India. The plant was identified and authenticated by Dr. S.R. Kshirsagar, plant Taxonomist, Dept. of Botany SSVPS College, Dhule, Maharashtra.

Preparation of Leaves and Pods Powder

The fresh plant leaves were washed thoroughly and carefully with distilled water and air dried for 7 days. Leaves was crushed and blended in Mixer for size reduced to mesh size #40 and used for physicochemical analysis. The powdered stored in closed container and utilized for extraction. 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.

Physicochemical evaluation of crude drug

The dried powder of moringa oleifera leaves & pods were standardized for physicochemical parameters as per standard methods (Ayurvedic Pharmacopeia). The following procedures were used to determine the different ash values and extractive values of Moringa oleifera powder.

Total ash:

Accurately weighed 2 to 3 g powder of the Moringa oleifera was taken in a silica crucible, which was previously ignited and weighed. The powdered Moringa Oleifera scattered at the bottom of the crucible and incinerated by gradually increasing the heat not exceeding 450°C until free from carbon. After complete incineration, crucibles cooled in desiccator and weighed. The percentage of total ash was calculated the with reference to the air-dried drug in triplicate for each selected roots using formulae mentioned below.

Total ash (%) = $\frac{\text{Weight of ash} \times 100}{W}$

W

Where, W = Weight of the plant parts powder in g.

Acid-insoluble ash:

The 25 ml of dilute hydrochloric acid added to the crucible containing total ash and boiled gently for 5 minutes. The content was filtered through ash less filter paper, washed with hot water until it got free from acid. The filter paper containing insoluble content was transferred to same silica crucible and further ignited. The crucible was allowed to cool in a desiccator for 30 minutes and then weighed. The percentage of Acid insoluble ash was calculated the with reference to the air-dried drug in triplicate for each selected leaves & pods using formulae mentioned below.

Acid insoluble ash (%) = $\frac{\text{Weight of ash} \times 100}{W}$

W

Where, W = Weight of the plant parts powder in g.

Water Soluble ash:

The 25 ml of water added to the crucible containing total ash and boiled gently for 5 minutes. The content was filtered by passing through ash less filter paper, transferred to original crucible, and ignited to constant weight. The ash produced weighed and subtracted from the weight of total ash to get weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug. The percentage of water-insoluble ash was calculated the with reference to the air-dried drug in triplicate for each selected leaves & pods using formulae mentioned below.

Water soluble ash (%) = $\frac{\text{Weight of ash} \times 100}{W}$

W

Where, W = Weight of the plant parts powder in g.

Loss on Drying (moisture content):

About 5 g of powdered Moringa Oleifera was accurately weighed in a Petri dish and kept in a hot air oven maintained at temperature of 105-110°C. After cooling in desiccators, the loss in weight was recorded. This procedure was repeated until constant weight was obtained. The percentage loss on drying was calculated with reference to the initial weight of crude drug.

Loss on drying (%) = $\frac{\text{Loss in weight} \times 100}{W}$

W

Where, W = Weight of the plant parts powder in gm.

PH determination:

To determine the pH, the extract was dissolved in 10 ml of pure water. A digital pH meter was used to determine the pH. The pH is measured 3 times.

Extractive values

Alcohol Soluble Extractive value



A coarsely powdered, air-dried 5 g of *Moringa Oleifera* was macerated in a 250 ml conical flask with a stopper. Add 100 ml of alcohol of the specified strength. Shake the flask frequently during the first 6 hours. Allow it to sit for 18 hours without being disturbed, then filter quickly to avoid alcohol loss. Pipette out 25ml of the filtrate and dry it in a tared flat-bottomed shallow dish. Then they dried it at 105°C before weighing. The percentage of ethanol soluble extractive calculated the with reference to the air- dried drug in triplicate for each *Moringa Oleifera* using formulae mentioned below.

$$\text{Alcohol soluble extractive (\%)} = \frac{\text{Weight of extract in 25 ml} \times 4 \times 100}{W}$$

Where, W = Weight of the plant parts powder in g.

Water Soluble Extractive value

A method similar to that used to estimate the alcohol soluble extractive value was used to calculate the water-soluble extractive value. The only change was that instead of alcohol, chloroform water was utilized as the extraction medium.

$$\text{Water-soluble extractive (\%)} = \frac{\text{Weight of extract in 25 ml} \times 4 \times 100}{W}$$

Where, W = Weight of the plant parts powder in g.

Ether Soluble Extractive Value

Transfer a suitably weighed quantity (depending on the fixed oil content) of the air dried, crushed drug to an extraction thimble, extract with Solvent ether (or petroleum ether, b.p. 40° to 60°) in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filter the extract quantitatively into a tared evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105° to constant weight. Calculate the percentage of ether-soluble extractive with reference to the air-dried drug.

$$\text{Ether-soluble extractive (\%)} = \frac{\text{Weight of extract in 25 ml} \times 4 \times 100}{W}$$

Where, W = Weight of the plant parts powder in g.

Antioxidant Activity

Antioxidants are thought to be crucial in the body's defence mechanism against free radicals and reactive oxygen species (ROS), which are dangerous by products of regular cell aerobic respiration. Increasing dietary antioxidant consumption might support appropriate antioxidant status and, as a result, the normal physiological operation of a living system

Mechanism of Action :

Antioxidants stop the electron-stealing process by giving one of their own electrons to free radicals in order to neutralise them. Since they are stable in both forms, antioxidants cannot produce free radicals by giving electrons to other molecules. These become both scavengers and housekeepers by removing free radicals from the body before they have a chance to cause damage. They may thus be described as chemicals that can stabilise or quench free radicals.

In-vitro Antioxidant Study

Determination of DPPH Radical Scavenging Activity

Principle : A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The structure of DPPH & its reduction by an antioxidant are shown below :



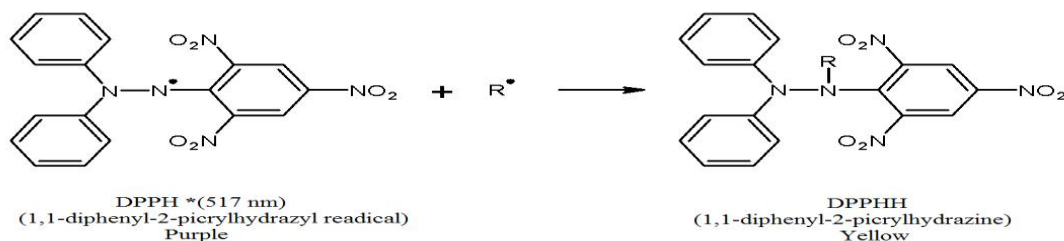


Fig 3- Structure of DPPH & its reduction by an antioxidant

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm & is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. the resulting decolorization is stoichiometric with respect to number of electron captured. The change in the absorbance produced at 517 nm has been used as a measure of radical scavenging activity.

Chemicals : DPPH (2,2-diphenyl-1-picrylhydrazyl) Ascorbic acid

Reagents Preparation :

DPPH Solution (0.1mM) : DPPH Solution was prepared by dissolving 33 mg of DPPH in one liter of analytical grade methanol & kept in amber colored bottle to protect from sunlight.

Ascorbic Acid : 100 µg/ml stock solution was prepared by dissolving 10 mg of ascorbic acid in 100 ml of distilled water. From this 20, 40, 60, 80 & 100 µg/ml of ascorbic acid solution prepared.

Sample Preparation : A stock solution of concentration 1 mg/ml was prepared by adding 10 mg of methanolic extracts of moringa oleifera leaves & pods in 10 ml methanol & solution of various concentrations of methanolic extracts (20, 40, 60, 80 & 100 µg/ml) were prepared.

Calculation :

$$\text{Inhibition\%} = \frac{(\text{Control Absorbance} - \text{Sample Absorbance})}{\text{Control}}$$

Where, Abs control is the absorbance of DPPH radical + methanol Abs sample is the absorbance of DPPH radical + extract/sample

The antioxidant effects of each extract were expressed in terms of IC₅₀ (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the graph after plotting the percentage DPPH inhibition versus extract concentration.

Determination of Total Antioxidant Activity

Principle :

Phosphomolybdenum assay used to determine the total antioxidant capacity is based on the reduction of Mo (VI) to Mo (V) by the extract & subsequent formation of a green phosphate/Mo (V) complex at acid pH(9).

Chemicals : Ascorbic acid Ammonium Molybdate Sodium Phosphate Sulphuric acid

Reagent Preparation :

For Phosphomolybdenum Reagent : 28 mm of Sodium Phosphate

(A) : 84 mg of Sodium Phosphate in 25 ml of distilled water. 4 mm of Ammonium molybdate

(B) : 124 mg of Ammonium molybdate in 25 ml of distilled water. 0.6 mm of Sulphuric acid

(C) volume make up to 100 ml of distilled water.

Sample Preparation : A stock solution of concentration 1 mg/ml was prepared by adding 10 mg of methanolic extracts of moringa oleifera leaves & pods in 10 ml methanol & solution of various concentrations of methanolic extracts (20, 40, 60, 80 & 100 µg/ml) were prepared.



Result & Discussion

The physicochemical evaluation of the herbal crude drug is an important parameter in detecting adulteration or improper handling of drugs. The physicochemical parameters like ash value, moisture content, extractive value & pH value was determined as per the standard procedure.

Ash:

Ash values of MOL & MOP were found to be 11.03 ± 0.75 & 7.5 ± 1.72 % respectively indicating the good sources of minerals. Ash content was recorded in this study which was comparatively high when compared to values obtained in similar research with ash content values of 8.05-10.38 %. Slightly ash content was found to be high in present study.

Acid-insoluble ash value-

Acid-insoluble ash value of MOL & MOP was found to be 4.30 ± 0.41 & 3.70 ± 0.31 respectively.

water-soluble ash-

water-soluble ash of MOL & MPO was found to be 8.5 ± 0.97 & 2.15 ± 1.03 respectively. It represents the total part of ash that is soluble in water.

extractive value -

The extractive value by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drug.

Water soluble extractive value-

WSE value of MOL & MOP powder was found to be 21.4 ± 2.43 & 62.46 ± 2.26 % respectively, it indicates that polar constituent is high in pods.

Alcohol soluble extractive value-

ASE value of MOL & MOP powder was found to be 14.2 ± 4.53 & 13.65 ± 2.58 % respectively.

Ether soluble extractive value-

The present study revealed that ESE value was high in MOL 18 ± 2.88 as compared to MOP 1.17 ± 0.59 . it reaved that nonpolar constituent are low in Pods.

Moisture Content-

fresh and dried Moringa leaves contain 73.8 ± 4.96 and 6.30 % moisture content whereas MOP contain 83.45 ± 4.21 & 22.02 % respectively.

PH value-

Present study indicates Moringa PH was 6.7 & 5.6 in MOL & MOP.

Parameters	Leaves	Pods
Ash Value (%)		
Total Ash	11.03 ± 0.75	7.5 ± 1.72
Acid-insoluble Ash	4.30 ± 0.41	3.70 ± 0.31
Water soluble Ash	8.5 ± 0.97	2.15 ± 1.03
Extractive Value (%)		
Water Soluble	21.4 ± 2.43	62.46 ± 2.26
Alcohol Soluble	14.2 ± 4.53	13.65 ± 2.58
Ether Soluble	18 ± 2.88	1.17 ± 0.59
Loss on Drying (%)		
On fresh bases	73.8 ± 4.96	6.30 %
On dry bases	83.45 ± 4.21	22.02 %
pH value	6.7	5.6

Values are expressed as mean \pm SEM (n=3)

Table.2. Physicochemical analysis of Moringa oleifera Leaves & Pods



Estimation of Antioxidant Potential :

The leaves of the *Moringa oleifera* tree have been reported to possess antioxidant activity, primarily attributed to their high content of polyphenols

74.50 ± 4.05 mg GAE/g . Extracts derived from leaves as compared to Pods of *Moringa oleifera* have demonstrated strong antioxidant activity against free radicals. This antioxidant activity helps prevent oxidative damage to important biomolecules within the body, such as proteins, lipids, and DNA.

Determination of DPPH Radical Scavenging Activity :

A comparative study indicated that mature *Moringa oleifera* leaves extract exhibited better values of enzymatic & non-enzymatic antioxidants. In the DPPH (2,2-Diphenyl- 1-picrylhydrazyl) free radical scavenging activity test, both mature & tender leaf extracts showed significant reduction of DPPH radicals. The scavenging activity was suggested to be attributed to its hydrogen donating ability & was seen more in the mature leaf extract (Razis, Ibrahim et al. 2014)

Concentration (µg/ml)	Absorbance	% Inhibition	SEM	IC50
Standard				
20	0.4111	21.61	28.66±5.26	25.547
40	0.4150	25.34		
60	0.4349	29.15		
80	0.4583	33.03		
100	0.4812	34.2		
Leaves				
20	0.2521	58.93	72.29±12.33	2.0280
40	0.2321	62.35		
60	0.1349	70.52		
80	0.1017	82.22		
100	0.0983	87.45		
Pods				
20	0.2721	55.67	73.08±13.20	4.0697
40	0.2724	65.45		
60	0.1693	72.42		
80	0.2244	87.45		
100	0.077	87.45		

Table 3. Free Radical Scavenging Activity by DPPH

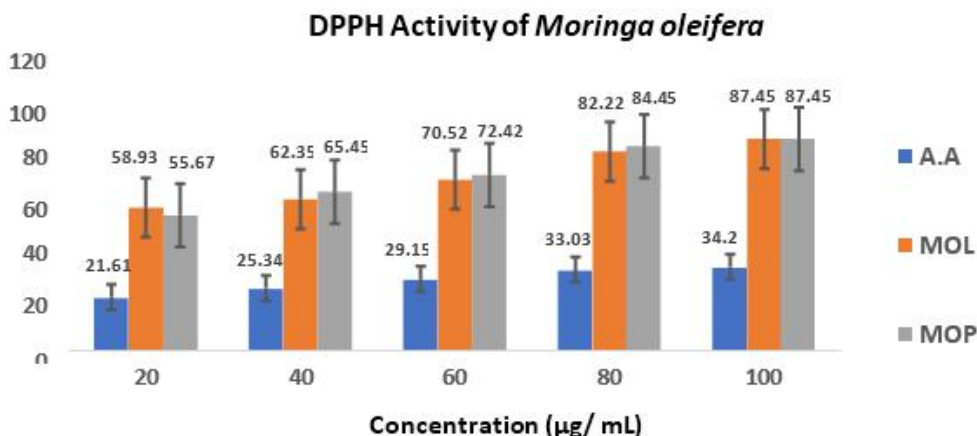
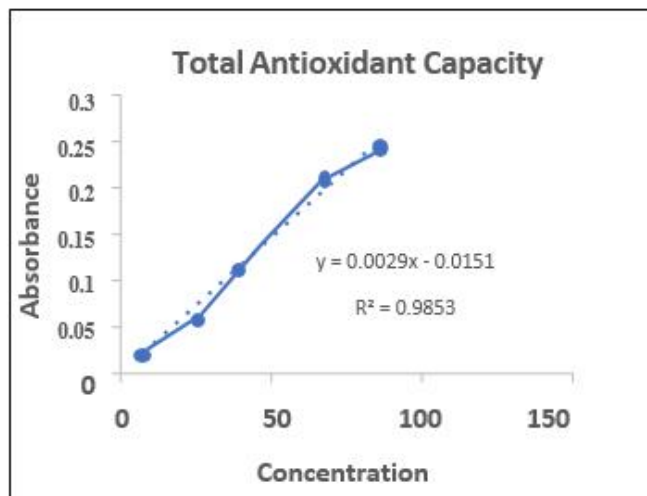


Fig 4 - DPPH Activity of *Moringa oleifera*



Concentration (µg/ml)	Absorbance
20	0.0470
40	0.0886
60	0.1639
80	0.2325
100	0.2663
MOL	0.0913
MOP	0.1265

Table 4. Total Antioxidant Activity



II. CONCLUSION

The present study highlights the significant nutraceutical potential of *Moringa oleifera*, emphasizing its rich phytochemical composition and strong antioxidant properties. Physicochemical analysis of the leaves and pods revealed valuable insights into their mineral content, extractive values, and moisture levels, establishing a scientific basis for their use in herbal medicine and nutrition. The leaves demonstrated notably higher antioxidant activity than the pods, likely due to their elevated polyphenol content, making them particularly effective in neutralizing free radicals and preventing oxidative stress. These findings support the traditional use of *Moringa oleifera* in promoting health and preventing various diseases. Further research, including clinical studies, is recommended to fully explore its therapeutic potential and to standardize formulations for use in functional foods and pharmaceutical applications.

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