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**Research Article**

# Pharmacognostical and Phytochemical Analysis of *Moringa Oleifera* Leaves

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## Abstract

*Moringa oleifera* Lam. (Family: Moringaceae), commonly known as the drumstick tree, is a nutritionally and therapeutically important medicinal plant. Its leaves contain diverse bioactive compounds with antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and hepatoprotective properties. Pharmacognostical and phytochemical standardization is vital to ensure authenticity, purity, and safety of herbal raw materials.

In this study, fresh leaves of *M. oleifera* were collected, authenticated, shade-dried, and powdered. Pharmacognostical evaluation included macroscopic and microscopic examinations, powder microscopy, and physicochemical analysis (loss on drying, total ash, acid-insoluble ash, water-soluble ash, and extractive values). Successive extraction was performed with ethyl acetate, methanol, and distilled water, followed by preliminary phytochemical screening using standard qualitative tests.

Diagnostic features observed were dorsiventral leaf anatomy, multicellular covering trichomes, paracytic stomata, lignified xylem vessels, and abundant calcium oxalate crystals. Physicochemical parameters were within pharmacopoeial limits, confirming good quality of the crude drug. Phytochemical screening revealed alkaloids, flavonoids, phenolics, tannins, glycosides, carbohydrates, proteins, amino acids, steroids, terpenoids, and saponins. Methanolic extract showed the richest phytochemical profile, aqueous extract contained abundant polar constituents (saponins, carbohydrates), while ethyl acetate extract was rich in steroids and terpenoids.

The pharmacognostical and phytochemical findings provide reliable diagnostic markers and quality control parameters for M. oleifera leaves. The presence of diverse bioactive constituents supports its medicinal significance and offers a scientific basis for its continued use in herbal formulations and future pharmacological research.

**Keywords:** *Moringa oleifera*, Apiaceae, Ajowan Leaves, Constituents, Pharmacological Activities.

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## 1. Introduction

Medicinal plants were the potent source of many pharmacological activities. Amongst that the plants with anthelmintic action have attained a great interest due the capability of the plant and its compound to treat a disease that causes major economic loss and reduced livestock production to the livestock holders. The pathogenic infection causes the severe effect of mortality and other problems that were uncontrolled due to the anthelmintic resistance that is developed in the host organism [1]. Even though, many synthetic drugs were manufactured; they produce more side effect than the efficacy of treatment [2].

Herbal drugs imply knowledge and practice of herbal healing for the prevention, diagnosis, and elimination of physical, mental, or social imbalance. The costs for health care are rising at an alarming rate throughout the world. At the same time, the world market for phytopharmaceuticals is growing progressively [3]. The World Bank estimates that trade in medicinal plants, botanical drug products and raw materials are growing at an annual rate of 5 to 15 % [6].

*Moringa oleifera* (L.) sprague is an annual herbaceous plant bearing the greyish brown fruits or seeds. An erect, glabrous or minutely pubescent, branched annual, up to 90 cm tall, cultivated almost throughout India [7].

Stems striate; leaves rather distant, 2-3 pinnately divided segments linear, ultimate segments 1.0-2.5 cm long; flowers in terminal or seemingly-lateral pedunculate, white, small; fruits ovoid, muricate, aromatic cremocarps 2-3 mm long, compounds umbels, grayish brown; mericarp compressed, with distinct ridges and tubercular surface, one-seeded. Flowers and fruits bearing from January – April [8].

Distribution and habitat: It belongs to the family 'Apiaceae' comprising 270 genera and species, mostly grown in the temperate regions of the world but species which are cultivated in tropics regions. Ajwain is grown in Iran, Egypt, Afghanistan and India (largely in Uttar Pradesh, Bihar, Madhya Pradesh, Punjab, Rajasthan, Bengal, Tamil Nadu and Andhra Pradesh). It is generally grown in October November and harvested in May -June. Though the plant is widely cultivated, it is indigenous to Egypt where it grows as a common weed in the fields [9].

**2.1 Medicinal uses:**

In Indian system of medicine, ajwain is administered for stomach disorders, a paste of crushed fruits is applied externally for relieving colic pains; and a hot and dry fomentation of the fruits is lapped on the chest to cure asthma. Ajwan-ka-arak (aqueous extract) is popular preparation for diarrhoea [10]. Therapeutic uses of T. ammi fruits include stomachic, carminative, expectorant, antiseptic, amoebiasis and antimicrobial activity. It also cures abdominal tumor, abdominal pains and piles [11]. It's also prescribed to comfort dipsomania, hysteria, sore throat; many ajowan ayurvedic formulations are available which is given to overcome infections with worms. It is also used for relieving flatulence, dyspepsia, spasmodic disorders, flatulence, common cold, acute pharyngitis, sore and congested throat [12].

**2.2 Adulteration**

Ajowan seed is available both as whole and in ground form. It adulterated by addition of exhausted or spent seed (from which oil or oleoresin has been extracted) excess stems, chaff and earth or dust [13]. The oil is also adulterated with ajowan chaff oil. The range of essential oil is 2–4% and it should contain thymol ranging from 35 to 60%. If chaff oil is added, the thymol content will reduce to below 35% [14]. The oleoresin may be adulterated by adding synthetic saturated acid. Detection of these adulterants can be done by gas chromatography or by thin layer chromatography coupled with high-performance liquid chromatography [15]. The adulteration at any level can be detected by using the specifications as explained separately for whole seed, powdered seed, volatile oil and oleoresin 6. The seeds are sometimes adulterated with ban ajwain [Seselidiffusum (Roxb. ex. Sm.)] or randhuni [Apium graveolens (Linn.) Sprague] [16]. The adulteration can be detected by thin layer chromatography using benzene: petrol (1:7). Reported phytoconstituents: Ajwain seed possessed fibre (11.9%), carbohydrates (38.6%), tannins, glycosides, moisture (8.9%), protein (15.4%), fat (18.1%), saponins, flavone and mineral matter (7.1%) containing calcium, phosphorous, iron and nicotinic acid [17].

**Figure 1:** ***Moringa oleifera*** **leaves**

## 2. Material and Methods

**2.1 Collection of Plant Materials**:

The leaves of *Moringa oleifera* were collected from the available graphical sources. The plant drugs were identified, collected and stored for further use [18].

**2.2 Preparation of Extracts:**

The plant leaves powder of the *Moringa oleifera* were extracted with ethyl acetate, methanol and water using as solvent respectively. A total of 50gm of individual plant powder of the *Moringa oleifera* was taken and mixed with 250 ml distilled water (1:5) in a round bottom flask and gently refluxed for ½ hour separately [19]. The residue was removed by filtration through Whatmann No.1 filter paper and the aqueous extract was concentrated used on a rotary evaporator to get solid yield extract [20].

**2.3 Physicochemical Investigations:**

Six samples of plant powder of *Moringa oleifera* were subjected to determination of physicochemical parameters such as loss on drying, ash values, pH value, aqueous and methanolic extractive values were carried out according to the methods recommended by the World Health Organization [21].

**2.4 Determination of pH range**

The pH of different formulations in 1% w/v (1g: 100ml) of water-soluble portions of plant powder of *Moringa oleifera* were determined using standard simple glass electrode pH meter [22].

**2.5 Loss on drying / Moisture content (Gravimetric determination):**

Separately placed about 1.0g of whole plant powder of the *Moringa oleifera* in an accurately weighed moisture disc. For estimation of loss on drying, it was dried at 105°C

for 5 hours in an oven, cooled in a desiccator for 30 minutes, and weighed without delay. The loss of weight was calculated as the content in mg per g of air-dried material [23].

**2.6 Determination of water and methanol-extractable matter:**

Separately placed about 5.0g of whole plant powder of the *Moringa oleifera* in an accurately weighed, glass stoppered conical flask. For estimation of hot water-extractable matter, 100ml of distilled water was added to the flask and weighed to obtain the total weight including the flask [24]. The contents were shaken well and allowed to stand for 1 hour. A reflux condenser was attached to the flask and boiled gently for 1 hour; cooled and weighed. The flask was readjusted to the original total weight with distilled water and it was shaken well and filtered rapidly through a dry filter [25]. Then 25 ml of the filtrate was transferred to an accurately weighed, tarred flat-bottomed dish (Petri dish) and evaporated to dryness on a water-bath. Finally, it was dried at 105°C for 6 hours in an oven, cooled in a desiccator for 30 minutes, and weighed without delay. Same procedure was followed using ethanol instead of distilled water to determine extractable matter in ethanol. The extractable matter was calculated as the content of active ingredient in mg per g of air-dried material [26].

**2.7 Determination of total ash**:

Two grams of the whole plant powder of *Moringa oleifera* was placed in a previously ignited (350°C for 1 hour) and tared crucible accurately weighed [27]. Dried material was spread in an even layer in the crucible and the material ignited by gradually increasing the heat to 550°C for 5 hours in a muffle furnace until it was white, indicating the absence of carbon. Cooled in a desiccator and weighed. Total ash content was calculated in mg per g of air-dried material [28].

**2.8 Determination of acid-insoluble ash:**

Twenty-five (25) ml of hydrochloric acid (~70g/l) TS was added to the crucible containing the total ash, covered with a watch-glass and boiled gently for 5 minutes. The watch-glass was rinsed with 5 ml of hot water and this liquid added to the crucible. The insoluble matter was collected on an ash less filter-paper (Whatmann 41) and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter was transferred to the original crucible, ignited by gradually increasing the heat to 550°C for 3 hours in a muffle furnace to constant weight. Allowed the residue to cool in a suitable desiccator for 30 minutes, and then weighed without delay. Acid-insoluble ash content was calculated as mg per g of air dried material [29].

**2.9 Determination of water-soluble ash**

Twenty- five (25) ml of water was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 minutes. Insoluble matter was collected on an ash less filter paper. Washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C in a muffle furnace. Allowed the residue to cool in a suitable desiccator for 30 minutes, and then weighed without delay. The weight of the residue was subtracted in mg from the weight of total ash. Water-soluble ash content was calculated as mg per g of air-dried material [30].

**2.10 Determination of sulfated ash**

Ignited a suitable crucible (silica) at 550°C to 650°C for 30 minutes, cooled the crucible in a desiccator (silica gel) and weighed it accurately. One gram of the plant powder of the *Moringa oleifera* was placed in a previously ignited crucible, ignited gently at first, until the substance was thoroughly white. Cooled and moistened the sample with a small amount (usually 1 ml) of sulfuric acid (~1760 g/l) TS, heated gently at a temperature as low as practicable until the sample is thoroughly charred. After cooling, moistened the residue with a small amount (usually 1 ml) of sulfuric acid (~1760 g/l) TS, heated gently until white fumes were no longer evolved, and ignited at 800°C ± 25°C until the residue is completely incinerated. Ensure that flames were not produced at any time during the procedure. Cooled the crucible in a desiccator, weighed accurately. This was repeated until the sample reaches a constant weight and calculated the percentage of residue [31].

**2.11 Microscopic Analysis of *Moringa oleifera* Leaves**

Fresh leaves of *Moringa oleifera* were collected, washed with distilled water, and small pieces containing the midrib were selected for microscopic examination. Thin transverse sections (T.S.) were prepared manually using a sharp razor blade. The sections were cleared with chloral hydrate solution, stained with 1% safranin, and mounted in 50% glycerin on clean glass slides. The prepared slides were examined under a compound light microscope using 10× and 40× objectives. Diagnostic anatomical features such as the upper and lower epidermis, palisade and spongy parenchyma, vascular bundles, xylem vessels, phloem, calcium oxalate crystals, trichomes, and stomata were observed and photographed for documentation.[32]

## 3. Results and Discussion

**3.1 Physicochemical Investigation:**

Physicochemical parameters were determined as per guidelines of WHO, air dried coarse powdered sample of *Moringa oleifera* was subjected for determination of physicochemical parameters such as pH, foreign organic matter, methanol soluble extractives, water soluble extractives, total ash content, acid insoluble ash, water soluble ash, loss on drying and % moisture content were determined. The average physicochemical parameters of the *Moringa oleifera* course powder ware tabulated in table 1.

### 3.2 Microscopic Analysis of *Moringa oleifera* Leaves

The transverse section of *Moringa oleifera* leaf exhibited a dorsiventral structure with a single-layered upper and lower epidermis covered by a cuticle. A single layer of elongated palisade cells was observed beneath the upper epidermis, while the lower region consisted of loosely arranged spongy parenchyma containing intercellular spaces. The midrib contained a prominent collateral vascular bundle with xylem oriented towards the adaxial surface and phloem towards the abaxial surface. Collenchymatous tissues were present below both epidermal layers in the midrib region. Calcium oxalate crystals and multicellular covering trichomes were observed in the mesophyll. Powder microscopy revealed characteristic epidermal fragments with paracytic stomata, lignified fibers, spiral xylem vessels, calcium oxalate crystals, palisade tissue fragments, and covering trichomes, confirming the identity of *Moringa oleifera* leaves.

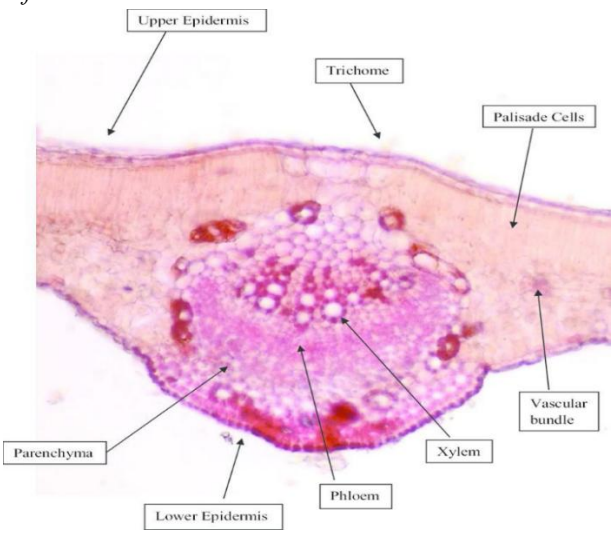


**Figure 2: Microscopic Analysis of *Moringa oleifera* Leaves**

### 3.3 Extraction of Plant Drug:

The plant leaves powder of the *Moringa oleifera* were extracted with ethyl acetate, methanol and water using as solvent respectively. The solvent was removed and practical yield was found and recorded. The findings were tabulated in table 2.

**Table 1: Physicochemical Parameters of *Moringa oleifera* plant**

| S. No. | Parameters | Values |
|---|---|---|
| 1 | pH range | 3.25±0.01 |
| 2 | Loss on drying | 8.20±0.10 |
| 3 | Methanol soluble extractive value | 15.56±0.20 |
| 4 | Water soluble extractive value | 13.20±0.50 |
| 5 | Total ash value | 4.25±1.10 |
| 6 | Water soluble ash | 2.10±0.05 |
| 7 | Acid insoluble ash | 1.35±0.20 |
| 8 | Sulphated ash | 1.20±0.10 |

**Table. 2: Extractive values of *Moringa oleifera***

| Solvent | Yield (g) | % Yield |
|---|---|---|
| Ethyl acetate | 2.5 | 5% |
| Methanol | 2.0 | 4% |
| Water | 3.5 | 7% |

**Table 3: Preliminary Phytochemical screening of plant *Moringa oleifera***

| S. No. | Phytochemical Constituent | Test Performed | EAE | ME | WE |
|---|---|---|---|---|---|
| 1 | Alkaloids | Dragendorff's Test | + | ++ | + |
| 2 | Alkaloids | Wagner's Test | + | ++ | + |
| 3 | Carbohydrates | Molisch's Test | + | ++ | ++ |
| 4 | Reducing sugars | Fehling's Test | - | + | ++ |
| 5 | Glycosides | Keller-Killiani Test | + | ++ | + |
| 6 | Cardiac glycosides | Legal's Test | - | + | + |
| 7 | Flavonoids | Shinoda Test | + | +++ | ++ |
| 8 | Phenolic compounds | Ferric Chloride Test | + | +++ | ++ |
| 9 | Tannins | Ferric Chloride Test | - | ++ | ++ |
| 10 | Saponins | Foam Test | - | ++ | +++ |
| 11 | Proteins | Biuret Test | - | + | ++ |
| 12 | Amino acids | Ninhydrin Test | - | + | ++ |
| 13 | Steroids | Salkowski Test | ++ | + | + |
| 14 | Terpenoids | Liebermann-Burchard Test | ++ | ++ | + |
| 15 | Fixed oils & fats | Spot Test | + | - | - |
| 16 | Gums & Mucilage | Ruthenium Red Test | - | + | ++ |

+++ = Strongly Present; ++ = Moderately Present; + = Present; − = Absent

**EAE:** Ethyl Acetate Extract**; ME:** Methanol Extract**; WE:** Water Extract

## 4. Conclusion

The present study successfully established the pharmacognostical and preliminary phytochemical profile of *Moringa oleifera* Lam. leaves, providing valuable parameters for their authentication, identification, and quality evaluation. Macroscopic examination revealed characteristic morphological features, while microscopic analysis demonstrated distinct anatomical characteristics, including a dorsiventral leaf structure, paracytic stomata, multicellular covering trichomes, collateral vascular bundles, lignified xylem vessels, and calcium oxalate crystals. These diagnostic features can serve as reliable

markers for distinguishing *Moringa oleifera* leaves from adulterants and substitutes.

Physicochemical evaluation indicated that the crude drug possessed acceptable quality with respect to moisture content, ash values, and extractive values, supporting its suitability for use as a medicinal raw material. Preliminary phytochemical screening of the ethyl acetate, methanolic, and aqueous extracts confirmed the presence of several important secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, terpenoids, steroids, carbohydrates, proteins, and amino acids. Among the three extracts, the methanolic extract exhibited the richest phytochemical profile, indicating that methanol is the most effective solvent for extracting a broad range of bioactive constituents from *Moringa oleifera* leaves.

The presence of these phytochemicals provides scientific evidence supporting the well-documented pharmacological properties of *Moringa oleifera*, such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, cardioprotective, and anticancer activities. The findings of this investigation contribute to the establishment of pharmacognostic standards and quality control parameters for *Moringa oleifera* leaves and may facilitate their standardization in herbal formulations.

Overall, this study provides a comprehensive scientific basis for the authentication and quality assessment of *Moringa oleifera* leaves. Further investigations involving quantitative phytochemical estimation, chromatographic fingerprinting (HPTLC, HPLC, or LC–MS), isolation and characterization of bioactive compounds, and pharmacological and toxicological evaluations are recommended to validate their therapeutic potential and promote their safe and effective utilization in pharmaceutical and nutraceutical applications.

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