Pharmacognostic Standardization, Physico and Phytochemical Evaluation of Aerial Parts of *Mentha arvensis* Linn.

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ABSTRACT

The present study deals with the macroscopic and microscopical studies of aerial parts of *Mentha arvensis* Linn. Microscopically, aerial parts showed glandular trichomes, helical to spiral xylem, palisade tissues with columnar cells, diacytic stomata. Powder microscopical examination showed the presence of glandular and uni to multi celled trichomes, helical to spiral xylem vessel, stomatal epidermal cells, abundant xylem vessels with pitted thickenings, abundant thin walled parenchymatius cells, epidermis with cuticle and collenchymatous cells, parenchymatous cells with reddish tannin contents. Physicochemical parameters and preliminary phytochemical studies of the powdered aerial parts were also carried out. Total ash was approximately sixteen and four times more than acid insoluble and water soluble ash, respectively. Water soluble extractive was slightly higher than ethanol soluble extractive. T.L.C. of petroleum-ether, chloroform and ethanol extract showed eight spots, nine spots and six spots, respectively. Phytochemically, it exhibited alkaloids, glycosides, steroids and sugars. These findings might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

Keywords: *Mentha arvensis* Linn, Physicochemical parameters, Phytochemical studies, Aerial parts.

INTRODUCTION

*Mentha arvensis* Linn belonging to the family Labiatae is a common edible and aromatic perennial herb which is cultivated throughout India. The aromatic leaves are used widely for flavouring foods and beverages.¹ It is an erect aromatic herb that grows up to 60 cm in height with suckers; the stem is cylindrical and the leaves are simple and opposing type. It is used as a contraceptive ², carminative, anti-spasmodic, anti peptic ulcer agent, and has been given to treat indigestion, skin diseases, coughs and colds in folk medicine.³ The main aim of the present work is to study the macro, microscopic and some other pharmacognostic characters and physico-chemical standards of aerial parts of *M arvensis* Linn which could be used for the proper identification of this drug.

MATERIALS AND METHODS

Plant material

The plant specimens for the study were collected from a farmer of Mangala, Bilaspur (Chhattisgarh, India) 22°06'35.83"N and 82°08'06.23"E and were positively identified and authenticated by the Dr. H. B. Singh, Scientist F and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources, Near Pusa Gate, New Delhi, India. A voucher specimen no. is submitted to the RHMD, NISCAIR. Reference no. is NISCAIR/ RHMD/Consult/-2008-09/1195/226, dated 31/03/2009. Care was taken to select healthy fully grown plant with normal organs. The samples of different organs were cut suitably and removed from the plant and thoroughly washed with water to remove the adherent impurities and dried in sunlight.

Macroscopic characterization

Macroscopical studies of leaf and stem were done by naked eye and shape, color, taste and odor of leaf and stem were determined and reported.

Microscopic characterization

Sectioning: Selected samples were stored in a solution containing formalin (5 ml), acetic acid (5 ml) and 70% v/v ethyl alcohol (FAA) (90 ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the method. ⁴ Infiltration of the specimens was carried by gradual addition of paraffin wax
(50-60°C m.p.) until tertiary-Butyl alcohol solution attained supersaturation. The specimens were casted into paraffin blocks. The paraffin-embedded specimens were sectioned with the help of Senior Rotary Microtome, RMT-30 (Radical Instruments, India). The thickness of the sections was kept between 10 and 12 μm. The dewaxing of the sections was carried out as per the procedure described by Johanson. [5]

The section was stained with phloroglucinol -hydrochloric acid (1:1) and mounted in glycerin. Powder (60) of the dried aerial parts was used for the observation of powder microscopical characters. The powdered drug was separately treated with glycercine, chloral hydrate and water to determine the presence of various tissues. [6]

**Photomicrograph:** Microscopic descriptions of selected tissues were supplemented with micrographs. Photographs of different magnifications were taken with Nikon Lab Photo 2 (Two) Microscopic unit. For normal observations, bright field was used. For the study of crystal, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property under polarized light they appear bright against dark background. [7]

**Physico-chemical evaluations**

Physicochemical parameters of powdered drug were determined [8] and reported as total ash, water-soluble ash and acid-insoluble ash values. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components. The moisture content and pH was also determined.

**Preliminary phytochemical screening**

Coarse powder of the drug (25 g) was subjected to soxhlet for successive solvent extraction. Extract were concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents. [5-10]

**RESULTS**

**Macroscopical study**

**Leaves:** Leaves of _M. arvensis_ were 2.5 cm long, shortly petiole, and oblong, ovate or lanceolate, obtusely or acutely serrate, cuneate at the base, sparsely hairy or almost glabrous. It had strongly aromatic and characteristic odor, and slightly pungent and slightly bitter taste (Fig. 1).

**Stem:** It was short, branched with short hairs, dense and as matures turns black. Stems were quadramular, dark green to brown colored. It had hairy surface, strong and aromatic odor, glandular trichomes, many layers of collemchymatous cells near the ridge (angular) region, uni to biseriate medullary rays and, also the presence of collateral and conjoint vascular bundles.

**Diagnostic characters:** It showed the presence of brown to green colored; quadrangular stem with hairy surface, strong and aromatic odor, glandular trichomes, many layers of collemchymatous cells near the ridge (angular) region, uni to biseriate medullary rays and, also the presence of collateral and conjoint vascular bundles.

**Physicochemical Parameters**

_M. arvensis_ aerial part’s powder showed the presence of total ash 11.4 % w/w, acid-insoluble ash 0.70 % w/w, water-soluble ash 2.51 % w/w, water-soluble extractive 22.30 % w/w, alcohol-soluble extractive 16.15 % w/w, moisture content 7.2% and pH- 6.5 (Table 1).

**Preliminary Phytochemical Studies**

Phytochemical analysis showed the presence of Steroid in chloroform extract. Alcohol extract showed positive report for alkaloids, glycosides and sugars (Table 2). T. L. C. of Petroleum-ether (60-80°C) extract of drug on Silica gel 60 F254 pre coated sheets using Benzene: Ethanol (19:1) showed eight spots in iodine vapor. In the chloroform extract, using Chloroform: Methanol (19:1), nine spots and in ethanol extract, using Toluene: Ethyl acetate (93:7) solvent system, only six spots were observed using same viewing medium (Table 3).

**Table 1: Phytochemical analysis of aerial parts of Mentha arvensis Linn.**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Physicochemical parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total Ash</td>
<td>11.4 % w/w</td>
</tr>
<tr>
<td>2</td>
<td>Acid insoluble ash</td>
<td>0.70 % w/w</td>
</tr>
<tr>
<td>3</td>
<td>Water soluble ash</td>
<td>2.51 % w/w</td>
</tr>
<tr>
<td>4</td>
<td>Water soluble extractive</td>
<td>22.30 % w/w</td>
</tr>
<tr>
<td>5</td>
<td>Ethyl alcohol soluble extractive</td>
<td>16.15 % w/w</td>
</tr>
<tr>
<td>6</td>
<td>Moisture content</td>
<td>7.2 %</td>
</tr>
<tr>
<td>7</td>
<td>pH</td>
<td>6.5</td>
</tr>
</tbody>
</table>

* w/w: weight/weight.
Table 2: Phytochemical analysis of aerial parts of Mentha arvensis Linn.

<table>
<thead>
<tr>
<th>Test for constituents</th>
<th>Petroleum ether extract</th>
<th>Chloroform extract</th>
<th>Ethyl alcohol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Steroid</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Terpene</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Glycoside</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Sugars</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Sapogenin</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Tannin</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Color &amp; Consistency</td>
<td>Colorless oily</td>
<td>Very light</td>
<td>Yellow gum</td>
</tr>
</tbody>
</table>

* Positive: present, Negative: absent

Table 3: TLC pattern of various extracts of Mentha arvensis Linn aerial parts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extracts</th>
<th>Adsorbent</th>
<th>Solvent system</th>
<th>Viewing medium</th>
<th>Rf Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Petroleum-ether 60-80°C</td>
<td>Silica gel 60 F 254 pre coated sheets</td>
<td>Benzene: Ethanol (19:1)</td>
<td>Iodine vapor</td>
<td>0.24, 0.37, 0.46, 0.57, 0.70, 0.76, 0.83, 0.96</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform</td>
<td>Silica gel 60 F 254 pre coated sheets</td>
<td>Chloroform: Methanol (19:1)</td>
<td>Iodine vapor</td>
<td>0.06, 0.28, 0.40, 0.48, 0.58, 0.66, 0.77, 0.84, 0.88</td>
</tr>
<tr>
<td>3.</td>
<td>Ethanol</td>
<td>Silica gel 60 F 254 pre coated sheets</td>
<td>Toluene: Ethyl acetate (93:7)</td>
<td>Iodine vapor</td>
<td>0.06, 0.08, 0.29, 0.57, 0.65, 0.94</td>
</tr>
</tbody>
</table>

* Rf: Retention Factor.

DISCUSSION

The macroscopic study of aerial parts indicated that its colour, odor and taste may be an important characteristic feature for identifying the plant. The anatomy of the leaf and stem was studied by taking transverse section. Transverse section of the leaf showed dorsiventral nature, single layered upper epidermis with covering glandular trichomes, ‘C’ shaped vessel embedded with radially arranged xylem tissues, thin walled and polygon cell Phloem cells and Xylem with helical to spiral vessels. T. S. of the leaf through laminar region showed Palisade tissues with columnar cells and spongy parenchyma with intercellular spaces, Lower epidermis with diacytic type stomata, covering and glandular trichomes. T. S. of the stem showed quadrangular in outline, epidermis covered by thin cuticle, collateral and conjoint vascular bundles near the plain region and uniseriate medullary rays.

Microscopic study of powder showed fragments of glandular and uniseriate celled trichomes, helical to spiral xylem vessel, epidermal cells with stomata, abundant xylem vessels with pitted thickenings, abundant thin walled parenchymatous cells, epidermis with cuticle and...
Total ash was approximately, sixteen and four times more than acid insoluble and water soluble ash respectively. Water soluble extractive was slightly higher than ethanol soluble extractive.

Phytochemically, it was found to contain alkaloids, glycosides, steroids and sugars. T. L. C. of Petroleum-ether extract using Benzene: Ethanol (19:1), showed eight spots. In the chloroform extract, using Chloroform: Methanol (19:1), nine spots and in ethanol extract, using Toluene: Ethyl acetate (93:7), only six spots were observed using iodine vapor as a viewing medium.

The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. The moisture content of the drug is not too high, thus it could discourage bacterial, fungal or yeast growth, as the general requirement for moisture content in crude drug is not more than 14% w/w. [11] The ash values, extractive values and moisture content of leaf and stem were determined. The results are depicted in Table 2. Pharmacognostic standardization including physico-chemical evaluation in Table-1 and 2 is meant for identification, authentication, and detection of adulteration and also compilation of quality control standards of crude drugs. [12] Since the plant, Mentha arvensis Linn is useful in traditional medicine for the treatment of various ailments, it is important to standardize it for use as a drug.

The Pharmacognostic constants for aerial parts of this plant, the diagnostic microscopic features and the numerical standards reported in this work could be used to fix the quality standards of this drug.

ACKNOWLEDGMENT
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REFERENCES