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

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ABSTRACT
The present study deals with the macroscopical 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

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India)
$22^0$06’35.83”N and $82^0$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, 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.

Macroscopical 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.

Microscopical 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 glycerine, 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. [9-10]

RESULTS

Macroscopical study

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

Stem: It was short, branched with short hairs, dense and as matures turns black. Stems were quadrangular, dark green to brown colored. It had hairy surface, strong and aromatic smell and slightly bitter taste. Stem measures 10 to 15 cm and more, 0.5 to 0.6 mm in breadth (Fig. 1).

Microscopical Study

T. S. of the leaf: Leaf showed dorsiventral in nature (Fig. 2.1). Upper epidermis was single layered, covered by thin cuticle and showed covering glandular trichomes (Fig. 2.2). Eight to ten layered ground tissue consisting of small rounded parenchymatous tissue, and one to three layered collenchymatous layers without intercellular spaces were observed. In centre 'C' shaped or half moon shaped vascular bundle was embedded with radially arranged xylem tissues which were collateral, conjoint and closed. Phloem cells were thin walled and polygonal, and Xylem showed helical to spiral vessels (Fig. 2.3).

T. S. of the leaf through laminar region: It showed dorsiventral in nature. Upper epidermis was covered with thin cuticle and showed covering glandular trichomes. Palisade tissues showed one layer of columnar cells and below this loosely arranged spongy parenchyma with intercellular spaces were observed. Lower epidermis was single layered, showed many stomata, covering and glandular trichomes. Stomata were of diacytic type on both sides of the leaf but more in lower side of the leaf. Simple starch grains and oil globules were present in cells (Fig. 2.4 & 2.5).

T. S. of the stem: Stem was quadrangular in outline (Fig. 3.1). Outer layer epidermis was well developed and covered by thin cuticle. Some of the epidermal cells showed glandular trichomes (Fig. 3.5). Epidermis was followed by six to ten layers of cortex region, where upper two to four layers of cells were chlorenchymatous and remaining were parenchymatous thin walled, closely arranged (Fig. 3.2 & 3.3). Towards the angular region, Stem showed many layered, closely arranged collenchymatous layer of cells. Vascular bundles near the ridge region were continuous, whereas near the plain region vascular bundles were collateral and conjoint. Medullary rays were uniseriate (Fig. 3.4). Pith was large, many layered, closely arranged with thin walled parenchymatous cells (Fig. 3.1).

Powder macroscopy: Leaf powder (Vegetative part) was dark green in color. It had strongly aromatic smell and slightly pungent taste.

Powder microscopy: Microscopic study of powder revealed the presence of fragments of glandular trichomes, unicellular trichomes, two to four celled trichomes, Parenchymatous cells, helical to spiral shaped xylem vessel, epidermal cells with stomata. Abundant xylem vessels with pitted thickenings, abundant thin walled parenchymatius cells, Epidermis with cuticle and collenchymatous cells, parenchymatous cells with reddish tannin contents were also observed.

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 collenchymatous 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 F_{254} 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: Physicochemical analysis of aerial parts of *Mentha arvensis* Linn.

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

* w/w: weight/weight.

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

Test for constituents	Petroleum ether extract	Chloroform extract	Ethyl alcohol extract
Alkaloid	Negative	Negative	Positive
Steroid	Negative	Positive	Negative
Terpene	Negative	Negative	Negative
Flavanoid	Negative	Negative	Negative
Glycoside	Negative	Negative	Positive
Sugars	Negative	Negative	Positive
Saponin	Negative	Negative	Negative
Tannin	Negative	Negative	Negative
Color & Consistency	Colorless oily	Very light yellow oil	Yellow gum

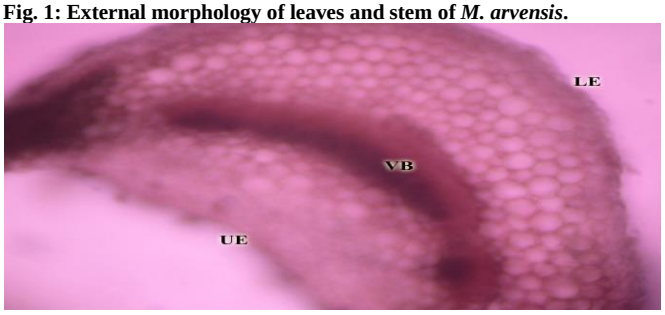
* Positive: present, Negative: absent

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

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

* R_f: Retention Factor.

Fig. 1: External morphology of leaves and stem of *M. arvensis*.



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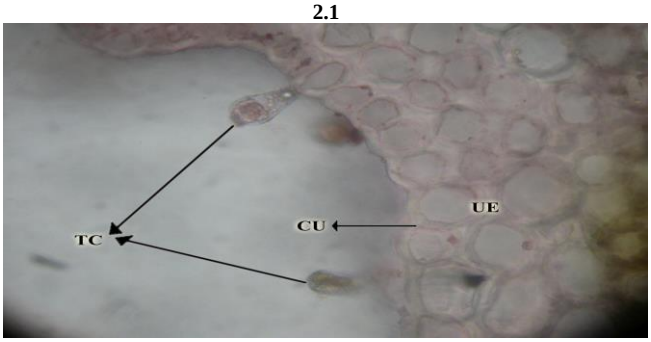

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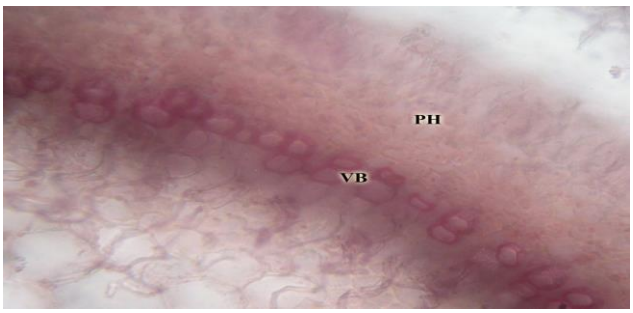

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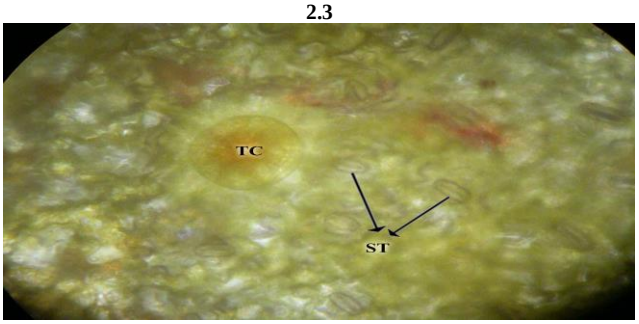

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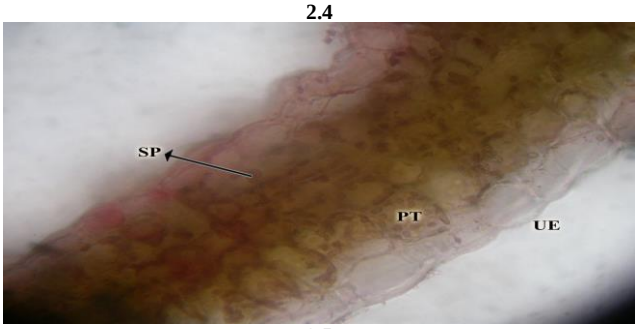

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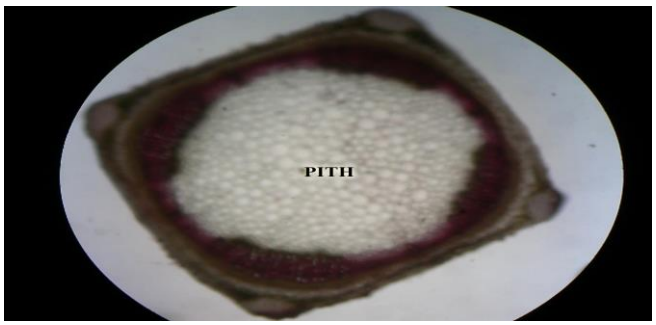
Fig. 2: Microscopical view of T. S. of *M. arvensis* leaf.
*** 2.1: Microscopical view at 10xX10x. [UE: Upper Epidermis, VB: Vascular Bundle, LE: Lower Epidermis]**
*** 2.2 and 2.3: Microscopical view at 10xX40x. [TC: Trichomes, CU: Cuticle, UE: Upper Epidermis, VB: Vascular Bundle, PH: Phloem]**
*** 2.4: Microscopical view of stomata at 10xX40x. [ST: Stomata, TC: Trichomes]**
*** 2.5: Microscopical view of laminar region at 10xX40x. [SP: Spongy Parenchyma, PT: Palisade Tissue, UE: Upper Epidermis]**

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 shaped vascular bundle embedded with radially arranged xylem tissues, thin walled and polygonal 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 **s**tem showed quadrangular in out line, 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 uni to multi 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



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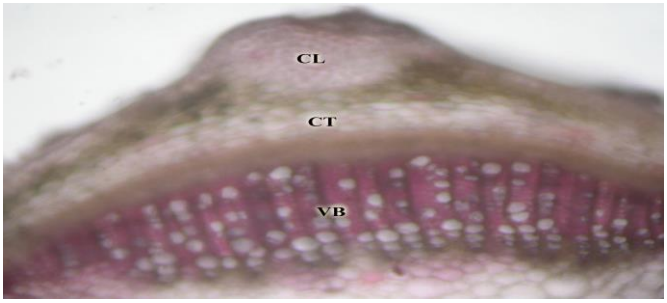

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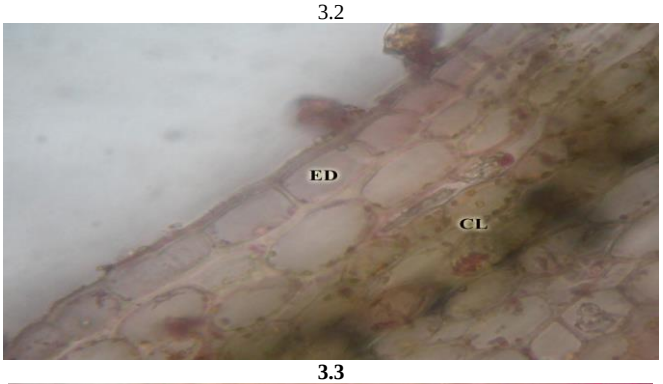

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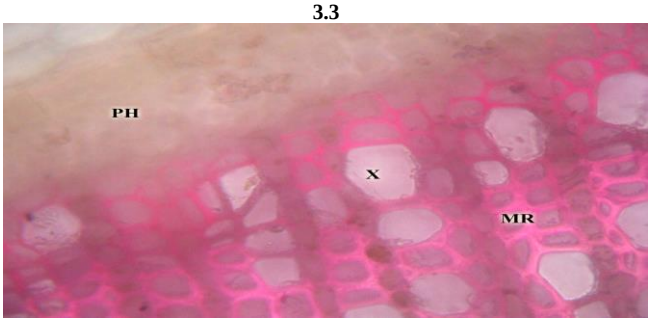

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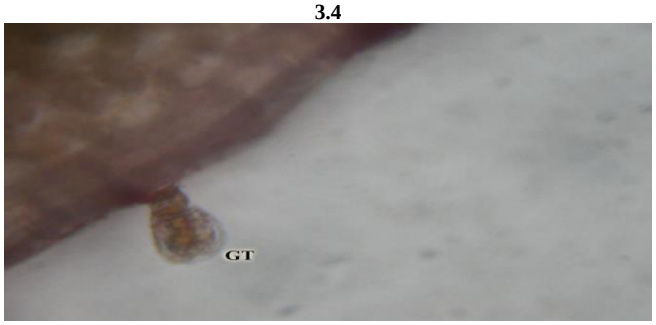

3.5

Fig. 3: Microscopical view of T. S. of *M. arvensis* stem.
*** 3.1: Microscopical view at 10xX10x.**
*** 3.2: Microscopical view of angular region at 10xX10x. [CL: Collenchyma, CT: Cortex, VB: Vascular Bundle]**
*** 3.3, 3.4 and 3.5: Microscopical view at 10xX40x. [ED: Epidermis, CL: Chlorenchyma, PH: Phloem, X: Xylem, MR: Medullary Ray, GT: Glandular Trichome]**

collenchymatous cells, parenchymatous cells with reddish tannin contents.

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, fungi 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.

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