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Pharmacognostic evaluation of leaves of *Ocimum basilicum linn*: the Lamiaceae family

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ABSTRACT

Pharmacognostic investigation of the leaves of *Ocimum basilicum L* was carried out to determine its macromorphological, micromorphological, and preliminary phytochemical screening profiles. The anatomy of the leaves of *Ocimum basilicum* reveals single, wide and bowl shaped vascular strand, diacytic type of stomata; peltate types of glandular trichomes seen on the epidermis of the lamina. The phloem is seen as small discrete masses and xylem elements are angular and narrow. Under powder microscopy epidermal trichomes are seen predominantly. Preliminary phytochemical analysis shows the presence of triterpenoids, flavanoids and polyphenols. These observations could be of immense help to validate the several medicinal properties of Ocimum basilicum L and would also add value in the botanical identification and standardization of the leaves of the plant in the crude form for neutraceutical purposes.

Keywords: *Ocimum basilicum L*, macromorphological, micromorphological and phytochemical screening

INTRODUCTION

Medicinal plants are a blessing to humanity. Traditional medicinal preparations are being used since the times of 'Charaka' and 'Sushruta'. One such perennial herb with therapeutic potential is 'Ocimum basilicum L' popularly known as 'Sweet basil' used in both 'Ayurvedic' and 'Unani' system of medicine (Alia, 2012). It is a culinary herb that belongs to the 'Lamiaceae family' and is well distributed throughout India. O.basilicum is known for its antioxidant, antimicrobial, antifibrotic and anticancer properties (Politeo, 2007). The aromatic leaves of O.basilicum contain a rich reservoir of phenolic compounds, flavanoids and volatile oils (monoterpenoids and sesquiterpenoids) (Juliani, 2002). It is used as a treatment modality for various ailments such as poor digestion, nausea, migraine, depression, insomnia, kidney malfunction and skin infections (Muafia Shafique, 2011; Biljana, 2011). Inspite of the various medicinal uses attributed to this plant; there are not many pharmacognostical reports on the leaves of this plant in particular. Hence, our work deals with morphological (macroscopic and microscopic characteristics) and preliminary phytochemical screening of the leaves of basil from Tuticorin, Tamil Nadu. This information could be of immense help to researchers working with the leaves of *O.basilicum* in in-vitro and in-vivo experiments.

MATERIALS AND METHODS

Collection of Specimen: The leaves of O.basilicum were collected from Tuticorin, Tamil Nadu. The plant was taxonomically identified by Dr.P.Jayaraman, Plant Anatomy Research Centre, Chennai, Tamil Nadu, India. The macroscopic features were described by Dr.D.Chamundeeswari, Principal, College of Pharmacy, Sri Ramachandra University, Chennai. Healthy plants were collected in the month of April and processed for evaluation. Different parts of the leaves were sectioned with 15mm Blood pressure blade and immediately fixed in FAA (Formalin (5ml) + Acetic acid (5ml) + 70% Ethyl alcohol (90ml). After fixing the specimen for 24hours; they were dehydrated in graded series of Tertiary-Butyl alcohol (TBA) following which the specimens were infiltrated with paraffin wax(melting point 58-60ºC) and casted into paraffin blocks.

Sectioning: Rotary microtome was used to section the paraffin embedded specimens. The section thickness was 10-12µm.The dewaxed sections were stained with Toluidine blue and Safranin. Paradermal sections were taken for studying venation pattern, trichome distribution and stomatal morphology. The leaf sections were then cleared with 5% Sodium Hydroxide (NaOH) or epidermal peeling by partial maceration by Jeffrey's maceration fluid. Temporary preparations mounted with glycerine were made for cleared materials. Clearing with NaOH was carried out for powdered materials which were later mounted in glycerine medium after staining. Different cell components were studied and measured.

Photomicrographs: Micrographs of tissues were supplemented along with microscopic description wherever necessary. Photographs were taken with Nikon lab 2 microscopic unit at different magnifications. Bright field microscopy was used for normal observations.

Preliminary phytochemical analysis: Shade dried and powdered plant samples were extracted with ethanol via the maceration technique over 24 hours, 48 hours and 72 hours. The pooled extracts were further filtered and concentrated using rotary flash and water bath. The leaf extracts were subjected to preliminary qualitative biochemical tests for identification of various phytochemical constituents as per standard procedures (Harbone, 2005; Kokate, 2003).

RESULTS AND DISCUSSION

Morphological /Macroscopic characteristics (Fig A&B): Leaves – simple, spathulate, oblong to pandurate, acute towards the apex, slightly laviolate in shape. Mature leaves measuring 8.4cm in length (petiole to apex); 2.3cm (middle) x 0.3cm (base) x 0.1cm (apex) in width , petiole – 1.5cm. The immature leaves measuring 3.2cm in length (petiole to apex), 1.4cm (middle) x 0.3cm (base) x 0.1cm (apex) in width, and petiole- 1.2cm. The margins are straight and entire on one side and dentate and serrated on the other. The texture is hairy and spongy. The leaves have a simple, alternate and non anastomosing venation reaching the margins. Upper surface is dark green in colour when compared to the lower. Dotted glandular trichomes are more on the lower surface when compared to the upper surface. The mid rib is prominent on the lower side of the leaf when compared to the upper. The petiole is winged. The leaves are strongly aromatic and pleasant; they are warm, aromatic and slightly pungent in taste.

Microscopic Characteristics (Fig 1-a, b): The leaf consists of adaxial concavity and abaxial prominent midrib with the lamina directed towards upper side (Fig.1-a). The midrib is somewhat bowl shaped in sectional view (Fig.1-b).It is 1.5mm wide and 60µm thick. The epidermal layer is thin and the epidermal cells are small, thick walled and have eclinate outer walls. The ground tissue is parenchymatous and the cells are small, polygonal and compact. The vascular strand is single, wide and bowl shaped (Fig.1-b).It is 150µm thick and 750µm wide. The vascular strand consists of several short, three or four cells long, angular narrow xylem elements with wide parenchymatous gaps in between. The phloem elements are located along the lower end of the xylem strand. The phloem is seen in small discrete masses.

Lamina (Fig.1-c): The lamina is dorsi-ventral. It consists of distinct dorsal and ventral sides. The adaxial epidermis is slightly thick and the cells are elliptical and cylindrical; the cell wall is thin and smooth. The abaxial epidermis includes thin and squarish small cells. The mesophyll tissue is differentiated into adaxial band of single layer of cylindrical palisade cells which are compactly arranged. The abaxial zone consists of four or five layers of small, spherical or lobed loosely arranged spongy parenchyma cells. The lamina is 150µm thin.

Epidermal cells and stomata (Fig.2-a, b): The epidermal tissue of the lamina was studied from the paradermal sections. The epidermal cells, as seen in surface are large and have highly undulate anticlinal walls, so that cells appear amoeboid in outline (Fig.2-a). Stomata are seen only on abaxial epidermis. The stomata are diacytic type (Fig.2-b). The guard cells have two subsidiary cells which lie on the opposite poles; the common walls of the two subsidiary cells lie at right angles to the long axis of the guard cells. The guard cells are 20 X 30µm in size.

Glandular trichomes (Fig.3-b): Peltate types of glandular epidermal trichomes are frequently seen on the epidermis of the leaf. The trichomes have a short stock – cell which is buried in circular shallow pits of the epidermis. At the tip of the stalk- cell, a plate of circular darkly stained secretory cells is present. The epidermal cells surrounding the cell the bears the stalk of the gland form a circular radiating rosette of cells. The secretory body of the trichome is 50µm in diameter.

Venetian (Fig.3-a; 4-a, b): Venetian pattern of the lamina was studied from paradermal sections (Fig. 3-a) and from the cleared and stained lamina (Fig. 4-a, b). The Venetian pattern is sparsely reticulate. The vein-islets are wide and polygonal in outline. Vein-terminations are present in almost all islets. The terminations are long and slender; they are slightly wavy and are either unbranched or branched once forming two equal branches (Fig.4-b).

Petiole (Fig 5), (Fig.6) and (Fig.7): Petiole was studied at proximal part (Fig.5), middle part (Fig.6) and distal part (Fig.7).The proximal petiole is boat shaped with shallow concavity on the adaxial side. It is 1.1mm thick and 2.5mm wide (Fig.5-a). It consists of epidermal layer of small thick walled cells. The ground tissue is homogenous and parenchymatous, polygonal cells. The vascular system includes a median, large, bowl shaped strand and three smaller, less prominent strands on each wing of the petiole. Xylem elements of the main strand are small, angular and occur in short vertical strands. Phloem elements are in small discrete strands. (Fig.5-b)

The middle part of the petiole is deeply concave on the adaxial side (Fig.6-a). The structure of the petiole is basically similar to that of the proximal part of the petiole. There is a wide, bowl shaped collateral main vascular strand and three smaller strands located in the wing portions (Fig.6-b).

The distal part of the petiole has long unequal wings. The wings are foliar in structure. They are folded on the adaxial side forming deep narrow vertical canal. (Fig.7-a)The midrib proper has thick, collateral shaped vascular strand and three circular strands in each lateral part of the main strand. (Fig.7-b)

Epidermal trichomes (Powder Microscopy): The leaf powder was examined under the microscope to study the components found in the powder. Epidermal trichomes were frequently seen in the powder. Two types of trichomes are seen in the powder

(i) **Non-glandular trichomes (Fig.8-c) :** The non-glandular trichomes are non-secretory covering type. They occur on the surface or along the margins of the lamina. The trichomes are either unicellular or multicellular, uniserate and unbranched. The trichome is broader at the base and tapering at the tip (Fig.8-c)

(ii) **Glandular trichomes (Fig 8-a; 9-a):** Peltate types of glandular trichomes are seen in the powder. The glands are seen attached on the lamina (Fig. 8-a, b; 9-a) The gland is circular plate with a central one called stalk; at the tip of the stalk occurs unicellular spherical or hemispherical glandular body (Fig.10-a, b) The glandular trichomes either small or large, both the types occur side by side (Fig.9-a) The larger gland is 120µm in diameter; the smaller gland is 30µm in diameter.

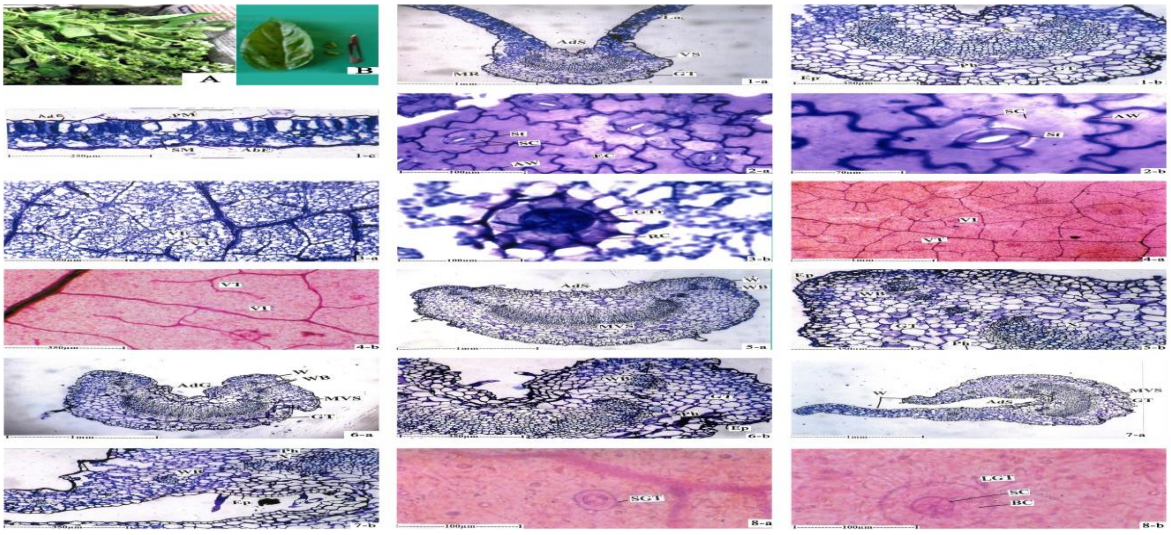
Preliminary phytochemical screening: In the preliminary phytochemical screening the ethanolic extracts showed the presence of Triterpenoids, flavanoids, glycosides, carbohydrate, polyphenols and mild amounts of tannin. These secondary metabolites have been proven in literature to be responsible for various therapeutic activities of medicinal plants (Aron, 2013). This article briefly highlights on the pharmacognostic features and screens phytochemical constituents of the leaves of *O.basilicum L*; this may render a great help in broadening its economical, pharmacological and botanical importance. The above work could also be utilized in establishing a standardized monograph of the plant.

Table.1.Phytochemical analysis of *Ocimum basilicum Linn* (OB)

Phytochemical constituents	Color Indicating the presence of active constituents	*Ocimum basilicum Linn*
Triterpenoids	Pink	+
Flavonoids	Dark yellow	+
Steroids	Green	-
Glycosides	Dark green	+
Carbohydrates	Blue	+
Polyphenols	Bluish green; Red	+
Tannins	White precipitate	Mild +

Note: The above mentioned color coding signifies the presence or absence of active principles in the plant extract.

Figure 1-8. Showing the microscopical characteristics of stem and leaf of *Ocimum basilicum*



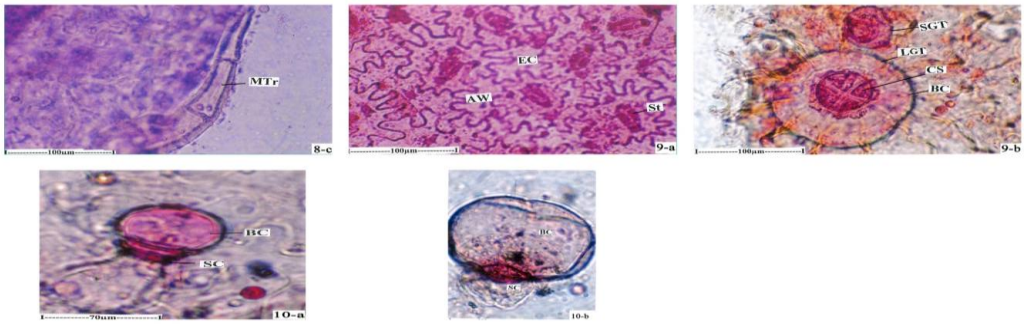


Figure 9-10. Showing the microscopical characteristics of stem and leaf of *Ocimum basilicum*

Note: Fig 1-a: TS of leaf through midrib; 1-b: TS of leaf through midrib – enlarged; 1-c: TS of lamina; 2-a: Paradermal section of the leaf epidermis showing stomata; 2-b: Single stoma – enlarged; 3-a: Paraderm section showing the venation of the lamina; 3-b Glandular trichome in surface view; 4-a: Venation pattern of the lamina; 4-bVein islets and Vein terminations – enlarged; 5-a: TS of petiole through proximal region; 5-b: TS of proximal petiole – enlarged; 6-a: TS of the middle part of the petiole; 6-b: TS of the middle part of the petiole – A sector enlarged; 7-a: TS of the distal part of the petiole; 7-b: TS of the distal part of the petiole – A sector enlarged; 8-a: Lamina showing smaller glandular trichome; 8-b: Large vascular bundle; 8-c: Marginal non-glandular trichome; 9-a: Epidermal peeling of the leaf in surface showing stomata; 9-b: Smaller and larger glands in surface view; 10-a: Isolated smaller glandular trichome; 10-b: Larger glandular trichome.

Abbreviations: AbE: Abaxial epidermis; AdE: Adaxial Epidermis; AdS: Adaxial side; Ep: Epidermis; GT:Ground tissue; La: Lamina; MR: Midrib; SM: Spongy mesophyll; VS: Vascular strand; X: Xylem; AW: Anticlival walls; EC: Epidermal cells; SC: Subsidiary cells; St:Stomata; Gtr: Glandular trichome; RC: Rosette cells; VI: Vein islets; VT: Vein termination; W: Wing; WB: Wing bundle; AdG: Adaxial Groove; MVS: Median vascular strand; BC: Body cell; LGT: Larger glandular trichome; MTr: Marginal non glandular trichome; SC: Stalk cell; SGT: Smaller glandular trichome.

CONCLUSION

O.basilicum L belonging to the Lamiaceae family also referred to as the 'King of Herbs' has been used tremendously as traditional medicine for various ailments. The leaves are rich in essential oils and secondary metabolites of therapeutic importance. Further quantification and screening of these compounds could help researchers establish a standardized drug for combating various disease processes such as diabetes, cancer and inflammatory conditions. To the best of our knowledge this study is first of its kind that primarily focuses on the leaves of *O.basilicum L*.

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