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Pharmacognostic and phytochemical studies of *Ocimum americanum*

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ABSTRACT

The present study deals with the pharmacognostic and phytochemical studies of leaves of Ocimum americanum. It includes collection, identification, microscopical and phytochemical evaluation of leaves of O. americanum. The preliminary phytochemical studies indicate the presence of volatile oils, flavonoids, carbohydrates, phytosterols, tannins and fixed oils. The sections were taken and cellular structures were studied. The T.S of leaf shows the presence of epidermis, parenchymatous cells, collateral vascular bundle, lateral vein, glandular trichomes which are peltate type and sessile. Powder microscopy of the leaf shows the presence of epidermal peelings and multicellular unbranched trichomes.

KEYWORDS *Ocimum americanum*, Lamiaceae, Volatile oils, flavanoids.

INTRODUCTION

Ocimum americanum Linn commonly called as Ocimum canum belongs to the Family: Lamiaceae (Labiatae) [1].it is generally distributed throughout India, in fields of waste lands, Plains and lower hills of India[2]. It is Common in wastelands, by arable lands; and paleotropic.The plant is a pubescent erect much branched herb, 15-60 cm high with sub-quadrangular striate branches[3]. Leaves are elliptic-lanceolate, entire, glabrous and gland dotted strongly aromatic herb; branchlets puberulous, terete to four- angular[4]. Leaves are elliptic-lanceolate, entire or faintly toothed, almost glabrous, gland-dotted[5]. Flowers are small, white, pink or purplish, in more or less closely set whorls in spiciform racemes[6].Seeds are having Nutlets with narrowly ellipsoid, punctulate black.,[7].The main chemical constituents are Volatile oils include methyl cinnamate, methylheptenone, methylnonylketone, d-camphor, citral, Ocimin, methylchavicol, linalool, nevadensin, salvigenin, beta-sitosterol, betulinic, ursolic, oleanolic acids, flavanoids, pectolinarigenin-7-methylether and nevadensin. Polysaccharides

composed of xylose, arabinose, rhamnose and galacturonic acids [8]. The main uses of *Ocimum americanum* are antimicrobial, antioxidant, anthelmintic and anti diabetic[9]

EXPERIMENTAL SECTION

The plant selected for the present study is *Ocimum americanum* Linn belongs to the family Lamiaceae was collected and authontificated by the botanist Dr.P.Jayaraman, M.Sc., Ph.D. Director Plant Anatomy Research center (PARC), Tambaram, Chennai.

Microscopical study of the leaves [10-21]

Collection of specimen

The plant specimens for the proposed study were taken to select healthy plants and normal organs[10]. The required samples of leaves were cut and removed from the plant and fixed in FAA (formalin -5ml+acid acid 5ml +70%ethyl alcohol 90ml). After 24hrs of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol as per the schedule given by sass, 1940 .infiltrations of the specimens was carried by gradual addition of paraffin wax (mp58-60) until TBA solution attained super saturation [11].The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. the thick nests of the section were 10-12 micro meter[12]. Dewaxing of the section was by customary procedure (Johnson, 1940)[11]. The section was stained with Toluidine blue as per the method published by O'Brien et al (1964)[15].since Toluidine blue is a polychromatic stain the staining results were remarkably good, and some phytochemical reactions were also obtained[16]. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and fast green and I+KI(for starch)[13,14]

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5%NaoH (OR) epidermal pealing by partial maceration employing Jeffery's maceration fluid (Sass, 1940)[21] were prepared. Glycerin mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaoH and mounted in glycerin medium after staining. Different cell components were studied and measured[20].

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units[17]. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells polarized light microscope was employed. Since these structures have birefrigent property, under polarized, UV light they appear bright against dark back ground[18]. Magnifications of the figures are indicated by the scale bars. Descriptive terms of anatomical features are as given in the standard anatomy books (Esau, 1964)[19].

Identification of the plant constituents by phytochemical tests: [22, 23]

Ethanolic extract, chloroform extract, pet ether extract and aqueous extract are subjected to various preliminary phytochemical analysis to test for the presence or absence of various phytoconstituents by the following tests.

1. Test for alkaloids:
To the extract dilute hydrochloric acid will be added and filtered. The filtrate will be treated with various alkaloidal reagents

a) Mayer's test:
The filtrate will be treated with Mayer's reagent: appearance of cream colour indicates the presence of alkaloids.

b) Dragendroff's test:
The filtrare will be treated with Dragendroffs reagent: appearance of reddish brown precipitate indicates the presence of alkaloids.

c) Hager's test:
The filtrate when treated with Hager's reagent, appearance of yellow colour precipitate indicates the presence of alkaloids.

2) Test for carbohydrates and reducing sugar
The small quantities of the filtrate will be dissolved in 4ml of distilled water and filtered. The filtrate will be subjected to

a) Molisch's test:
A small portion of the filtrate will be treated with Molisch's reagent and sulphuric acid. Formation of a violet ring indicates the presence of carbohydrates.

b) Fehling's test:
The extract will be treated with Fehling's reagent A and B. The appearance of reddish brown colour precipitate indicates the presence of reducing sugar.

c) Benedict's test:
The extract will be treated with Benedict's reagent; appearance of reddish orange colour precipitate indicates the presence of reducing sugar.

d) Barfoed's test:
The extract will be treated with barfoed's reagent and heated. Appearance of reddish orange colour precipitate indicates the presence of non reducing sugars.

3) Test for steroids:
Libermann burchard's test:
The extract will be treated with 3ml of acetic anhydride, few drops of glacial acetic acid followed by a drop of concentrated sulphuric acid. Appearance of bluish green colour indicates the presence of steroids.

4) Test for proteins:
a) Biuret test:
The extract will be treated with copper sulphate solution, followed by addition of sodium hydroxide solution; appearance of violet colour indicates the presence of proteins.

b) Millon's test:
The extract will be treated with Millon's reagent; appearance of pink colour indicates the presence of proteins.

5) Test for tannins:
The extract will be treated with 10% lead acetate solution; appearance of white precipitate indicates the presence of tannins.

6) Test for phenolic compounds:
a) The extract will be treated with neutral ferric chloride solution; appearance of violet colour indicates the presence of phenolic compounds.
b) The extract will be treated with 10% sodium chloride solution; appearance of cream colour indicates the presence of phenolic compounds.

7) Test for flavonoids:
a) 5ml of extract will be hydrolyzed with 10%sulphuric acid and cooled. Then, it will be extracting with diethyl ether and divided in to three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution will be added to the first, second and third test tubes respectively. In each test tube. Development of yellow colour demonstrated the presence of flavonoids.

b) Shinoda's test:
The extract will be dissolved in alcohol, to which few magnesium turnings will beaded followed by concentrated HCL drop wise and heated, and appearance of magenta colour shows the presence of flavonoids.

8. Test for gums and mucilage:
The extract was treated with 25 ml of absolute alcohol, and filtered. The filtrate will examine for its swelling properties.

9. Test for glycosides
When a pinch the extract was treated with glacial acetic acid and few drops of ferric chloride solution, followed by the addition of conc. Sulphuric acid, formation of ring at the junction of two liquids indicates the presence of glycosides.

10. Test for saponins
Foam test
About 1 ml of the extract was diluted to 20 ml of with distilled water and shaken well in a test tube. The formation of foam in the upper part of test tube indicates the presence of saponins.

11. Test for Triterpenoids
The substance was warmed with tin and thionyl chloride. Pink colour indicates the presence of triterpenoids.

RESULTS

Anatomy of the leaf
The leaf has prominent and thick abaxial midrib and lateral veins. The lamina is thin with wide shallow glandular pits (Fig:1 and 2) the midrib is 350 µm thick and 400µm wide. It consists of a short, semi-circular adaxial part and wide, thick abaxial .The epidermis is thin and continuous comprising small, thick walled squarish cells. The ground tissue abaxial part is collenchymatous and adaxial part is parenchymatous. The cells are angular, thin walled and compact.

The **vascular system** includes a single, horizontally, oblong, collateral vascular bundle. The bundle is 70µm thick and 180µm wide. The vascular bundle consists of about 10 short, parallel line of xylem elements which are angular and thick walled. The metaxylem elements are 20µm in diameter. Phloem elements occur in small clusters of three to five elements along the lower end of xylem segment (Fig: 3)

Lateral vein (Fig: 4): the lateral vein is slightly concave on the adaxial side and prominently semi-circular on the abaxial side. It is about 200µm in thickness. The abaxial part consists of thick walled epidermal layer and two layers of wide, compact, parenchyma cells. The vascular strand of the lateral vein comprises two short lines of xylem elements and small group of phloem elements.

Leaf margin (Fiig: 5and7) the marginal part of the lamina is semi-circular and the epidermal cells are dilated and angular in out line. The cuticle is very thick. The mesophyll tissue consists of palisade and spongy parenchyma similar to middle part of the lamina.

Lamina (Fig: 6-8): lamina is thin and uneven in surface due to the presence of shallow pit (Fig: 4 and 6). The abaxial epidermis is broad with thick walled, rectangular cells. The cells are 20µm in thickness. The abaxial epidermis is thin with narrow, rectangular (Fig: 8). The mosophyll tissue consists of a single upper layer of narrow, loosely arranged vertical cylinders of palisade cells and two or three layers lobed spongy parenchyma cells.

Both on the lower and upper epidermal layer there are wide, shallow pits in which sessile, peltate glandular trichomes are situated (Fig: 6,7).

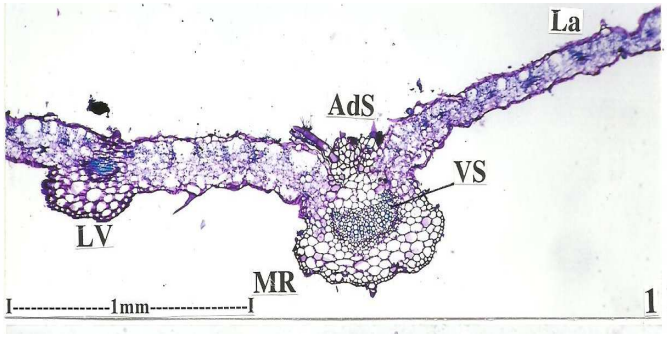

Fig.1: T,S of leaf through midrib with lamina

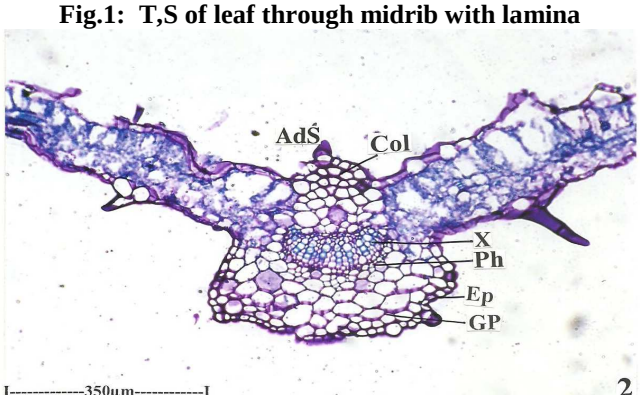

Fig. 2: T.S of midrib with lamina magnified

(Ads- Adaxial side; Col-Collenchyma; Ep-Epidermis; GP-Ground parenchyma; La- lamina; Lv- Lateral vein; MR-midrib;Ph-phloem; Vs-vascular strand ; x- xylem).

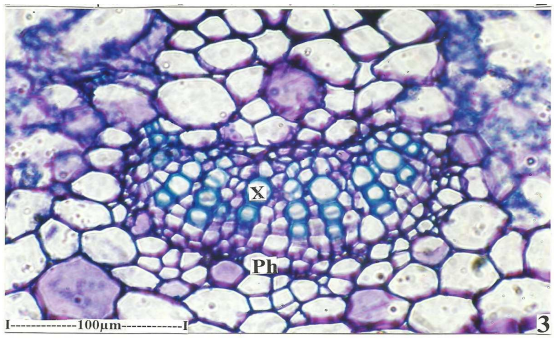

Fig.3: T.S of midrib vascular bundle magnified

(Ads- Adaxial side; Col-Collenchyma; Ep-Epidermis; GP-Ground parenchyma; La- lamina; Lv- Lateral vein; MR-midrib;Ph-phloem; Vs-vascular strand ; x- xylem).

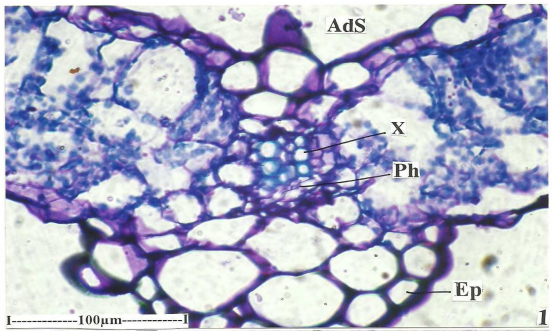

Fig.4: t.s. Of lamina through lateral vein.

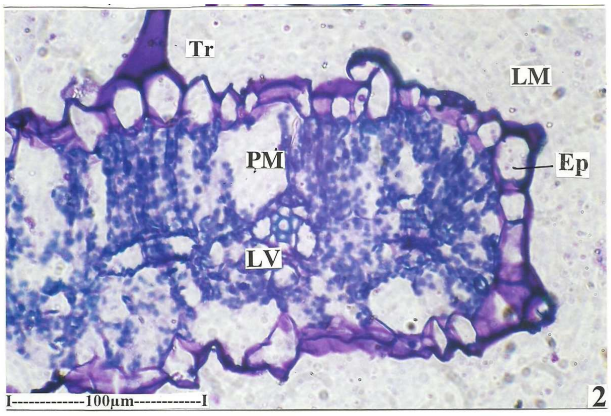

Fig.5: T.S. of leaf margin.

(Ads- adaxial side; EP-Epidermis; LM-Leaf margin; Lv-Lateral vein; Ph-Phloem; PM-Palisade mesophyll; Tr-trichome; X- Xylem).

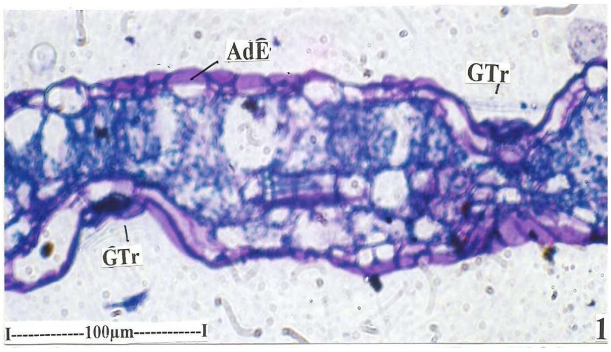

Fig.6: T.S. of lamina showing glandular trichomes on the abaxial and adaxial side.

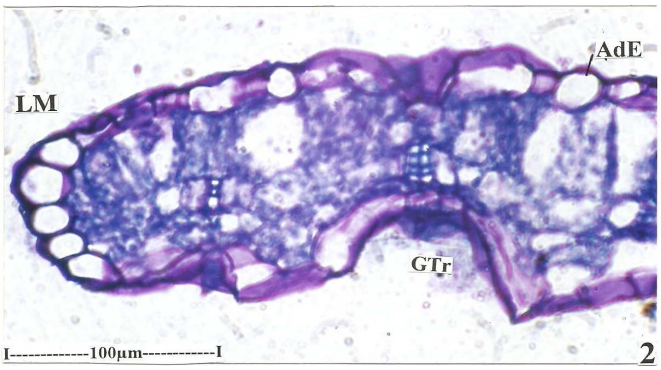

Fig.7: T.S. of lamina with glandular trichome on the abaxial epidermis
(AbE-Abaxial epidermis; AdE-Adaxial epidermis; GTr-Glandular trichome; LM- Leaf margin; LV-Lateral vein; Tr-Trichome; PM-Palisade mesophyll; SM-Spongy mesophyll)

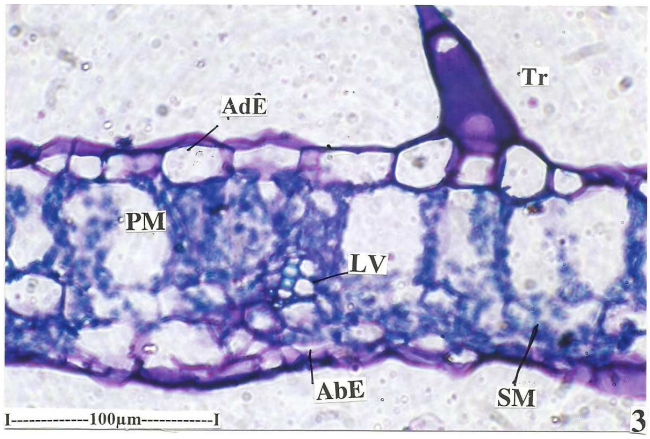

Fig.8: T.S.of lamina with showing non-glandular trichome on the adaxial epidermis.
(AbE-Abaxial epidermis; AdE-Adaxial epidermis; GTr-Glandular trichome; LM- Leaf margin; LV-Lateral vein; Tr-Trichome; PM-Palisade mesophyll; SM-Spongy mesophyll)

Powder microscopy of the leaf: The leaf powder when examined under the microscope exhibit the following inclusions.

Epidermal peeling (Fig: 9): thin pieces of epidermal peelings are visible in the powder. In surface view the epidermal cells have thin, highly, wavy anticlinal walls; the epidermal cells appear amoeboidal in outline (Fig: 9) Stomata are also seen in the powder. Stomata are "diacytic" type.

Covering or non-glandular trichomes (Fig: 10, 11):

Multicellular, uniseriate, unbranched trichomes are frequently seen in the powder. They are three to five cells. The basal cell is wider, and the upper cells are gradually narrow and tapering in to pointed tip. The basal cell is 40µm thick and the terminal cells are up to 10µm thick. The surface of the cell wall have minute cuticular echinate out growths (Fig: 10,11).

Glandular trichome (Fig: 12, 13):

Glandular trichomes are also frequently seen in the powder. They are peltate type and sessile. The glands are embedded in the epidermal pits. The gland has short, horizontally oblong basal cell and semi-circular body comprising four to eight secretory cells. The glandular trichome is 80µm wide.

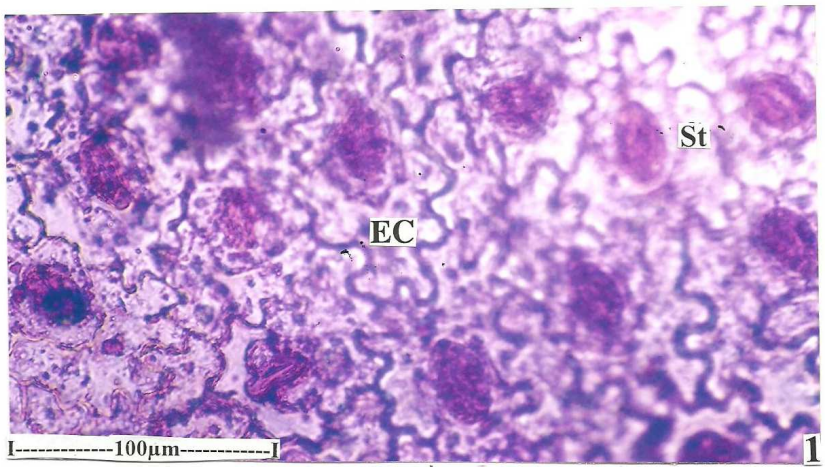

Fig.9: Fragment of abaxial epidermis with stomata

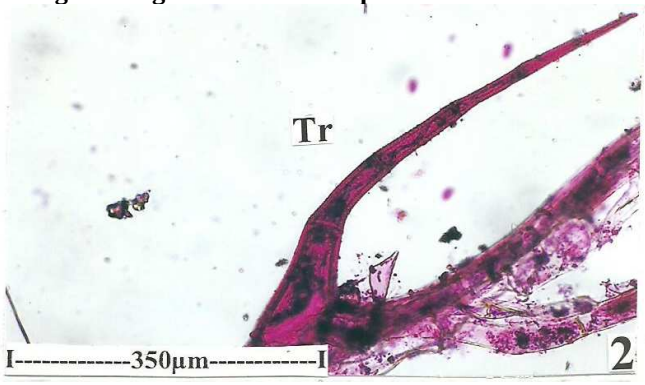

Fig.10: Non-glandular trichome in the leaf powder
(BC- Basal cell of trichome; EG-Epidermal cell; St-Stomata; TC-Tip cell of trichome; Tr-Trichome

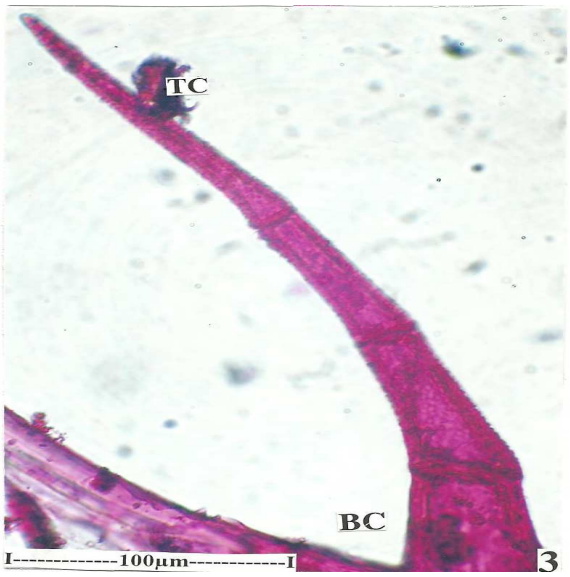

Fig.11: Non-glandular trichome in the leaf powder magnified
(BC- Basal cell of trichome; EG-Epidermal cell; St-Stomata; TC-Tip cell of trichome; Tr-Trichome).

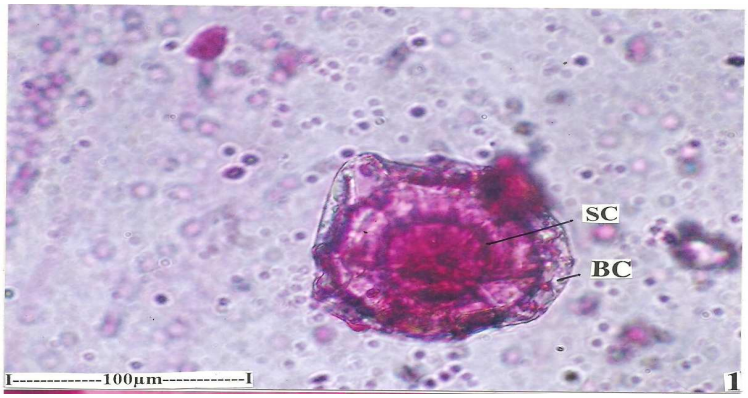

Fig.12: A detected glandular trichome in surface view

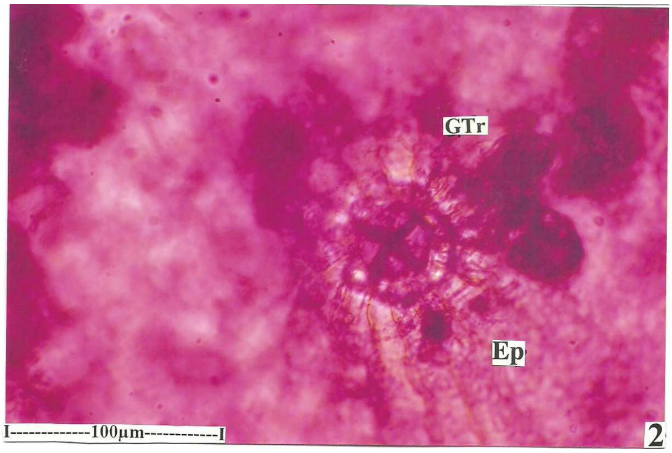

Fig.13: A gland attached on the epidermal pit.
(BC-Basal cell; Ep- Epidermis; GTr-Glandular trichome; Sc-Secretory cell)

Preliminary Phytochemical Screening of Different Extracts of Leaves of *Ocimum americanum* Linn

S.No	Test	Alcoholic Extract	Aqueous Extract	Chloroform Extract	Petroleum Ether Extract
1	alkaloids	-	-	-	-
2	carbohydrates	+	+	-	-
3	glycosides	-	-	-	-
4	phytosterols	-	-	+	+
5	fixed oils	-	-	+	+
6	saponins	-	-	-	-
7	tannins	+	-	+	-
8	proteins and amino acids	-	-	-	-
9	gums and mucilage	-	-	-	-
10	flavanoids	+	+	-	-
11	terpenoids	-	-	+	+

(-)=Absent
(+) =Present

DISCUSSION

Pharmacognostic studies

The plant O.americanum was pharmacognostically identified by studying its microscopical characters with main focus on its leaf. The leaf has prominent and thick abaxial midrib and

lateral veins. The lamina is thin with wide shallow glandular pits (Fig: 1) the midrib is 350 µm thick and 400µm wide. It consists of a short, semi-circular adaxial part and wide, thick abaxial .The epidermis is thin and continuous comprising small, thick walled squarish cells. The ground tissue abaxial part is collenchymatous and adaxial part is parenchymatous. The cells are angular, thin walled and compact. The vascular system (Fig: 3) includes a single, horizontally, oblong, collateral vascular bundle. The lateral vein (Fig: 4) is slightly concave on the adaxial side and prominently semi-circular on the abaxial side. The marginal part **(**Fig: 5 and 7) of the lamina is semi-circular and the epidermal cells are dilated and angular in out line. Lamina is thin and uneven in surface due to the presence of shallow pit (Fig: 4 and 6). The abaxial epidermis is broad with thick walled, rectangular cells. The cells are 20µm in thickness. The adaxial epidermis is thin with narrow, rectangular.

Powder analysis

Powder microscopy of the leaf (Fig: 9-13) shows that (Fig: 9) thin pieces of epidermal peelings are visible in the powder. Multicellular, uniseriate, unbranched trichomes (Fig: 10, 6) are frequently seen in the powder. Glandular trichomes (Fig: 4, 12) are also frequently seen in the powder. They are peltate type and sessile.

CONCLUSION

The pharmacognostic studies include the microscopic characters and powder analysis for the leaves of *Ocimum americanum* was performed. It shows the presence of epidermis, parenchymatous cells, collateral vascular bundle, lateral vein, glandular trichomes and multicellular unbranched trichomes. The preliminary phytochemical studies for different extracts of *Ocimum americanum* show the presence of volatile oils, flavonoids, phytosterols, carbohydrates and tannins.

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