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PHARMACOGNOSTIC, PRELIMINARY PHYTOCHEMICAL AND TLC FINGER PRINT PROFILE INVESTIGATION ON *OCIMUM GRATISSIMUM*

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ABSTRACT

Pharmacognostic evaluation, preliminary phytochemical investigation and chromatographic profile on leaf of *Ocimum gratissimum* was carried out to determine macroscopic, microscopic, physical parameters, phytochemical constituents and standardization of mobile phase for thin layer chromatography. A macroscopic study indicates plant was 190-210 cm long, Stem erect, much branched, quadrangular woody at base and Bark grayish brown in color. Leaves are lanceolate to oblong. Length 13.5-15cm, width 5.2-6.8 cm, margin crenate-serrate, surface gland dotted and pubescent on both sides, venation pinnate. Microscopic study in leaf and stem showed presence of multicellular covering and glandular trichomes, collenchymatous cells, vascular bundles, palisade cells and spongy tissues. Finger print profiles of various extracts were analyzed in different mobile phases. Seven spots were seen in methanolic (MeOH) extract in a mobile phase of CHCl₃: MeOH (17:3) and eight spots in a mobile phase of CHCl₃: Ethylacetate: GAA in a ratio of (7:2:1). Preliminary phytochemical investigation showed the presence of alkaloids, saponins, sterols, tannins, carbohydrates and flavonoids in aqueous extract, alkaloids, saponins sterols, triterpenoids, carbohydrates and flavonoids in methanolic extract, Sterols, triterpenoids, carbohydrates and flavonoids in chloroform extract and sterols and carbohydrates in petroleum ether extract. These findings will be useful towards establishing Pharmacognostic standards on identification, purity, quality and classification of plant, which is gaining relevance in plant drug research.

Introduction

Genus *Ocimum* (Family: Lamiaceae) is an important source of many essential oils and aroma chemicals useful in perfumery and cosmetic industries. The genus is well represented in the warmer parts of both the hemispheres from sea level to 1800 m. The maximum numbers of species are from the tropical rain forests of Africa. *Ocimum* species have been used in traditional systems of medicine. In Ayurveda, the plant has been used in vomiting, fits, "vata", "kapha" and skin diseases, erysipelas, inflammations. In Unani system of medicine, the plant has been used as carminative, aphrodisiac, useful in diseases of brain, heart, liver and spleen, removes foul breath, strengthens the gums, good for griping and piles. The inflorescence has been prescribed for snake bite. A decoction of the mucilaginous seeds is used as a demulcent.¹ It is also used in aches of ear and stomach, as an anesthetic, antiseptic, arthritis,

bactericide, bronchitis, cold, cough, delirium, diaphoretic, dysentery, dysmenorrhea, ejaculation, epistaxis, fever, flu, intoxicant, itch, lumbago, nausea, ophthalmic disorders, pneumonia, purgative, insect repellent, stimulant, vermifuge, vertigo. Topical formulation of *Ocimum* oil is used in the management of *Acne vulgaris*.² The plant is used as a potential phytotherapeutic agent in some fungal diseases and for the control of fungi in the environment³, antihypertensive⁴. *Ocimum gratissimum* have also been reported as sedative⁵, cytotoxic⁶, antidiarrhoeal⁷, blood coagulating agent⁸. It is also useful in the treatment of mental illness⁹, malaria.¹⁰ The present study has been focused to provide some pharmacognostic characters which might be helpful in separation of *Ocimum gratissimum* from other *Ocimum* species.

Materials and Methods

Plant Material

Plant of *O. gratissimum* were collected from the nursery of National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab, India, in the month of April. The material was authenticated with existed specimen (912/96) by Dr. H.B Singh, Head of Raw Materials Herbarium & Museum, National Institute of Science Communication and Information Resources and a voucher specimen was deposited at Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, Punjab, India for future references.

Preparation of Plant Extract

The aerial part of plant material was shade dried and grinded mechanically. Powder was extracted in a soxhlet apparatus for 10-14 h with petroleum ether, chloroform, methanol and water successively.

The extracts were dried using Buchi rotavapour and kept in deep freezer for further studies.

Pharmacognostic Investigation

The macroscopic and microscopic studies were determined using prescribed method.¹¹ The leaves of plant were boiled with suitable solvent for surface studies and quantitative microscopically observation of leaf was done using compound microscope.

Physio-chemical parameters

Physio-chemical values such as the percentage of total ash, acid insoluble ash, water-soluble ash, water and alcohol soluble extractive values were calculated as per the Indian Pharmacopoeia.¹²

Preliminary phytochemical investigations

Qualitative tests in the petroleum ether, chloroform, methanol and aqueous extracts for the different phytochemical constituents, as

glycosides, flavonoids, alkaloids, carbohydrates, saponins, phytosterols, amino acids, tannins, triterpenoids, steroids and phenols were carried out.¹³

TLC Fingerprint profile

1mg of each extract was dissolved in respective solvents for Thin layer chromatography. 10µl of the respective sample was applied on pre coated TLC plates (Merck). The TLC plates were kept in in Kamag chromatographic chamber which is fully saturated with mobile phase. The mobile phase was changed according to polarity of solvent. The TLC plates were visualized using UV chamber. The R_f value of various extracts in the different mobile phases were calculated.

Results

Pharmacognostic Evaluation

Macroscopy

- Plant was 190-210 cm tall
- Stem erect, much branched, quadrangular woody at base
- Bark greyish brown in color
- Branches opposite, quadrangular, green and pubescent when young (Fig1)
- Leaves opposite, simple, green, lanceolate to oblong. Length 13.5-15cm, width 5.2-6.8 cm, petiole 4-5.3 cm, margin crenate-serrate, surface gland dotted and pubescent on both side, venation pinnate, petiole light green in color and pubescent (Fig.2)
- Inflorescence verticillaster, arranged in a terminal, branched racemes (10-17 cm) in close whorls
- Flower small, irregular, white
- Fruit in form of a small capsule containing 4 nutlets
- Nutlets subglobose and brown in color



Fig 1: *Ocimum gratissimum* L. Plant

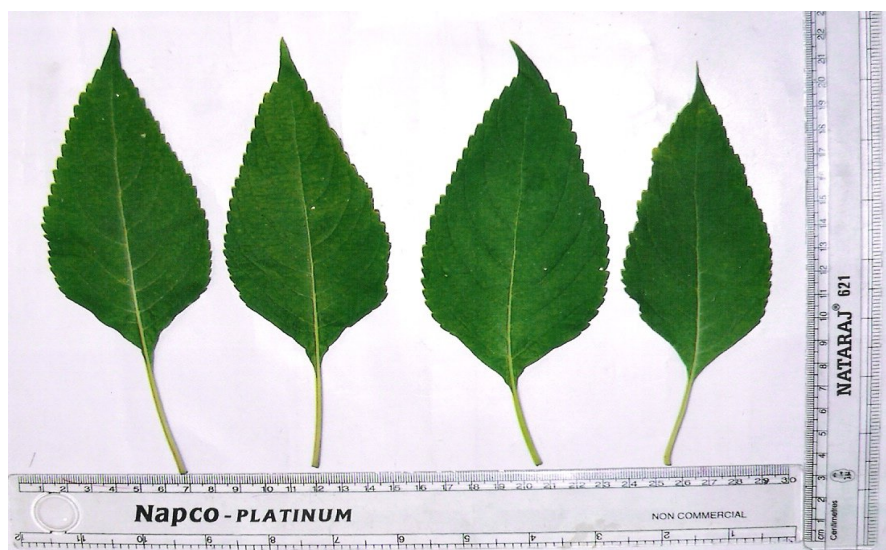


Fig 2: Leaves of *Ocimum gratissimum* L.

Microscopy

A transverse section of leaf (Fig.3) showed

- Single layered epidermis
- Multicellular covering trichomes and glandular trichomes
- Collenchymatous cells
- Vascular bundle consisting of xylem and phloem
- Palisade cells and spongy tissue

A transverse section of stem (Fig.4) showed

- Shape of section was rectangular
- Compressed bark cells followed by single layered epidermis
- Multicellular covering trichomes and glandular trichomes
- Collenchymatous cells
- Vascular bundle contains xylem and phloem
- Spongy tissue was present at centre

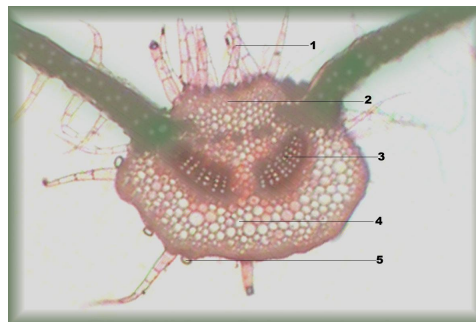


Fig 3: T.S of leaf of *Ocimum gratissimum* L.

1-Multicellular covering trichomes, 2-Collenchymatous cells, 3-Vascular bundle containing xylem & phloem, 4-Spongy tissue, 5-Glandular trichome

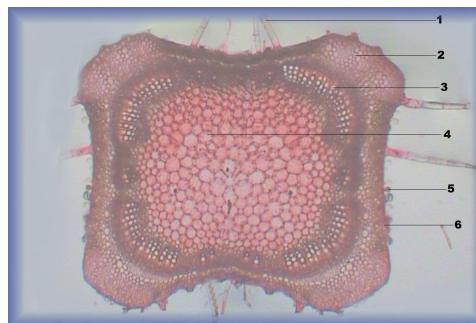


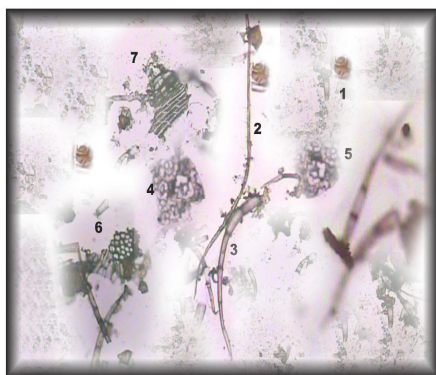
Fig 4: T.S of stem of *Ocimum gratissimum* L.

1-Multicellular covering trichome, 2-Collenchymatous cells, 3-Vascular bundle containing xylem & phloem, 4-Spongy tissue, 5-Glandular trichome, 6-Bark

Powder Microscopy

Table 1: Characteristics of Powder Material of *Ocimum gratissimum* L.

Characters	Observation (Fig.5)
Color	Greenish
Odor	Specific
Taste	Characteristic
Stomata	Diacytic
Trichomes	Glandular trichomes with multicellular head Multicellular covering trichomes
Vessels	Spiral vessels
Epidermal cell	Wavy walled epidermal cells were present
Collenchyma	Collenchymatous cells with intercellular spaces
Fiber	Thin walled fibers with pointed end



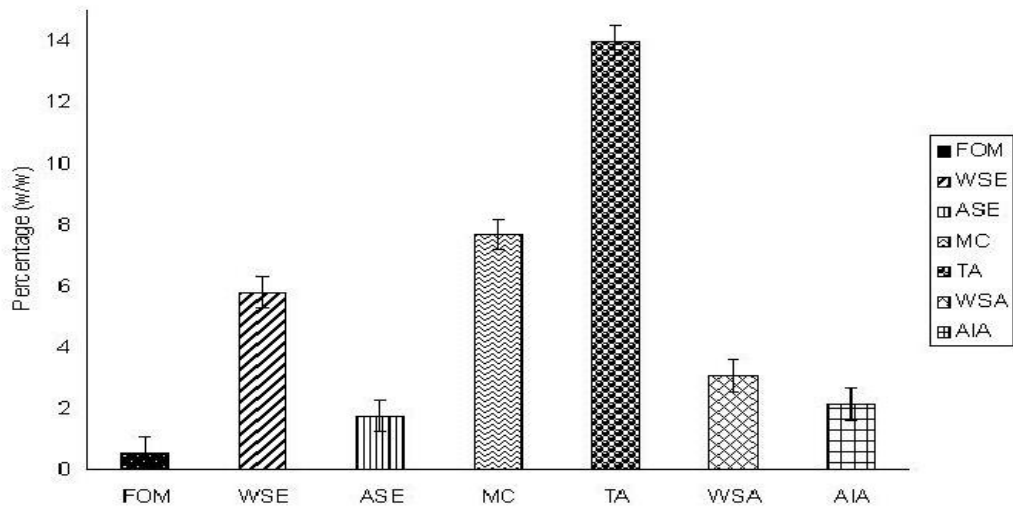
1-Glandular trichome with multicellular head, 2-Thin walled fiber with pointed end, 3-Multicellular covering trichome, 4-Diacytic stomata, 5-Wavy walled epidermal cells, 6-Collenchymatous cell with intercellular spaces, 7-Vessels

Fig 5: Powder Microscopy of *Ocimum gratissimum* L.

Determination of leaf surface data

Table 2: Leaf Surface Data

Leaf constant	Observations
Vein-islet number (per sq. mm)	6.66
Vein termination number (per sq. mm)	13.33
Stomatal number (per sq. mm)	
Upper surface	26
Lower surface	57
Stomatal index	
Upper surface	15.61
Lower surface	28.75
Palisade ratio	
Upper surface	9.57
Lower surface	7.93



Physio-chemical parameters

Physio-chemical values such as the percentage of total ash, acid insoluble ash, water-soluble ash, water and alcohol soluble extractive values are summarized in fig 6.

Fig.6 Physio-chemical parameters of *Ocimum gratissimum* L

FOM-Foreign organic matters
WSE- Water Soluble Extractive Value

ASE-: Alcohol Soluble Extractive Value
MC- Moisture content, TA-Total ash

WSA-Water soluble ash value

AIA-Acid insoluble ash value

TLC Finger print Profile

Finger print profiles of various extracts were analyzed in different mobile phases. Seven spots were seen in methanolic (MeOH) extract in a mobile phase CHCl₃: MeOH (17:3) and eight spots in a

mobile phase CHCl₃: Ethylacetate: GAA in a ratio of (7:2:1). The R_f values of various extracts in different mobile phases are summarized in table 3.

Preliminary phytochemical investigation

Preliminary phytochemical investigation showed the presence of alkaloids, saponins, sterols, tannins, carbohydrates and flavonoids in aqueous extract, alkaloids, saponins sterols, triterpenoids, carbohydrates and flavonoids in methanolic extract, Sterols, triterpenoids, carbohydrates and flavonoids in chloroform extract and sterols and carbohydrates in petroleum ether extract.

Discussion and conclusion

The quantitative determination of some pharmacognostic parameters is useful for setting standards for crude drugs. The physical constant evaluation is an important parameter in detecting adulteration or improper

handling of the drug. Various ash values are important to determine purity of the drug i.e. the presence or absence of foreign inorganic matter.¹⁴ Since the plant *Ocimum gratissimum* is useful in the traditional medicine for the treatment of some ailment, it is important to standardize it for use as a drug. The pharmacognostic constants for the leaf stem and aerial parts of this plant, the diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable

monograph for its proper identification. TLC finger print profile may be useful to identify various constituents. Different phytochemical detected in the plant useful for treating different ailments and having potential of providing useful drugs of human use.

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Table 3: R_f Values of Various Extracts in Different Mobile Phases

Extracts	Mobile phase	Number of spots & R _f Values
Pet. Ether Ext.	Petroleum ether: Toluene (9:1)	No spots
	Petroleum ether:Toluene: Ethylacetate (3:1:1)	(5) 0.28, 0.40, 0.62, 0.76, 0.95
CHCl ₃ Ext.	Chloroform: Methyl alcohol (9:1)	(5) 0.25, 0.49, 0.50, 0.86, 0.93
	Toluene: Ethylacetate: Acetone (8:1:1)	(1) 0.89

	Toluene: Ethylacetate: GAA (35: 4:1)	(3) 0.57,0.70,0.89
MeOH Ext.	Chloroform: Methyl alcohol (17:3)	(7) 0.37,0.15,0.30,0.42, 0.60,0.75,0.93
	CHCl ₃ : Ethylacetate: GAA (7:2:1)	(8) 0.72,0.11,0.47,0.63, 0.75,0.79,0.87,0.9
	Ethylacetate:GAA (9:1)	(4) 0.40,0.87,0.91,0.96

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