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PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDIES ON A RELIGIOUS HERB *OCIMUM TENUIFLORUM* L.

A. Ashwini [1], Kavitha C. Sagar [2] and Vijay Danapur [*1]

Department of Phytomedicine [1], Vriksha Vijnan Private Limited, 10th Main, 3rd Cross, BHCS Layout, Chikkalsandra, Bengaluru - 560061, Karnataka, India.
Department of PG studies in Botany [2], VSK University, Vinayaka Nagar, Allipur Road, Ballari - 583105, Karnataka, India.

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Correspondence to Author:
Vijay Danapur

CEO & Director,
Department of Phytomedicine,
Vriksha Vijnan Private Limited,
10th Main, 3rd Cross, BHCS Layout,
Chikkalsandra, Bengaluru - 560061,
Karnataka, India.

E-mail: drvijay.danapur@gmail.com

ABSTRACT: "The Queen of Herbs" – Tulsi (*Ocimum tenuiflorum*), is the most sacred herb of India, also known as the holy Basil, is ubiquitous in Hindu tradition. Tulsi has been revered in India for over five thousand years as a healing balm for body, mind, and spirit. It is known to bestow an amazing number of health benefits. Modern scientific research offers impressive evidence that Tulsi reduces stress, enhances stamina, relieves inflammation, lowers cholesterol, eliminates toxins, protects against radiation, prevents gastric ulcers, lowers fevers, improves digestion, and provides a rich supply of antioxidants and other nutrients. Tulsi is especially effective in supporting the heart, blood vessels, liver, and lungs and also regulates blood pressure and blood sugar. In the present study, the preliminary phytochemical studies revealed the presence of secondary metabolites *viz*., alkaloids steroids, phenols, flavones, *etc*. The Pharmacognostic profile of this plant is also studied in detail and confirms the results of IHP and API.

INTRODUCTION: *Ocimum tenuiflorum* L. is an annual herb belonging to the mint family, having at least 150 varieties. At least two types of *Ocimum tenuiflorum*, also known as *Ocimum sanctum*, Tulsi, or Holy Basil from the family Lamiaceae, has been described as the "Queen of plants" and the "mother medicine of nature" due to its perceived medicinal qualities [1]. The green type (Sri Tulasi) is the most common; the second (Krishna Tulasi) bears purple leaves.

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The plant is an erect, herbaceous, much-branched, softly hairy annual. The leaves are elliptic-oblong, acute or obtuse, entire or serrate, pubescent on both sides, and minutely gland-dotted; the flowers are in close whorled racemes, purplish or crimson.

Eshwar *et al.*, 2016 [18] worked on *Ocimum sanctum* (Linn.) extract, which demonstrated an anti-microbial activity against *Actinobacillus actino-mycetemcomitans* and *P. gingivalis*. The maximum antimicrobial potential was observed at the 6% concentration level. It was concluded that 8% concentration of *O. sanctum* (tulsi) extract showed the maximum antimicrobial activity against *A. actinomycetemcomitans* and *P. gingivalis.* Eugenol, a major component of the volatile oil present in *Ocimum tenuiflorum*, and compounds cirsilineol,

isothymusin, isothymonin, rosmarinic acid, demonstrated good antioxidant activity at 10-microM concentrations in a study by Kelm MA *et al.,* 2000 [2]. Anti-inflammatory activity or cyclo-oxygenase-1 inhibitory activity of Eugenol demonstrated 97% when assayed at 1000-microM concentrations. Compounds cirsilineol, cirsimaritin, isothymonin and rosmarinic acid displayed 37, 50, 37, 65, and 58% cyclooxygenase-1 inhibitory activity, respectively. Eugenol and compounds cirsilineol, cirsimaritin, apigenin, and rosmarinic acid demonstrated cyclooxygenase-2 inhibitory activity at slightly higher levels when assayed at 1000-microM concentrations. These results support traditional uses of *O. tenuiflorum* L.

Singh S *et al.,* 1996 [3] studied the significant anti-inflammatory activity of the fixed oil present in *Ocimum tenuiflorum* against carrageenan- and other different mediator-induced paw edema in rats.

The findings inferred that *Ocimum tenuiflorum* to be a useful anti-inflammatory agent, which blocks both the pathways, *i.e.,* cyclooxygenase and lipoxygenase, or arachidonic acid metabolism. Singh S *et al.,* 1997 [4] evaluate the anti-inflammatory activity of fatty acids suggested that linolenic acid present in *O. sanctum* fixed oil has the capacity to block both the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism and could be responsible for the anti-inflammatory activity of the oil. Singh S 1998 [5] studied comparative evaluation of the anti-inflammatory potential of fixed oil against PGE2, leukotriene, and arachidonic acid-induced paw edema in other species of *Ocimum*, *viz. O. basilicum* and *O. americanum* also containing linolenic acid in varying proportions, showed significant inhibition. The fixed oil of *O. basilicum* containing a maximum percentage of linolenic acid showed higher protection.

Caps HT2, a herbal Ayurvedic medicine formulation was containing methanolic extracts of *Ocimum tenuiflorum* along with many selected plants, was studied by Mary Nk *et al.,* 2003 [6] showed that the anti-inflammatory action of the formulation was significant ($p < 0.001$) with acute and chronic inflammations induced by carrageenan and formalin respectively in rats.

Godhwani S *et al.,* 1987 [7] studied the anti-inflammatory activity of *Ocimum tenuiflorum* that inhibited acute as well as chronic inflammation in rats as tested by carrageenan-induced pedal edema and croton oil-induced granuloma and exudate, respectively gave the anti-inflammatory response of 500 mg/kg of methanol extract and aqueous suspension was comparable to the response observed with 300 mg/kg of sodium salicylate.

A negative study was reported by Lampronti I *et al.,* 2005 [8], that the high amount of extracts from *Paederia foetida, Cassia sophera, Hygrophila auriculata,* or *Ocimum tenuiflorum* were unable to inhibit NF-kappaB/DNA interactions, which could be of interest in search of compounds active in inflammatory diseases, for which inhibition of NF-kappaB binding activity without toxic effects should be obtained.

Singh S *et al.,* 2007 [9] studied the biological activities of the seeds of Holy Basil containing the fixed oil. The oil possesses anti-inflammatory activity due to dual inhibition of arachidonate metabolism supplemented by antihistaminic activity. The anti-inflammatory activity is not dependent on the pituitary-adrenal axis. The oil possesses antipyretic activity due to prostaglandin inhibition and peripherally acting analgesic activity. The oil has been found to be effective against formaldehyde or adjuvant-induced arthritis and turpentine oil-induced joint edema in animals. Lipoxygenase inhibitory, histamine antagonistic, and antisecretory activities of the oil contribute towards an antiulcer activity. The oil can inhibit the enhancement of vascular capillary permeability and leucocyte migration following an inflammatory stimulus. The LD_{50} of the oil is 42.5 ml/kg, and long-term use of oil at 3 ml/kg dose does not produce any untoward effects in rats. The oil contains a-linolenic acid, an omega-3 fatty acid, which on metabolism produces eicosapentaenoic acid, and the same appears to be responsible for the biological activity.

The oil has hypotensive, anticoagulant, and immunomodulatory activities. The antioxidant property of the oil renders metabolic inhibition, chemo-prevention, and hypo-lipidaemic activity. The presence of linolenic acid in the oil imparts antibacterial activity against *Staphylococcus*

aureus. The oil alone or in combination with cloxacillin, a beta-lactamase resistant penicillin, has been found to be beneficial in bovine mastitis, an inflammatory disorder resulting from staphylococcal infection. The existence of anti-inflammatory, analgesic, and antibacterial activities in a single entity *i.e.*, fixed oil appears to be unique.

In view of the promising results reported so far, an attempt is made to study detailed pharmacognostic and phytochemical studies of *Ocimum tenuiflorum* L.

MATERIALS AND METHODS:

Collection: Aerial parts of *Ocimum tenuiflorum* L. are collected in and around Bangalore and the Voucher specimen was deposited in herbarium Dept of Botany, with no HGUG-535.

Preliminary Phytochemical Tests: The qualitative phytochemical tests were carried out for phenols, flavonoids, steroids, triterpenes, diterpenes, lactones, tannins, lignins, saponins, alkaloids following the methods of Gibbs (1974) [10], Kleipool (1952) [11], Peach and Tracey (1959) [12].

TLC Studies: TLC fingerprinting profile carried as per (Stahl E 1965) [13].

Physicochemical and Fluorescence Studies:

Organoleptic Characters: The present investigation comprises studies on both physical and sensory characteristics such as color, sensation, taste, oily stain, and mucilage of the species under study.

Determination of Total Ash: Two grams of powdered drug was incinerated in a sintered silica crucible by gradually increasing heat up to 450 ºC until the drug is free from carbon and then cooled. This ash kept in a dessicator for 15-20 min. and weighed using Anamed Electronic balance, India and noted down the readings [14].

$$\% \text{ ash} = z\text{-}x \times 100 / y$$

Where, weight of empty crucible = x, weight of plant material = y, weight of crucible + ash = z, weight of ash = z-x.

Determination of Acid Insoluble Ash: Total ash obtained was boiled for 15 min. in 25 ml of hydrochloric acid and filtered to collect the insoluble matter on Whatman filter paper and ignited in a sintered crucible. It was allowed to cool and then kept in a desiccator for 15 min. The residue was weighed in Anamed Electronic balance, and the acid-soluble ash was calculated using the formula.

$$\% \text{ acid insoluble ash} = z\text{-}x \times 100 / y$$

Where, weight of empty crucible = x weight of plant material = y, weight of crucible + ash = z, weight of ash = z-x.

Determination of Extractive Values: Hundred gram of powdered plant material of both plants under study were extracted with ethanol (95%) and water using cold extraction. Thus obtained extracts were allowed to dry to room temperature. After complete evaporation, weight, nature, and colour of the extracts were recorded [15].

% Extractive value = Weight of the residue obtained × 100 / Weight of plant material taken

Fluorescence Studies: The fluorescent study of the powder of *Ocimum tenuiflora* leaves was treated with chemicals such as Ethanol, Methanol, Toluene, HNO_3, concentrated H_2SO_4, concentrated HCl, and Water. The powdered materials gave different color reactions with different chemicals, and fluorescent colors of treated and untreated drugs were observed under visible and UV light, and the observations were noted [16].

Histological Studies: For the purpose of studying the microscopical characters, freehand sections were used. These sections were washed in tap water and stained with saffranine for further observations, and Powder microscopy was also observed & photographed using Magnus microscope [17].

Powder Microscopy Studies: The powdered plant material was soaked in 10% Nitric acid overnight. The sample is washed with distilled water the following day. Slides are prepared by staining the soaked plant material with saffranine and observed under a microscope, and the images were captured.

RESULTS AND DISCUSSION:

Preliminary Phytochemical Tests: The results indicated the presence of all the 6 metabolites tested by showing a positive response to the tests conducted.

TABLE 1: SHOWING THE PRESENCE/ABSENCE OF SECONDARY METABOLITES

Test	Observation	Inference
Shinoda test	Yellowish-brown color	+
Salkowski test	Red color	+
Salkowski test	Yellow color	+
Foam test	Foam	+
Meyer's test	Creamy white precipitate	+
Labat test	Olive green color	+

TLC Studies: In the present study when the sample was observed under visible light, showed two bands. When observed under short/long UV light, after spraying with 10% Ethanolic H_2SO_4 it showed 5 bands respectively. When sprayed with anisaldehyde 8 bands were seen.

TABLE 2: SHOWING R_F VALUES IN DIFFERENT MEDIUM OF OBSERVATION

S. no.	Observation	R_f Value							
A	Under Visible light		0.23			0.488			
B	10%H2SO4			0.283	0.386		0.735	0.792	0.962
C	Anisaldehyde	0.05	0.24	0.29	0.4	0.47	0.59	0.66	0.84
D	Short UV 254nm		0.22	0.31		0.48	0.59	0.62	
E	Long UV 366nm	0.14		0.28		0.47	0.5	0.69	

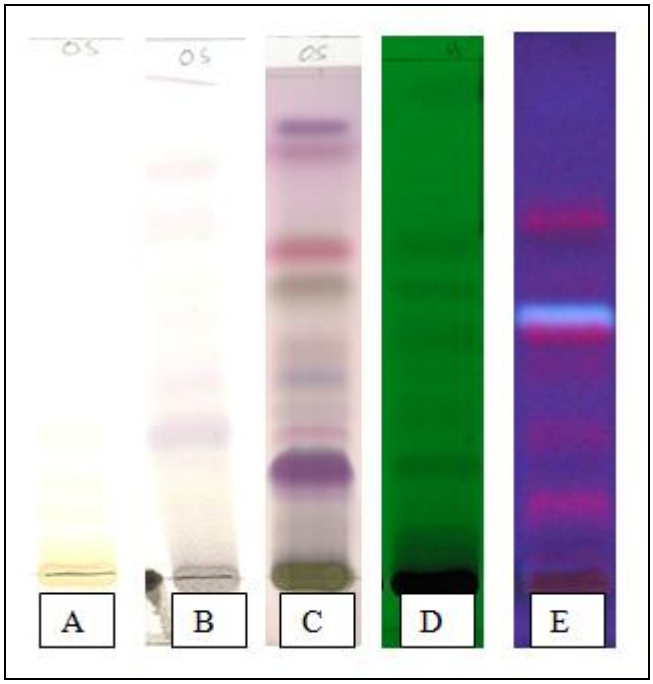

FIG. 1: SHOWING TLC PROFILE

Physico-chemical and Fluorescence Studies:

Organoleptic Characters: The above-mentioned Organoleptic properties are unique for the particular plant.

TABLE 3: SHOWING ORGANOLEPTIC FEATURES

S. no.	Color	Texture	Odor	Taste
Observation	Brownish green	Coarse powder	Clove like aroma	Astringent

Physicochemical: Physicochemical parameters conform with the results mentioned in API & IHP.

TABLE 4: SHOWING PHYSICOCHEMICAL FEATURES

Parameter	Total Ash content (%)	Acid insoluble ash (%)	ASE (%)	WSE (%)
Observation	18.254	8.145	3.6	10.00

Fluorescence Studies: The powdered material gave different color reactions with different chemicals; these color reactions are unique to the plant.

TABLE 5: SHOWING COLOUR CHANGES WHEN OBSERVED UNDER FLUORESCENT LIGHT

S. no.	Solvent	Visible	UV
1	Ethanol	Green	Black-green
2	Methanol	Orange	Black
3	Toulene	Green	Black
4	HNO_3	Reddish-brown	Reddish black
5	H_2SO_4	Dark brown	Black
6	HCl	Olive green	Greenish black
7	Water	Citrine	Creamish brown

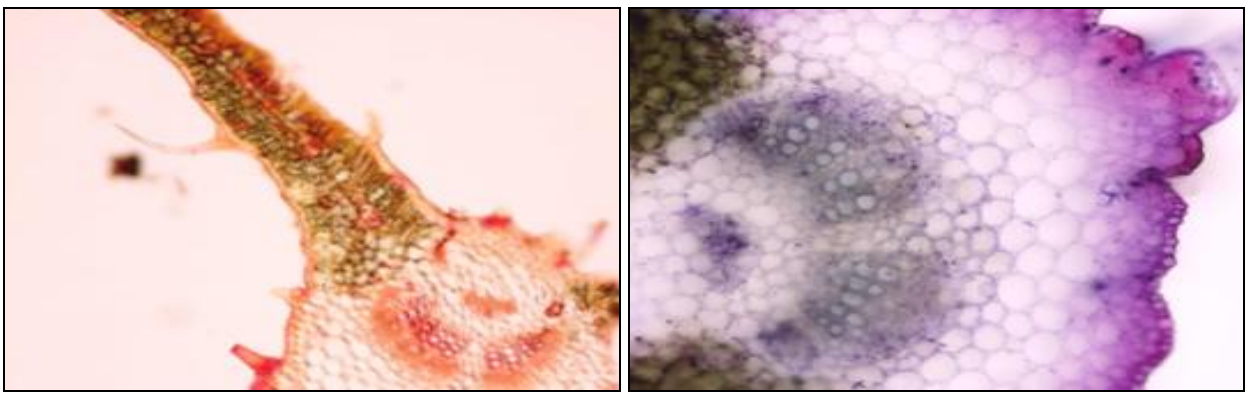

FIG. 2: SHOWING TS OF LEAF

Histology Characters:

1. TS of dorsiventral structure. One layered epidermal cells with a thin cuticle
2. Surface preparation shows many glands, glandular trichomes, and few stomata
3. Lower epidermis also shows some glands trichomes; these are stalkless and with uni- or bicellular head, often very long
4. The glands consisting of few cells containing essential oils and these glands are often found in the depression of the upper and lower epidermis
5. Palisade is of one layer, and spongy parenchyma is of 5-6 layers with intercellular spaces and oleo-resin contents.
6. Midrib shows the vascular bundle in the center in which the xylem vessels are arranged in the form of an arc.
7. The phloem is on the dorsal side of the xylem.
8. The ventral side of the midrib is provided with a collenchymatous region, and the epidermis shows different types of trichomes.

Powder Microscopy:

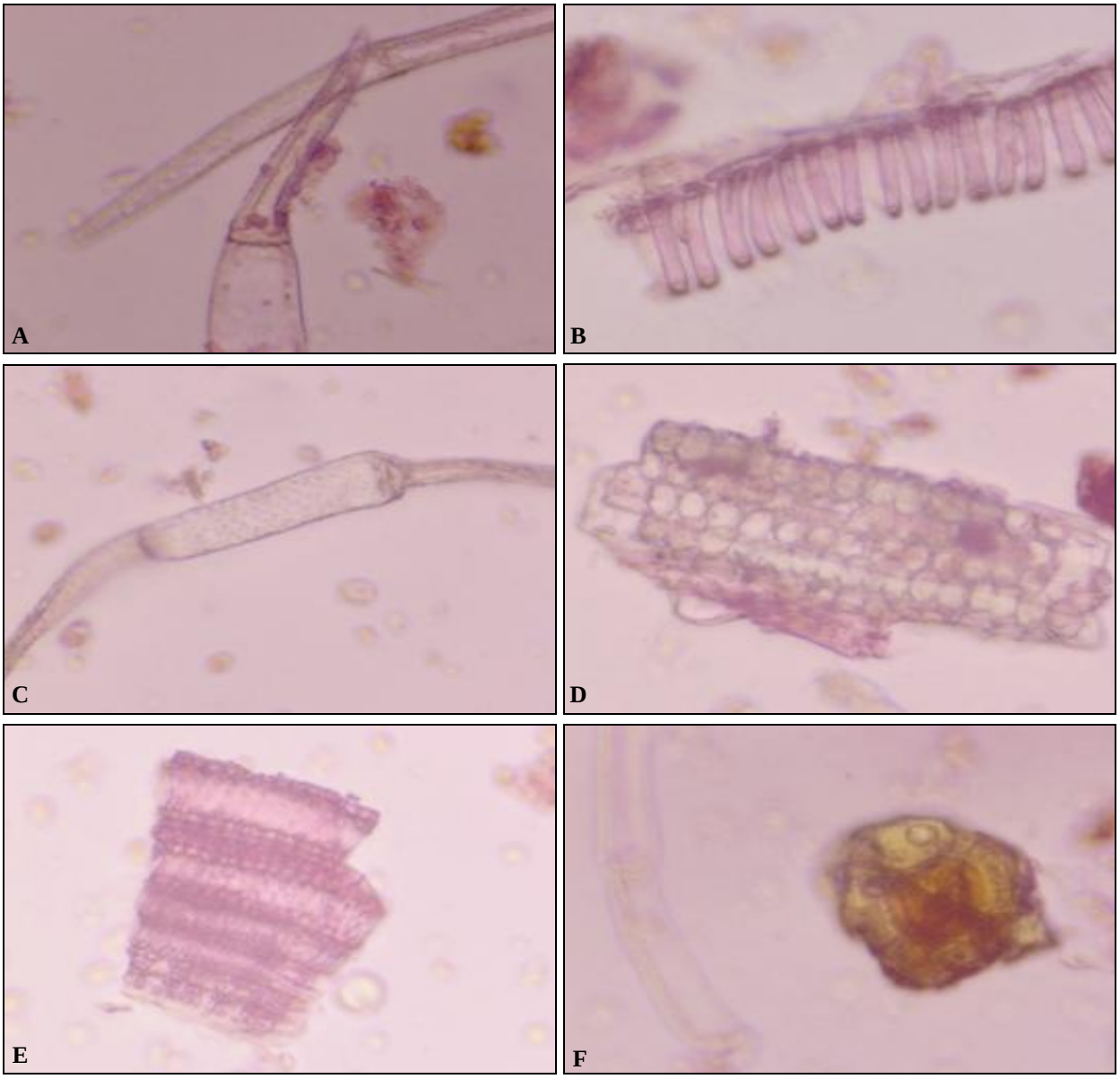

FIG. 3: SHOWING POWDER MICROSCOPY

Powder characteristics of *Ocimum tenuiflorum* L.:

A- Covering trichomes

B- Part of the group of vessels from the stem,

C- Covering trichomes with starch granules,

D -Inner epidermis of the corolla,

E-Multicellular gland in the lower epidermis,

F -Multicellular gland on the epidermis of the leaf

CONCLUSION: The *Ocimum tenuiflorum* L. is in use for more than 5000 years as a medicine. The efficacy of this plant may be attributed to the group of secondary metabolites present in it. The collected sample exhibits almost all the original properties of Holy Basil by showing similar results that of standard texts *viz*., Ayurvedic Pharmacopoeia of India (API) and Indian Herbal Pharmacopoeia (IHP). The TLC profile can be used as its fingerprint profile. The Anatomical and powder microscopical studies are unique.

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CONFLICTS OF INTEREST: Nil

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