https://dx.doi.org/10.4314/dujopas.v11i1c.30
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 11 No. 1c March 2025*

Relationships Between Glandular Trichomes and Essential Oil Production in *Ocimum basilicum* and *Ocimum sanctum* (LAMIACEAE)

Sunday Adebunmi Adeniran[2*], Abdullahi Alanamu AbdulRahaman[1,2], Asrar Muhammed Khan[1]

[1] Biochemistry, Biotechnology and Molecular Biology (BBMB) Laboratory,
Department of Biological Sciences,
Faculty of Sciences,
The COMSATS Institute of Information Technology (CIIT),
Islamabad,
Pakistan.

[2] Applied Plant Anatomy and Wood Technology (APAWT) Laboratory,
Department of Plant Biology,
Faculty of Life Sciences,
University of Ilorin,
PMB 1515,
Ilorin,
Nigeria,

Email: adeniran.sa@unilorin.edu.ng

Abstract

Trichomes on the leaves, stem and flowers of Ocimum basilicum and O. sanctum were studied. The seeds of the plant materials were collected from Nigeria and Pakistan, and planted at the Laboratory of the Department of Biological Sciences, COMSATS Institute of Information Technology (CIIT), Islamabad, Pakistan. Three trichomes types are present in the two species, namely multicellular-non glandular, peltate glandular and capitate glandular trichomes, and this formed the basis for essential oil extraction from these parts using Soxhlet extraction methods. Oils extracted were screened for the presence of alkaloids, terpenoids, phenol, tannins, saponins and flavonoids. There is a correlation between the density of the glandular trichomes within and between the three parts of the two plants. The density of the glandular trichomes is higher in the plant parts that produce higher essential oil. Percentage oil yield is higher in the leaves of O. sanctum (19.85%) and O. basilicum (13.58%), followed by the flowers and stem in the two plants. In line with the percentage oil yields, the density of the glandular trichomes also followed a similar pattern; with higher density in the leaves (86.6mm^2 and 21.50mm^2) for Ocimum sanctum and O. basilicum respectively, followed by the flowers and stem. Comparing the two species, O. sanctum has a higher oil yield and higher density of glandular trichomes in all its parts than O. basilicum.

Keywords: Anatomy, essential oils, morphology, Lamiaceae, trichomes

**Author for Correspondence*

INTRODUCTION

Plants are natural gifts of nature whose useful potentials in many folds have not been fully exploited. There are sundry efforts to exploit these potentials in all possible ways. Trichomes are essential for plant development and are found in many species. Factors such as environmental conditions, hormones, transcription factors, and non-coding RNA influence trichome growth and development (Wang et al., 2021) and the production of essential oils and other secondary metabolites. These compounds are not only vital for plant defense but also have widespread uses in medicine, food, cosmetics, and agriculture (Ramos Da silva et al., 2021; Tissier 2012).

The Lamiaceae family, in particular, has been widely utilized since ancient times due to its rich content of secondary metabolites, particularly essential oils (Ramos Da silva et al., 2021). These oils, characterized by their diverse chemical compositions, are primarily produced and stored in glandular trichomes, often referred to as "natural biofactories" (Mahmoud et al., 2021). Glandular trichomes are found in approximately one-third of all plant species (Fahn, 1988) and serve critical ecological functions, such as protecting against biotic and abiotic stressors (Glas et al., 2012), attracting pollinators (Zhou et al., 2021), and deterring herbivores (Gostin & Popescu, 2023).

Within the Lamiaceae family, glandular trichomes are primarily categorized into two types: peltate and capitate (Naidoo et al., 2021; Muravnik, 2020). Peltate trichomes consist of a basal cell, a foot cell, and a secretory head composed of multiple glandular cells arranged in a single layer. These trichomes store essential oils within a subcuticular space, enabling their controlled release (Haratym & Weryszko-Chmielewska, 2017). Capitate trichomes, on the other hand, exhibit greater structural diversity, featuring a basal cell, one or more stalk cells of varying length, and a glandular head composed of one to four (or occasionally more) secretory cells (Maleci & Giuliani, 2006). Both glandular and non-glandular trichomes contribute significantly to the taxonomic classification of Lamiaceae species (Giuliani et al., 2024; Siadati et al., 2020; Tozin et al., 2016).

The essential oils produced by glandular trichomes have long been used in industries such as perfumery, cosmetics, pharmaceuticals, and food flavoring. Terpenoids, or isoprenoids, are the primary constituents of these oils and are valued for their aromatic properties and biological activities (Tissier, 2012). Despite their economic importance, the regulatory mechanisms governing terpenoid biosynthesis remain poorly understood, presenting a promising area for future research. Recent advances in molecular biology and genetic engineering have enabled scientists to study the genes and proteins responsible for trichome metabolism, paving the way for optimized essential oil production through classical breeding and targeted genetic modification (Glas, 2012).

Meanwhile, there are many plant species (e.g., tobacco and tomato in Solanaceae and *Gossypium* and *Salvia* in Lamiaceae) in the wild which are currently neglected for years, despite their potential to produce valuable compounds (Zhigila et al., 2015). Majority of these plants are rich in many natural products such as aromatic oil. Research works into discovery of more plants that are in the wild which are potential producers of these oils in abundance will definitely be advantageous economically. Many products can also be produced subsequently from such discoveries. Many of these so-called "useless" plants possess chemical potentials that could contribute significantly to industrialization.

In this study, the aim of the study is to identify various types of glandular trichomes and their exudates from various plant species that can be potential sources of production for many industries. If this is achieved, certainly many industries will spring up and subsequently the rate of unemployment will drop especially in Nigeria. Wild plant species with potential industrial-useful glandular trichomes will be identified for tapping in industrial production of different types of products. Thus, there is today an increasing interest in understanding the chemistry of glandular trichome exudates and taking advantage of their potential uses in many plants as possible.

The aim of this research work is to determine the quantity of essential oil contents in *Ocimum basilicum* and *O. sanctum* in relation to the density of the glandular trichomes and to isolate and characterize the oils' chemical composition.

MATERIALS AND METHODS

Cultivation and Sample Collection- Seeds of *O. basilicum* and *O. sanctum* were collected from Nigeria and Pakistan (Table 1) and planted in clay pots at the Laboratory of the Department of Biological Sciences, COMSATS Institute of Information Technology (CIIT), Islamabad, Pakistan. Leaves, stems and flowers were harvested and used for the anatomical studies and extraction of oils. Specimens for oil extractions were collected between 11 am and 2 pm for the best yield of the oils (Turner et al., 1980).

Table 1: Species names and place of collection of the samples used for the study

Species	Family	Common name	Local names	Place of collection
Ocimum basilicum L.	Lamiaceae (Labiatae)	Basil, Common basil, Holy basil, Sweet basil, Basilie, Clove basil, Garden basil, Roman basil,	Efirin (Yoruba); Tulsi, Kashmalay, Niazbo, Barbra, Babrai (Pakistani); Basilic, Basilic commun, Herbe royale (Frence); Basilikum, basilinkraut, konigskraut (German); Basilico (Italian)	Ilorin, Nigeria; Hattar, and Haripur, KPK, Pakistan
Ocimum sanctum L.	Lamiaceae (Labiatae)	Sweet basil, holy basil, tulasi, tulsi, thulasi, tulsli, tulasi, Madura-tala	Sri tulsi [green-leaved holy basil], Krishna tulsi [purple-leaved holy basil] (India)	Hattar, Haripur, KPK, Pakistan

For clarity of the morphological parts, i.e. leaf, stem and flower were observed for the presence of the trichomes using a stereomicroscope (model IM-SZ-500 IREMCO GmbH, Schwarzenbek/Germany). Observations were recorded with photographs using a digital camera.

Anatomical studies

Anatomical specimens (leaves, stem, and flowers) were fixed in 10% formal acetic acid (FAA). The leaf epidermises were removed using hand sectioning method. The specimens were then stained with safranin O and counter-stained with iodine. Excess stain was removed with distilled water or wiped off with tissue paper. Each specimen was mounted in glycerol or DPX and observed under a compound microscope (Nikon model YS100, Nikon Corporation, Japan) using 35 fields of view at x10 objectives as quadrats. Observations were recorded with tables and photographs using a digital Olympus camera (Olympus Stylus VR-370, Olympus Europa Holding GmbH/Olympus Imaging America Inc./Olympus Imaging Corp., Tokyo). The trichome occurrence, distribution and type were counted and noted

(Dilcher 1974). The trichome density was determined as the number of stomata per square millimeter.
The mean trichome size or area was determined using this formula:
l x b x k
Where l= length, b= breadth and k= Franco's constant = 0.79 (Franco 1939).

Extraction of essential oils
Specimens (leaves, inflorescences and stems) were collected between 11am and 2pm for the best yield of the oils (Turner et al., 1980). Fresh plant specimens were shade-dried for two weeks before the extraction of the oils (Plate 1). Dried specimens were separated into leaves, stems and inflorescences and weighed using electronic weighing balance (Atlas Precision Scale, Log of Portable Weighing Balance, ATL.300G/0.01G, Mettler-Teledo Ltd, Leicester UK). Later they were then ground with blender. Oils were extracted from the ground leaf, stem and inflorescence using a Soxhlet extractor. 95% ethanol (335 ml of ethanol and 15 ml of distilled water = 350 ml solution) was used as solvent in a Soxhlet apparatus, at temperature 64^{o}C. The Soxhlet extractor was placed into a flask containing the extraction solvent (95% ethanol). The Soxhlet was then equipped with a condenser and heated to reflux. The solvent vapour travelled up a distillation arm, and flooded into the chamber housing the thimble of solid. The chamber containing the solid material was slowly filled with warm solvent. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. After many cycles, the desired compound was concentrated in the distillation flask. After extraction, the liquid extractant was poured inside Petri dish and covered with perforated foil paper and transferred to the Fume Hood chamber (or Fume cupboard or Fume closet) for evaporation of the solvent (ethanol) and capturing and removal of other air- borne hazardous substances generated during laboratory experiments (e.g. gases, vapours, aerosols and particulates or dust) to obtain pure oil extract. The obtained semi solid oil was scraped from the Petri dish using a flat spatula into a falcon tube. The oil was weighed using Shimadzu weighing balance (Shimadzu TX323L, Shimadzu Corporation, Japan).

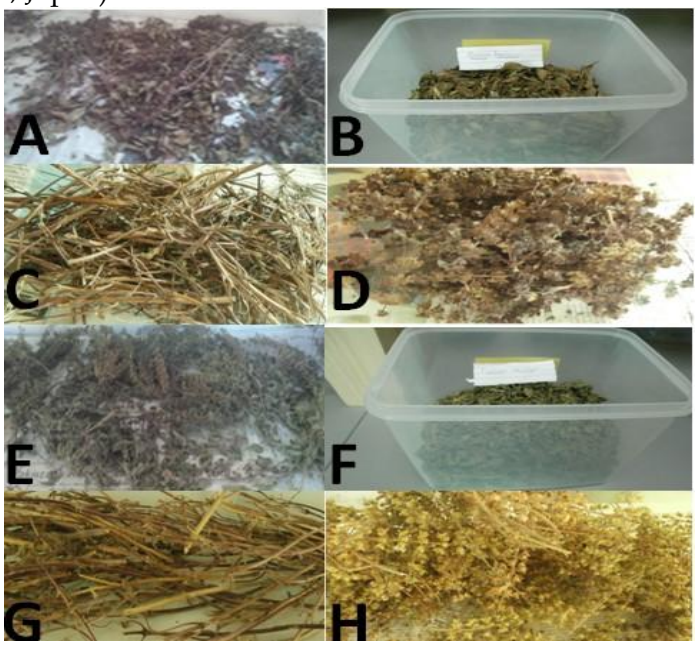


Plate 1: Shade drying of whole plant of *Ocimum basilicum* (a), leaves (b), stem (c) and inflorescence (d); whole plant of *Ocimum sanctum* (e), leaves (f), stem (g) and inflorescence (h)

Characterisation of oil extracted from the *Ocimum* species
Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids, flavonoids, phenols, terpenoids, saponins and tannins. Titration of chemicals was done using an Adjustable-volume pipette or Pro electronic pipette (Eppendorf Ag, Hamburg, Germany).

Determination of alkaloids
Using Mayer's test where the filtrates were treated with Mayer's reagent (1.36g mercuric chloride, 5.00g potassium iodide and 100ml water). Formation of a yellow cream precipitate indicates the presence of alkaloids.

Determination of flavonoids
Using lead acetate test, extracts were treated with a few drops of lead acetate solution (5% lead acetate and 5ml water). Formation of a yellow colour precipitate indicates the presence of flavonoids.

Determination of terpenoids
0.2g of the extract was mixed with 2ml of chloroform and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish-brown colouration of the inner face was an indication of the presence of terpenoids

Determination of phenols
Using the ferric chloride test, extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Determination of saponins
About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

Determination of tannins
A small quantity of the extract was mixed with 3ml water and heated on a water bath (Memmert GmbH+Co.KG, Schwabach FRG, Germany) at 60^{o}C for 25 minutes. The mixture was filtered and ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

Determination of oils and resins
Extracts were applied on filter paper. Development of translucent appearance on the paper indicates the presence of oils and resins.

Oil yield
The percentage oil yield was calculated as:

$$\% \text{ Oil yield} = \frac{\text{Weight of oil extracted}}{\text{Weight of the plant used}} \times 100$$

Statistical Analysis of Data - Data generated on the type of trichomes was subjected to statistical analysis using SPSS. The data generated from the trichome densities, size, and percentage oil yield were analyzed using analysis of variance (ANOVA) to determine the significant difference. Duncan's multiple range test was used to separate the means.

RESULTS

The morphological features (including the observations with the stereomicroscope) and anatomical evidence have shown that all the plant parts (namely leaf, stem and flower) are pubescent i.e. having hairs. Close examinations have shown that these hairs are indeed the trichomes. There are evidences of the presence of both trichome types in all parts of the plant used for extraction of oils.

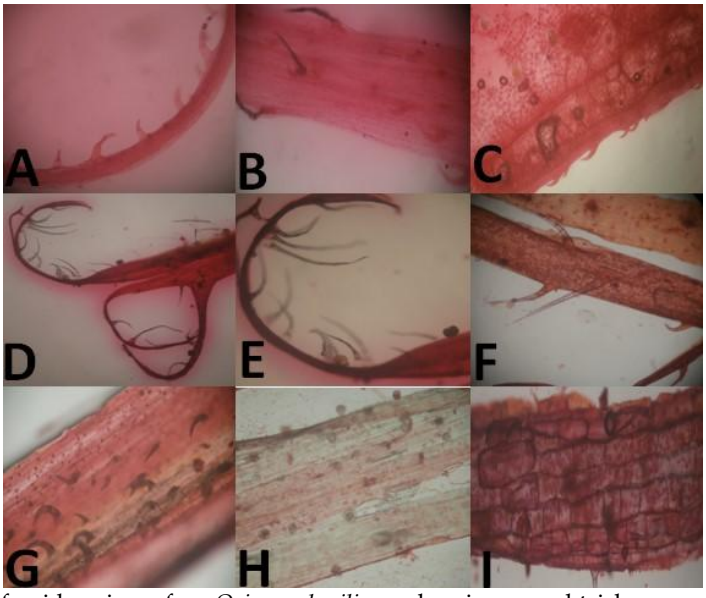
Stem – The microscopic observation of the stem showed that both glandular and non-glandular (i.e. multicellular uniseriate) trichomes are present. Stems are hairy in the two species.

Leaf – Midrib is whitish, veins are light green oppositely arranged. The leaf is pubescent on the abaxial surface (hairy is hirsute or coarse in *O. sanctum*). Trichomes sparsely occur on the adaxial surface.

Flower – Floral surfaces are pubescent (i.e. hairy or hirsutely, dense or coarse pubescent in *O. sanctum*) showing the presence of unicellular, uniseriate trichomes especially on the sepal, petal and pedicel. Trichomes are whitish with high density on both abaxial and adaxial surfaces of the sepals and pedicel than on the petals. The flower is hermaphrodite with male and female organs on the same flower. The sepals and petals are not distinguishable in *O. sanctum*, hence are referred to as perianths. The ovary is superior and a four chamber loculi with each locule containing one ovule.

Trichome anatomy

Trichomes (glandular [peltate and glandular] and non glandular [multicellular-uniseriate]) are present on the leaf, stem and flowers of the studied species (Plates 2; Table 2). Meanwhile, there are variations in the trichomes frequency, density and size between the three parts studied, namely leaf, stem and flower within each species and between the two species. Epidermal cells and anticlinal cell walls are rectangular, irregular, straight to wavy (Table 2). The presence and distribution of the trichomes are based on the three parts of each of the two plants as follow:

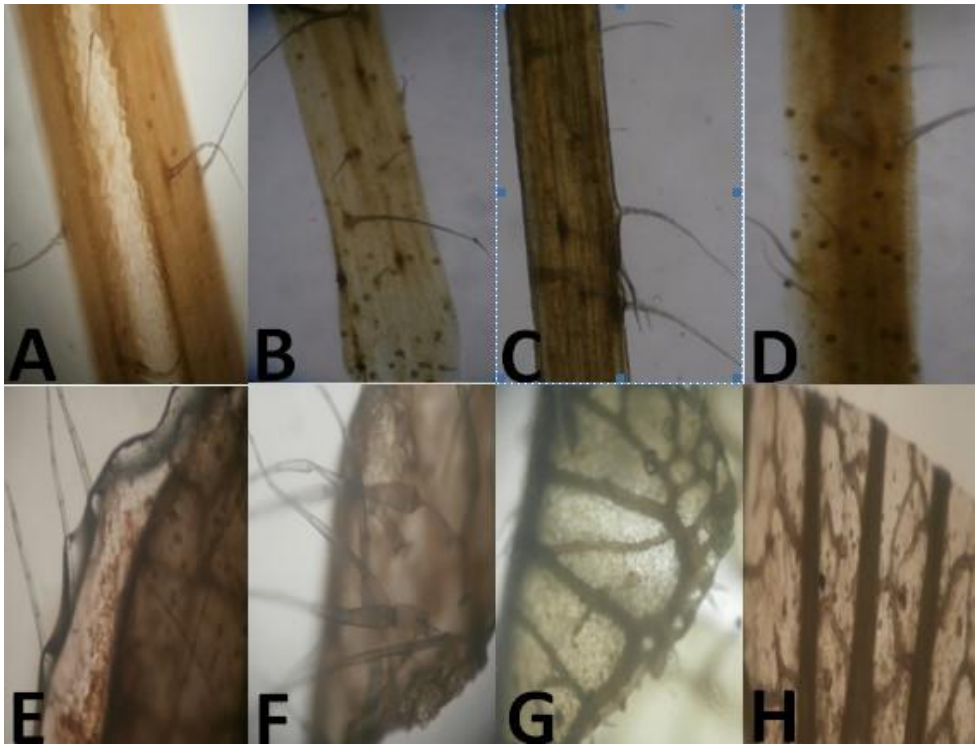


Plates 2: A-C: Adaxial leaf epidermis surface *Ocimum basilicum* showing round trichomes and tiny stomata, C-F: Abaxial leaf epidermis surface of *Ocimum sanctum* showing glandular and multicelular trichomes and tiny stomata, G-I: Stem epidermis of *Ocimum basislicum* showing glandular and non-glandular trichomes

Leaf epidermis: Two types of trichomes were present on the leaf surfaces, namely multicellular, non-glandular and glandular trichomes (i.e. peltate and glandular). The latter is often observed along the midrib and the edge of leaves. The non-glandular trichomes are multicellular and unbranched. The multicellular, non-glandular trichomes are the most commonly occurred types found on the leaves, in *O. basilicum* (Plate 2). While, non-glandular and capitate glandular trichomes are abundant along the midribs and sometimes along the veins on the leaf surfaces of *O. sanctum* (Plates 2a-c; Table 2).

Stem epidermis: The microscopic observation of the stem showed that both glandular and non-glandular trichomes are present (Plates 2 and 3; Table 2).

Floral epidermis: Glandular and multicellular non-glandular trichomes are present in the two species (Plate 3; Table 2), especially on the sepals, petals and pedicels.



Plates 3: **A-D**: Stem epidermis of *Ocimum sanctum* showing glandular and non-glandualr trichomes, **E, F**: Flower anatomy (sepal) of *Ocimum basilicum* showing multicellular, non-glandular trichomes, glandular trichomes and stomata, **G, H**: Flower anatomy (sepal) of *Ocimum sanctum* showing multicellular, non-glandular trichomes, glandular trichomes and stomata

Table 2: Trichome character of the epidermises of *Ocimum basilicum* and *Ocimum sanctum*

Species	Trichome types	Trichome frequency (%)	Trichome density (mm^2)	Mean glandular trichome density (mm^2)	Trichome size (µm)	Epidermal cell	Anticlinal cell wall pattern
			Leaf epidermis				
Ocimum basilicum	MNT	100	20.5	21.5	20.5	Rectangular, Irregular	Highly wavy; straight in midrib and veins
	PG	100	17.5		8.33		
	CG	100	4		9		
Ocimum sanctum	MNT	100	18.5	86.67	35.8	Rectangular, Irregular	Wavy; straight in midrib and veins
	PG	100	74		4.83		
	CG	75	12.67		2		
			Stem epidermis				
Ocimum basilicum	MNT	100	24.33	10	41	Rectangular with thick walls, irregular	Straight
	PG	100	3.67		6.00.00		
	CG	70	6.33		3.5		
Ocimum sanctum	MNT	83.33	2.33	28.1	68.2	Rectangular, irregular	Straight
	PG	100	25.83		5		
	CG	100	2.5		3		
			Flower epidermis				
Ocimum basilicum	MNT	100	20.3	16.8	45.32	Rectangular	
	PG	98	10.2		4		
	CG	76.4	6		4		
Ocimum sanctum	MNT	10	3	33.17	34.2	Rectangular	Wavy
	PG	90	17.5		4.83		
	CG	92.03	15.67		2.3		

Keys: MNT = Multicellular, non-glandular trichomes, UNG = Unicellular, non-glandular trichomes, PG = Peltate glandular trichomes, CG = Capitate glandular trichomes

Extraction of oil

The weight of oils obtained from the two species varies within and between the species; for *O. basilicum* parts (42.64g leaf, 21.27g inflorescence, and 19.36g stem) and the *O. sanctum* parts (40g leaf, 26.46g inflorescence and 22.30g stem). The distinct odours and flavours perceived in the plant as a whole are also evidenced in the extracted oils. The qualitative features such as alkaloids, flavonoids, terpenoids, phenols, saponins and tannins were present and absent in some oils (Table 3). All the compounds are present in the stems of both plants. Terpenoids and alkaloids are absent in the leaves of *O. basilicum* and *O. sanctum* respectively, while alkaloid are absent in the inflorescence of *O. basilicum*.

Table 3: Quantitative features of oils in *Ocimum basilicum* and *Ocimum sanctum*

Species	Part used (g)	Oil extracted (g)	Colour and scent of oil	Alkaloids	Flavonoids	Terpenoids	Phenols	Saponins	Tannins
Ocimum basilicum	Leaf (42.64)	5.79		+	+	-	+	+	+
	Stem (19.36)	1.34		+	+	+	+	+	+
	Inflorescence (21.27)	1.99		-	+	+	+	+	+
Ocimum sanctum	Leaf (40)	7.94	Black and scented	-	+	+	+	+	+
	Stem (22.30)	2.54		+	+	+	+	+	+
	Inflorescence (26.46)	3.75		+	+	+	+	+	+

Keys: + = present, - = absent, g = amount of ground plant parts used for oil extraction in gram

Percentage oil yield is higher in the leaves followed by the flowers and stem of the two *Ocimum* plants. In line with the percentage oil yields, the density of the glandular trichomes also followed a similar pattern i.e. higher glandular trichome density in the leaves followed by the flowers and stem. Comparing the two species, *O. sanctum* has a higher oil yield and higher density of glandular trichomes in all its parts than in *O. basilicum* (Table 4).

Table 4: Correlations between the essential oil yield and trichome density in plant parts of *Ocimum basilicum* and *Ocimum sanctum*

Species	Plant part used	Dry weight (g)	Oil yield (g)	Percentage oil yield (%)	Trichome type	Trichome density (mm^2)	Mean glandular trichome density (mm^2)
Ocimum basilicum	Leaf	42.64	5.79	13.58	PG	17.5	21.5
					CG	4	
					MNT	20.56	
	Stem	19.36	1.34	6.92	PG	3.67	10
					CG	6.33	
					MNT	24.33	
	Inflorescence	21.27	1.99	9.36	PG	10.2	16.2
					CG	6	
					MNT	20.3	
Ocimum sanctum	Leaf	40	7.94	19.85	PG	74	86.67
					CG	12.67	
					MNT	18.5	
	Stem	22.3	2.54	11.39	PG	25.83	28.13
					CG	2.5	
					MNT	2.33	
	Inflorescence	26.46	3.75	14.17	PG	14.5	33.17
					CG	12.67	
					MTN	3	

Keys: MNT = Multicellular, non-glandular trichomes, UNG = Unicellular, non-glandular trichomes, PG = Peltate glandular trichomes, CG = Capitate glandular trichomes

DISCUSSION

The two species of *Ocimum, namely O. basilicum and O. sanctum, are fragrantly scented, indicating their potential ability to produce* aromatic or essential oils. Haas et al., (2024) reported that the members of the family Lamiaceae are known for possessing of chemical compounds called monoterpenes, which give distinctive and pleasant odours and flavours to the plant, especially in the leaves of many species. Meanwhile, chemicals such as methyl chavicol, eugenol and linalool (Ambrose et al., 2016), geranial (for rose flavour), thymol (for thyme flavour), camphor, trans-methyl cinnamate (for cinnamon flavour) and citral (lemon) (Yaldiz et al., 2023) are responsible for various flavours in *Ocimum* species. The two species studied in this work were found to be highly flavoured and fragrant in all the three parts studied, and it is an indication of presence of essential oils in the plant parts. Additionally, the presence of trichomes, especially the glandular types on these parts (sepals and petals), is also an indication of essential oils in the two species. Glandular trichomes have been implicated as the main factories behind the secretion of essential oils in the plant species of the family Lamiaceae (Gostin & Blidar, 2024; Feng et al., 2021; Zhigila et al., 2015; Jia et al., 2013; Glas 2012; Tissier 2012).

There are a series of works on the anatomy of *Ocimum* species which are documented (Ahmed, 2024; Parida et al., 2020; Abd et al., 2014; Venkateshappa and Sreenath, 2013). Earlier the taxonomic value of leaf epidermal characters in some species of the genus *Ocimum* was studied by Ahmed (2024). Parida et al., (2020) examined the leaf epidermal features of six *Ocimum* species, and identified an amphistomatic leaf (i.e. presence of stomata on both leaf surfaces) with the occurrence of glandular and non-glandular unicellular

trichomes, and stomatal complex types. So also, Gul et al., (2019) classified trichomes in Lamiaceae into glandular (GTs) and nonglandular (NGTs) types, each with distinct subtypes. GTs include sessile capitate, subsessile capitate, and barrel and sunken types, while NGTs are dendritic, stellate, conical, falcate, simple, and 1-6 cells long with varied surface ornamentation. NGTs are dominant on both leaf surfaces, with species like *Vitex negundo* and *Isodon rugosus* distinguished by their twisted NGTs on the abaxial surface. Notably, observations of the leaf, stem, and flower epidermises under a stereomicroscope revealed a substantial presence of trichomes across the leaves, stems, and inflorescences. Based on this evidence, oils were subsequently extracted from the three plant parts. Though there is variation in the density of these trichomes which were later correlated to the amount of the essential oils extracted from each part of each plant.

Essential oils are complex mixtures of biological active substances used for fragrance and traditional medicine since long time. It contains mainly triterpenes and aromatic compounds; the chemical composition of the essential oils varies with seasonal, geographical and climatic conditions. Recently there are more researches focused on their chemical profiles and medicinal properties. Due to their antimicrobial, antifungal, insecticidal, larvicidal and antioxidant properties, they are used as alternatives for synthetic chemical products to reduce cost and side effects. Phytochemicals like flavonoids, tannins, terpenoids and saponins are reported to be present in the leaves and stems of *Ocimum* species (Abd et al., 2014). Shafqatullah et al., (2013) reported the presence of alkaloids, flavonoids, terpenoids, saponins and tannins in the genus as confirmed in this study. Terpenoids were not detected in the leaves of the *O. basilicum*. The compound, terpenoids (or isoprenoids) are a very large group of chemical constituents of essential oils in plants. They are important due to their pleasing and aromatic odour, which makes the essential oils important ingredients in pharmaceutical, perfumery, cosmetics and many other industries (Biswas and Foster, 2009).

Though there are two types of trichomes that are present in the two *Ocimum* species (glandular and non-glandular), it is the glandular trichomes (which are further of two types namely peltate and capitate) that actually produced the oils. The non-glandular trichomes are multicellular-uniseriate and non-glandular i.e. not secretory. The secretion of essential oils has been linked to the presence of glandular trichomes in plants because they are believed to be the chemical manufacturers of the plants (Zahran et al., 2020; Rehman et al., 2016; Zhigila et al., 2015; Jia et al., 2013; Glas 2012; Tissier, 2012; Bhasin, 2012).

The essential oil occurs in the secondary tissues, glands or trichomes in the leaves and are usually responsible for the characteristic pleasant odours and flavours, which thus, accounted in large extent for their value and determine their uses. Further to this, the characteristic distinct and pleasant odours and flavours are also perceived in the extracted oils as well as in the fresh and dried plant samples.

Apart from the fact that the glandular trichomes secreted essential oils, their density or abundance has an effect on the amount of essential oil produced. This was reported for many plants such as papermint, lima bean, Lavender and tomato (Zahran et al., 2020; Hazzoumi et al., 2019; Rehman et al., 2016; Bhasin et al., 2012; Pinto et al., 2007; Behnam et al., 2006). Realizing the essence of trichomes in the secretion of the essential oils, Zuzarte et al. (2010) developed a protocol for in vitro micropropagation of *Lavandula pedunculata*. The protocol was assessed to be worthy of note because the trichomes and essential oils of

micropropagated plantlets are identical to those produced by the field-growing plants. They, therefore, suggested the use of micropropagation for large-scale multiplication of essential oils-producing plants with the aim of avoiding over exploitation of natural resources.

Extraction of oils from the parts of the two *Ocimum* species studied was ascribed to the presence of trichomes on those parts. The density of trichomes on those parts can be correlated to the amount of oil extracted from each part. The trichome density may be related to the age of the plant. The types of glandular trichomes and their pattern of distribution on the leaf surfaces in the young and older leaves of *O. basilicum* were investigated by Werker et al., (1993), and was discovered that there is a higher density of the glandular trichomes on the young meristematic leaves and meristematic regions of the older leaves. They also observed that there were no new trichomes on the mature leaves but instead the trichome density is on decrease. The outcome of this is that the essential oil differs in percentage of some of its components between the young and mature leaf regions. Also, density of trichomes and essential oil yield from the shoots of three vegetative stages under the control and salt conditions leaves of *Origanum majorana* L. Scanning electron showed trichome density decreased with leaf maturity but increased under salt stress (Olfa et al., 2016). Here it was observed that there is a correlation between the essential oil and the number of trichomes before the bloom, but the correlation was negative during and after the bloom.

In a study where the density of glandular trichomes in the leaves and inflorescence of *Lippia origanoides* was correlated with the essential oil production, it was observed that there are higher glandular trichomes on the bracts and sepals followed by petals and leaves, and subsequently there was a higher yield of essential oil in the inflorescence than in the leaf (Tozin and Marques, 2015).

Bearing all of these in mind, we are suggesting tissue culturing and genetic manipulations of genes responsible for trichomes production in *O. basilicum* and *O. sanctum* in order to increase the number of trichome production, and subsequently increasing essential oil secretion of the plants.

In conclusion, the plant part used (leaf, stem and flower) extracted essential oils in the two species of *Ocimum* and three types of trichomes were present. The amount of the oils extracted vaired with the plant parts with a higher density of glandular trichomes (i.e. peltate and capitate) producing higher amounts of oils., namely multicellular-non glandular trichome, peltate glandular trichome and capitate glandular trichome. A correlation between the density of the glandular trichomes and essential oil produced within and between the *O. basilicum* and *O. sanctum*; with a higher percentage oil yield and higher density of glandular trichomes in he leaves followed by the flowers and stems. Comparing the two species, *O. sanctum* has a higher oil yields and higher density of glandular trichomes in all its three parts (i.e. leaf, stem and flower) than in *O. basilicum*.

ACKNOWLEDGEMENT
The authors are grateful to The World Academy of Sciences (TWAS), for the advancement of science in the developing countries, Trieste, Italy and COMSATS Institute of Information Technology (CIIT), Islamabad, Pakistan, for provision of fellowship; Mr Muhammad Rashid, Garden Manager at Qarshi Industries (Pvt.), Limited, Hattar, Haripur, KPK, Pakistan for providing fresh specimens of *Ocimum basilicum* and *O. sanctum* for this work. Also, we

acknowledge the immense contribution of Dr. Humaira Fasi Shaheen of Department of Biological Sciences, COMSATS Institute of Information Technology (CIIT), Islamabad, Pakistan, for identification of plant samples, and isolation of essential oil.

REFERENCES

Abd, M., Nassar, E., Usama, M., & Azoz, S. (2014): Anatomical and Phytochemical Studies on *Ocimum basilicum* L. Plant (Lamiaceae). *International Journal of AdvancedResearch,* (2)11, 204-226

Ahmed, W. (2024): Morphological and anatomical studies of *Ocimum basilicum* L. cultivatedin Al-Marj city, Libya *Global Libyan Journal*. 1-13

Ambrose, D. C. P., Manickavasagan, A. & Naik, R. (Eds) (2016): Leafy Medicinal Herbs: Botany, Chemistry, Postharvest Technology and Uses. CPI Group (UK) Ltd., Croydon.

Bhasin, M. (2012): Ocimum-Taxonomy, medicinal potentialities and economic value of essential oil, *Journal of Biosphere* 1: 48-50

Behnam, S., Farzaneh, M., Ahmadzadeh, M. & Tehrani, A. S. (2006): Composition and Antifungal activity of essential oils of *Mentha piperita* and *Lavendula angustifolia* on post harvest phytopathogens. *Commun Agric Appl Biol Sci*., 71: 1321 – 1326.

Biswas, K. K. & Foster, A. J. (2009): Essential oil production: relationship with abundance of Glandular trichomes in aerial surface of plants. *Acta Physiology Plantarum*, 31: 13- 19.

Dilcher, D. L. (1974): Approaches to the identification of angiosperm leaf remains. *Botanical Review*, 40(1): 1-57.

Fahn, A. (1988). Secretory Tissues in Vascular Plants. *New Phytol. 108*, 229–257

Feng, Z., Bartholomew, E.S., Liu, Z., Cui, Y., Dong, Y., Li, S., Wu, H., Ren, H., & Liu, X. (2021). Glandular Trichomes: New Focus on Horticultural Crops. *Hortic. Res. 8*, 158.

Franco, C. (1939): Relation between chromosome number and stomata in *Coffea*. *Botanic Gazette*,100: 817– 818.

Giuliani, C., Bottoni, M., Spada, A., Falsini, S., Santagostini, L., Pieracci, Y., Flamini, G., Milani, F. & Fico, G. (2024): Micromorphological and Phytochemical Insights on *Phlomis fruticosa* L. Cultivated at the G.E. Ghirardi Botanical Garden (Lombardy, Northern Italy). *Flora, 314*, 152505.

Glas, J. J., Schimmel, B. C. J., Alba, J. M., Escobar-Bravo, R., Schuurink, R.C. & Kant, M. R. (2012): Plant glandular trichomes as targets for breeding or engineering of Resistance to herbivores. *International Journal of Molecular Science*, 13(12): 17077 – 17103.

Gostin, I. N., & Blidar, C. F. (2024): Glandular Trichomes and Essential Oils Variability in Species of the Genus *Phlomis* L.: A Review. *Plants*, *13*(10), 1338.

Gostin, I.N. & Popescu, I.E. (2023): Evaluation of the Essential Oils Used in the Production of Biopesticides: Assessing Their Toxicity toward Both Arthropod Target Species and Beneficial Pollinators. *Agriculture*, *14*, 81.

Gul, S., Ahmad, M., Zafar, M., Bahadur, S., Sultana, S., Ashfaq, S., Ullah, F., Kilic, O., Hassan, F. U. & Siddiq, Z. (2019): Foliar epidermal anatomy of Lamiaceae with special emphasis on their trichomes diversity using scanning electron microscopy. *Microsc Res Tech.*;82(3):206-223.

Haas, R.A, Crişan, I., Vârban, D. & Vârban R. (2024): Aerobiology of the Family Lamiaceae: Novel Perspectives with Special Reference to Volatiles Emission. Plants (Basel).

Haratym, W. & Weryszko-Chmielewska, E. (2017): Ultrastructural and Histochemical Analys of Glandular Trichomes of *Marrubium vulgare* L. (Lamiaceae). *Flora*, *231*,

11-20.
Hazzoumi, Z., Moustakime, Y., & Khalid, A. (2019): Essential Oil and Glandular Hairs: Diversity and Roles. Intechopen Sidi Mohamed Ben Abdellah University
Jia, P., Liu, H., Gao, T. and Xin, H. (2013): Glandular trichomes and essential oil of *Thymus quinquecostatus*. The Scientific World Journal, Artilce ID 387952, 8 pages.
Mahmoud, S.S., Maddock, S. & Adal, A.M. (2021): Isoprenoid Metabolism and Engineering in Glandular Trichomes of Lamiaceae. *Front. Plant Sci.*, *12*, 699157.
Maleci Bini, L.; Giuliani, C. (2006). The Glandular Trichomes of The Labiatae. A Review. *Acta Hortic.*, *723*, 85–90.
Muravnik, L.E. (2020): The Structural Peculiarities of the Leaf Glandular Trichomes: A Review. In *Plant Cell and Tissue Differentiation and Secondary Metabolites* Ramawat, K.G., Ekiert, H.M., Goyal, S., Eds.; Reference Series in Phytochemistry; Springer International Publishing: Cham, Switzerland, pp. 1–35.
Naidoo, Y., Dladla, T., Dewir, Y.H., Gangaram, S., Naidoo, C.M. & Rihan, H.Z. (2021): The Micromorphology and Histochemistry of Foliar Mixed Indumentum of *Leucas lavandulaefolia* (Lamiaceae). *Plants*, *10*, 1767
Olfa, B., Tarchoune, I., Chebbi, M., Nasri Nasrabadi, M., (2016): Essential oil and trichome density from Origanum majorana L. shoots affected by leaf age and salinity. *Bioscience Journal.* (32): 238-245
Parida, S., Mohapatra, B. & Mahalik, G. (2020). Anatomical Study of Six Ocimum Species: The Valuable Method Used in Indian Systems of Medicines. *International Journal of Ayurvedic Medicine* (11)278-283
Pinto, D. M., Blande, J.D., Nykanen, R., Dong, W. X.D., Nerg, A. M. and Holopainen, J. K. (2007): Ozone degrades common herbivore-induced plant volatiles: does this affect herbivore prey location by predators and parasitoids? *Journal of Chem Ecol.*, 33: 683–694.
Ramos Da Silva, L.R., Ferreira, O.O., Cruz, J.N., De Jesus Pereira Franco, C., Oliveira Dos Anjos, T., Cascaes, M.M., Almeida Da Costa, W., Helena De Aguiar Andrade, E.& Santana De Oliveira, M. (2021). Lamiaceae Essential Oils, Phytochemical Profile, Antioxidant, and Biological Activities. *J. Evid. Based Complement. Altern. Med.*, 6748052.
Rehman, R., Hanifand, M., Mushtaq, Z., Mochona, B., Qi, X. (2016). Biosynthetic Factories of Essential Oils: The Aromatic Plants. Natural Products Chemistry and Research (4); 227
Shafqatullahi, M. K., Asadullah, K. and Khan, F. A. (2013): Comparative analyses of *Ocimum sanctum* stem and leaves for phytochemicals and inorganic constituents. *Middle-East Journal of Scientific Research*, 13(2): 236 – 240.
Siadati, S.; Salmaki, Y.& Bräuchler, C. (2020). Trichome Morphology Provides Phylogenetically Informative Signal for Generic Delimitation in Tribe Marrubieae (Lamiaceae). *Flora*, *273*, 151720.
Tissier, A. (2012): Glandular trichomes: what comes after expressed sequence tags? *The Plant Journal*, 70: 51 – 68.
Tozin, L. R. S. & Marques, M. O. M. (2015): Glandular trichome density and essential oil composition in leaves and inflorescences of *Lippia origanoides* Kunth (Verbenaceae) in the Brazilian Cerrado. *Ann Braz Acad Sci.*, 87(2): 943 – 953.
Tozin, L.R.S., De Melo Silva, S.C. & Rodrigues, T.M. (2016). Non-Glandular Trichomes in Lamiaceae and Verbenaceae Species: Morphological and Histochemical Features Indicate More than Physical Protection. *N. Z. J. Bot.* 2016, *54*, 446–457.

Turner, J. C., Hemphill, J. K. & Mahlberg, P. G. (1980): Trichomes and cannabinoid content of developing leaves and bract of *Cannabis sativa* L. (Cannabiaceae). *American Journal of Botany*, 67:1397–1406.

Venkateshappa, S. M. & Sreenath, K. P. (2013): Some species of Lamiaceae – comparative anatomical studies. *Indo American Journal of Pharmaceutical Research*, 3(11): 9249 – 9254.

Wang, X., Shen, C., Meng, P., Guo-Fei, T., & Lv, L. (2021): Analysis and review of trichomes in plants. BMC Plant Biology 21

Yaldiz, G., Camlica, M., Pradhan, Y., Ali, A. (2023): Chemical Characterization, Biological Activities, and Some Medicinal Uses of Different Sweet Basil (*Ocimum basilicum* L.) Genotypes. In: Arunachalam, K., Yang, X., Puthanpura Sasidharan, S. (eds) Natural Product Experiments in Drug Discovery. Springer Protocols Handbooks. Humana, New York, NY.

Zahran, E.M., Abdelmohsen, U. R., Khalil, H. E., Desoukey, S.Y., Ahmed, M. & Kamel, M.S. (2020): Diversity, phytochemical and medicinal potential of the genus *Ocimum* L. (Lamiaceae) 19(17)

Zhigila, D. A., Sawa, F. B. J. & Abdul, S. D. (2015): Systematic significance and Pharmaceutical potentials of trichomes in accessions of *Sesamum indicum* L. Pedaliaceae. *Journal of Medicinal Plants Research*,9(11): 385 – 394.

Zhou, S.; Han, C.; Zhang, C.; Kuchkarova, N.; Wei, C.; Zhang, C.; Shao, H. (2021). Allelopathic, Phytotoxic, and Insecticidal Effects of *Thymus proximus* Serg. Essential Oil and Its Major Constituents. *Front. Plant Sci. 12*, 689875.

Zuzarte, M. R., Dinis, A. M., Cavaleiro, C., Salguieiro, L. R. and Canhoto, J. M. (2010): Trichomes, essential oils and *in vitro* propagation of *Lavandula pedunculata*. *Industrial Crops and Products*, 32:580 – 587.