LEAF EPIDERMAL MICROSCOPY, CHEMO-MICROSCOPY AND GC-MS ANALYSES OF THREE *OCIMUM* SPECIES FROM NIGERIA

ABSTRACT

Comparative analyses of the leaf epidermal microscopy, chemo-microscopy and GC-MS analysis of essential oils from the leaves of three *Ocimum* species (*Ocimum basilicum* L.; *Ocimum canum* Sims. and *Ocimum gratissimum* L.) belonging to the family Lamiaceae were analyzed. The leaf epidermal microscopy revealed anomocytic stomata in all the species studied. *O. basilicum* has anomocytic stomata on both surfaces and were more aboundant on the lower surface; cell walls are wavy on the upper surface and have glandular trichomes on both surfaces. *O. canum* also has anomocytic stomata on both surfaces; cell walls are wavy and trichomes are glandular and non-glandular occurring on both surfaces but occurring more on the upper surface. The non-glandular trichomes are cone-shaped with pointed tip. *O. gratissimum* has anomacytic stomata on both surfaces occurring more on the lower surface; the cell walls are curved on both surfaces and glandular trichomes occur on both surfaces which are more abundant on the lower surface. The glandular trichomes are radially flagellated in all the species studied. The chemo-microscopic analyses were positive for lignin, cellulose, tannins, mucilage, starch, calcium oxalate, oils and protein in all the species studied. The GC-MS analyses of the leaf essential oils revealed 35 compounds for *O. basilicum*, 49 compounds for *O. canum* and 34 compounds for *O. gratissimum* with 3-allyl-6-methoxyphenol being the most abundant in *O. basilicum* (34.42%); 1-Cyclopentene-1-methanol,2-methyl-5-1-methyl, the most abundant in *O. canum* (29.56%) and thymol being the most abundant in *O. gratissimum* (48.04%).

Keywords: *Ocimum gratissimum*, Microscopy, Analysis, Nigeria

INTRODUCTION

The Genus *Ocimum* belongs to Lamiaceae family and comprised of about 200 species of herbs and shrubs and is distributed over Asia, Africa, Central and Southern America. *Ocimum* is cultivated for its essential oil which displays many potent pharmacological application (mosquito repellent, antimicrobial, antioxidant, wound healing, anti-melanoma, and radio-protective) culinary, perfume for herbal toiletries, aromatherapy treatment and as flavoring agent. The study was done to compare the leaf epidermal microscopy, chemo-microscopy, and GC-MS analyses of *Ocimum basilicum* L. *Ocimum canum* Sims*;* and *Ocimum gratissimum* L. leaves.

Ocimum basilicum L. (Sweet basil), the Mediterranean type and the most popular species, is assumed to originate from India, Africa, and/or the Middle East. It is the most common basil type in the Western hemisphere and has the greatest economic importance. It is utilized as a food ingredient, remedy, cosmetic ingredient, and for ornamental purpose.

Ocimum canum Sims is a semi-woody plant of about 40 cm high it is an aromatic plant and native to tropical Africa (Steel, 2006). It is popularly called African basil, but known as "Efinrin elewe dudu" in south-western Nigeria where it is used locally for the treatment of gastrointestinal problems, piles, dysentery, as an infusion for nursing mothers and as insect repellant. The leaf is used for the treatment of diabetes in Ghana (Hogarh, 1996; Nyarko *et al.*, 2002). The plant is reported to be rich in volatile essential oils of therapeutic importance (Ekundayo *et al.*, 1989). The leaves of *O. canum* are used in the preparation of delicious local soups as well as flavoring agent in yam and cocoyam porridges in the Yoruba tribe of Nigeria. The plant is also used as a local condiment because of its aromatic properties (Bassole *et al.*, 2005). Despite its nutritive and therapeutic values, this plant has not been scientifically examined for its nutritive components. *O. canum* is used specially for treating various types of diseases and lowering blood glucose and it also treats cold, fever, parasitic infestations on the body and inflammation of joints and headaches (Ngassoum *et al.*, 2004). Essential oil from the leaves of *O. canum* poses antibacterial and insecticidal properties (Bassole *et al.*, 2003).

Ocimum canum is used medicinally in Africa to treat conjunctivitis, malaria, and headache, and has been used as an analgesic and rubefacient (Ngassoum 2004, Nyarko 2002). It also has been used to manage diabetes mellitus in Ghana Nyarko 2002. Ethnopharmacology studies document its use in treating dysuria in Iran (Naghibi 2005). The essential oils of the plant species have been used mainly for antipyretic purposes and for treating respiratory diseases on the eastern coast of Africa (Martins 1999, Ngassoum 2004).

Ocimum gratissimum is indigenous to tropical areas especially India and it is also in West Africa. In Nigeria, it is found in the Savannah and coastal areas. It is cultivated in Ceylon, South Sea Islands, and also within Nepal, Bengal, Chittagong and Deccan. It is known by various names in different parts of the world. In India it is known by its several vernacular names, the most commonly used ones being Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), Nimma tulasi (Kannada). In the southern part of Nigeria, the plant is called "effinrin-nla" by the Yoruba speaking tribe. It is called "Ahuji" by the Igbos, while in the Northern part of Nigeria; the Hausas call it "Daidoya". *O. gratissimum* is grown for the essential oils in its leaves and stems. Eugenol, thymol, citral, geraniol and linalool have been extracted from the oil (Dhifi *et al.*, 2016). Essential oils from the plant have been reported to possess an interesting spectrum of antifungal properties. The anti-nociceptive property of the essential oil of the plant has been reported (Rabelo *et al.*, 2003. The whole plant and the essential oil are used in traditional medicine especially in Africa and India. The essential oil is also an important insect repellent. Eugenol and thymol extracted from the oil are substitutes for clove oil and thyme oil. The essential oil is also used in perfumery. This species is often planted as ornamental, culinary and medicinal plant. In Asia, a tea is made from the leaves (Arueya *et al.,* 2015). Leaves are also eaten in salads and used as a condiment for sauces, soups or meat (Arueya *et al.*, 2015). It is also planted for hedges and as a mosquito repellent (Oyen and Nguyen, 1999; Orwa *et al.*, 2009; Useful Tropical Plants, 2018; PROSEA, 2018; PROTA, 2018; USDA-ARS, 2018).

MATERIALS AND METHODS

Plant Material:

The plant material of *Ocimum canum* and *Ocimum grattissimum* (leaves) were purchased from Karmo market, Abuja while *Ocimum basilicum* was collected from the garden, National Institute of Pharmaceutical Research and Development (NIPRD) Idu, Abuja Nigeria.

Leaf Epidermal Microscopy

Leaf epidermal preparation followed the methods of Ayodele and Olowokudejo (1997), cited by Ugbabe and Ayodele (2008). Slides were labeled appropriately and examined under the light microscope (ACCU-SCOPE 3025 Microscope Series) while photographs of the micro morphological features were taken using the camera (Industrial Design Camera E31SPM12000KPA) with magnifications x100 and x400. Terminologies are based on Metcalfe and Chalk (1979).

Chemo-Microscopy

A chemo-microscopic study of the comminuted dried leaf sample was carried out using methods to test for the presence of different metabolites (African Pharmacopoeia, 1986; Evans, 2002).

The collected leaves of *Ocimum canum, Ocimum gratissimum and Ocimum basilicum* were air dried and finely crushed into powder with a mortar and a pestle. A microscope slide was prepared by sprinkling the finely crushed particle on different slides and reagent. Phloroglucinol, conc. Hydrochloric acid, 66% sulphuric acid N/50 Iodine, 1% picric acid, millions reagent and Sudan IV reagent was used to test for the presence of Lignin, cellulose, tannins, starch, calcium Oxalate, Oil and Protein.

GC-MS Analysis

Essential Oil Extraction

Freshly collected leaves of *Ocimum canum, Ocimum gratissimum, and Ocimum basilicum* were extracted for three hours using the Clevenger type apparatus. The extracted oils was then dried over anhydrous Sodium sulphate and stored in small vials and tightly sealed and kept for GC-MS analysis. The oils were sent to Schimadzu Training Center for analytical Institute (STC), Lagos for analysis.

Gas Chromatography–Mass Spectrometry (GC- MS) Analysis

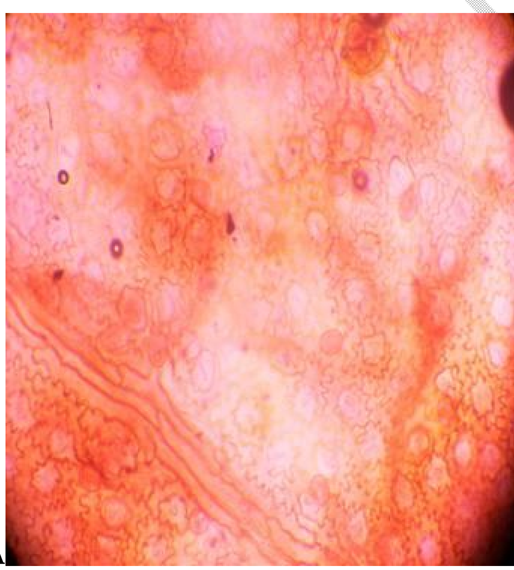
The methods of Okhale *et al*., 2018 were used where freshly collected leaves of *Ocimum canum, Ocimum gratissimum, and Ocimum basilicum* samples were chopped separately into pieces and each subjected to hydro-distillation for 4 hours using Clevenger-type apparatus. The essential oil obtained was dried over anhydrous sodium sulphate and used immediately for GC-MS using Shimadzu QP-2010 GC with QP-2010 mass selective detector [MSD, operated in the EI mode (electron energy =70Ev), scan range = 45400 amu, and scan rate = 3.99 scan/sec], and Shimadzu GCMS solution data system. The GC column was HP-5MS fused silica capillary with a 5% phenylpolymethylIsililoxane stationary phase, length 30 m, internal diameter 0.25 mm and film thickness 0.25 µm. The program used for GC oven temperature was isothermal at 60^{o}C, increased from 60^{o}C to 180^{o}C at rate of 10^{o}C/min, held at 180^{o}C for 2 minutes; increased from 180^{o}C at a rate of 15^{o}C/min, then held at 280^{o}C for 4 minutes. The injection port temperature was 250^{o}C. The ionization of sample components was performed in the electron impact mode (70eV). Injector temperature was 250^{o}C. The injection port temperature was 250°C while detector temperature was 280°C. Diluted sample (1/100 in hexane, v/v) of 1.0 µL was injected using auto sampler and in the split mode with ratio of 20:80. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Spectral

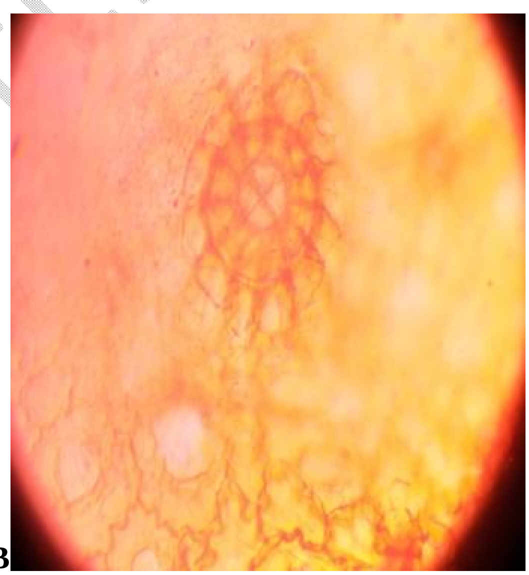
Library (NIST 11). The percentages of each component were reported as raw percentages based on the total ion current without standard.

RESULTS

Leaf Epidermal Microscopy

Figures 1 and 2 showed the leaf epidermis qualitative characters of the three species of *Ocimum.* It revealed Anomocytic stomata in all the species studied. *O. basilicum* has anomocytic stomata on both surfaces and more aboundant on the lower surface; cell walls are wavy on the upper surface and have glandular trichomes on both surfaces. *O. canum* also has anomocytic stomata on both surfaces; cell walls are wavy and trichomes are glandular and non-glandular occurring on both surfaces but more on the upper surface. The non-glandular trichomes are cone-shaped with pointed tips. *O. gratissimum* has anomacytic stomata on both surfaces but occurring more on the lower surface; the cell walls are curved on both surfaces and glandular trichomes occur on both surfaces but more abundant on the lower surface. The glandular trichomes are radially flagellated in all the species studied (Figures 1, 2 & 3).

A 

B 

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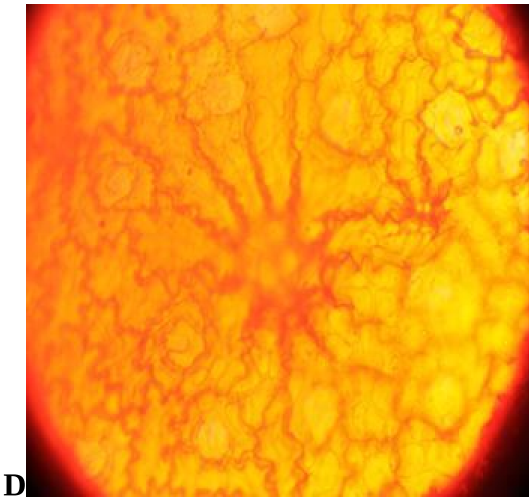

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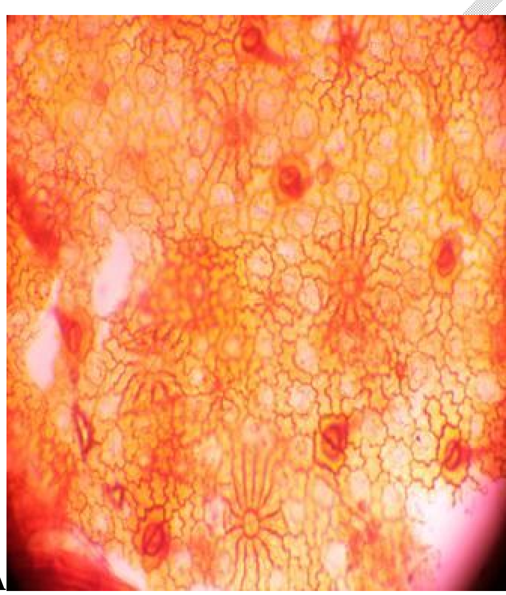
Figure 1. Leaf epidermal microscopy of *Ocimum basilicum*

A) Lower surface X 100. Anomocytic stomata, wavy cell walls and glandular capitate trichome
B) Lower surface X 400. Anomocytic stomata and multicellular glandular capitate trichome
C) Upper surface X 100. Multicellular Glandular capitate trichomes and no stomata.
D) Upper surface X400. Wavy cell shape and glandular trichome



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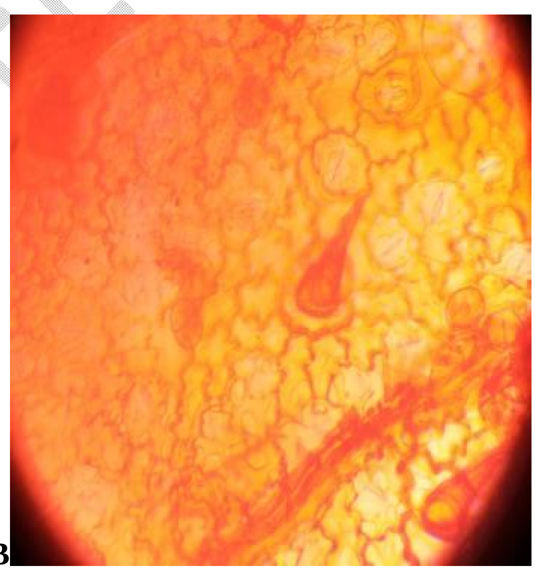

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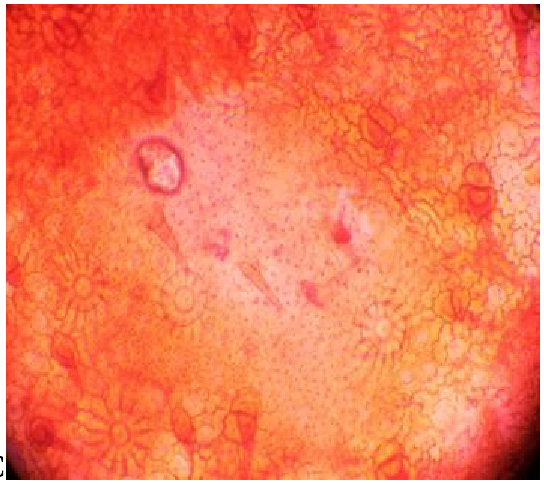
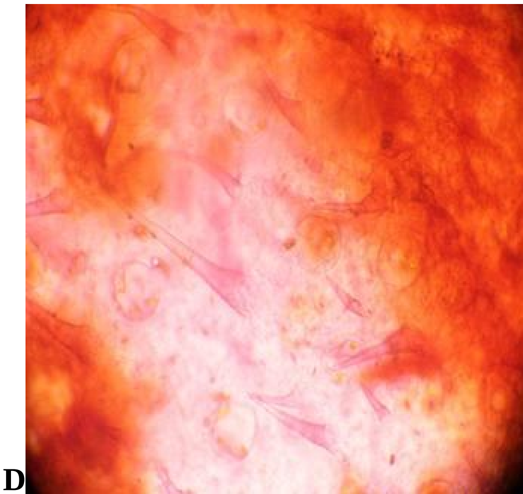
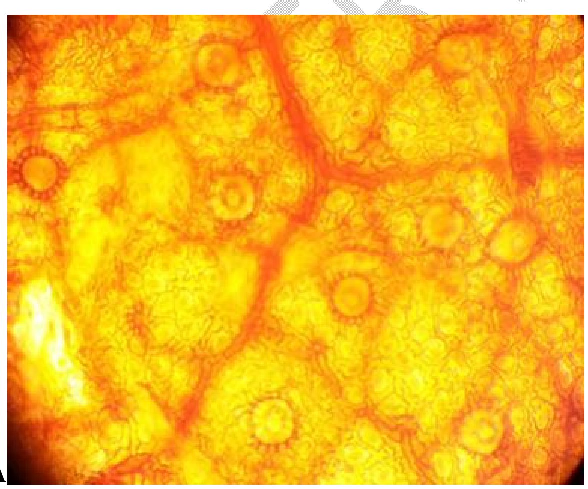
C  D 

Figure 2. Leaf epidermal microscopy of three *Ocimum canum*

A) Lower surface X 100. Anomocytic stomata, wavy cell wall, glandular and non-glandular trichomes
B) Lower surface X 400. Ancmoytic stomata, wavy cell shape and non-glandular cone-shaped trichome
C) Upper surface X 100. Flagellated glandular and non-glandular cone-shaped trichomes; wavy cell shape
D) Upper surface X 400. Glandular and non-glandular cone-shaped trichomes.

A  B 

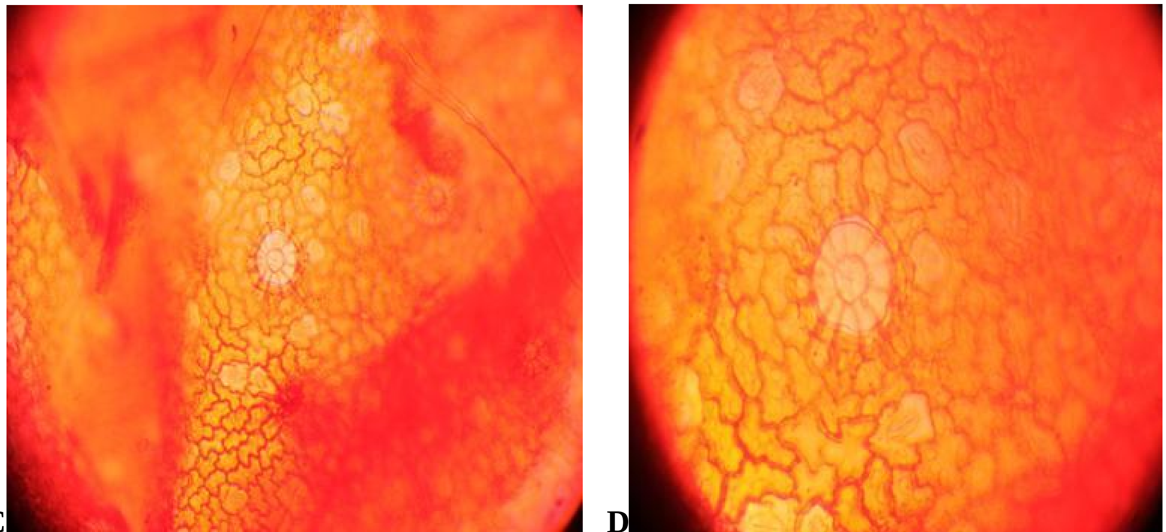


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Figure 3. Leaf epidermal microscopy of *Ocimum gratissimum*

A) Lower surface x100. Anomocytic stomata, Flagellated glandular trichomes and wavy cell wall
B) Lower surface x400. Anomocytic stomata, Flagellated glandular trichomes and wavy cell wall
C) Upper surface x100. Anomocytic stomata, Flagellated glandular trichomes and wavy cell wall
D) Upper surface x400. Anomocytic stomata, Flagellated glandular trichomes and wavy cell wall.

Chemo-microscopy

The chemo-microscopic analyses were positive for Lignin, Cellulose, Tannins, Mucilage, Starch, Calcium oxalate, oils and Protein in all the species studied (Table 1).

Table 1: Chemo-microscopy of three *Ocimum* species

TEST	*Ocimum basilicum*	*Ocimum canum*	*Ocimum gratissimum*
LIGNIN	+	+	+
CELLULOSE	+	+	+
TANNINS	+	+	+
MUCILAGE	+	+	+
STARCH	+	+	+
CALCIUM OXALATE	+	+	+
OIL	+	+	+
PROTEIN	+	+	+

GC-MS Analysis

The GC-MS analyses of the three species studies revealed 35 compounds for *O. basilicum*, 49 compounds for *O. canum* and 34 compounds for *O. gratissimum* with 3-Allyl-6-methoxyphenol being the most abundant in *O. basilicum* (34.42%); 1-Cyclopentene-1-methanol,2-methyl-5-1-methyl, the most abundant in *O. canum* (29.56%) and Thymol being the most abundant in *O. gratissimum* (48.04%).

Table 2. Chromatographic profile of *Ocimum basilicum* leaf oil

S/N	Names of compound	Retention Time	% Composition
1.	1,8-Cineole	6.724	2.37
2.	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	6.992	0.81
3.	p-Mentha-1,5-diene Menthadiene	7.133	0.26
4.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methy	7.198	0.39
5.	Bicyclo[2.2.1]heptan-2-one, 1,3,3-trimethyl-	7.373	0.68
6.	1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	7.621	20.88
7.	Ethyl (2E)-2-(1,7,7-trimethylbicyclo[2.2.1]hept-2-ylidene)hydrazinecarboxylate	8.056	1.57
8.	endo-Borneol	8.410	0.60
9.	Terpinen-4-ol	8.560	4.15
10.	alpha.-Terpineol	8.734	0.69
11.	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, acet	9.067	0.09
12.	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, ace	9.781	0.63
13.	3-Allyl-6-methoxyphenol	10.514	34.42
14.	alfa.-Copaene	10.942	0.08
15.	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-meth	11.059	2.44
16.	1H-Cyclopenta [1,3]cyclopropa[1,2]benzene, oct	11.117	0.46
17.	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-	11.280	1.03

	pentenyl)-		
18.	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	11.388	1.55
19.	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	11.510	10.25
20.	gamma.-Muurolene	11.626	0.63
21.	Humulene	11.722	0.77
22.	(+)-epi-Bicyclosesquiphellandrene	11.792	0.61
23.	beta.-copaene	11.968	2.63
24.	Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl-	12.035	0.21
25.	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimeth	12.114	0.53
26.	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, (1S,4S,7R)-	12.186	0.87
27.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1α,4aβ,8aα)-	12.253	1.66
28.	1H-3a,7-Methanoazulene, octahydro-3,8,8-trimethyl-6-methylene-, [3R-(3α,3aβ,7β,8aα)]-	12.318	0.90
29.	Cubedol	12.425	0.12
30.	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-, [1R-(1α,3α,4β)]-	12.531	0.83
31.	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]-	12.834	0.26
32.	Caryophyllene oxide	12.892	0.08
33.	Cubedol	13.175	0.60
34.	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	13.373	5.17
35.	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR (1aα,4β,4aβ,7α,7aβ,7bα)]-	13.501	0.74

Table 3. Chromatographic profile of *Ocimum canum* leaf oil

S/N	Names of compound	Retention Time	% Composition
1.	alpha.-Pinene	5.533	3.89
2.	2,2-dimethyl-3-methylidenebicyclo[2.2.1]heptane	5.710	0.40
3.	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	6.010	0.15
4.	Cyclohexene, 4-methylene-1-(1-methylethyl)-	6.068	0.09
5.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	6.211	2.23
6.	p-Mentha-1,5-diene Menthadiene 1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-	6.423	0.07
7.	(+)-4-Carene	6.582	0.56
8.	1-Methyl-4-(propan-2-yl)benzene	6.626	8.69
9.	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)	6.744	3.90
10.	gamma.-Terpinene	7.107	3.79
11.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2β,5α)-	7.198	7.61
12.	(+)-4-Carene	7.503	0.72
13.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1a,2a,5a)-	7.581	7.89
14.	1-Octen-3-yl-acetate	7.665	1.53
15.	2-Cyclohexen-1-ol, 1-methyl-	7.894	0.76

	4-(1-methylethyl)-		
16.	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)-	8.050	1.13
17.	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl	8.122	0.33
18.	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	8.455	0.18
19.	1-Cyclopentene-1-methanol, 2-methyl-5-(1-met	8.608	29.56
20.	3-Cyclohexene-1-methanol, α,α4-trimethyl-	8.733	0.87
21.	1,4-Cyclohexadiene-1-methanol, 4-(1-methylethyl)-	8.791	1.22
22.	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, acetate, (1R,2R,4S)-	9.072	0.69
23.	1,4-dihydroxy-p-menth-2-ene	9.418	0.73
24.	1,4-dihydroxy-p-menth-2-ene	9.588	0.69
25.	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate	9.783	0.91
26.	6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methyl acetate	10.186	0.76
27.	alfa.-Copaene	10.941	0.16
28.	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1a,2b,4b)]-	11.058	0.21
29.	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	11.283	0.11
30.	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trim	11.392	4.98
31.	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	11.506	7.17
32.	(E)-.beta.-Famesene	11.625	0.36
33.	Humulene	11.719	0.44
34.	gamma.-Muurolene	11.968	1.08
35.	1,2,4-Metheno-1H-indene, octahydro-1,7a-dimethyl-5-(1-methylethyl)-, [1S-	12.115	0.14

	(1α,2α,3aβ,4α,5α,7aβ,8S)]-		
36.	3.beta.-Acetoxy-bisnor-5-cholenamide	12.258	0.36
37.	Beta.-copaene	12.314	0.66
38.	Trans-Sesquisabinene hydrate	12.493	0.09
39.	Caryophyllene oxide	12.602	0.17
40.	Caryophyllene oxide	12.885	1.24
41.	tau.-Muurolol	13.111	0.50
42.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1a,4aa,8aa)-	13.372	0.61
43.	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1aα,4β,4aβ,7α,7aβ,7bα)]-	13.496	1.24
44.	cis-4,7,10,13,16,19-Docosahexanoic acid	13.631	1.17
45.	Cyclooctasiloxane, hexadecamethyl-	13.840	0.07
46.	Butyl 5,8,11,14,17-eicosapentaenoate	13.954	0.29
47.	Caryophyllene oxide	14.499	0.10
48.	n-Propyl 5,8,11,14,17-eicosapentaenoate	14.599	0.15
49.	Caryophyllene oxide	14.685	0.19

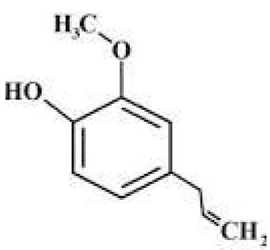
Table 4. Chromatographi**c** profile of *Ocimum gratissimum* leaf oil

S/N	Name of compound	Retention Time	% Composition
1.	alpha.-Pinene	5.532	0.98
2.	2,2-dimethyl-3-methylidenebicyclo[2.2.1] heptane	5.711	0.11
3.	3-Isopropyl-6-methylenecyclohex-1-ene	6.008	0.66
4.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene	6.066	0.47
5.	**Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-**	6.221	1.93

	methylene-, (1S)-		
6.	p-Mentha-1,5-diene Menthadiene 1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-	6.426	0.26
7.	3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene	6.520	0.18
8.	Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl-, (1S,6R)-	6.579	1.86
9.	Benzene, 1-methyl-3-(1-methylethyl)-	6.624	13.91
10.	Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	6.744	0.73
11.	1,3,6-Octatriene, 3,7-dimethyl-, (E)-;	6.841	0.50
12.	p-Mentha-1,4-diene	7.108	14.15
13.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2β,5α)-	7.194	0.65
14.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1α,2β,5α)-	7.609	1.03
15.	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, endo-	8.419	0.32
16.	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-;	8.601	0.73
17.	5-methyl-2-propan-2-ylphenol	9.826	48.04
18.	alfa.-Copaene	10.941	0.26
19.	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1a,2b,4b)]-	11.057	0.29
20.	Bicyclo[5.2.0]nonane, 2-	11.385	1.93

	methylene-4,8,8-trimethyl-4-vinyl-		
21.	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	11.500	0.18
22.	Humulene	11.717	0.33
23.	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	12.031	3.75
24.	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2α,4aα,8aβ)]-Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)-	12.120	1.33
25.	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1aα,7α,7aα,7bα)]-	12.332	0.71
26.	Caryophyllene oxide	12.888	2.65
27.	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-	13.129	0.26
28.	2-Adamantanol, 2-(bromomethyl)-	15.972	0.09
29.	Cyclohexene, 2-ethenyl-	16.225	0.72

	1,3,3-trimethyl-		
30.	2,5,5,8a-Tetramethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-ol	16.399	0.28
31.	3-Adamantan-1-yl-butan-2-one	16.497	0.09
32.	Retinoic acid	17.042	0.08
33.	Cyclohexane, 1,1-bis(5-methyl-2-furyl)-	17.231	0.18
34.	Benzoic acid, 4-[N'-(4,7,7-trimethyl-3-oxo-bic	18.484	0.36



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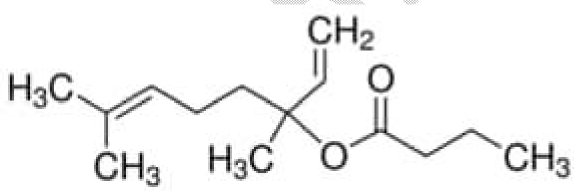
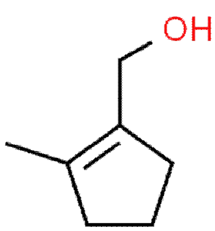


Linalyl butanoate



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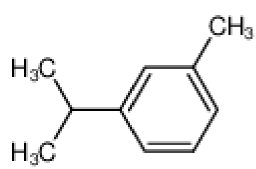

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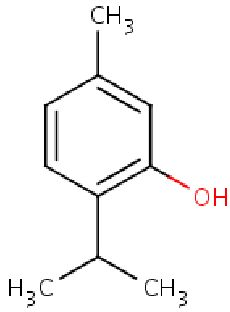

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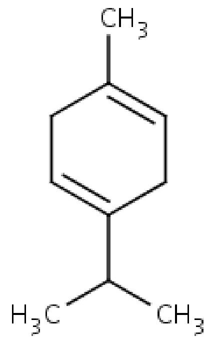
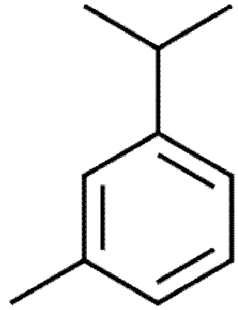
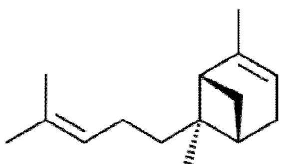


gamma-Terpinene



β-Cymene



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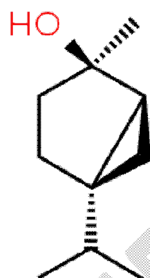
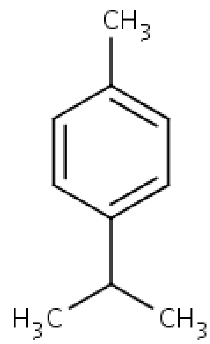


cis-4-Thujanol



p-cymene

Figure 4. Structure of some common compounds in the study

Table 5. Compounds Common to the three species of *Ocimum*

S/N	Compound	% Composition in *Ocimum basilicum*	% Composition in *Ocimum canum*	% Composition in *Ocimum gratissimum*
1	alpha.-Phellandrene	0.26	0.07	0.26
2	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methy	0.39	7.61	1.03
3	alfa.-Copaene	0.08	0.16	0.26
4	Caryophyllene oxide	0.08	1.24	2.65
5	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-m	1.03, 10.25	7.17	0.18
6	Humulene	0.77	0.44	0.33
7	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-meth	2.44	0.21	0.29
8	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trime	1.55, 0.53	4.98	1.93

Table 6. Compounds Common to *Ocimum basilicum* and *Ocimum canum*

S/N	Compound	% Composition	
		Ocimum basilicum	*Ocimum canum*
1.	(+)-2-Bornanone	1.57	1.13
2.	alpha.-Terpineol	0.69	0.87
3.	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, acet	0.09	0.69
4.	Bicyclo[2.2.1]heptan-2-ol, 1,7,7 trimethyl-, ace	0.63	0.91
5.	gamma.-Muurolene	0.63	1.08
6.	beta.-copaene	2.63	0.66
7.	Naphthalene,	1.66	0.61

1,2,3,4,4a,5,6,8aoctahydro-7-m

Table 7. Compounds Common to *Ocimum basilicum* and *Ocimum gratissimum*

S/N	Compound	% Composition	
		Ocimum basilicum	***Ocimum gratissimum***
1.	Endo-Borneol	0.6	0.32
2.	Terpinen-4-ol	4.15	0.73

Table 8. Compounds common to *Ocimum cranum* and *Ocimum gratissimum*

S/N	Compound	% Composition	
		Ocimum canum	***Ocimum gratissimum***
1.	alpha.-Pinene	3.89	0.98
2.	Camphene	0.4	0.11
3.	B4.icyclo[3.1.1]heptane, 6,6-dimethyl-2-methyle	2.23	1.93
4.	gamma.-Terpinene	3.79	14.15
5.	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl	0.11	0.18

DISCUSSION

All the species studied had anomocytic stomata in this type, the accessory or subsidiary cells are five in number. Stomata are used for the exchange of gases in between the plant and atmosphere

(Scranton, 2001). To facilitate this function, each stoma opens in a sub-stomatal chamber or respiratory cavity. Evaporation of water also takes place through the stomata (Bade, 2009).

Stomata are minute pores on the surface of green plants that are involved in the exchange of water and carbon dioxide between the plants and its atmosphere. It can be easily seen under a microscope. A single pore is called the stoma, which is found in the epidermis of leaves, stems, and other organs of the plant. Thousands of stomata are there on the surface of the leaves. Stomata help in the process of transpiration and photosynthesis which are the most essential process for the survival of a plant (Peter and Graham 2007). These processes are carried out through well-defined structures and procedures.

Trichomes simply referred to as hair outgrowths of epidermal cells in organisms including plants. Plant trichomes have long been known for their multiple beneficial roles, ranging from protection against insect herbivores and ultraviolet light to the reduction of transpiration (Kim, 2019). Trichomes are widely distributed on the surface of different tissues in different plants, exhibiting various morphologies. Trichomes are generally divided into single-celled or multicellular, branched or un-branched, and glandular or non-glandular based on different characteristics and functions. Trichomes also have different shapes, such as head, star, hook and scale (Wang *et al.,* 2021).

Lignin in plants adds comprehensive strength and stiffness to the plant’s cell wall and is believed to play a role in evolution of terrestrial plants by helping them withstand the compressive force of gravity (Melissa, 2022). Lignin also waterproofs the cell wall facilitating the upward transport of water in xylem tissues. Lignin has anti-fungal properties and is often rapidly deposited in response to injury by fungi, protecting the plant’s body from the diffusion of fungal enzyme and toxins (Melissa, 2022).

Cellulose is a complex carbohydrate or polysaccharide. It is the basis structural component of plants (James, 2019). Cell wall is non-digestible by humans and a food for herbivorous animals (Brett, 1990.). Tannins are a group of phenolic compounds in woody flowering plants that are important deterrents to herbivores (Jorma, 2021). They occur in roots, barks, wood, leaves and fruits of plants. They are used in tanning leather, dying fabrics and making ink (Lina and Maria, 2018). Tannins are acidic and have astringent taste. They are used in clarification of wine and beer (Susana 2020). Mucilage is a water-soluble viscous material characterized by a light color, which is part of the fiber. It is formed by some specialized secretory cells of the plant endosperm and its function is to prevent excessive dehydration (Dhingra *et al.,* 2012).

Ocimum canum is an underutilized medicinal plant that is used for the treatment of gastrointestinal problems and also for the preparation of local soups. The leaves have high carbohydrate content, ash, crude fat and crude fiber, but very low in protein and high concentration of calcium with appreciable levels of potassium, sodium, phosphorous and magnesium (Aluko *et al.,* 2012). In addition, the plant was found to be a good source of iron, zinc and manganese. Furthermore, the concentrations of cadmium and lead, which are toxic metals were very low, while the vitamin C content of the leaves was found to be high (Aluko *et al.,* 2012).

Essential oils of the leaves of *O. canum* possess antibacterial (Janssen *et al*, 1989) and insecticidal (Bassole *et al*., 2003b) properties. The chemical compositions of the leaves of *O. canum* reported here differ from those observed by Philoppe *et al.* 2013, and Tamal *et al.,* 2015. In Tamil *et al.,* 2015 the GC-MS analysis of hydro-distilled oil revealed the presence of 36 compounds in *O. canum* and of which camphor was identified as a major compound which was accounted to be 39.77%, followed by limonene (8.67%), naphthalene (7.37%), valencene (5.80%), caryophyllene (5.60%), a-pinene (5.59%), camphene (5.20%) and myrtenyl acetate (2.74%). Similarly Martins *et al.* (1999) and Chagonda *et al*. (2000) also reported camphor as a major compound in certain species of *Ocimum cimum* including *O. canum*, *O. gratissimum* and *O. minimum* with varied percentage of camphor. This variation may be due to environmental and physiological factors. On the contrary the essential oil of several chemotypes of *O. canum* has been reported with a wide range of major compounds like eugenol, citral, b-caryophyllene and methyl cinnamate (Ekundayo *et al*., 1989; Choudhary *et al*., 1998; Sanda *et al*., 1998; Chalchat *et al*., (1999). The GC-MS analyses of the three species in this study revealed 35 compounds for *O. basilicum*, 49 compounds for *O. canum* and 34 compounds for *O. gratissimum*. In *O. basilicum* the most abundant compound is m-Eugenol (34.42%) followed by lindalyl butanoate (20.88%) and alpha-bergamotene (10.25%). In *O. canum* the most abundant compound is 1-cyclopentaene-1-metanol, 2-methyl-5-(met. (29.56) followed by p-Cymene (8.9%), followed by Sabinene hydrate (7.89%) and cis-4-Thyjanol (7.61%) and alpha-berganotene (7.17%). In *O.*

gratissinum, Thymol (48.04) is the most abundant followed by gamma-terpinene (14.15%) and m-cymene (13.91%).

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