

# 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 GCMS analysis of essential oils from three *Ocimum* species were analyzed. *Ocimum* belong to the family Lamiaceae. Leaf epidermal microscopy revealed anomocytic stomata in the species studied. *Ocimum basilicum* has anomocytic stomata on both surfaces but were more abundant on the lower surface; cell walls were wavy on the upper surface and had glandular trichomes on both surfaces. *Ocimum canum* had anomocytic stomata on both surfaces; cell walls were wavy and trichomes were 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. *Ocimum gratissimum* has anomocytic 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. Lignin, tannins, cellulose, mucilage, starch, calcium oxalate, oils and proteins were observed in all 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%). The leaf epidermal microscopy and the chemo-microscopy can be used for the standardization of the plant. Chemical composition of the three *Ocimum* species can be used for the treatment of several diseases.

*Keywords:* *Ocimum* species; microscopy; analysis; Nigeria.

## 1. INTRODUCTION

“The Genus *Ocimum* comprised of about 200 species distributed over Asia, Africa, Central and Southern America” [1,2]. “*Ocimum* is cultivated for its essential oil with many potent pharmacological application and culinary, perfume for herbal toiletries, aromatherapy treatment and as flavoring agent” [3]. 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* is the most popular species and is assumed to originate from India, Africa, and/or the Middle East [4]. 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 [5].

*Ocimum canum* is used in Africa to treat malaria, and headache, and has been used as an analgesic and rubefacient [6,7]. It also has been used to manage diabetes mellitus in Ghana [7]. Ethnopharmacology studies document its use in treating dysuria in Iran [8]. 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 [9,6]. “*Ocimum canum* is an underutilized medicinal plant that is used for the treatment of gastrointestinal problems and also for the preparation of local soups” [10]. “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” [10]. “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" [10].

"*Ocimum gratissimum* is indigenous to tropical areas especially India and it is also in West Africa" [11]. "In Nigeria, it is found in the Savannah and coastal areas [11]. It is cultivated in Ceylon, South Sea Islands, and also within Nepal, Bengal, Chittagong and Deccan" [12]. "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)" [12]. "In the southern part of Nigeria, the plant is called "effirin-nla" by the Yoruba speaking tribe" [13]. "It is called "Ahuji" by the Igbos, while in the Northern part of Nigeria; the Hausas call it "Daidooya". *Ocimum gratissimum* is grown for the essential oils in its leaves and stems. Eugenol, thymol, citral, geraniol and linalool have been extracted from the oil" [14]. "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" [15]. "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" [15]. "Eugenol and thymol extracted from the leaf are substitutes for clove oil and thyme oil" [15]. "The essential oil is also used in perfumery" [15]. "This species is often planted as ornamental, culinary and medicinal plant. In Asia, a tea is made from the leaves" [16]. "Leaves are also eaten in salads and used as a condiment for sauces, soups or meat" [16]. "It is also planted for hedges and as a mosquito repellent" [17,18].

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

*Ocimum canum* and *Ocimum gratissimum* were purchased while *Ocimum basilicum* was collected from a garden.

### 2.2 Leaf Epidermal Microscopy

The methods of Ayodele and Olowokudejo [19] were adopted for the leaf epidermal microscopy. 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 [20].

### 2.3 Chemo-Microscopy

The collected leaves 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 for the test [21].

### 2.4 GC-MS Analysis

#### 2.4.1 Essential oil extraction

The Methods of Okhale *et al.*, 2018 were used for the extraction of the oils. The oils were sent in vials to Shimadzu Training Center for analytical Institute (STC), Lagos for analysis.

#### 2.4.2 Gas chromatography–Mass spectrometry (GC- MS) analysis

The methods of Okhale *et al.*, 2018 were used where freshly collected leaves of *O. canum*, *O. gratissimum*, and *O. 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% phenylpolymethylsiloxane 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 60oC, increased from 60oC to 180oC at rate of 10oC/min, held at 180oC for 2 minutes; increased from 180oC at a rate of 15oC/min, then held at 280oC for 4 minutes. The injection port temperature was 250oC. The ionization of sample components was performed in the electron impact mode (70eV). Injector temperature was 250oC. 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

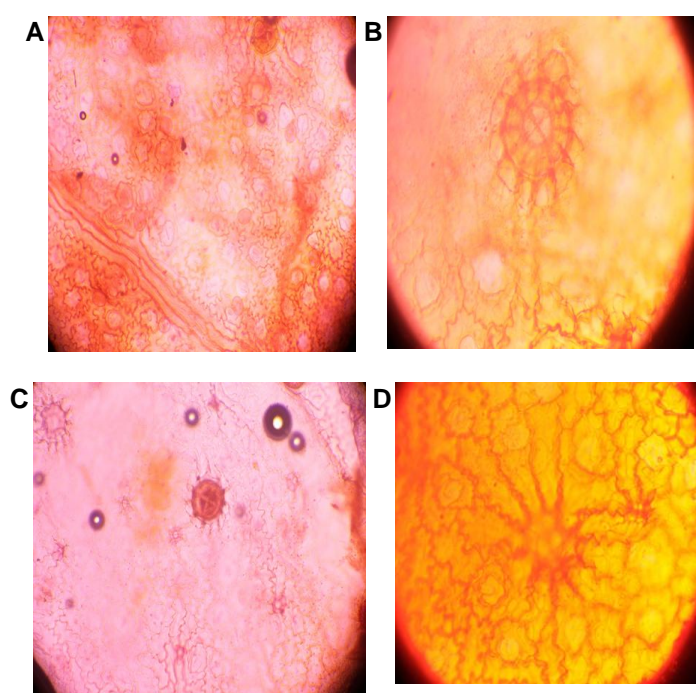
comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 11). The percentages of each component were reported as raw percentages based on the total ion current without standard.

### 3. RESULTS

#### 3.1 Leaf Epidermal Microscopy

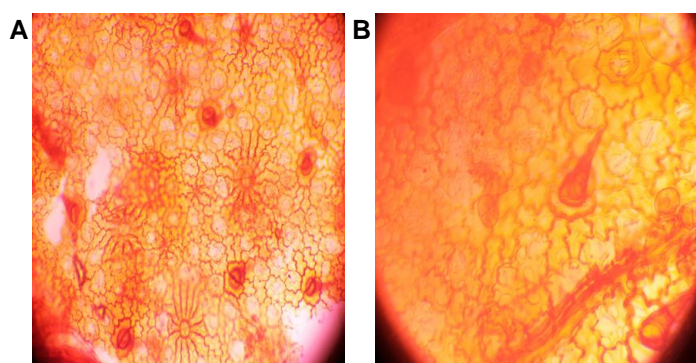
The three species of *Ocimum* revealed Anomocytic stomata. *Ocimum basilicum* has anomocytic stomata on both surfaces and more abundant on the lower surface; cell walls are wavy on the upper surface and have glandular

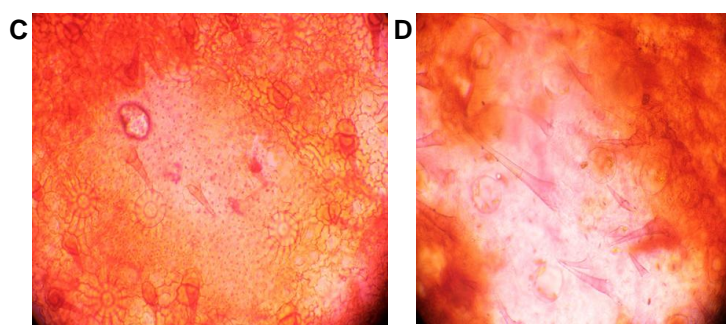
trichomes on both surfaces. *Ocimum canum* also has anomocytic stomata on both surfaces; cell walls are wavy and trichomes are glandular and non-glandular occurring on both surfaces but more on the upper surface. The non-glandular trichomes are cone-shaped with pointed tips. *Ocimum gratissimum* has anomocytic 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 (Figs. 1, 2 & 3).



**Fig. 1. Leaf epidermal microscopy of *Ocimum basilicum***

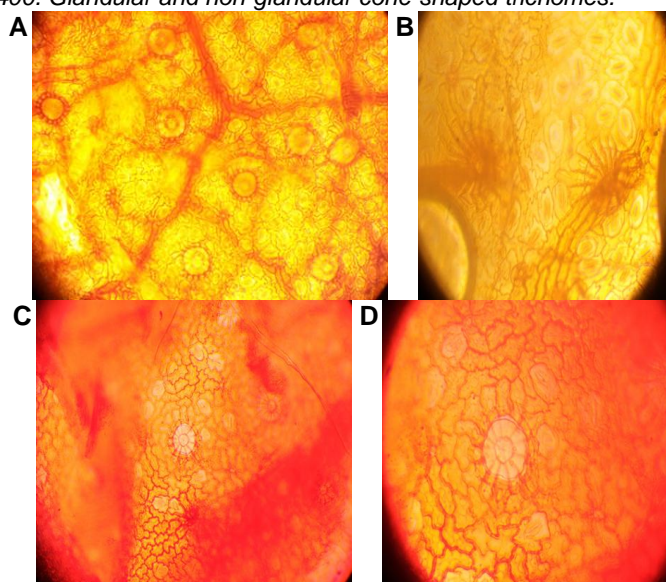
- A) Lower surface X 100. Anomocytic stomata, wavy cell walls and glandular capitate trichome
- B) Lower surface X 400. Anomocytic stomata and multicellular glandular capitate trichome
- C) Upper surface X 100. Multicellular Glandular capitate trichomes and no stomata.
- D) Upper surface X400. Wavy cell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





**Fig. 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. Anomocytic 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.



**Fig. 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.

### 3.2 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	<i>Ocimum basilicum</i>	<i>Ocimum canum</i>	<i>Ocimum gratissimum</i>
Lignin	+	+	+
Cellulose	+	+	+
Tannins	+	+	+
Mucilage	+	+	+

Starch	+	+	+
Calcium	+	+	+
Oxalate			
Oil	+	+	+
Protein	+	+	+

### 3.3 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%).

#### 4. 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” [22]. “To facilitate this function, each stoma opens in a sub-stomatal chamber or respiratory cavity. Evaporation of water also takes place through the stomata” [23].

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” [24]. These processes are carried out through well-defined structures and procedures.

**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-methyl-	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-methyl-	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-pentenyl)-	11.280	1.03
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-trimethyl-	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)-, (1 $\alpha$ ,4 $\alpha$ ,8 $\alpha$ )-	12.253	1.66
28.	1H-3a,7-Methanoazulene, octahydro-3,8,8-trimethyl-6-	12.318	0.90

	methylene-, [3R-(3 $\alpha$ ,3 $\alpha\beta$ ,7 $\beta$ ,8 $\alpha$ )]-		
29.	Cubedol	12.425	0.12
30.	Cyclohexanemethanol, 4-ethenyl- $\alpha,\alpha$ ,4-trimethyl-3-(1-methylethenyl)-, [1R-(1 $\alpha$ ,3 $\alpha$ ,4 $\beta$ )]-	12.531	0.83
31.	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1 $\alpha\alpha$ ,4 $\alpha\alpha$ ,7 $\beta$ ,7 $\alpha\beta$ ,7 $\beta\alpha$ )]-	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 (1 $\alpha\alpha$ ,4 $\beta$ ,4 $\alpha\beta$ ,7 $\alpha$ ,7 $\alpha\beta$ ,7 $\beta\alpha$ )]-	13.501	0.74

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

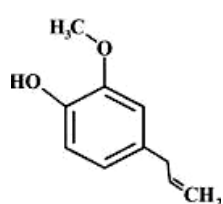
S/N	Names of compound	Retention Time	% Composition
1.	alpha.-Pinene	5.533	3.89
2.	2,2-dimethyl-3methylidenebicyclo[2.2.1]heptane	5.710	0.40
3.	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	6.010	0.15
4.	Cyclohexene, 4-methylene-1-(1-methylethyl)-	6.068	0.09
5.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	6.211	2.23
6.	p-Mentha-1,5-diene Menthadiene	6.423	0.07
7.	1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-	6.582	0.56
8.	(+)-4-Carene	6.626	8.69
9.	1-Methyl-4-(propan-2-yl)benzene	6.744	3.90
10.	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (R)	7.107	3.79
11.	gamma.-Terpinene	7.198	7.61
12.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1 $\alpha$ ,2 $\beta$ ,5 $\alpha$ )-	7.503	0.72
13.	(+)-4-Carene	7.581	7.89
14.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1 $\alpha$ ,2 $\alpha$ ,5 $\alpha$ )-	7.665	1.53
15.	1-Octen-3-yl-acetate	7.894	0.76
16.	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-	8.050	1.13
17.	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)-	8.122	0.33
18.	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)	8.455	0.18
19.	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	8.608	29.56
20.	1-Cyclopentene-1-methanol, 2-methyl-5-(1-met	8.733	0.87
21.	3-Cyclohexene-1-methanol, $\alpha$ , $\alpha$ 4-trimethyl-	8.791	1.22
22.	1,4-Cyclohexadiene-1-methanol, 4-(1-methylethyl)-	9.072	0.69
23.	Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, acetate, (1R,2R,4S)-	9.418	0.73
24.	1,4-dihydroxy-p-menth-2-ene	9.588	0.69
25.	1,4-dihydroxy-p-menth-2-ene	9.783	0.91
26.	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate	10.186	0.76
27.	6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methyl acetate	10.941	0.16
28.	alfa.-Copaene	11.058	0.21
29.	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1 $\alpha$ ,2 $\beta$ ,4 $\beta$ )]-	11.283	0.11
30.	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	11.392	4.98
31.	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trim	11.506	7.17
32.	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	11.625	0.36
33.	(E)-.beta.-Famesene	11.719	0.44
34.	Humulene	11.968	1.08
35.	gamma.-Muurolene	12.115	0.14
36.	1,2,4-Metheno-1H-indene, octahydro-1,7 $\alpha$ -dimethyl-5-(1-methylethyl)-, [1S-(1 $\alpha$ ,2 $\alpha$ ,3 $\alpha\beta$ ,4 $\alpha$ ,5 $\alpha$ ,7 $\alpha\beta$ ,8S)]-	12.258	0.36
37.	3.beta.-Acetoxy-bisnor-5-cholenamide	12.314	0.66
38.	Beta.-copaene	12.493	0.09
39.	Trans-Sesquisabinene hydrate	12.602	0.17
40.	Caryophyllene oxide	12.885	1.24
41.	Caryophyllene oxide	13.111	0.50
42.	tau.-Muurolol	13.372	0.61
42.	Naphthalene, 1,2,3,4,4 $\alpha$ ,5,6,8 $\alpha$ -octahydro-7-		

S/N	Names of compound	Retention Time	% Composition
43.	methyl-4-methylene-1-(1-methylethyl)-, (1a,4aa,8aa)- 1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7- tetramethyl-, [1aR-(1a $\alpha$ ,4 $\beta$ ,4a $\beta$ ,7 $\alpha$ ,7a $\beta$ ,7b $\alpha$ )]-	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

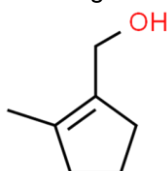
**Table 4. Chromatographic profile of *Ocimum gratissimum* leaf oil**

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- methylene-, (1S)-	6.221	1.93
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)-, (1 $\alpha$ ,2 $\beta$ ,5 $\alpha$ )-	7.194	0.65
14.	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1- methylethyl)-, (1 $\alpha$ ,2 $\beta$ ,5 $\alpha$ )-	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-methylene-4,8,8- trimethyl-4-vinyl-	11.385	1.93
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-(1methylethenyl)-, [2R-(2 $\alpha$ ,4 $\alpha$ ,8a $\beta$ )]-Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2- (1-methylethylidene)-, (4aR-trans)-	12.120	1.33

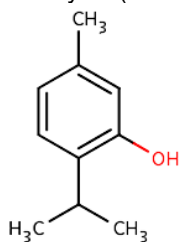
S/N	Name of compound	Retention Time	% Composition
25.	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1a $\alpha$ ,7 $\alpha$ ,7a $\alpha$ ,7b $\alpha$ )]-	12.332	0.71
26.	Caryophyllene oxide	12.888	2.65
27.	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-	13.129	0.26
28.	2-Adamantanol, 2-(bromomethyl)-	15.972	0.09
29.	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	16.225	0.72
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



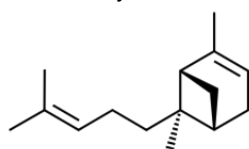
m-Euginol



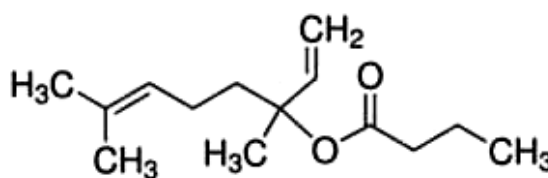
1-Cyclopentene-1-methanol, 2-methyl-5-(1-met)



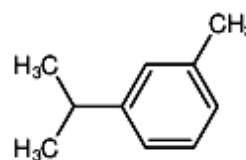
Thymol



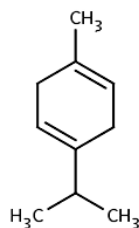
alpha-Bergamotene



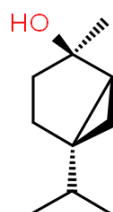
Linalyl butanoate



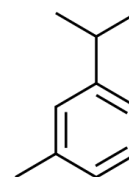
Benzene, 1-methyl-3-(1-methylethyl)-



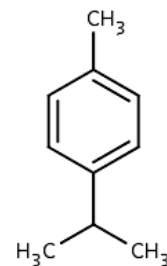
gamma-Terpinene



cis-4-Thujanol



$\beta$ -Cymene



p-cymene

**Fig. 4. Structure of some common compounds in the study**  
**Table 5. Compounds Common to the three species of *Ocimum***

S/N	Compound	% Composition <i>Ocimum basilicum</i>	% Composition <i>Ocimum canum</i>	% Composition <i>Ocimum gratissimum</i>
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	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	4.98	1.93

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

S/N	Compound	% Composition <i>Ocimum basilicum</i>	% Composition <i>Ocimum canum</i>
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, 1,2,3,4,4a,5,6,8a octahydro-7- m	1.66	0.61

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

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

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

S/N	Compound	% Composition	
		<i>Ocimum canum</i>	<i>Ocimum gratissimum</i>
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

“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” [25]. “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 unbranched, and glandular or non-glandular based on different characteristics and functions. Trichomes also have different shapes, such as head, star, hook and scale” [26].

“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” [27]. “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” [27].

Cellulose is a complex carbohydrate or polysaccharide. It is the basis structural component of plants [28]. Cell wall is non-digestible by humans and a food for herbivorous animals [29]. “Tannins are a group of phenolic compounds in woody flowering plants that are important deterrents to herbivores” [30]. “They occur in roots, barks, wood, leaves and fruits of plants and are used in tanning leather, dying fabrics and making ink” [30]. “Tannins are acidic and have astringent taste. They are used in clarification of wine and beer” [31]. “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” [32].

“*Ocimum canum* is an underutilized medicinal plant that is used for the treatment of gastrointestinal problems and also for the preparation of local soups” [10]. “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” [10]. “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” [10].

“Essential oils of the leaves of *O. canum* possess antibacterial (Janssen *et al.*, 1989) and insecticidal [33] properties”. “The chemical compositions of the leaves of *O. canum* reported here differ from those” observed by Philoppe *et al.* 2013, and Tamil *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%),  $\alpha$ -pinene (5.59%), camphene (5.20%) and myrtenyl acetate (2.74%)”. Similarly Martins *et al.* [9] and Chagonda *et al.* [34] also reported “camphor as a major compound in certain species of *Ocimum* 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,  $\beta$ -caryophyllene and methyl cinnamate” [35,36,37,38]. 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 linalyl butanoate (20.88%) and  $\alpha$ -bergamotene (10.25%). In *O. canum* the most abundant compound is 1-cyclopentane-1-methanol, 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  $\alpha$ -bergamotene (7.17%). In *O. gratissimum*, Thymol (48.04) is the most abundant followed by  $\gamma$ -terpinene (14.15%) and *m*-cymene (13.91%) [39-42].

Eight compounds: ( $\alpha$ -Phellandrene; Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methyl- $\alpha$ -Copaene; Caryophyllene oxide; Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-*m*); Humulene; Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methyl and Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl) are present in the three species of *Ocimum* studied (Table 5). *Ocimum basilicum* and *O. canum* had seven compounds: (+)-2-Bornanone;  $\alpha$ -Terpineol; Bicyclo[2.2.1]heptan-2-ol, 1,3,3-trimethyl-, acet; Bicyclo[2.2.1]heptan-2-ol, 1,7,7 trimethyl-, acet;  $\gamma$ -Murolene;  $\beta$ -copaene and Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-*m* in common (Table 6). *Ocimum basilicum* and *O. gratissimum* had two compounds in common: Endo-Borneol and Terpinen-4-ol (Table 7) While *O. canum* and *O. gratissimum* had five compounds:  $\alpha$ -Pinene; Camphene; Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methyl-;  $\gamma$ -Terpinene and Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl) in common (Table 8).

## 5. CONCLUSION

In conclusion, the leaf epidermal microscopy and the chemo-microscopy in this study can be used for the identification and standardization of the plant. The chemical composition of the essential oils from the three *Ocimum* species could serve as a good source for food and crude drug preparation for the treatment of several diseases.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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