Review Article

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 (*Ocimumbasilicum* L.; *Ocimumcanum*Sims. and *Ocimumgratissimum*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: *Ocimumgratissimum*, Microscopy, Analysis, Nigeria

INTRODUCTION

The Genus *Ocimum* belongs to Lamiaceae family and comprised of about200 species of herbs and shrubs and is distributed over Asia, Africa, Central andSouthern America (Reference). *Ocimum*is cultivated for itsessential oil which displays many potent pharmacologicalapplication (mosquito repellent, antimicrobial, antioxidant,wound healing, anti-melanoma, and radioprotective) culinary,perfume for herbal toiletries, aromatherapy treatment and as flavoring agent (Reference). The study was done to compare the leaf epidermal microscopy, chemo-microscopy, and GC-MS analyses of *Ocimumbasilicum* L. *Ocimumcanum*Sims*;* and *Ocimumgratissimum*L.leaves.

Ocimumbasilicum L. (Sweet basil), the Mediterranean type and the most popular species, is assumed to originate from India, Africa, and/or the Middle East(Reference). 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(Reference). *Ocimumcanum*Sims is a semi-woody plant of about 40 cm high it is an aromatic plant and native to tropical Africa (Steel, et al missing2006). It is popularly called African basil, but known as "Efinrinelewedudu" 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(Reference). 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(Reference). 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).

Ocimumcanum is used medicinally in Africa to treat conjunctivitis, malaria, and headache, and has been used as an analgesic and rubefacient (Ngassoum 2004, et al missing, Nyarkoet al missing2002). It also has been used to manage diabetes mellitus in Ghana (Nyarkoet al missing 2002).Ethnopharmacology studies document its use in treating dysuria in Iran (Naghibiet al missing2005). 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 et al missing1999, Ngassoumet al missing2004).

Ocimumgratissimum is indigenous to tropical areas especially India and it is also in West Africa (Reference). In Nigeria, it is found in the Savannah and coastal areas (Reference). It is cultivated in Ceylon, South Sea Islands, and also within Nepal, Bengal, Chittagong and Deccan (Reference). 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), Nimmatulasi (Kannada) (Reference). In the southern part of Nigeria, the plant is called "effinrin-nla" by the Yoruba speaking tribe (Reference). 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(Reference).Eugenol andthymol extracted from the oil are substitutes for clove oil and thyme oil(Reference). The essential oil is also used in perfumery(Reference). 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 – Not mentioned in reference).

MATERIALS AND METHODS

Plant Material:

The plant material of *Ocimumcanum* and *Ocimumgrattissimum* (leaves) were purchased from Karmo market, Abuja while *Ocimumbasilicum*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 methodsto test for the presence of different metabolites (African Pharmacopoeia, 1986; Evans, 2002).

The collected leaves of *Ocimumcanum, Ocimumgratissimum and Ocimumbasilicum* 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 *Ocimumcanum, Ocimumgratissimum, and Ocimumbasilicum* were extracted for three hours using the Clevenger type apparatus(Reference). The extracted oils was then dried over anhydrous Sodium sulphate and stored in small vials (Reference)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 *Ocimumcanum, Ocimumgratissimum, and Ocimumbasilicum*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 surfacesbut 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 trichomesoccur 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).

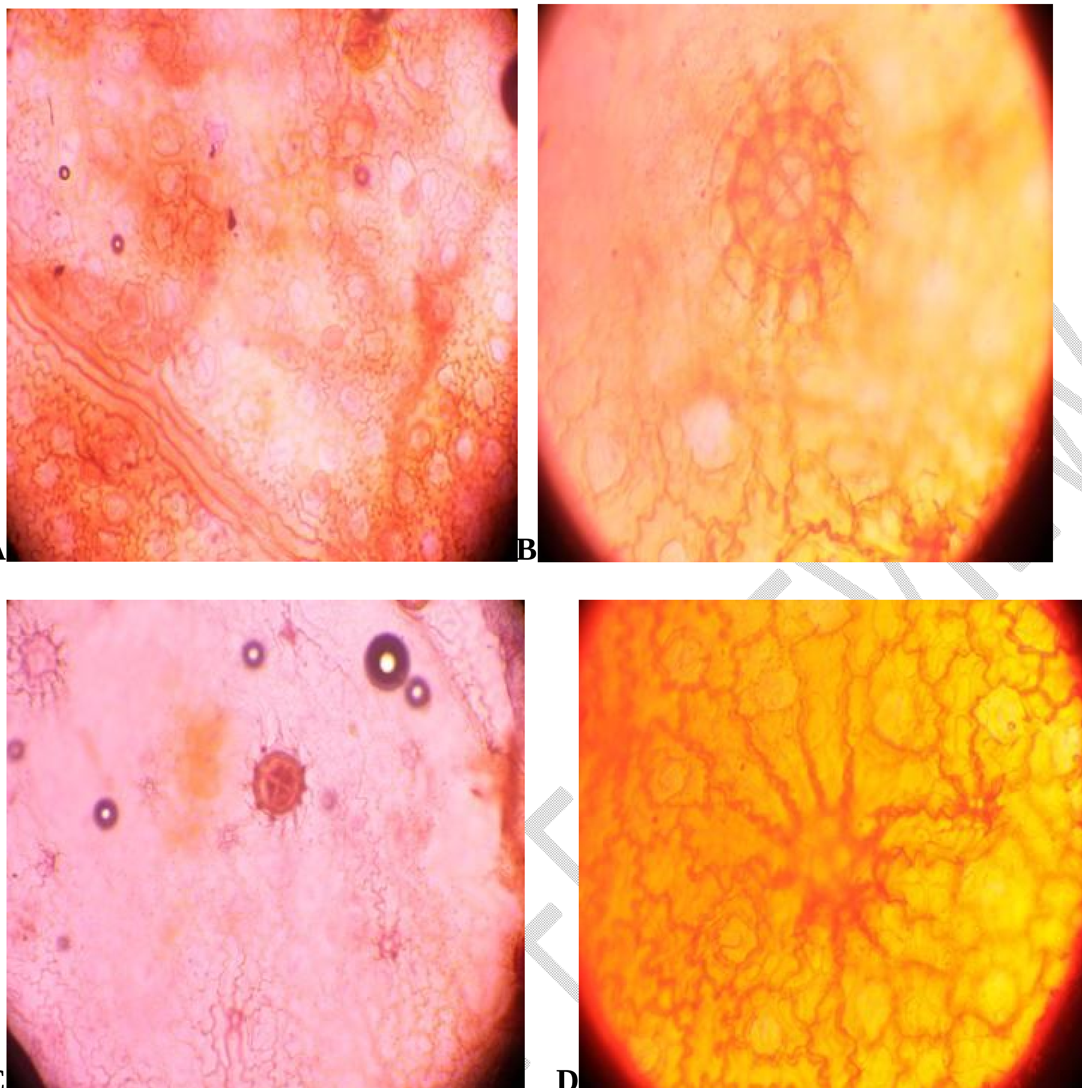
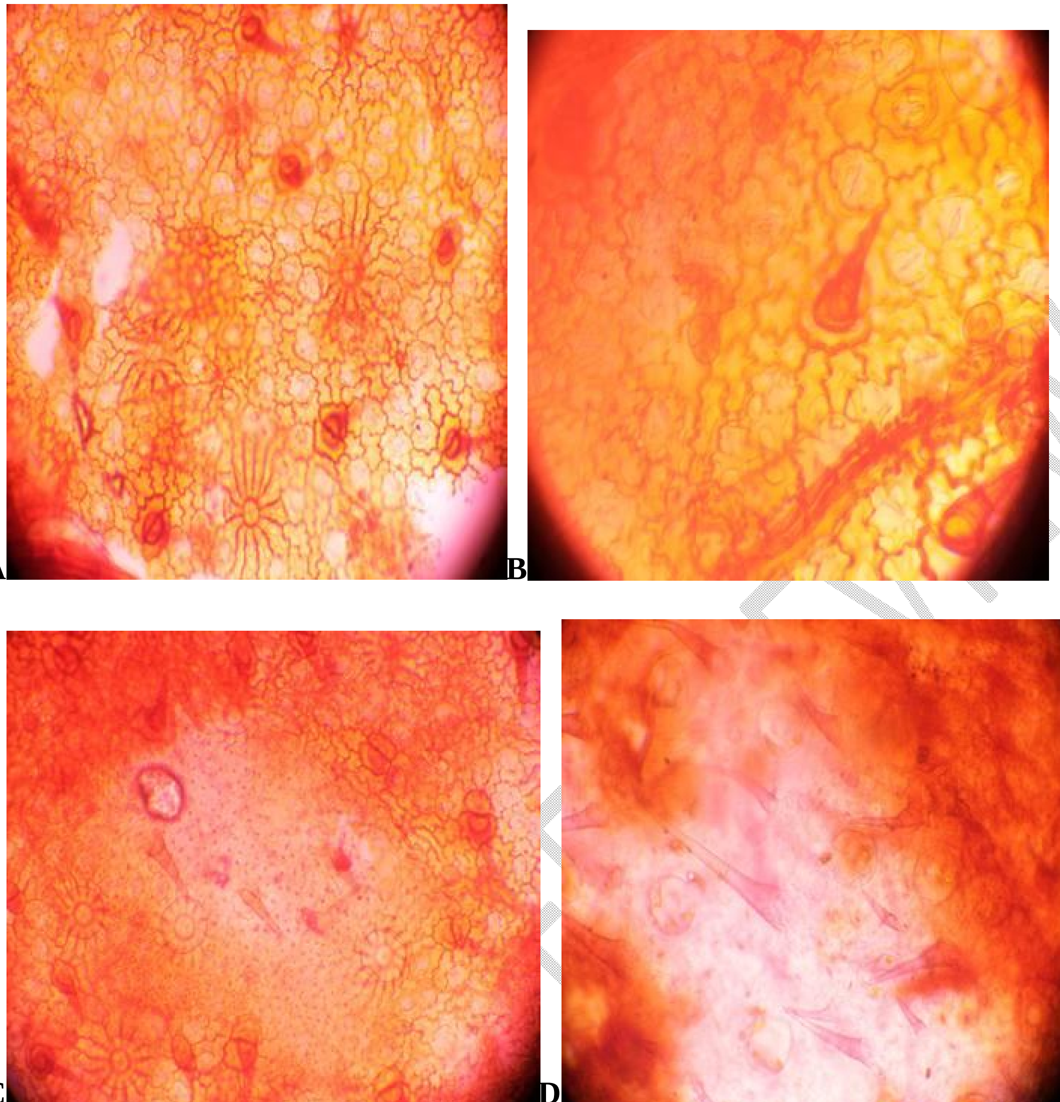


Figure 1. Leaf epidermal microscopy of *Ocimumbasilicum*

A) Lower surface X 100. Anomocytic stomata, wavy cell walls and glandular capitatetrichome
B) Lower surface X 400. Anomocytic stomata and multicellular glandular capitatetrichome
C) Upper surface X 100. Multicellular Glandularcapitatetrichomes and nostomata.
D) Upper surface X400. Wavy cell shape and glandular trichome



A B

C D

Figure 2. Leaf epidermal microscopy of three *Ocimumcanum*

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.

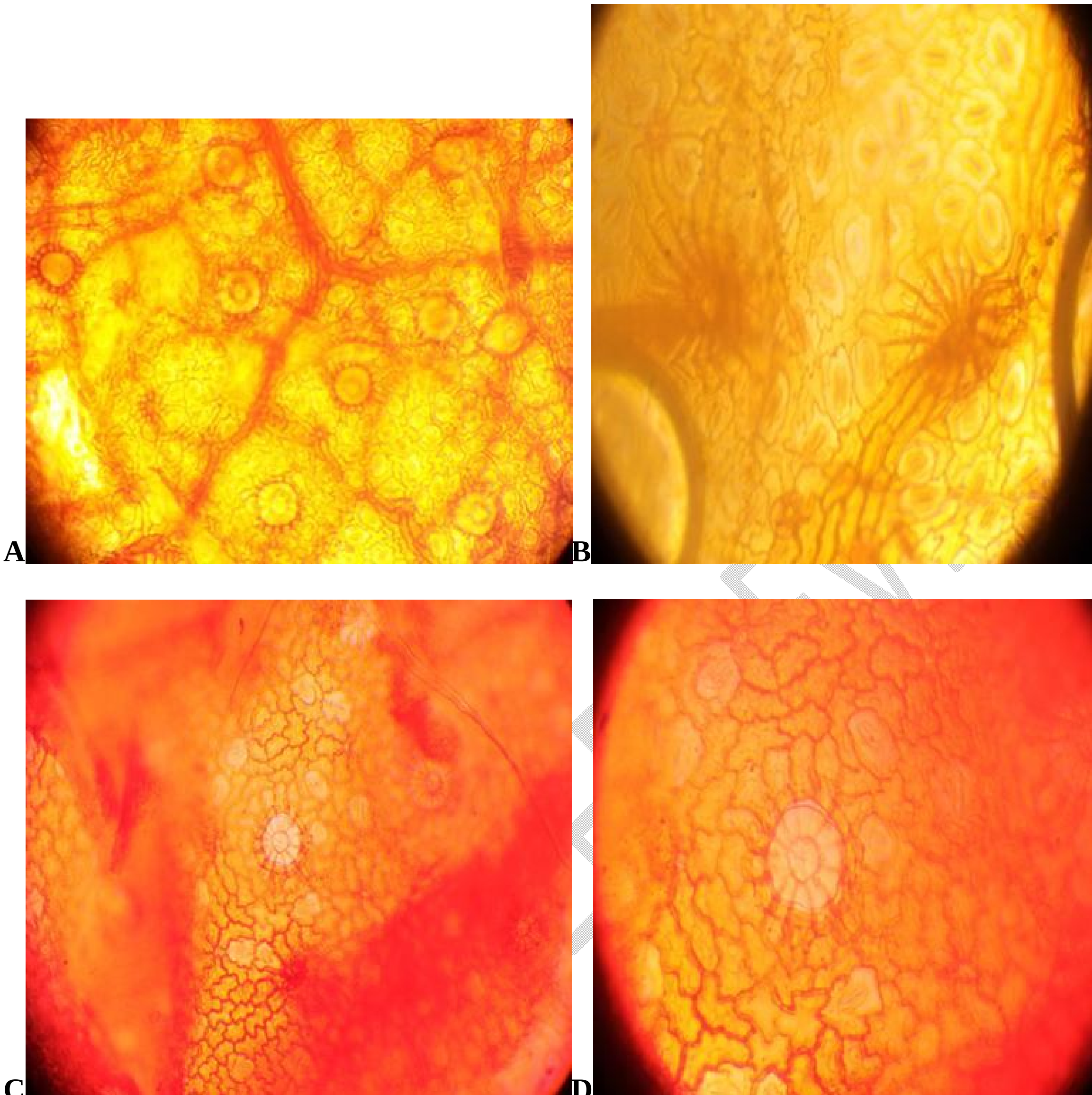


Figure 3. Leaf epidermal microscopy of*Ocimumgratissimum*

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 speciesstudied (Table 1).

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

TEST	*Ocimumbasilicum*	*Ocimumcanum*	*Ocimumgratissimum*
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 *Ocimumbasilicum*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-	8.056	1.57

	trimethylbicyclo[2.2.1]hept-2-ylidene)hydrazinecarboxylate		
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-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-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*Ocimumcanum*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-4-(1-methylethyl)-	7.894	0.76
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-(1α,2α,3aβ,4α,5α,7aβ,8S)]-	12.115	0.14
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

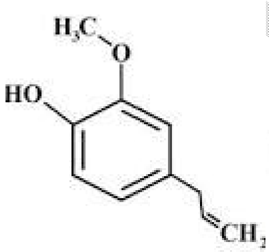
49.	Caryophyllene oxide	14.685	0.19

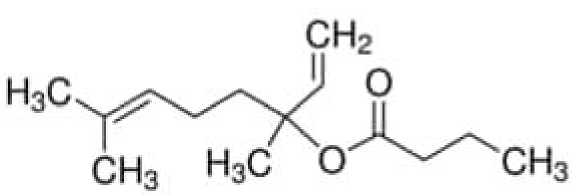
Table 4. Chromatographic profile of *Ocimumgratissimum*leaf oil

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)-, (1α,2β,5α)-	7.194	0.65
14.	Bicyclo[3.1.0]hexan-2-ol,	7.609	1.03

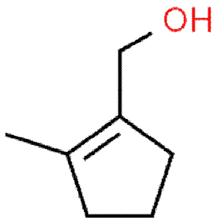
	2-methyl-5-(1-methylethyl)-, (1α,2β,5α)-		
15.	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, endo-	8.419	0.32
16.	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-;	8.601	0.73
17.	5-methyl-2-propan-2-ylphenol	9.826	48.04
18.	alfa.-Copaene	10.941	0.26
19.	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [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-(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

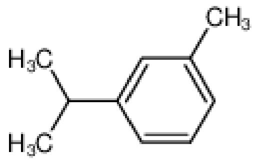
25.	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1aα,7α,7aα,7bα)]-	12.332	0.71
26.	Caryophyllene oxide	12.888	2.65
27.	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-	13.129	0.26
28.	2-Adamantanol, 2-(bromomethyl)-	15.972	0.09
29.	Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	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



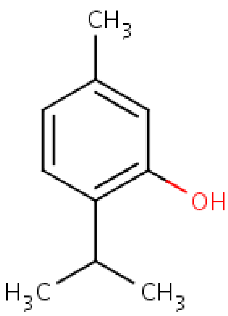


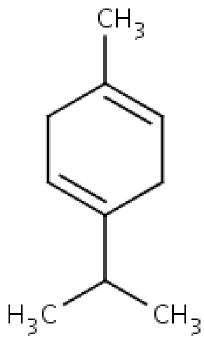
m-EuginolLinalylbutanoate

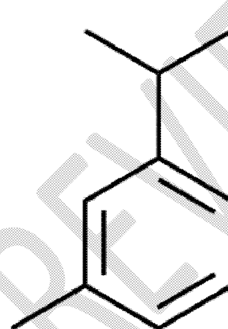




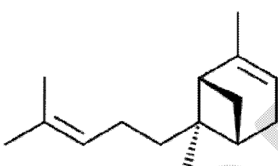
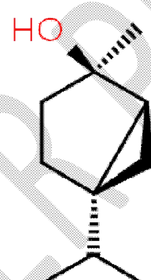
1-Cyclopentene-1-methanol, 2-methyl-5-(1-metBenzene, 1-methyl-3-(1-methylethyl)-

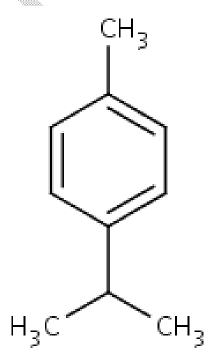






Thymol gamma-Terpineneβ-Cymene



alpha-Bergamotene 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 *Ocimumbasilicum*	% Composition in *Ocimumcanum*	% Composition in *Ocimumgratissimum*
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.CompoundsCommon to*Ocimumbasilicum* and *Ocimumcanum*

S/N	Compound	% Composition	
		Ocimumbasilicum	***Ocimumcanum***
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.CompoundsCommon to*Ocimumbasilicum* and *Ocimumgratissimum*

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

Table 8.Compounds common to*Ocimumcranum* and *Ocimumgratissimum*

S/N	Compound	% Composition	
		Ocimumcanum	***Ocimumgratissimum***
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(Discussion needs to be completely re-written over. It is written as an extension of Introduction. No discussion has been done comparing the results reported in this manuscript with previously found studies, just some lines from other studies have been referenced and mentioned)

All the species studied had anomocytic stomatain 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 – not listed in reference).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– not listed in reference).

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).

Ocimumcanum is an underutilized medicinal plant that is used for the treatment of gastrointestinal problems and also for the preparation of local soups(Reference).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 cadmiumand 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).(If this is from the present study, why is a reference mentioned here?)

(A mere mention of one line of the results found and going on to elaborate the results of other studies cannot be considered as a discussion.) Essential oils of the leaves of *O. canum* possess antibacterial (Janssen *et al*, 1989) and insecticidal (Bassole*et al*., 2003b) properties. Thechemical compositions of the leaves of *O. canum*reported here differ from those observed byPhilippe*et al.* 2013, and Tamal*et al.*, 2015– not listed in reference. InTamil *et al.*– not listed in reference,2015the 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 *Ocimumcimum*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 inthis 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 lindalylbutanoate (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%).

REFERENCES

1. João RM Almeida, Tobias Modig, AnneliPetersson, BärbelHähn-Hägerdal, Gunnar Lidén, Marie F Gorwa-Grauslund (2007). Increased tolerance and conversion of inhibitors in lignocellulosichydrolysates by *Saccharomyces cerevisiae*. Journal of Chemical Technology and Biotechnology. Pp. 340-349.
2. Steel, P., &Konig, C. J. (2006). Integrating Theories of Motivation. Academy of Management Review, 31, 889-913. http://dx.doi.org/10.5465/AMR.2006.22527462
3. Hogarh NJ (1996). Effect of *Ocimumcanum* aqueous extract on experimental diabetes mellitus. B.Sc Research Project Report, Department of Biochemistry, University of Ghana, Accra.
4. Nyarko AK, Asare-Anane H, Ofosuhene M, Andy ME (2002). Extract of *Ocimumcanum* lowers blood glucose and facilitates insulin release by isolated pancreatic β- islets cells. Phytomedicine 9:346-351.
5. Ekundayo, J A; Aisueni, N; Benka-Coker, M O (1989). TheEffects of drilling fluids in some waste and burrow pits inwestern operational areas of Shell Petroleum DevelopmentCompany of Nigeria Limited on the soil and water quality ofthe areas. Environmental Consultancy Service Group,Consultancy Services Unit, University of Benin, Benin City,Nigeria.
6. Bassole HN, Nebie R, Savadogo A, Ouattara CT, Barro N, Traore SA (2005).Composition and antimicrobial activities of the leafand flower essential oils of *Lippiachevalieri* and*Ocimumcanum* from Burkina Faso. African Journal of Biotechnology Vol. 4 (10), pp. 1156-1160, October 2005Available online athttp://www.academicjournals.org/AJBISSN 1684–5315 © 2005 Academic Journals

7. Ngassoum, M.B., Ousmaila, H., Ngamo, L.T., Maponmetsem, P.M.,Jirovetz, L., Buchbauer, G., 2004. Aroma compounds of essentialoils of two varieties of the spice plant *Ocimumcanum* Sims. Fromnorthern Cameroon. J. Food Comp. Anal. 17, 197–204.
8. Dhifi, W.; Bellili, S.; Jazi, S.; Bahloul, N.; Mnif, W. Essential Oils' Chemical Characterization and Investigation of Some Biological Activities: A Critical Review. Medicines 2016, 3, 25. https://doi.org/10.3390/medicines3040025.
9. M Rabelo [1] , E P Souza, P M G Soares, A V Miranda, F J A Matos, D N Criddle(2003). Antinociceptive properties of the essential oil of *Ocimumgratissimum* L. (Labiatae) in mice.Braz J Med Biol Res. 2003 Apr;36(4):521-4. doi: 10.1590/s0100-879x2003000400016. Epub 2003 Apr 8.
10. Gibson Lucky Arueya, OlayemiIrewolede and OluwatosinAdaramoye (2015).Physicochemical Characterization and Enhancement of the Antioxidant Potential of*Ocimumgratissimum* Enriched Pepper Soup Mix.Advance Journal of Food Science and Technology 7(6): 408-415, 2015ISSN: 2042-4868; e-ISSN: 2042-4876
11. Bassole, H.N, GuelbeogoW.M. NebieR., CostantiniC., SagnonN.F., Z.I. Kabore, S.A. TraoreOvicidal and larvicidal activity against *Aedesaegypti* and *Anopheles gambiae* complex mosquitoes of essential oils extracted from three spontaneous plants of Burkina Faso Parasitology, 45 (2003), pp. 23-26.
12. Oyen PA, Nguyen XD (1999). *Ocimumgratissimum* L. In: Plant. Resources of South-East Asia. Essential oil Plants.Meroon J. Food Comp. Anal., 17, pp. 197-204
13. Orwa C, A Mutua, Kindt R, Jamnadass R, S Anthony. 2009. Agroforestrydatabase: a tree reference and selection guide version 4.0http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp
14. Ayodele, A.E. and Olowokudejo, J.D. (1997) Systematic Importance of Leaf and Epidermal Characters in West African Species of Family Myrtaceae. Boletim da SociedadeBroteriana, 68, 35-72.
15. Ugbabe GE, Ayodele AE (2008). Foliar epidermal studies in the family BignoniaceaeJuss. in Nigeria. African Journal of Agricultural Research 3(2): 154-166
16. Metcalfe CR, Chalk L. (1979). Anatomy ofDicotyledons. Vol. I. Second Edition. Oxford University Press, New York.

17. African PharmacopoeiaAfrican Pharmacopoeia (1986). General Methods for Analysis. OAU/SRTC Scientific Publications Lagos. pp. 137-149

18. Evans MC, (2002). Trease and Evans pharmacognosy. 15th Edition. WB Sanders. London. 183-393.

19. Okhale SE, Ugbabe GE, Oladosu PO, Ibrahim JA, Egharevba HO, (2018). Chemical constituents and antimicrobial activity of the leaf essential oil of *Ixoracoccinea* L (Rubiaceae) collected from North Central Nigeria. International Journal of Bioassays 7.5 pp. 5630-5637.

20. Peter J. Franks and Graham D. Farquhar (2007). The Mechanical Diversity of Stomata and Its Significancein Gas-Exchange Control.Plant Physiology, January 2007, Vol. 143, pp. 78–87, www.plantphysiol.org Ó 2006 American Society of Plant Biologists.

21. Kim, K.W. Plant trichomes as microbial habitats and infection sites. Eur J Plant Pathol 154, 157–169 (2019). https://doi.org/10.1007/s10658-018-01656-0

22. Wang, X., Shen, C., Meng, P. (2021). Analysis and review of trichomes in plants. BMC Plant Biol 21, 70 (2021). https://doi.org/10.1186/s12870-021-02840-x

23. DevinderDhingra& Mona Michael &Hradesh Rajput &R. T. Patil (2012).Dietary fibre in foods: a review.Journal of Food Science Technology 49(3):255–266

24. AlukoB. T.;OloyedeO. I. and AfolayanA. J. (2012).Phytochemical and nutrient compositions of the leavesof *Ocimumcanum* Sims.African Journal of Biotechnology Vol. 11(63), pp. 12697-12701, 7 August, 2012Available online at http://www.academicjournals.org/AJBDOI: 10.5897/AJB11.3418ISSN 1684–5315 © 2012 Academic Journals.

25. M.I. Scranton, M.A. de Angelis, in Encyclopedia of Ocean Sciences (Third Edition), 2001.

26. D.L. Bade, in Encyclopedia of Inland Waters, 2009.

27. James N. BeMiller, in Carbohydrate Chemistry for Food Scientists (Third Edition), 2019.

28. Brett, C.T. Physiology and Biochemistry of Plant Cell Walls. London: Unwin Hyman, 1990.

29. Jorma Kim, Sofia Gripenberg, MaaritKaronen, Juha-PekkaSalminen (2021).Seed tannin composition of tropical plants. 187 112750. Available online 10 April 20210031-9422/©.

30. Susana Soares, Elsa Brandão, Carlos Guerreiro, SóniaSoares, NunoMateus, and Victor de Freitas 2020. Tannins in Food: Insights into the Molecular Perception of Astringency and Bitter Taste.Molecules; 25(11): 2590.
31. Janssen A M, Scheffer JJC, Ntezurubanza L, Baerhem-Svendsen A(1989). Antimicrobial activities of some Ocimum species grown inRwanda. J. Ethnopharmacol. 26: 57-63.
32. Bassole IHN, Guelbeogo WM, Nebie R, Costantini C, Sagnon NF,Kabore ZI, Traore, SA (2003 b). Ovicidal and larvicidal activityagainst Aedesaegypti and Anopheles gambiae complex mosquitoesof essential oils extracted from three spontaneous plants of BurkinaFaso. Parassitologia 45: 23-26.
33. Philippe Belong, Patrick AkonoNtonga, Eric- MoïseBakwoFils, GisèleAurelieFokoDadji, Joseph LebelTamesse (2013). Chemical composition and residue activities of *Ocimumcanum* Sims and *Ocimumbasilicum* L essential oils on adult female Anopheles funestus ss. Journal of Animal &Plant Sciences. Vol.19, Issue 1: 2854-2863. http://www.m.elewa.org/JAPS; ISSN 2071-7024.
34. Martins, A.P., Salgueiro, L.R., Vila, R., Tomi, F., Canigueral, S.,Casanova, J., Proenca da cunha, A., Adzet, T., 1999. Compositionof the essential oil of *Ocimumcanum*, *O. gratissimum* and *O. minimum*. PlantaMedica. 65, 187–189.
35. Chagonda, L.S., Makanda, C.D., Chalchat, J.C., 2000. The essentialoils of *Ocimumcanum* Sims (basilic camphor) and *Ocimumurticifolia* Roth from Zimbabwe. Flavour Frag. J. 15, 23–26.
36. Ekundayo, O., Laakso, I., Hiltunen, R., 1989. Constituents of thevolatile oil from leaves of *Ocimumcanum* Sims. Flavour Fragrance Journal. 4,17–18.
37. Choudhary, R., Kharya, M.D., Dixit, V.K., Varma, K.C., 1998. Roleof phytohormone on the cultivar and essential oil of *Ocimumcanum*sims. A potential source of citral. Indian Perfumery 33, 224–227.
38. Sanda, K., Koba, K., Namb, P., Gaset, A., 1998. Chemical investigation of *Ocimum*species growing in Togo. Flavour FragranceJournal 13,226–232.
39. Chalchat, J.C., Garry, R.P., Sidibe, L., Harama, M., 1999. Aromaticplants of Mali (II): chemical composition of essential oil of *Ocimumcanum* Sims. Journal of Essential Oil Research 11, 473–476.
40. Nyarko AK, Asare-Anane H, Ofosuhene M, Addy ME, Teye K, Addo P. Aqueous extract of *Ocimumcanum* decreases levels of fasting blood glucose and free radicals and

increases antiatherogenic lipid levels in mice. *VasculPharmacol*. 2002;39(6):273-279.14567064

41. Naghibi F, Mosaddegh M, Motamed SM, Ghorbani A. Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. *Iran J Pharmaceutical Res*. 2005; 4(2):63-79.