Asian Plant Research Journal

Volume 11, Issue 2, Page 10-23, 2023; Article no.APRJ.97088
ISSN: 2581-9992

Leaf Epidermal Microscopy, Chemo-Microscopy and GC-MS Analyses of Three *Ocimum* Species from Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/APRJ/2023/v11i2206

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/97088

Original Research Article

Received: 02/02/2023
Accepted: 04/03/2023
Published: 08/04/2023

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 anomacytic

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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 "effinrin-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 grattissimum* 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 Schimadzu 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% 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 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 aboundant 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 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 (Figs. 1, 2 & 3).

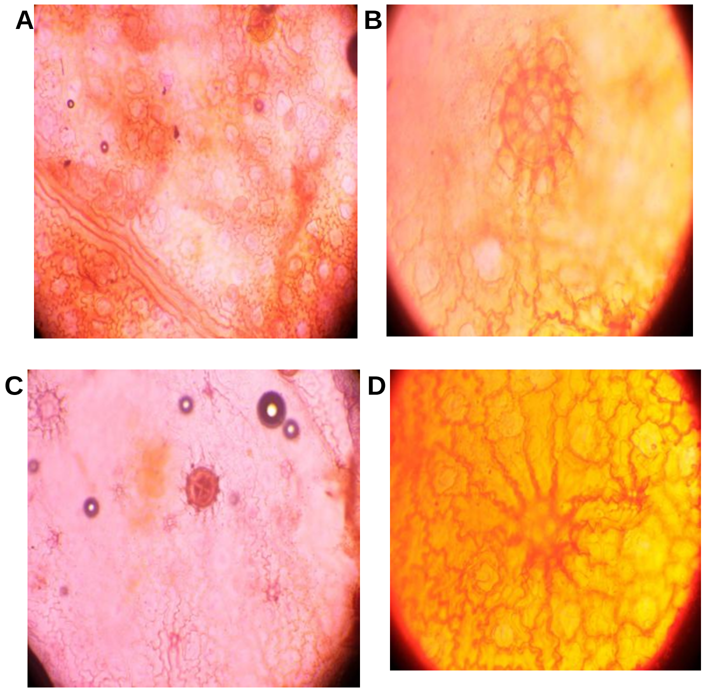


Fig. 1. Leaf epidermal microscopy of *Ocimum basilicum*

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

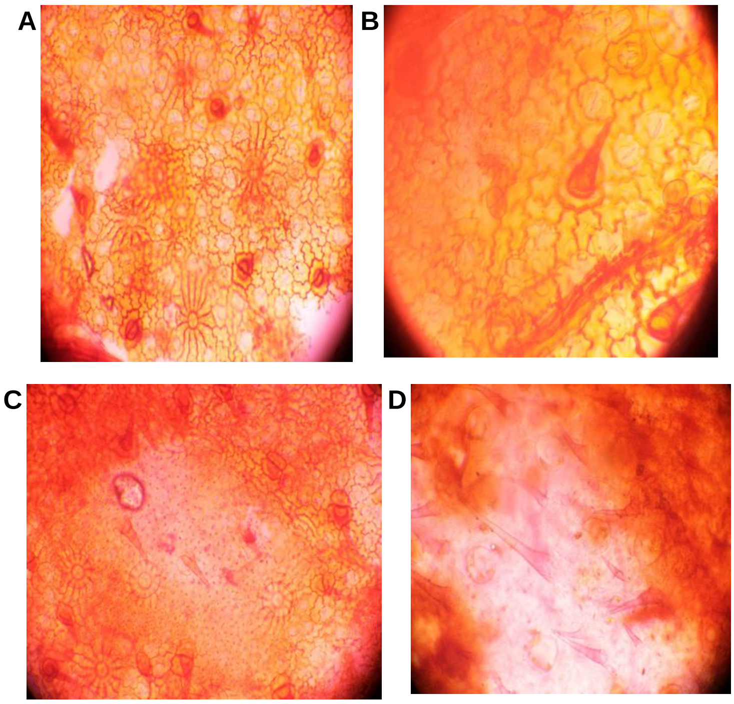


Fig. 2. Leaf epidermal microscopy of three *Ocimum canum*

A) Lower surface X 100. Anomocytic stomata, wavy cell wall, glandular and non-glandular trichomes
B) Lower surface X 400. 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.

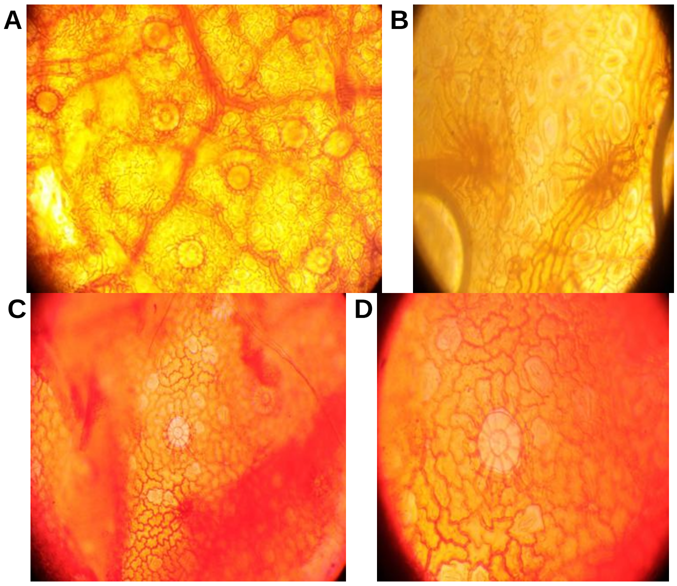


Fig. 3. Leaf epidermal microscopy of *Ocimum gratissimum*

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	*Ocimum basilicum*	*Ocimum canum*	*Ocimum gratissimum*
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-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-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 *Ocimum canum* leaf oil

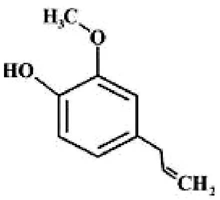
S/N	Names of compound	Retention Time	% Composition
1.	alpha.-Pinene	5.533	3.89
2.	2,2-dimethyl-3methylidenebicyclo[2.2.1]heptane	5.710	0.40
3.	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	6.010	0.15
4.	Cyclohexene, 4-methylene-1-(1-methylethyl)-	6.068	0.09
5.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	6.211	2.23
6.	p-Mentha-1,5-diene Menthadiene 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

S/N	Names of compound	Retention Time	% Composition
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

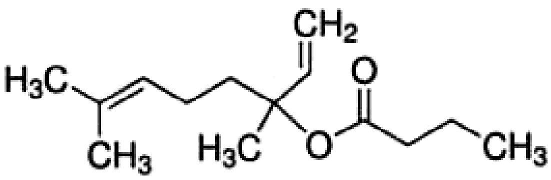
Table 4. Chromatographic profile of *Ocimum gratissimum* leaf oil

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)-, (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-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-(2α,4aα,8aβ)]-Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-	12.120	1.33

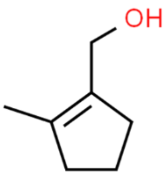
S/N	Name of compound	Retention Time	% Composition
	(1-methylethylidene)-, (4aR-trans)-		
25.	1H-Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b-octahydro-1,1,7,7a-tetramethyl-, [1aR-(1aα,7α,7aα,7bα)]-	12.332	0.71
26.	Caryophyllene oxide	12.888	2.65
27.	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-	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



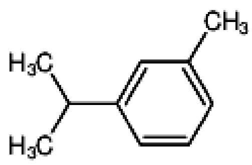
m-Euginol



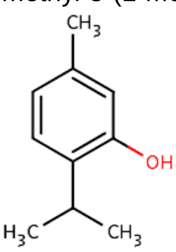
Linalyl butanoate



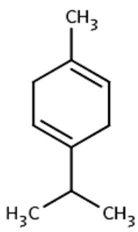
1-Cyclopentene-1-methanol, 2-methyl-5-(1-met)



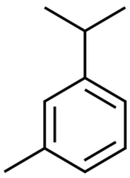
Benzene, 1-methyl-3-(1-methylethyl)-



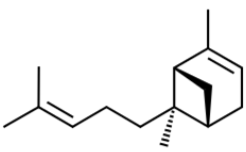
Thymol



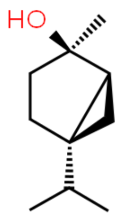
gamma-Terpinene



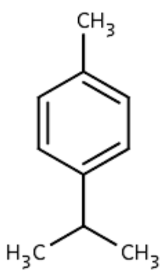
β-Cymene



alpha-Bergamotene



cis-4-Thujanol



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 *Ocimum basilicum*	% Composition *Ocimum canum*	% Composition *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	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	
		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, 1,2,3,4,4a,5,6,8aoctahydro-7-m	1.66	0.61

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 canum* 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

"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 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" [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%), a-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, b-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 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%) [39-42].

Eight compounds: (Alpha.-Phellandrene; Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methy; alfa.-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-meth and Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trime) are present in the three species of *Ocimum* studied (Table 5). *Ocimum bascilicum* 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-, ace; gamma.-Muurolene; beta.-copaene and

Naphthalene, 1,2,3,4,4a,5,6,8aoctahydro-7-m in common (Table 6). *Ocimum bascilicum* 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; B4.icyclo [3.1.1]heptane, 6,6-dimethyl-2-methyle; 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.

ACKNOWLEDGEMENT

We acknowledge the DG and all members of TMC NIPRD for providing the enabling environment for the research work. We appreciate all members of staff of MPR & TM Department for their support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Runyoro D, Ngassapa O, Vagionas K, Aligiannis N, Graikou K, Chinou I. Chemical composition and antimicrobial activity of the essential oils of four Ocimum species growing in Tanzania. Food Chem. 2010;119(1):311-6.
   DOI: 10.1016/j.foodchem.2009.06.028
2. Irvine FR. Woody plants of Ghana with special reference to their uses. London: Oxford University Press; 1961.
3. Pavithra KHG, Narase Gowda PN, Prasad MP. Studies on antimicrobial activity of Ocimum species of Karnataka against clinical isolates. Int J Pure App Biosci. 2019;7(4):245-54.
   DOI: 10.18782/2320-7051.7732
4. Maddi R, Amani P, Bhavitha S, Gayathri T, Lohitha T. A review on Ocimum species: *Ocimum americanum L., Ocimum basilicum L., Ocimum gratissimum L*. and *Ocimum tenuiflorum L*. Int J Res Ayurveda Pharm. 2019;10:41-8.
5. Keita SM, Vincent C, Schmit JP, Belanger A. Essential oil composition of *Ocimum basilicum* L.,*O. gratissimum L.* and *O. suave L.* in the Republic of Guinea. Flavour Fragr J. 2000;15(5):339-41.
   DOI:10.1002/1099-1026(200009/10)15:5<339::AID-FFJ922>3.0.CO;2-H.
6. Ngassoum MB, Ousmaila H, Ngamo LT, Maponmetsem PM, Jirovetz L, Buchbauer G. Aroma compounds of essential oils of two varieties of the spice plant Ocimum canum Sims. From northern Cameroon. Journal of Food Composition and Analysis. 2004;17(2):197-204.
   DOI: 10.1016/j.jfca.2003.08.002.
   Food Comp J. Anal;17:197-204.
7. Nyarko AK, Asare-Anane H, Ofosuhene M, Addy ME. Extract of *Ocimum canum* lowers blood glucose and facilitates insulin release by isolated pancreatic β- islets cells. Phytomedicine. 2002;9(4):346-51.
   DOI: 10.1078/0944-7113-00124
   PMID 12120816.
8. Naghibi F, Mosaddegh M, Motamed MM, Ghorbani A. Labiatae family in folk medicine in Iran: From ethnobotany to pharmacology. Iran J Pharm Res. 2005; 4:63-79.
9. Martins AP, Salgueiro LR, Vila R, Tomi F, Cañigueral S, Casanova J, et al. Composition of the essential oils of Ocimum canum, O. gratissimum and O. minimum. Planta Med. 1999;65(2):187-9.
   DOI: 10.1055/s-2006-960465
   PMID 17260257.
10. Aluko BT, Oloyede OI, Afolayan AJ. Phytochemical and nutrient compositions of the leaves of Ocimum canum Sims. Afr J Biotechnol. 2012;11(63):12697-701.
    DOI: 10.5897/AJB11.3418 ISSN 1684-5315
11. Imosemi. Innocent O. A Review of the Medicinal values, Pharmacological actions, Morphological effects and Toxicity of *Ocimum gratissimum* Linn. European Journal of Pharmaceutical and Medical Research. EJPMR. 2020;7(7):29-40.
12. Joshi RK. GC–MS analysis of the essential oil of *Ocimum gratissimum* L. growing desolately in South India. Acta Chromatographica. 2017;29(1):111-9.
    DOI: 10.1556/1326.2017.29.1.10
13. Ilori M, Sheteolu AO, Omonibgehin EA, Adeneye AA. Antidiarrhoeal activities of *O. gratissimum* (Lamiaceae). J Diarrhoeal Dysentery Res. 1996;14:283-5.

14. Dhifi W, Bellili S, Jazi S, Bahloul N, Mnif W. Essential oils' chemical characterization and investigation of some biological activities: A critical review. Medicines (Basel). 2016;3(4):25.
DOI: 10.3390/medicines3040025, PMID 28930135.
15. Rabelo M, Souza EP, Soares PM, Miranda AV, Matos FJ, Criddle DN. Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice. Braz J Med Biol Res. 2003;36(4):521-4.
DOI: 10.1590/s0100-879x2003000400016. PMID 12700832.
16. Lucky AG, Irewolede O, Adaramoye O. Physicochemical characterization and enhancement of the antioxidant potential of *Ocimum gratissimum* enriched pepper soup mix. Adv J Food Sci Technol. 2015. ISSN: 2042-4868; e-ISSN: 2042-4876;7(6):408-15.
17. Oyen PA, Nguyen XD. *Ocimum gratissimum* L. In: plant. Resources of South-East Asia. Essential oil Plants. Meroon J. Food Anal, compiler. 1999;197-204.
18. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Agroforestry database: A tree reference and selection guide version 4.0; 2009.
Available:http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp
19. Ayodele AE, Olowokudejo JD. Systematic importance of leaf and epidermal characters in West African species of family Myrtaceae. Bol Soc Broteriana. 1997;68:35-72.
20. Metcalfe CR, Chalk L. Anatomy of dicotyledons. 2nd ed. Vol. I. New York: Oxford University Press; 1979.
21. African pharmacopoeia African pharmacopoeia. General methods for analysis. OAU/SRTC. Lagos: Scientific Publishing. 1986;137-49.
22. Scranton MI, de Angelis MA. Encyclopedia of ocean sciences. 3rd ed; 2001.
23. Bade DL. Encyclopedia of inland waters; 2009.
24. Franks PJ, Farquhar GD. The mechanical diversity of stomata and its significance in gas-exchange control. Plant Physiol, 2007; 143(1):78-87 Ó 2006.
DOI: 10.1104/pp.106.089367, PMID 17114276.
25. Kim KW. Plant trichomes as microbial habitats and infection sites. Eur J Plant Pathol. 2019;154(2):157-69.
DOI: 10.1007/s10658-018-01656-0
26. Wang X, Shen C, Meng P, Tan G, Lv L. Analysis and review of trichomes in plants. BMC Plant Biol. 2021;21(1):70.
DOI: 10.1186/s12870-021-02840-x, PMID 33526015.
27. Mili M, Hashmi SAR, Ather M, Hada V, Markandeya N, Kamble S, et al. Novel lignin as natural-biodegradable binder for various sectors—a review. J Appl Polym Sci. 2022;139(15):51951.
DOI: 10.1002/app.51951
28. BeMiller JN. Carbohydrate chemistry for food scientists. 3rd ed; 2019.
29. Brett CT. Physiology and biochemistry of plant cell walls. London: Unwin Hyman; 1990.
30. Kim J, Gripenberg S, Karonen M, Salminen JP. Seed tannin composition of tropical plants. Phytochemistry. 2021;187:112750. Available online 10 April 2021 0031-9422/©.
DOI: 10.1016/j.phytochem.2021.112750, PMID 33845405.
31. Soares S, Brandão E, Guerreiro C, Soares S, Mateus N, de Freitas V. Tannins in food: insights into the molecular perception of astringency and bitter taste. Molecules. 2020;25(11):2590.
DOI: 10.3390/molecules25112590
PMID 32498458.
32. Dhingra D, Michael M, Rajput H, Patil RT. Dietary fibre in foods: A review. J Food Sci Technol. 2012;49(3):255-66.
DOI: 10.1007/s13197-011-0365-5
PMID 23729846.
33. Bassolé IHN, Guelbeogo WM, Nébié R, Costantini C, Sagnon NF, Kabore ZI, et al. Ovicidal and larvicidal activity against *Aedes aegypti* and *Anopheles gambiae* complex mosquitoes of essential oils extracted from three spontaneous plants of Burkina Faso. Parassitologia. 2003b; 45(1):23-6. PMID 15270540.
34. Chagonda LS, Makanda CD, Chalchat JC. The essential oils of *Ocimum canum* Sims (basilic camphor) and *Ocimum urticifolia* Roth from Zimbabwe. Flavour Fragr J. 2000;15(1):23-6.
DOI:10.1002/(SICI)1099-1026(200001/02)15:1<23::AID-FFJ866>3.0.CO;2-W
35. Ekundayo O, Laakso I, Hiltunen R. Constituents of the volatile oil from leaves

of *Ocimum canum* Sims. Flavour Fragr J. 1989;4(1):17-8.
DOI: 10.1002/ffj.2730040104

36. Choudhary R, Kharya MD, Dixit VK, Varma KC. Role of phytohormone on the cultivar and essential oil of Ocimum canum Sims. A potential source of citral. Indian Perfumery. 1998;33: 224-7.
37. Sanda K, Koba K, Nambo P, Gaset A. Chemical investigation of Ocimum species growing in Togo. Flavour Fragr J. 1998;13(4):226-32.
DOI: 10.1002/(SICI)1099-1026(1998070)13:4<226::AID-FFJ717>3.0.CO;2-H.
38. Chalchat JC, Garry RP, Sidibé L, Harama M. Aromatic plants of Mali (II): Chemical Composition of Essential Oils of Ocimum canum Sims. J Essent Oil Res. 1999; 11(4):473-6.
DOI: 10.1080/10412905.1999.9701188
39. Evans MC. Trease and Evans pharmacognosy. 15th ed. London: W B Saunders. 2002;183-393.
40. Okhale SE, Ugbabe GE, Oladosu PO, Ibrahim JA, Egharevba HO. Chemical constituents and antimicrobial activity of the leaf essential oil of *Ixora coccinea* L. (Rubiaceae) collected from North Central Nigeria. Int J Bioassays. 2018;7.5.:5630-7.
41. Belong P, Ntonga PA. Eric- Moïse Bakwo fils, Gisèle Aurelie Foko Dadji, Joseph Lebel Tamesse. J Anim Plant Sci. Chemical composition and residue activities of Ocimum canum Sims and *Ocimum basilicum* L essential oils on adult female Anopheles funestus ss. 2013; 19(1):2854-63; ISSN 2071-7024.
42. Tamil Selvi M, Thirugnanasampandan R, Sundarammal S. Antioxidant and cytotoxic activities of essential oil of Ocimum canum Sims. from India. J Saudi Chem Soc. 2015;19(1):97-100.
DOI: 10.1016/j.jscs.2011.12.026

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