

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

ABSTRACT

Comparative analyses of the leaf epidermal microscopy, chemo-microscopy and GC-MS analysis of essential oils from the leaves of three *Ocimum* species (*Ocimum basilicum* L.; *Ocimum canum* Sims. and *Ocimum gratissimum* L.) belonging to the family Lamiaceae were analyzed. The leaf epidermal microscopy revealed anomocytic stomata in all the species studied. *O. basilicum* has anomocytic stomata on both surfaces and were more abundant 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 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. 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%). The leaf epidermal microscopy and the chemo-microscopy in this study can be used for the identification and standardization of the crude drug. 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.

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

INTRODUCTION

The Genus *Ocimum* belongs to Lamiaceae family and comprised of about 200 species of herbs and shrubs and is distributed over Asia, Africa, Central and Southern America (Runyoro *et al.*,

2010; Irvine, 1961).). *Ocimum* is cultivated for its essential oil which displays many potent pharmacological application (mosquito repellent, antimicrobial, antioxidant, wound healing, anti-melanoma, and radio-protective) culinary, perfume for herbal toiletries, aromatherapy treatment and as flavoring agent (Pavithra *et al.*, 2019) The study was done to compare the leaf epidermal microscopy, chemo-microscopy, and GC-MS analyses of *Ocimum basilicum* L. *Ocimum canum* Sims; and *Ocimum gratissimum* L. leaves.

Ocimum basilicum L. (Sweet basil), the Mediterranean type and the most popular species, is assumed to originate from India, Africa, and/or the Middle East (Maddi *et al.*, 2019). 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 (Keita *et al.*, 2000).

Ocimum canum Sims is a semi-woody plant of about 40 cm high it is an aromatic plant and native to tropical Africa (Steel, & Konig 2006). It is popularly called African basil, but known as "Efinrin elewe dudu" in south-western Nigeria where it is used locally for the treatment of gastrointestinal problems, piles, dysentery, as an infusion for nursing mothers and as insect repellent (Aluko *et al.*, 2012). 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 (Ekundayo *et al.*, 1989). The plant is also used as a local condiment because of its aromatic properties (Bassole *et al.*, 2005). Despite its nutritive and therapeutic values, this plant has not been scientifically examined for its nutritive components. *O. canum* is used specially for treating various types of diseases and lowering blood glucose and it also treats cold, fever, parasitic infestations on the body and inflammation of joints and headaches (Ngassoum *et al.*, 2004). Essential oil from the leaves of *O. canum* poses antibacterial and insecticidal properties (Bassole *et al.*, 2003).

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

have been used mainly for antipyretic purposes and for treating respiratory diseases on the eastern coast of Africa (Martins *et al.*, 1999, Ngassoum 2004).

Ocimum gratissimum is indigenous to tropical areas especially India and it is also in West Africa (Innocent, 2020). In Nigeria, it is found in the Savannah and coastal areas (Innocent, 2020). It is cultivated in Ceylon, South Sea Islands, and also within Nepal, Bengal, Chittagong and Deccan (Joshi, 2017). 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) (Joshi, 2017). In the southern part of Nigeria, the plant is called “effinrin-nla” by the Yoruba speaking tribe (Ilori, *et al.*, 1996). It is called “Ahuji” by the Igbos, while in the Northern part of Nigeria; the Hausas call it “Daidooya”. *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 (Rabelo *et al.*, 2003). Eugenol and thymol extracted from the oil are substitutes for clove oil and thyme oil (Rabelo *et al.*, 2003). The essential oil is also used in perfumery (Rabelo *et al.*, 2003). 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).

MATERIALS AND METHODS

Plant Material:

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

Leaf Epidermal Microscopy

Leaf epidermal preparation followed the methods of Ayodele and Olowokudejo (1997), cited by Ugbabe and Ayodele (2008). Slides were labeled appropriately and examined under the light

microscope (ACCU-SCOPE 3025 Microscope Series) while photographs of the micro morphological features were taken using the camera (Industrial Design Camera E31SPM12000KPA) with magnifications x100 and x400. Terminologies are based on Metcalfe and Chalk (1979).

Chemo-Microscopy

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

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

GC-MS Analysis

Essential Oil Extraction

Freshly collected leaves of *Ocimum canum*, *Ocimum gratissimum*, and *Ocimum basilicum* were extracted for three hours using the Clevenger type apparatus (Okhale *et al.*, 2018). The extracted oils was then dried over anhydrous Sodium sulphate and stored in small vials (Okhale *et al.*, 2018) and tightly sealed and kept for GC-MS analysis. The oils were sent to Shimadzu Training Center for analytical Institute (STC), Lagos for analysis.

Gas Chromatography–Mass Spectrometry (GC- MS) Analysis

The methods of Okhale *et al.*, 2018 were used where freshly collected leaves of *Ocimum canum*, *Ocimum gratissimum*, and *Ocimum basilicum* samples were chopped separately into pieces and each subjected to hydro-distillation for 4 hours using Clevenger-type apparatus. The essential oil obtained was dried over anhydrous sodium sulphate and used immediately for GC-MS using Shimadzu QP-2010 GC with QP-2010 mass selective detector [MSD, operated in the EI mode (electron energy =70Ev), scan range = 45400 amu, and scan rate = 3.99 scan/sec], and Shimadzu GCMS solution data system. The GC column was HP-5MS fused silica capillary with a 5% 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 60°C,

increased from 60°C to 180°C at rate of 10°C/min, held at 180°C for 2 minutes; increased from 180°C at a rate of 15°C/min, then held at 280°C for 4 minutes. The injection port temperature was 250°C. The ionization of sample components was performed in the electron impact mode (70eV). Injector temperature was 250°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 abundant on the lower surface; cell walls are wavy on the upper surface and have glandular trichomes on both surfaces. *O. canum* also has anomocytic stomata on both surfaces; cell walls are wavy and trichomes are glandular and non-glandular occurring on both surfaces but more on the upper surface. The non-glandular trichomes are cone-shaped with pointed tips. *O. gratissimum* has 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 (Figures 1, 2 & 3).

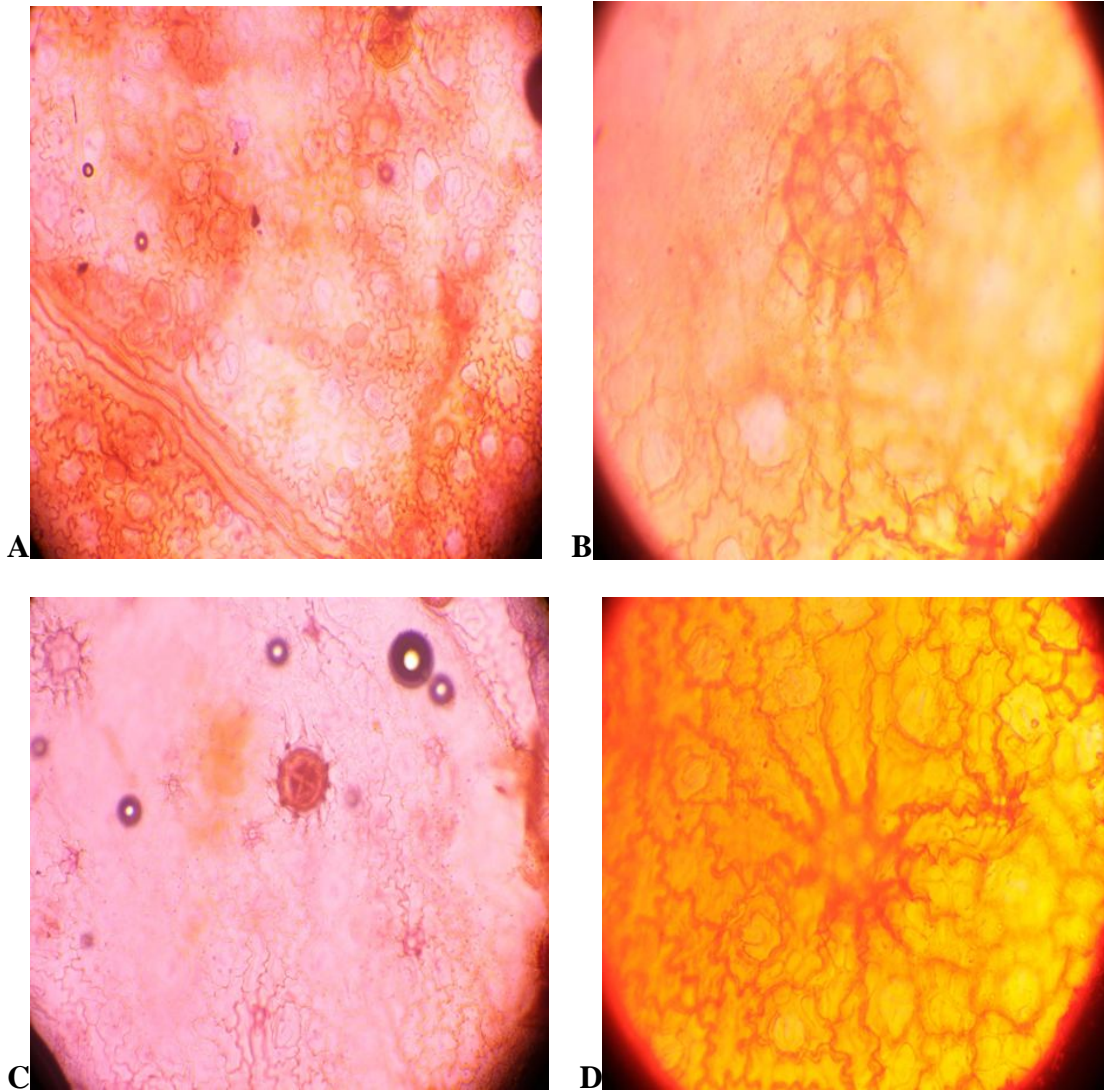


Figure 1. Leaf epidermal microscopy of *Ocimum basilicum*

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

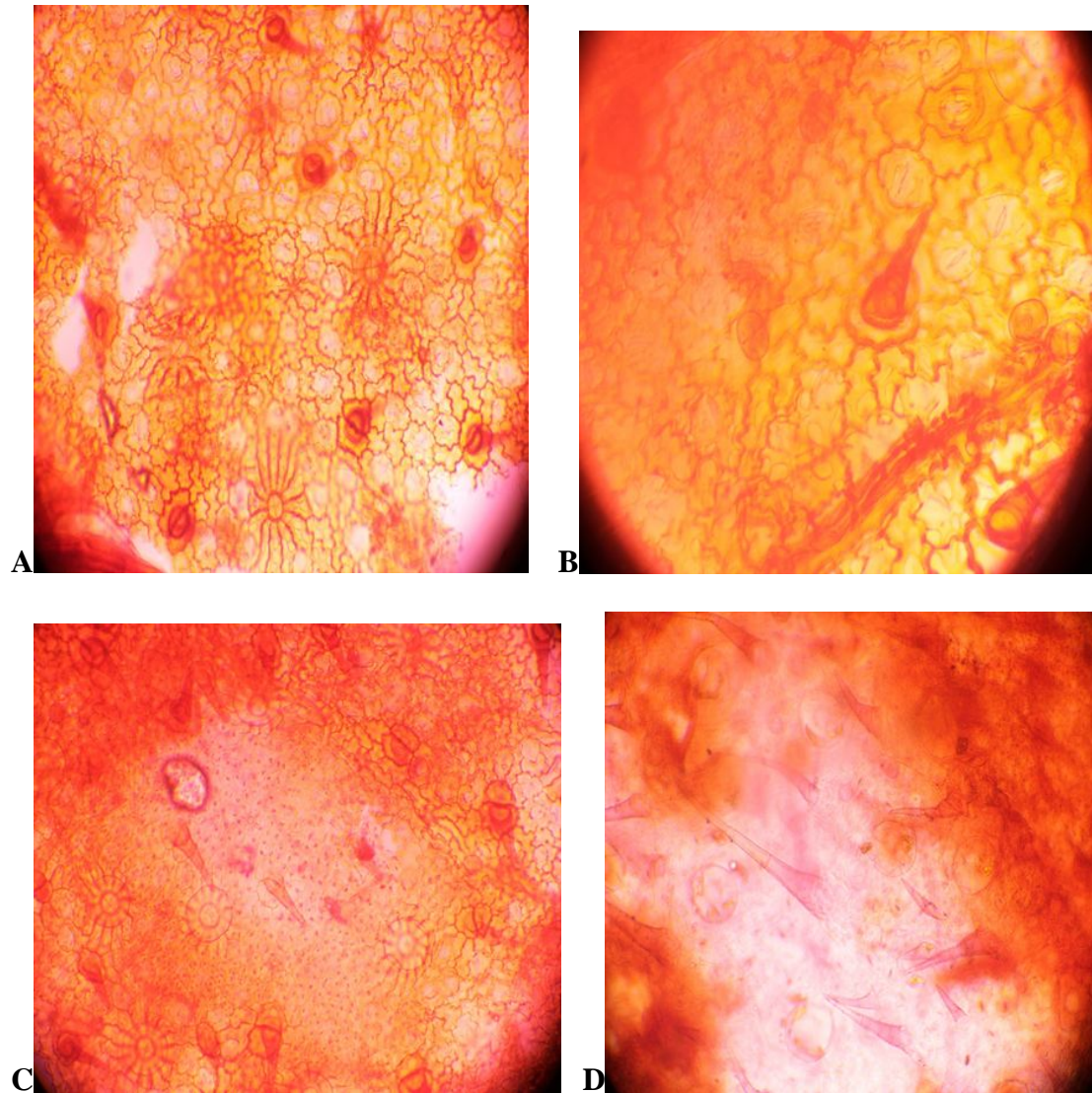


Figure 2. Leaf epidermal microscopy of three *Ocimum canum*

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

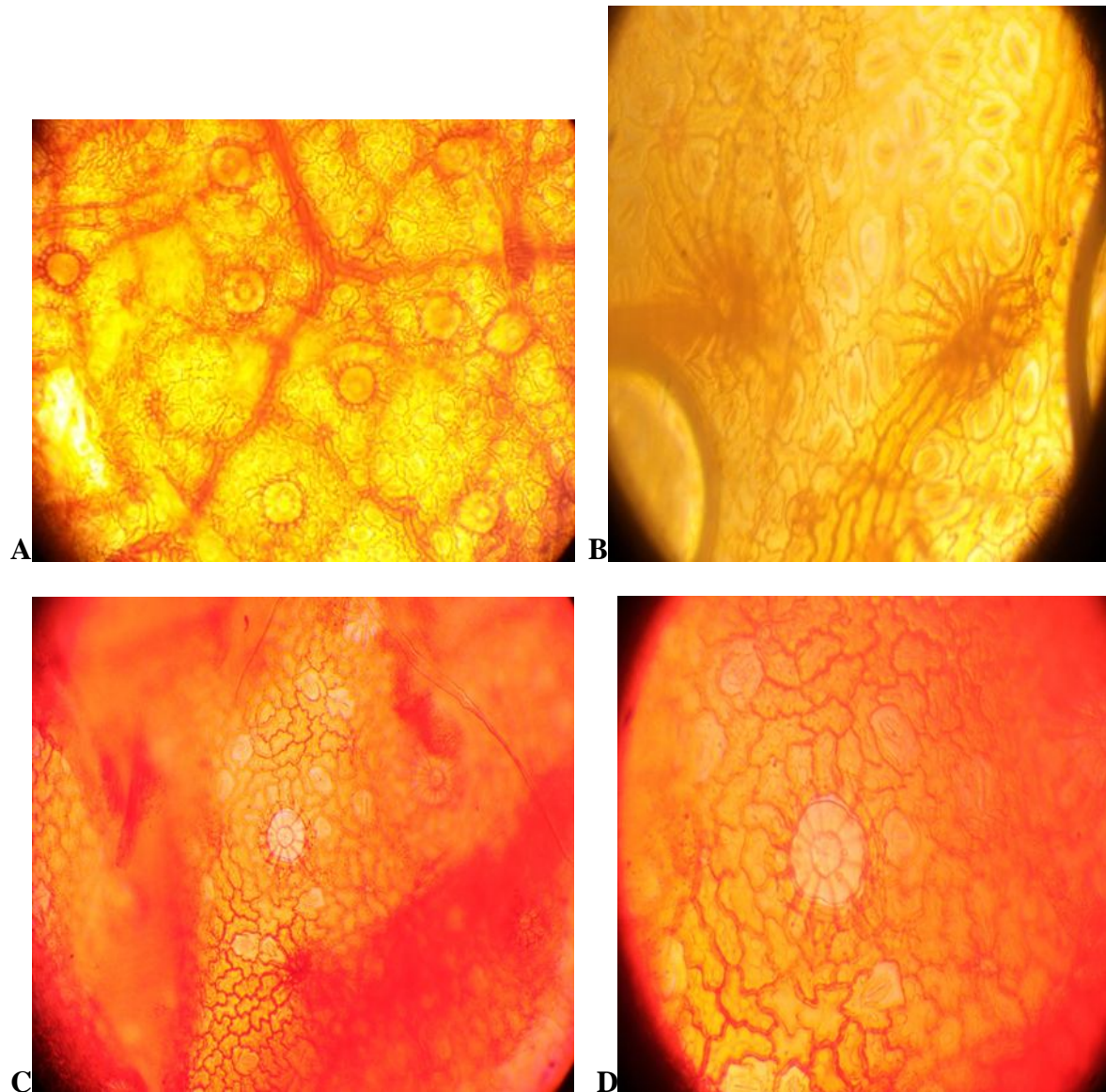


Figure 3. Leaf epidermal microscopy of *Ocimum gratissimum*

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

Chemo-microscopy

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

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

| TEST | <i>Ocimum basilicum</i> | <i>Ocimum canum</i> | <i>Ocimum gratissimum</i> |
|-----------------|-------------------------|---------------------|---------------------------|
| LIGNIN | + | + | + |
| CELLULOSE | + | + | + |
| TANNINS | + | + | + |
| MUCILAGE | + | + | + |
| STARCH | + | + | + |
| CALCIUM OXALATE | + | + | + |
| OIL | + | + | + |
| PROTEIN | + | + | + |

GC-MS Analysis

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

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

| S/N | Names of compound | Retention Time | % Composition |
|-----|--|----------------|---------------|
| 1. | 1,8-Cineole | 6.724 | 2.37 |
| 2. | 1,3,6-Octatriene, 3,7-dimethyl-, (Z)- | 6.992 | 0.81 |
| 3. | p-Mentha-1,5-diene Menthadiene | 7.133 | 0.26 |
| 4. | Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-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- | 8.056 | 1.57 |

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| | 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)-, (1 α ,4 β ,8 $\alpha\alpha$)- | 12.253 | 1.66 |
| 28. | 1H-3a,7-Methanoazulene, octahydro-3,8,8-trimethyl-6-methylene-, [3R-(3 α ,3 β ,7 β ,8 $\alpha\alpha$)]- | 12.318 | 0.90 |

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

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| 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)-, (1 α ,2 β ,5 α)- | 7.198 | 7.61 |
| 12. | (+)-4-Carene | 7.503 | 0.72 |
| 13. | Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1 α ,2 α ,5 α)- | 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 , α 4-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 |

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| 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-(1 α ,2 α ,3 $\alpha\beta$,4 α ,5 α ,7 $\alpha\beta$,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-(1 $\alpha\alpha$,4 β ,4 $\alpha\beta$,7 α ,7 $\alpha\beta$,7 $\beta\alpha$)]- | 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 |

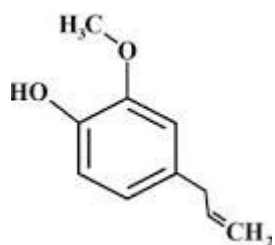
49. Caryophyllene oxide 14.685 0.19

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

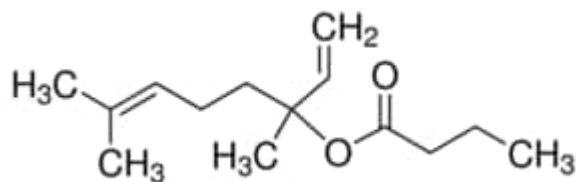
| S/N | Name of compound | Retention Time | % Composition |
|-----|--|----------------|---------------|
| 1. | alpha.-Pinene | 5.532 | 0.98 |
| 2. | 2,2-dimethyl-3-methylidenebicyclo[2.2.1]heptane | 5.711 | 0.11 |
| 3. | 3-Isopropyl-6-methylenecyclohex-1-ene | 6.008 | 0.66 |
| 4. | Bicyclo[3.1.1]heptane, 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 α ,2 β ,5 α)- | 7.194 | 0.65 |
| 14. | Bicyclo[3.1.0]hexan-2-ol, | 7.609 | 1.03 |

| | | | |
|-----|---|--------|-------|
| | 2-methyl-5-(1-methylethyl)-, (1 α ,2 β ,5 α)- | | |
| 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-(2 α ,4 α ,8 β)]-Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)-, (4aR-trans)- | 12.120 | 1.33 |

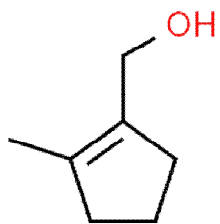
| | | | |
|-----|--|--------|------|
| 25. | 1H- Cyclopropa[a]naphthalene, 1a,2,3,5,6,7,7a,7b- octahydro-1,1,7,7a- tetramethyl-, [1aR- (1α,7α,7α,7β)]- | 12.332 | 0.71 |
| 26. | Caryophyllene oxide | 12.888 | 2.65 |
| 27. | 12- Oxabicyclo[9.1.0]dodeca- 3,7-diene, 1,5,5,8- tetramethyl- | 13.129 | 0.26 |
| 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 |



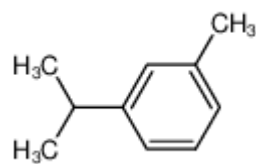
m-Eugenol



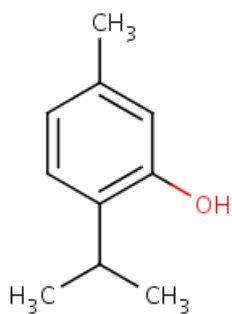
Linalyl butanoate



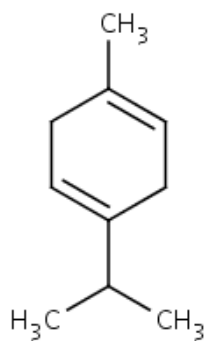
1-Cyclopentene-1-methanol, 2-methyl-5-(1-met



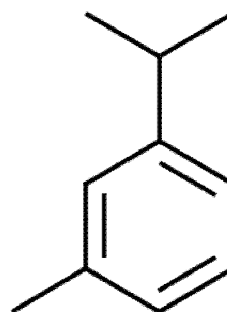
Benzene, 1-methyl-3-(1-methylethyl)-



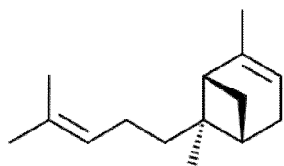
Thymol



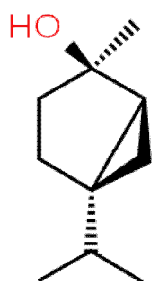
gamma-Terpinene



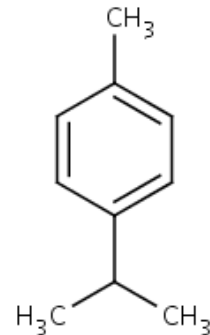
β-Cymene



alpha-Bergamotene



cis-4-Thujanol



p-cymene

Figure 10. Structure of some common compounds in the study

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

| S/N | Compound | % Composition in <i>Ocimum basilicum</i> | % Composition in <i>Ocimum canum</i> | % Composition in <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-methyl-) | 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-methyl-) | 10.25 | 7.17 | 0.18 |
| 6 | Humulene | 0.77 | 0.44 | 0.33 |
| 7 | Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methyl-) | 2.44 | 0.21 | 0.29 |
| 8 | Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl- | 1.55 | 4.98 | 1.93 |

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

| S/N | Compound | % Composition | |
|-----|---|-------------------------|---------------------|
| | | <i>Ocimum basilicum</i> | <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, | 1.66 | 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. | Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methyl | 2.23 | 1.93 |
| 4. | gamma.-Terpinene | 3.79 | 14.15 |
| 5. | Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl | 0.11 | 0.18 |

DISCUSSION

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

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

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

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

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

Cellulose is a complex carbohydrate or polysaccharide. It is the basis structural component of plants (James, 2019). Cell wall is non-digestible by humans and a food for herbivorous animals (Brett, 1990.). Tannins are a group of phenolic compounds in woody flowering plants that are important deterrents to herbivores (Jorma, 2021). They occur in roots, barks, wood, leaves and fruits of plants. They are used in tanning leather, dying fabrics and making ink (Lina and Maria, 2018). Tannins are acidic and have astringent taste. They are used in clarification of wine and

beer (Susana 2020). Mucilage is a water-soluble viscous material characterized by a light color, which is part of the fiber. It is formed by some specialized secretory cells of the plant endosperm and its function is to prevent excessive dehydration (Dhingra *et al.*, 2012).

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

Essential oils of the leaves of *O. canum* possess antibacterial (Janssen *et al.*, 1989) and insecticidal (Bassole *et al.*, 2003b) properties. The chemical compositions of the leaves of *O. canum* reported here differ from those observed by Philoppe *et al.* 2013, and 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%), α -pinene (5.59%), camphene (5.20%) and myrtenyl acetate (2.74%). Similarly Martins *et al.* (1999) and Chagonda *et al.* (2000) also reported camphor as a major compound in certain species of *Ocimum* 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, β -caryophyllene and methyl cinnamate (Ekundayo *et al.*, 1989; Choudhary *et al.*, 1998; Sanda *et al.*, 1998; Chalchat *et al.*, (1999). The GC-MS analyses of the three species in this study revealed 35 compounds for *O. basilicum*, 49 compounds for *O. canum* and 34 compounds for *O. gratissimum*. In *O. basilicum* the most abundant compound is m-Eugenol (34.42%) followed by linalyl butanoate (20.88%) and α -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-Thujanol (7.61%) and α -bergamotene (7.17%). In *O.*

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

In this study eight compounds (Alpha.-Phellandrene; Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methyl; alfa.-Copaene; Caryophyllene oxide; Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl; 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 basclicum* 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.-Muurolene; beta.-copaene and Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl in common (Table 6). *Ocimum basclicum* 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).

In conclusion, the leaf epidermal microscopy and the chemo-microscopy in this study can be used for the identification and standardization of the crude drug. 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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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, 311–316.
2. Irvine, F.R. (1961). *Woody Plants of Ghana with Special Reference to their Uses* London: Oxford University Press.

3. Pavithra, K.H.; Narase Gowda, P.N.; Prasad, M.P. Studies on Antimicrobial Activity of *Ocimum* Species of Karnataka against Clinical Isolates. *Int. J. Pure Appl. Biosci.* 2019, 7, 245–254.
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, 41–48.
5. Keita SM, Vincent C, Schmit JP, Belanger A (2000) Essential oil composition of *Ocimum basilicum* L., *O. gratissimum* L. and *O. suave* L. in the Republic of Guinea. *Flavour Fragr J* 15: 339-341.
6. Aluko B. T.; Oloyede O. I and Afolayan A. J. (2012) Phytochemical and nutrient compositions of the leaves of *Ocimum canum* Sims. *African Journal of Biotechnology* Vol. 11(63), pp. 12697-12701, 7 August, 2012 Available online at <http://www.academicjournals.org/AJB> DOI: 10.5897/AJB11.3418 ISSN 1684–5315 © 2012 Academic Journals
7. 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>
8. Hogarth NJ (1996). Effect of *Ocimum canum* aqueous extract on experimental diabetes mellitus. B.Sc Research Project Report, Department of Biochemistry, University of Ghana, Accra.
9. Nyarko AK, Asare-Anane H, Ofosuhene M, Andy ME (2002). Extract of *Ocimum canum* lowers blood glucose and facilitates insulin release by isolated pancreatic β - islets cells. *Phytomedicine* 9:346-351.
10. Ekundayo, J A; Aisueni, N; Benka-Coker, M O (1989). The Effects of drilling fluids in some waste and burrow pits in western operational areas of Shell Petroleum Development Company of Nigeria Limited on the soil and water quality of the areas. Environmental Consultancy Service Group, Consultancy Services Unit, University of Benin, Benin City, Nigeria.
11. Bassole HN, Nebie R, Savadogo A, Ouattara CT, Barro N, Traore SA (2005). Composition and antimicrobial activities of the leaf and flower essential oils of *Lippia chevalieri* and *Ocimum canum* from Burkina Faso. *African Journal of Biotechnology*

Vol. 4 (10), pp. 1156-1160, October 2005 Available online at <http://www.academicjournals.org/AJB> ISSN 1684–5315 © 2005 Academic Journals.

12. Bassole, H.N, Guelbeogo W.M. Nebie R., Costantini C., Sagnon N.F., Z.I. Kabore, S.A.(2003). Traore Ovicidal and larvicidal activity against *Aedes aegypti* and *Anopheles gambiae* complex mosquitoes of essential oils extracted from three spontaneous plants of Burkina Faso *Parasitology*, 45 (2003), pp. 23-26.
13. Ngassoum, M.B., Ousmaila, H., Ngamo, L.T., Maponmetsem, P.M., Jirovetz, L., Buchbauer, G., 2004. Aroma compounds of essential oils of two varieties of the spice plant *Ocimum canum* Sims. From northern Cameroon. *J. Food Comp. Anal.* 17, 197–204.
14. Naghibi, F., M. Mosaddegh, M.M. Motamed and A. Ghorbani, 2005. Labiatae family in folk medicine in Iran: From ethnobotany to pharmacology. *Iran. J. Pharm. Res.*, 4: 63-79.
15. Martins Ana Paula, Lúgia R. Salgueiro , Roser Vila , Félix Tomi , Salvador Cañigueral , Joseph Casanova , António Proença da Cunha , Tomás Adzet (2006). Composition of the Essential Oils of *Ocimum canum*, *O. gratissimum* and *O. minimum*. *Planta Med* 1999; 65(2): 187-189 DOI: 10.1055/s-2006-960465
16. Innocent O. Imosemi (2020). 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.
17. Joshi R.K. (2017). GC–MS Analysis of the Essential Oil of *Ocimum gratissimum* L. Growing Desolately in South India. *Acta Chromatographica* 29(2017)1, 111–119 DOI: 10.1556/1326.2017.29.1.10
18. Ilori, M., Sheteolu, A.O., Omonibgehin, E.A., Adeneye, A.A. (1996). Antidiarrhoeal activities of *O. gratissimum* (Lamiaceae). *Journal of Diarrhoeal Disentery Research* 14:283-285.
19. 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>.
20. Rabelo M; 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 *Ocimum gratissimum* L. (Labiatae) in mice. *Braz J Med Biol Res.* 2003 Apr; 36(4):521-4. doi: 10.1590/s0100-879x2003000400016. Epub 2003 Apr 8.

21. Arueya Gibson Lucky, Olayemi Irewolede and Oluwatosin Adaramoye (2015). Physicochemical Characterization and Enhancement of the Antioxidant Potential of *Ocimum gratissimum* Enriched Pepper Soup Mix. *Advance Journal of Food Science and Technology* 7(6): 408-415, 2015 ISSN: 2042-4868; e-ISSN: 2042-4876
22. Oyen PA, Nguyen XD (1999). *Ocimum gratissimum* L. In: Plant. Resources of South-East Asia. Essential oil Plants. Meroon J. Food Comp. Anal., 17, pp. 197-204
23. Orwa C, A Mutua, Kindt R, Jamnadass R, S Anthony. 2009. Agroforestry database: a tree reference and selection guide version 4.0 <http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp>
24. Ayodele, A.E. and Olowokudejo, J.D. (1997) Systematic Importance of Leaf and Epidermal Characters in West African Species of Family Myrtaceae. *Boletim da Sociedade Broteriana*, 68, 35-72.
25. Ugbabe GE, Ayodele AE (2008). Foliar epidermal studies in the family Bignoniaceae Juss. in Nigeria. *African Journal of Agricultural Research* 3(2): 154-166
26. Metcalfe CR, Chalk L. (1979). *Anatomy of Dicotyledons. Vol. I. Second Edition.* Oxford University Press, New York.
27. Okhale SE, Ugbabe GE, Oladosu PO, Ibrahim JA, Egharevba HO, (2018). Chemical constituents and antimicrobial activity of the leaf essential oil of *Ixora coccinea* L (Rubiaceae) collected from North Central Nigeria. *International Journal of Bioassays* 7.5 pp. 5630-5637.
28. African Pharmacopoeia African Pharmacopoeia (1986). *General Methods for Analysis.* OAU/SRTC Scientific Publications Lagos. pp. 137-149
29. Evans MC, (2002). *Trease and Evans pharmacognosy.* 15th Edition. WB Sanders. London. 183-393.
30. Scranton M.I., M.A. de Angelis, in *Encyclopedia of Ocean Sciences (Third Edition)*, 2001.
31. Bade D.L., in *Encyclopedia of Inland Waters*, 2009.
32. Peter J. Franks and Graham D. Farquhar (2007). The Mechanical Diversity of Stomata and Its Significance in Gas-Exchange Control. *Plant Physiology*, January 2007, Vol. 143, pp. 78–87, www.plantphysiol.org Ó 2006 American Society of Plant Biologists.
33. 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>

34. 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>.
35. Mili M, Hashmi SAR, Ather M, Hada V, Markandeya N, Kamble S, (2022). Novel lignin as natural-biodegradable binder for various sectors—A review. Journal of Applied Polymer Science. 2022;139(15):51951
36. James N. BeMiller, in Carbohydrate Chemistry for Food Scientists (Third Edition), 2019.
37. Brett, C.T. Physiology and Biochemistry of Plant Cell Walls. London: Unwin Hyman, 1990.
38. Jorma Kim, Sofia Gripenberg, Maarit Karonen, Juha-Pekka Salminen (2021). Seed tannin composition of tropical plants. 187 112750. Available online 10 April 2021 0031-9422/©.
39. Susana Soares, Elsa Brandão, Carlos Guerreiro, Sónia Soares, Nuno Mateus, and Victor de Freitas 2020. Tannins in Food: Insights into the Molecular Perception of Astringency and Bitter Taste. Molecules; 25(11): 2590.
40. Dhingra Devinder & Mona Michael & Hradesh Rajput & R. T. Patil (2012). Dietary fibre in foods: a review. Journal of Food Science Technology 49(3):255–266
41. Aluko B. T.; Oloyede O. I. and Afolayan A. J. (2012). Phytochemical and nutrient compositions of the leaves of *Ocimum canum* Sims. African Journal of Biotechnology Vol. 11(63), pp. 12697-12701, 7 August, 2012 Available online at <http://www.academicjournals.org/AJB> DOI: 10.5897/AJB11.3418 ISSN 1684–5315 © 2012 Academic Journals.
42. Bassole IHN, Guelbeogo WM, Nebie R, Costantini C, Sagnon NF, Kabore ZI, Traore, SA (2003 b). Ovicidal and larvicidal activity against *Aedes aegypti* and *Anopheles gambiae* complex mosquitoes of essential oils extracted from three spontaneous plants of Burkina Faso. Parassitologia 45: 23-26.
43. Janssen A M, Scheffer JJC, Ntezurubanza L, Baerhem-Svendsen A (1989). Antimicrobial activities of some *Ocimum* species grown in Rwanda. J. Ethnopharmacol. 26: 57-63.
44. Philippe Belong, Patrick Akono Ntonga, Eric- Moïse Bakwo Fils, Gisèle Aurelie Foko Dadji, Joseph Lebel Tamesse (2013). Chemical composition and residue activities of *Ocimum canum* Sims and *Ocimum basilicum* 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.

45. Tamil M. Selvi, Thirugnanasampandan R., Sundaramma S. (2015). Antioxidant and cytotoxic activities of essential oil of *Ocimum canum* Sims. from India. Journal of Saudi Chemical Society (2015) 19, 97–100. King Saud University. www.ksu.edu.sa
www.sciencedirect.com
46. Chagonda, L.S., Makanda, C.D., Chalchat, J.C., 2000. The essential oils of *Ocimum canum* Sims (basilic camphor) and *Ocimum urticifolia* Roth from Zimbabwe. Flavour Frag. J. 15, 23–26.
47. Ekundayo, O., Laakso, I., Hiltunen, R., 1989. Constituents of the volatile oil from leaves of *Ocimum canum* Sims. Flavour Fragrance Journal. 4, 17–18.
48. Choudhary, R., Kharya, M.D., Dixit, V.K., Varma, K.C., 1998. Role of phytohormone on the cultivar and essential oil of *Ocimum canum* Sims. A potential source of citral. Indian Perfumery 33, 224–227.
49. Sanda, K., Koba, K., Namb, P., Gaset, A., 1998. Chemical investigation of *Ocimum* species growing in Togo. Flavour Fragrance Journal 13, 226–232.
50. Chalchat, J.C., Garry, R.P., Sidibe, L., Harama, M., 1999. Aromatic plants of Mali (II): chemical composition of essential oil of *Ocimum canum* Sims. Journal of Essential Oil Research 11, 473–476.