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# Comparative studies of constituents and antibacterial activities of leaf and fruit essential oils of *Ocimium basilicum* grown in north central Nigeria

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## Abstract

Leaves and fruits (500g each) of *Ocimum basilicum* that were separately hydrodistilled, yielded 0.4 and 0.5% (v/w) of the oils respectively. Analyses of the oils using GC and GC-MS showed that, bulk of the oils were constituted by oxygenated monoterpenes. The principal constituents of the leaf and fruit oils were; linalool (61.7 and 62.9%), 1,8-cineole (17.2 and 18.7%), borneol (8.5 and 6.4%), eugenol (5.7 and 5.4%) and α-caryophyllene (4.3 and 4.0%). With the predominance of linalool in the oils, they are of linalool chemotypes. The antibacterial activities of the oils were evaluated on ten clinical bacterial isolates using disc diffusion method. The oils were found to inhibit three Gram-positive and three Gram-negative bacteria. The bacteria inhibited were *Bacillus megaterium*, *Bacillus cereus*, *Streptococcus pyogenes*, *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumonia*. *Bacillus megaterium* had the highest susceptibility with minimum inhibitory concentration (MIC) of 2.5 mg $ml^{-1}$. *Bacillus cereus*, *Streptococcus pyogenes*, *Serratia marcescens* and *Pseudomonas aeruginosa* showed resistance to the oils.

**Key words:** Linalool, 1, 8-cineole, β-pinene, α-caryophyllene, Gram-positive bacteria

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## 1. Introduction

Basil (*Ocimum basilicum L.*) is a pleasant smelling shrub which grows in several parts the world [1,2]. Essential oils extracted from leaves and flowers of the plant are used as flavors in pharmaceuticals, food and cosmetics [3,4]. Traditionally, the plant is used in the treatments of headache, cough, diarrhea, constipation, warts, worms and kidney malfunction [3]. Anti-inflammatory, anti-carcinogenic, anti-fungal and anti-bacterial activities of the plant extracts, confirmed its therapeutic potentials [5-11]. Insect repellant properties of the plant extracts have also been reported [12-14].

Phytochemical investigations of the plant showed the presence of tannins, steroids, terpenoids, flavonoids and cardiac glycosides [15]. Earlier work on the leaf essential oil of the plant from different part of the world revealed the existence of linalool, methyl chavicol, eugenol, estragole, thymol and p-cymene chemotypes, β-caryophylene [16,17]. The presence of 1,8-cineole in the essential oils of western Nigerian grown plants and other parts of the world were also documented [18-23].

It has been established that composition pattern of essential oils in different parts of some odoriferous plants varied significantly hence, could affects their biological activities [24,25]. It is on the basis of this, that we investigate the constituents and antibacterial activities of leaf and fruit essential oils of north central Nigerian grown *Ocimum basilicum.*

## 2. Materials and methods

### *2.1. Plant Materials*

Fruits and leaves of *Ocimum basilicum* were collected in Ilorin, Ilorin West Local Government Area of Kwara State, Nigeria. Identification was carried out at the herbarium of Forestry Research Institute of Nigeria, Ibadan, where voucher specimens were deposited.

### 2.2. Oil isolation

Pulverized leaves and fruits of *Ocimum basilicum* (500g) were separately hydrodistilled for 3 hours using Clevenger type apparatus according to the British pharmacopoeia specification [26]. The resulting oils were collected in a sealed glass tube and stored under refrigeration until analysis.

### 2.3. Gas Chromatography

GC analysis was performed on an Orion micromat 412 double focusing gas chromatography system fitted the two capillary column coated with CP-Sil 5 and CP-Sil 19 (fused silica, 25m x 0.25mm x 0.15 μm film thickness) and flame ionization detector (FID). The volume injected was 0.2 μL and the split ratio was 1:30. Oven temperature was programmed from 50-230$^{o}$C at 50/min. using hydrogen gas as carrier gas. Injector and Detector temperature were maintained at 200 and 250$^{o}$C respectively. Qualitative data were obtained by electronic integration of FID area percents without the use of correction factors.

### 2.4. Gas Chromatography/Mass Spectrometry

A Hewlett Packard (HP 5890A) GC interfaced with a VG analytical 70-250S double focusing mass spectrometer was used. Helium was the carrier gas at 1.2ml/min. The MS operating conditions were; ionization voltage 70ev, ion source 230°C. The GC was fitted with a 25m × 0.25mm, fused silica capillary column coated with CP-Sil 5. The film thickness was 0.15μm. The GC operating conditions were identical with those of GC analysis. The MS data were acquired and processed by online desktop computer equipped with disk memory. The percentage composition of the oil was computed in each case from GC peak areas. The identification of the component was based on the comparison of retention indices (determined relative to the retention time of series of n-alkanes) and mass spectra with those of authentic samples and with data from literature [27-29].

### 2.5. Antibacterial Activity Test

Antibacterial activities of the oils were carried out using the disc diffusion method [30-32].

#### 2.5.1. Organism

The test bacteria were clinical isolates obtained from the collection of microorganisms of the Department of Microbiology, University of Ilorin. They comprised of *Bacillus megaterium, Streptococcus pyogenes Staphylococcus epidermidis, Staphylococcus aureus,* and *Bacillus cereus* which were Gram-positive; and *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Serratia marcescens* and *Pseudomonas aeruginosa* which were Gram negative bacteria. The bacteria were refreshed by growing on Nutrient Agar plates for 18hrs at 37±1℃.

#### 2.5.2 Preparation of inoculum

Inoculum was prepared from fresh culture by transferring portion of bacterial culture on Nutrient Agar to sterile physiological saline with the aid of a wire loop. The inoculums were standardized to Mcfarland's scale 0.5.

#### 2.5.3. Preparation of discs

Discs (8 mm each) were prepared from Whatman filter paper No. 1 using perforating machine. The discs were wrapped in foil paper and sterilized in hot air oven at 150℃ for 1 hr. The essential oils were diluted with Tween 80 to obtain 40, 20, 10, 5, and 2.5 mg $ml^{-1}$ and the discs were loaded by immersing in different concentration of oils.

#### 2.5.4. Inoculation and incubation of the plates

The prepared Mueller-Hinton plates were inoculated with the ten bacteria strains. Soaked discs of varying concentration were removed with sterile forceps and placed firmly on the surface of each inoculated plates. The plates were incubated at 37$^{o}$C for 24 hrs. and observed for zones of inhibition after growth. The inhibition zones were measured with metric ruler and compared with inhi-bition zones produced by 100μg/ml of gentamicin. Minimum inhibitory con-centrations (MIC) were determined. The antibacterial tests were conducted in triplicates and zones of inhibition (mm) were expressed as the mean of three measurements.

## 3. Results and Discussion

Pulverized leaves and fruits (500g each) of *Ocimum basilicum* that were separately hydrodistilled, yielded 0.4 and 0.5 %( v/w) of the oils respectively. The yield from the leaves compared favorably well with the yield from the leaves of south west grown *Ocimum basilicum* in Nigeria [18]. Table 1 shows retention indices, relative percentages and identities of the constituents of the oils. A total of 39 and 41 compounds that represent 98.9 and 99.4% of the leaf and fruit oils were identified from their retention indices and mass spectra. Hydrocarbons and oxygenated monoterpenes constituted 1.1 and 87.6% of the leaf oil. The percentage composition of hydrocarbon sesquiterpenes and phenylpropanoids were 4.5 and 5.7% respectively. The most abundant hydrocarbon monoterpene in the oil was β-pinene (0.8%). Other notable hydrocarbons in the oil were; D-limonene (0.2%) and allo-ocimene (0.1%). Linalool (61.7%) was the most abundant oxygenated monoterpene in the oil. 1,8-cineole (17.2%), borneol (8.5%) and nerol (0.2%) were found in appreciable quantities. Linalyl acetate, isoartemesia ketone, fenchone, terpenen-4-ol, α-terpeneol, neral, geranial and geraniol were detected in trace amounts. The principal hydrocarbon sesquiterpene in the oil was α-caryophyllene (4.3%). β-bisabolene (0.2%) was found in appreciable quantity. α-copaene, β-elemene, trans-α-bergamotene, bicyclogermacrene, β-sesquiphellandrene, β-cary-ophyllene, 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl existed in trace amounts. Eugenol (5.7%) was the most abundant phenylpropanoid in the oil. Meanwhile, benzyl alcohol, cinnamide aldehyde, ethyl cinnamate, acetyl eugenol, elemicin, benzyl benzoate, thymyl methyl ether were found in trace amounts.

Hydrocarbon and oxygenated monoterpenes constituted 1.7 and 88.2% of the fruit oil. Percentage composition of hydrocarbon sesquiterpenes and phenylpropanoids were 4.1 and 5.4%. The most abundant hydrocarbon monoterpenes in the fruit oil was β-pinene (1.3%). Other notable hydrocarbons in the oil were; D-limonene (0.3%) and allo-ocimene (0.1%).

**Table 1:** Chemical Composition (%) of leaf and fruit essential oils of *Ocimum basilicum*

| Compound[a] | R1[b] | %Composition | | Mass Spectra Data |
|---|---|---|---|---|
| | | leaf | fruit | |
| Triclene | 922 | tr | tr | 136,121,105,93,77,67 |
| α-thujene | 926 | tr | tr | 136,121,115,105,91,77 |
| α-pinene | 933 | tr | tr | 136,121,105,93,79,67 |
| β-pinene | 976 | 0.8 | 1.3 | 136,121,107,93,79,67 |
| myrcene | 990 | tr | tr | 136,121,115,105,91,77 |
| car-2-ene | 1001 | tr | tr | 136,121,105,105,93,79,74 |
| D-limonene | 1027 | 0.3 | 0.2 | 136,121,107,93,79,67 |
| Benzyl alcohol | 1028 | tr | tr | 108, 91,89,77,51 |
| 1, 8 – cineole | 1029 | 17.2 | 18.7 | 154,139,125,108,93,81 |
| Cis-ocimene | 1035 | tr | tr | 105, 93, 79, 67, 53, 41 |
| Allo-ocimene | 1142 | 0.1 | 0.1 | 136, 121, 105, 93, 79, 74 |
| γ-terpinene | 1057 | tr | tr | 136, 121, 105, 93, 77, 65 |
| isoartemisia | 1062 | tr | tr | 91, 83, 69, 55, 41 |
| fenchone | 1058 | tr | tr | 109, 91, 81, 69, 53, 41 |
| linalool | 1098 | 61.7 | 62.9 | 139, 121, 109, 97, 93, 80 |
| borneol | 1162 | 8.5 | 6.4 | 136, 121, 110, 95, 81, 67 |
| terpinen-4-ol | 1175 | tr | tr | 154, 136, 125, 111, 98, 93 |
| α-terpineol | 1189 | 2.0 | tr | 136, 121, 107, 93, 81, 71 |
| cinnamaldehyde | 1214 | tr | tr | 131, 115, 103, 91, 87, 78 |
| nerol | 1226 | 0.2 | 0.2 | 139, 121, 111, 93, 81, 69 |
| thymol methyl ether | 1235 | tr | tr | 139, 123, 111, 93, 81, 69 |
| neral | 1238 | tr | tr | 135, 119, 109, 99, 95 81 |
| geraniol | 1253 | tr | tr | 139, 123, 111, 93, 81, 69 |
| linalyl acetate | 1255 | tr | tr | 135, 121, 105, 93, 80, 67 |
| geranial | 1268 | tr | tr | 152, 137, 123, 109, 99, 95 |
| eugenol | 1354 | 5.7 | 5.4 | 164, 149, 103, 77, 55 |
| α-copeane | 1375 | tr | tr | 105, 119, 161, 91, 81, 41 |
| β-elemene | 1391 | tr | tr | 105, 93, 79, 67, 53, 41 |
| β-caryophyllene | 1423 | tr | tr | 133, 105, 91, 79, 67, 41 |
| tran-α-bergamotene | 1435 | tr | tr | 119, 93, 79, 69, 55, 41 |
| α-caryophyllene | 1454 | 4.3 | 4.0 | 204, 147, 121, 93, 80 |
| ethyl cinnamate | 1461 | tr | tr | 176, 158, 147, 131,115,103 |
| bicyclicgermacrene | 1480 | tr | tr | 209, 204, 189, 161, 147,133 |
| β-bisabolene | 1509 | 0.2 | 0.1 | 204, 189, 176, 161,147, 133 |
| β-sesquiphel landrene | 1523 | tr | tr | 204, 161, 147, 133,120,105 |
| acetyl eugenol | 1523 | tr | tr | 164, 149, 137, 131,121,103 |
| 1, 6, 10-dodacatrien-3-ol, 3, 7, 11-trimethyl | 1534 | tr | tr | 161, 105, 91, 69, 41 |
| Elemicin | 1553 | tr | tr | 208, 193, 177, 165,150,133 |
| Viridiflorol | 1589 | - | tr | 205, 161, 109, 95, 43 |
| Torreyol | 1643 | - | tr | 161, 119, 105, 79, 43 |
| Benzyl benzoate | 1761 | tr | tr | 212, 194, 167, 152, 105, 91 |
| **TOTAL** | | **98.9** | **99.4** | |

[a]Compound are listed in order of elution from silica capillary column coated on CP-Sil 5; [b]retention indices on fused silica capillary column coated with CP-Sil 5; t= trace (<0.1%).

**Table 2:** Anti-bacterial activity of leaf and fruit essential oils of *Ocimum basilicum at* 40mg/ml

| Bacteria | Mean diameter Zone of inhibition (mm) by 100µg/disc gentamicin | Mean diameter Zone of inhibition (mm) by 40mg /ml of leaf oil | Mean diameter Zone of inhibition (mm) by 40mg /ml of fruit oil |
|---|---|---|---|
| Gram – positive | | | |
| *Bacillus megaterium* | 15 | 21.0 ± 0.2 | 18 ± 0.2 |
| *Bacillus cereus* | 13 | 16 ±0.3 | 17 ± 0.4 |
| *Streptococcus pyogenes* | 12 | 14 ± 0.1 | 10 ± 0.1 |
| *Staphylococcus epidermidis* | 10 | -- | -- |
| *Staphylococcus aureus* | -- | -- | -- |
| Gram – negative | | | |
| *Escherichia coli* | 30 | 32±0.4 | 28.0 ± 0.3 |
| *Proteus mirabilis* | 25 | 27.0 ± 0.2 | 26.0 ± 0.2 |
| *Klebsiella pneumonia* | 15 | 14.0 ± 0.1 | 18.0 ± 0.5 |
| *Serratia marcescens* | 12 | -- | -- |
| *Pseudomonas aeruginosa* | 10 | -- | -- |

Values are means of three determinations ± SD, --- Not active

**Table 3:** Anti-bacterial activity of leaf and fruit essential oils of *Ocimum basilicum at varying concentrations*

| | **Leaf** | | | | | **Fruit** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean diameter zone of inhibition (mm)** | | | | | **Mean diameter zone of inhibition (mm)** | | | | |
| **Bacteria** | 2.5mg/ml | 5mg/ml | 10mg/ml | 20mg/ml | 40mg/ml | 2.5mg/ml | 5mg/ml | 10mg/ml | 20mg/ml | 40mg/ml |
| *Bacillus megaterium* | 6±0.2 | 15±0.2 | 18±0.1 | 20±0.2 | 21±0.2 | - | 12±0.1 | 14±0.2 | 14±0.3 | 18±0.2 |
| *Bacillus cereus* | - | 7±0.3 | 11±0.2 | 13±0.1 | 16±0.3 | - | - | - | 13±0.2 | 17±0.4 |
| *Streptococcus pyrogenes* | - | - | 8±0.2 | 11±0.3 | 14±0.1 | - | 4±0.2 | 5±0.3 | 8±0.4 | 10±0.1 |
| Gram negative | | | | | | | | | | |
| *Escherichia coli* | 16±0.2 | 20±0.4 | 25±0.3 | 28±0.1 | 32±0.4 | 10±0.3 | 20±0.1 | 23±0.4 | 24±0.3 | 28±0.3 |
| *Proteus mirabi Lis* | - | 16±0.3 | 20±0.1 | 23±0.2 | 27±0.2 | - | 21±0.3 | 22±0.1 | 23±0.5 | 26±0.2 |
| *Klebsiella pneumonia* | - | 5±0.3 | 9±0.1 | 10±0.2 | 14±0.1 | - | - | 13±0.3 | 15±0.2 | 18±0.5 |

Values are means of three determinations ± SD, --- Not active

Linalool (61.7%) was the most abundant oxygenated monoterpene in the oil. 1,8-cineole (17.2%), borneol (8.5%) and nerol (0.2%) were found in appreciable quantities. Linalyl acetate, isoartemesia ketone, fenchone, terpenen-4-ol, α-terpeneol, neral, geranial and geraniol were detected in trace amounts. The principal hydrocarbon sesquiterpene in the oil was α-caryophyllene (4.3%). β-bisabolene (0.2%) was found in appreciable quantity. α-copaene, β-elemene, trans-α-bergamotene, bicyclogermacrene, β-sesquiphellandrene, β-caryophyllene, 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl existed in trace amounts. Eugenol (5.7%) was the most abundant phenylpropanoid in the oil. Meanwhile, benzyl alcohol, cinnamide aldehyde, ethyl cinnamate, acetyl eugenol, elemicin, benzyl benzoate, thymyl methyl ether were found in trace amounts. Hydrocarbon and oxygenated monoterpenes constituted 1.7 and 88.2% of the fruit oil. Percentage composition of hydrocarbon sesquiterpenes and phenylpropanoids were 4.1 and 5.4%. The most abundant hydrocarbon monoterpenes in the fruit oil was β-pinene (1.3%). Other notable hydrocarbons in the oil were; D-limonene (0.3%) and allo-ocimene (0.1%). Linalool (62.9%) was the most abundant oxygenated monoterpene in the fruit oil. 1,8-cineole (18.7%), borneol (6.4%) and nerol (0.2%) were found in appreciable quantities. Linalyl acetate, isoartemesia ketone, fenchone, terpinen-4-ol, α-terpineol, neral, geranial and geraniol were found in trace amounts. Predominant hydrocarbon sesquiterpenes in the oil was α-caryophyllene (4.0%). β-bisabolene (0.1%) was found in appreciable quantity. α-copaene, β-elemene, trans-α-bergamotene, bicyclogermacrene, β-sesquiphellandrene, β-car-yophyllene, viriflorol, torreyol, 1,6, 10-dodecatrien-3-ol,3,7,11-trimethyl existed in trace amounts. Eugenol (5.4%) was the most abundant pheylpropanoid in the oil. Meanwhile, benzyl alcohol, cinnamaldehyde, ethyl cinnamate, acetyl eugenol, elemicin, benzyl benzoate, thymyl methyl ether were not detected in significant amounts.

Qualitatively, the composition patterns of the oils were similar with respect to their constituents, except viridiflorol and torreyol that were not detected in the leaf oil. However, the quantities of the constituents vary significantly in the oils. For instance, linalool, 1,8-cineole, and β-pinene were found in higher proportions in fruit oil than leaf oil. On the other hand, β-caryophyllene and eugenol were of greater abundance in the leaf oil than fruit oil. Interestingly, benzyl alcohol, cinnamaldehyde, ethylcinnamate, acetyl eugenol, elemicin, benzyl benzoate, thymyl methyl ether were found in trace amounts in both oils. Comparison of the leaf oil with the leaf oil of South west Nigerian grown *Ocimum bacilicum* showed that the oils are of different chemotypes. The leaf oils obtained from the two locations are of linalool and methyl carvicol chemotypes respectively [18]. However, the oils shared similar composition pattern with respect to the notable constituents like linalool, eugenol and 1,8-cineole in the leaf essential oil of *Ocimum. basilicum* grown in Northern Carlifonia [33]. The sensitivity pattern of the bacteria to the oils is presented in Tables 2 and 3. The essential oils inhibited three of the Gram-positive bacteria with *Bacillus megaterium* showing highest susceptibility at 2.5 and 5.0 mg $ml^{-1}$ of the leaf and fruit oils respectively. The MIC to the leaf and fruit oils was 5.0 and 20.0 mg $ml^{-1}$ for *Bacillus cereus* and 10.0 and 5.0 mg $ml^{-1}$ for *Streptococcus pyogenes* respectively. *Staphylococcus epidermidis* and *Staphylococcus aureus* were resistant to the oil at all the concentrations tested.

Among the gram-negative bacteria, *Escherichia coli* showed the highest sensitivity with MIC at 2.5 mg $ml^{-1}$ of both leaf and fruit oils. *Proteus mirabilis* had the MIC of 5.0 mg $ml^{-1}$ for the two oils while that of *Klebsiella pneumoniae* was 5.0 and 10.0 mg $ml^{-1}$ respectively for the leaf and fruit oils. The other bacteria showed no susceptibility to the oils. The susceptibility obtained indicates that the leaf and fruit essential oils of *O. bassilicum* are active against the bacteria tested and do not discriminate between Gram positive and Gram negative and hence have therapeutic potentials for the ailment caused by the organisms. Similar inhibitory property of the oils to both Gram positive and Gram negative bacteria have been reported [34].

## References


[1] A. Akgul. (1993). Spice Science and Technology. Turkish Association Food Technologists Publication, No. 15, Ankara, Turkey.

[2] O. Baritaux, H. Richard, J. Touche and M. Derbesy. (1992). Effects of drying and storage of herbs and spices on the essential oil. Part I. Basil, *Ocimum basilicum L*. Flavour and Fragrance Journal, 7: 267-271.

[3] J.E. Simons, M.R. Morales, W.B. Phippen, R.F. Vieira and Z. Hao. (1999). A source of aroma compounds and a popular culinary and ornamental herb, in J. Janick (Ed.), perspectives on new crops and new uses. Alexandria, VA: ASHS Press, pp. 499-505.

[4] J. Javanmardi, A. Khalighi, A. Kashi, H.P. Bais and J.M. Vivanco. (2002). Chemical characterization of basil (*Ocimum basilicum L*.) found in local accessions and used in traditional medicines in Iran. Journal of Agricultural and food chemistry. 50: 5878-5883.

[5] A. Caceres, O. Cano, B. Samayo and L. Aguilar. (1990). Plant used in Guatemala for the treatment of gastrointestinal disorders. 1. Screening of 84 plants against anterobacteria. Journal of Ethnopharmacology. 30:55-73.

[6] M. Ndounga and J.M. Ouamba. (1997). Antibacteria and antifungal activities of essential oils of *Ocimum gratissimum* and *Ocimum basilicum* from Congo. Fitoterapia. 68: 190-191.

[7] A. Abdel-Sattar, V. Bankova, A. Kujumgiev, A. Galabov, A.Ignotavo, C. Todorova and S. Popov. (1995). Chemical composition and biological activity of leaf exudates from some Lamiaceae plants. Pharmazie. 50: 62-65.

[8] S. Dube, P.D. Upadhyay and S.C. Tripthi (1989). Antifungal, physio-chemical and insect repelling activity of the essential oil of *Ocimum basilicum*. Canadian Journal of Botany. 67: 2085-2087.

[9] R.R. Chattopahdyay, S.K. Sarkar, S. Ganguly and T.K. Basu. (1994). A comparative evaluation of

some anti-inflamatory agents of plant origin. Fitoterapia. 65: 146-148.

[10] M.S. Akhtar and M. Munir. 1989. Evaluation of the gastric anti-ulcerogenic effects of *Solanum nigrum*, *Brassica oleraceae* and *Ocimum basilicum* in rats. J. Ethnopharmacol. 27: 163-176.

[11] P. Agrawal, V. Rai and R.B. Singh. (1996). Randomized placebo-controlled, single blind trial of holy basil leaves in patient with noninsulin-dependent diabetes mellitus. International Journal of Clinical Pharmaceutical Therapeutics. 34: 406-409.

[12] S. Mohiuddin, R.A. Qureshi, M.A. Khan and M.K.A. Nasir. (1987). Laboratory investigations on the repellency of some plant oils to red flour beetle *Tribolium castan-eum* Herbst. Pakistan Journal of Science and Industrial Research. 30: 754-756.

[13] C. Regnault-Roger and A. Hamraoui. (1994). Inhibition of reproduction of *Acanthoscelides obtectus* say (caleoptera), a kidney bean (*Pbaseoulus vulgaris*) brunched, by aromatic essential oils. Crop protection. 13: 624-628.

[14] M. Bhamagar, K.K. Kapur, S. Jalees and S.K. Sharma. (1993). Laboratory evaluation of insecticidal properties of *Ocimum baslicum Linnaeus* and O. Sanctum L. plants essential oils and their major constituents against vector mosquito species. Journal of Entomological Research. 17: 21-26.

[15] K. Sekar, S. Thangaraj, S.B. Saravana, R. Hraisaranraj and K. Suresh. (2009). Phytochemical constituents and antioxidant activity of extract from the leaves of *Ocimum basilicum*. Journal of Phytology. 1(6): 408-413.

[16] L.M. Khatri, L.M., M.K.A. Nasir, R. Saleem and F. Noor. (1995). Evaluation of Pakistani sweet basil oil for commercial exploitation. Pakistan Journal of Science and Industrial Research. 38: 281-282.

[17] S.M. Keita, C. Vincent, J.P. Schemit and A. Belanger. (2000). Essential oil composition of *Ocimum basilicum L.*, *O. gratissum L.* and *O. suave L.* in the Republic of Guinea. Flavour and Fragrance Journal. 15: 339-341.

[18] O. Ekundayo, L. Laakso, B. Oguntimehin, J.I. Okogun, A.A. Elujoba and R. Hiltunen. (1987). Essential oil composition of two chemotypes of *Ocimum basilicum* from Nigeria. Acta Pharmaceutica Fennicae. 96: 101-106.

[19] Y.I. Sheen, Y.H. Tsaiou, and S.J. Tsai. (1991). Flavor characteristic compounds found in the essential oil of *Ocimum basilicum* L. with sensory evaluation and statistical analysis. Journal of Agriculture and Food Chemistry. 39: 939-943.

[20] M.J. Perez-Alonso, A. Velasco- Negueruela, M. Emin Duru, M. Harmanda and J.L. Esteban. (1995). Composition of the essential oil of *Ocimum basililcum, Var. glabratum* and *Rosmarinus officinalis* from Turkey. Journal of Essential Oil Research. 7: 73-75.

[21] Z. Fleisher and A. Flaisher. (1992). Volatiles of *Ocimum basilicum* traditionally grown in Israel. Aromatic plants of the holy land and the Sinai, part III. Journal of Essential Oil Research. 4: 97-99.

[22] Y.K.Hasegawa, K. Tajima, N. Toi and Y. Sugimura. (1997). Characteristic components found in the essential oil of *Ocimum basilicum L*. Flavour. Fragr.ance Journal. 12: 195-200.

[23] R.J. Grayer, G.C. Kite, F.J. Goldstone, S. Bryan, A. Paton and E. Putievsky. (1996). Infraspecific taxonomy and essential oil chemotypes in sweet basil, *Ocimum basilicum*. Phytochemistry. 43: 1033-1039.

[24] L.A. Usman, A.A. Hamid, O.C. George, O.M, Ameen, N.O. Muhammed, M.F. Zubair and A. Lawal.(2009). Chemical composition of rhizome essential oil of *Curcuma Longa L*. growing in north central Nigeria. World Journal of Chemistry. 4 (2): 178-181.

[25] M. Lahlou. (2004). Method to study the phytochemistry and bioactivity of essential oil. Phytotherapy Research. 18: 435-488.

[26] British pharmacopoeia II, (1980). 109, HM, stationary office, London.

[27] R.P. Adams. (1995). Identification of essential oil components by gas chromatography and mass spectrometry. Allured publisher Corperation, Carol Stream, Ilnois.

[28] D. Joulain and W.A. Koenig. (1998). The atlas of spectra data of sesquiterpene hydrocarbon. E. B. Verlay Hamburg, Germany.

[29] W. Jennings, T. Shibamoto. (1980). Qualitative analysis of flavor volatiles by gas capillary chromatography. Academic press, New York.

[30] J. Hudzick. (2012). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. http://www.microbelibrary.org/library/8-authors/3208-jan-hudzicki

[31] M.C. Lalitha. (2004). Manual on Antimicrobial Susceptibility Testing. Indian Association of Medical Microbiologists *www.ijmm.org/documents/Antimicrobial.doc*

[32] J.H. Jogersen and M.J. Ferraro. (2009). Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. *Clinical Infectious Diseases* 9 (11): 1749 – 1755.

[33] L. Seung-Joo, K. Umano, S. Takayuki and K. Lee. (2005). Identification of volatile components in basil (*Ocimum basilicum L.*) and Thyme leaves (*Thymus vulgaris*) and the antioxidant properties. Food Chemistry. 91. 131-137.

[34] S. Inouye, T. Takazawa and H. Yamaguchi. (2001). Antimicrobial activity of the essential oils and their major constituents against respiratory tract pathogens by gaseous contact. Journal of Antibacterial Chemotherapy. 47: 565-573.