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# Physiochemical investigation of essential oils from three *Cymbopogon* species cultivated in Sudan

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**Abstract**
*Cymbopogon* (Poaceae) represents an important genus of about 140 species that grow in tropical and subtropical regions around the world. They have diverse uses in pharmaceutical, cosmetics, food and flavor, and agriculture industries. The essential oils from *C. citratus* leaves, C. *nervatus* inflorescences, *C. proximus* leaves and *C. proximus* inflorescence were prepared using hydrodistillation method. The percentage yield and the physical properties of the oils were determined, The essential oils were analyzed for identification of their chemical composition using GC-MS technique. Piperitone (43.2, 45.8%), elemol (13.45, 14.43%), 4-carene (7.55, 9.75%), β - eudesmol (5.41, 4.32%), limonene (2.45, 4.03%) and α- eudesmol (2.61, 4.66%), β-elemene (2.24, 1.30%) were the main components identified in the essential oil of *C. proximus* leaves and inflorescence, respectively. The main constituents identified in *C. nervatus* inflorescence essential oil were α- verbenol (20%), trans-pincarveol (19.41%), trans- p- menthe-2,8- dien-ol (14.14), d-Limonene (8.49%), cis-piperitol (8.46%), cyclohexanol-2-methylene -5- (1-methyl ethenyl) (8.32%), 2-cyclo pentyl- cyclopentanone (7.28%) and carvone (4.58%), whereas the main identified constituents in *C. citratus* essential oil were citral, which is a mixture of the stereoisomer geranial (32.74%) and neral (26.23%), β-pinene (9.36%) and Geraniol (4.47%). The essential oils distilled from *Cymbopogon* species, *C. citratus* leaves, *C. proximus* leaves, *C. proximus* inflorescences and C. *nervatus* inflorescences, revealed different chemical composition, but they shared a common feature, that their major constituents were oxygenated monoterpenes among the other terpenoid classes

**Keywords:** *Cymbopogon,* Hydrodistillation, GC-MS, Piperitone, Citral

## 1. Introduction
*Cymbopogon* (Poaceae) represents an important genus of about 140 species that grow in tropical and subtropical regions around the world. On account of their diverse uses in pharmaceutical, cosmetics, food and flavor, and agriculture industries, *Cymbopogon* grasses are cultivated on a large scale, especially in the tropics and subtropics. [1-3] Among the several aromatic species belonging to the genus *Cymbopogon* the most important in terms of essential oil production are *C. citratus* (West Indian lemongrass), *C. flexuosus* (East Indian lemongrass) and *C. winterianus* (citronella). Also known to produce essential oils are *C. proximus* Stapf, (Halfa bar) and *C. nervatus* (Hochst) Chiov [1, 4].
*C. citratus* L. is one of the largely cultivated medicinal plants for its essential oils in parts of tropical and subtropical areas of Asia, Africa and America [5]. It contains 1 to 2% essential oil on a dry basis with widely variation of the chemical composition as a function of genetic diversity, habitat and agronomic treatment of the culture [6]. Lemongrass essential oil is characterized by a high content of citral (neral and geranial isomers (up to 80%) [7-9]. The minor compounds presented in significant percents in previously investigated lemongrass oil were myrcene, geraniol and geranyle acetate [10-12].
*C. nervatus* (Hochst) Chiov, represents one of the predominant wild grasses in the Central Sudan, East Central Sudanas well as Western Sudan [1, 13, 14]. Generally essential oil content of *C. nervatus* inflorescence in Sudan vary from 0.8 – 1.5% (Guenther 1950, Anand 2010) [1, 4]. Heiba and Rizk [15] observed that, the essential oil of *C. nervatus* was found to be of menthane-type; unusual menthadien-ols were the predominant components. El-Kamali [16] analyzed essential oil sample from inflorescence of *C. nervatus* collected during dry season in 2000 from Rufaa (Central Sudan), using GC-MS. He concluded that, the main constituents

of the essential oil, which represented about (1.3%) of the dried inflorescence, were: cis-p-mentha-1(7), 8-dien-2- ol (25.2%), trans-p-mentha-1(7)-8-dien-2-ol (22.9%), 2-(1-methyl-propyl)-cyclopentanone (11.3%), trans-carveol (9.6%) and trans-p-2,8-mentha-dien-1-ol (8.4%). Abushama *et al.* [17] investigated the essential oil composition and antifungal activity of *C. nervatus* and concluded that the volatile oil consisted mainly of oxygenated monoterpenes. Trans-p-Mentha-1(7), 8-dien-2-ol was identified as the major compound and presented 21.25%. Cycloheptane, 1, 3, 5-tris (Methylene), the second main compound, was also identified in the oil and presented 16.08%. Cis- p- Mentha- 1(7), 8-dien- 2- ol was the third main compound; its percentage was 9.83%, and P- mentha- Trans- 2, 8- Dien-1-ol constituted 9.83%. dl-Limonene was the fifth main compound constituting 7.37%.

*C. proximus* Stapf is distributed in Central and Northern Sudan and in Egyptian desert and the sandy coast of the Red Sea on the southern boundaries of Egypt. The chemical composition of *C. proximus* from Egypt was investigated using GC/MS system. A total of 19 constituents representing 95.47% of the oil were identified. Piperitone (72.44%), elemol (9.43%), α - eudesmol (4.34%), limonene (2.45%) and β- eudesmol (1.26%) were the main components comprising 88.92% of the oil [18].

This research was conducted to investigate the chemical composition of the essential oils from three plants belong to the Genus *Cymbopogon* cultivated in Sudan: *Cymbopogon citratus*, *C. proximus* and *C. nervatus*.

## 2. Materials and Methods

### Materials

### Plant materials

The three herbs parts used in the study (*C. citratus* leaves, C. *nervatus* inflorescences, *C. proximus* leaves and *C. proximus* inflorescence) were collected from Industrial Research and Consultancy Centre (IRCC) farm, Khartoum State, Sudan.

### Methods

### *E*ssential oil preparation and content determination

Essential oils were isolated from the different parts using the hydro-distillation method. For oil content determination, 100g from each sample were subjected to hydrodistillation for 4 h using Clevenger apparatus. The distilled essential oil was dried over anhydrous sodium sulphate, filtered and stored in a sealed vial at 4 °C. The yield of the oil (v/w %) was calculated based on the plant dry matter.

### Physical properties determination

The physical properties of the oils were determined according to the British Standards methods [19].

### GC-MS analysis of the oils

The essential oils were analyzed by gas chromatography coupled with mass spectrometry (GC-MS) using HP 6890 (GC) and HP 5973 (MSD). The sample was dissolved in dichloromethane (1%) and injected at 250 °C (Injector temperature) into a capillary column type HP-1, 30 ´ 0.25 mm ´ 0.25 µm, stationary phase (95% diethyl-5% diphenylsiloxane), using helium as a carrier gas at a flow rate of 1 ml/min. The injected volume was 1 µl and the injection mode used was splitless. The oven temperature was programmed from 45–280 °C at the rate of 4 °C/min. Detector temperature was 250 °C. The MS was operated in the El mode at 70 eV. The mass and scan range was set at *m/z* 30-500.

## 3. Results and Discussion

### Physiochemical analysis of *Cymbopogon* oils

The essential oil content of *C. citratus* leaves, C. *nervatus* inflorescences, *C. proximus* leaves and *C. proximus* inflorescence were found to be (1.7±0.08), (2.0±0.09), (0.9±0.02) and (3±0.2%), respectively. Their physical constants are shown in Table (1).

**Table 1:** Physical Properties of *Cymbopogon* essential oils

| Essential oil / Physical Property | *C. citratus* leaves | *C. nervatus* inflorescence | *C. proximus* leaves | *C. proximus* inflorescence |
|---|---|---|---|---|
| Specific Gravity, at 15° | 0.8847 ± 0.005 | 0.9405 ± 0.004 | 0.9169 ±0.005 | 0.9009± 0.005 |
| Refractive index, at 20° | 1.4849± 0.001 | 1.4946± 0.002 | 1.4831± 0.001 | 1.4947± 0.002 |
| Optical rotation, at 20° | −0.62° | +26°22′ | −59°22′ | −58°18 |
| Solubility | Soluble in 1 vol. of 75% alcohol | Soluble in 0.5 vol. of 80% alcohol | Soluble in 0.8 vol. of 70% alcohol | Soluble in 0.8 vol. of 70% alcohol |

The four *Cymbopogon* essential oils were analyzed using GC-MS technique. Identification of compounds from essential oils was based on comparison of their mass spectra with the, computer library of NIST or Wiley Registry of Mass Spectral Data.

GC-MS of the hydrodistilled essential oils of *C. proximus* leaves and inflorescence revealed detection and identification of twenty and nineteen compounds, respectively (Table 2).

**Table 2:** *C. proximus* essential oils constituents

| Peak No. | RT | Compound | Essential oil | |
|---|---|---|---|---|
| | | | *C. proximus leaves* | *C. proximus* inflorescence |
| 1 | 11.981 | p-cymene | 0.86 | 1.27 |
| 2 | 13.031 | 4-carene | 7.55 | 9.75 |
| 3 | 13.033 | 2-carene | 8.08 | - |
| 4 | 13.779 | o-cymene | 0.76 | - |
| 5 | 13.925 | Limonene | 3.33 | 4.03 |
| 6 | 14.006 | 1,8-cineole | 0.62 | - |
| 7 | 15.854 | (+)-fenchone | - | 0.53 |

| | | | | |
|---|---|---|---|---|
| 8 | 16.876 | p-menth-2-en-1-ol | 1.79 | 0.05 |
| 9 | 17.434 | Cis-p-mentho-2,8 dien-1-ol | 1.47 | 1.66 |
| 10 | 18.565 | Trans-2-carene-4-ol | - | 1.85 |
| 9 | 21.148 | Piperitone | 43.2 | 45.8 |
| 10 | 22.281 | 2-oxocineol | 0.56 | - |
| 11 | 24.590 | β-elemene | 2.24 | 1.30 |
| 12 | 25.355 | Caryophyllene | 1.69 | 1.70 |
| 13 | 27.036 | Epi-α-selinene | 0.71 | - |
| 14 | 27.248 | α-selinene | 0.52 | - |
| 15 | 27.517 | α-cuparene | 1.27 | 0.83 |
| 16 | 28.611 | Elemol | 13.45 | 14.43 |
| 17 | 29.415 | Caryophyllene oxide | 1.38 | 1.10 |
| 18 | 30.566 | γ-eudesmol | 2.49 | 2.55 |
| 19 | 30.984 | β- eudesmol | 5.41 | 4.32 |
| 20 | 31.046 | α- eudesmol | 2.61 | 4.66 |

The monoterpenes constitute about 68.22% of the oil, of which oxygenated fraction comprised (47.64%) whereas the hydrocarbon fraction represented about (20.58%), of the oil. The oxygenated sesquiterpenes represented about (25.34%). Piperitone (43.2, 45.8%), elemol (13.45, 14.43%), 4-carene (7.55, 9.75%), β - eudesmol (5.41, 4.32%), limonene (2.45, 4.03%) and α- eudesmol (2.61, 4.66%), β-elemene (2.24, 1.30%) were the main components identified in the essential oil of *C. proximus* leaves and inflorescence, respectively (Fig. 1). 2-carene which was detected in *C. proximus* leaves essential oil in considerable amount (8.08%), was not detected in *C. proximus* inflorescence essential oil. Also there was a noticeable difference in the minor constituents between the two oils.

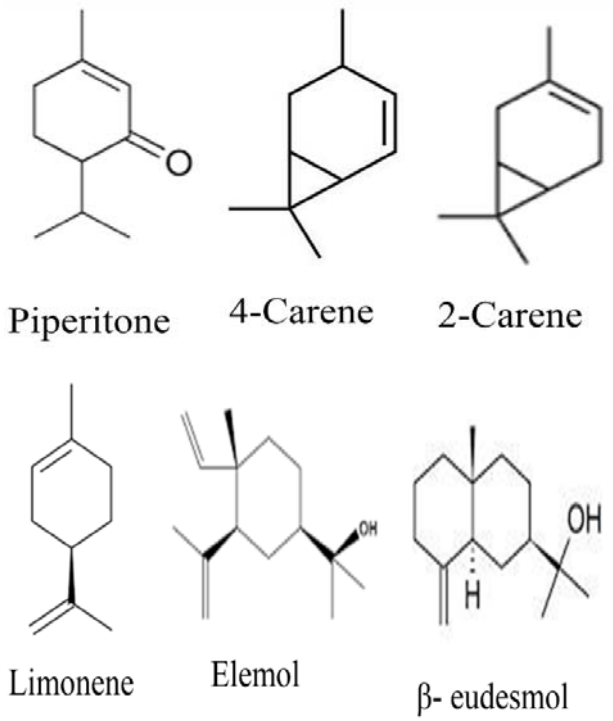



**Fig 1:** *C. proximus* essential oils main constituents

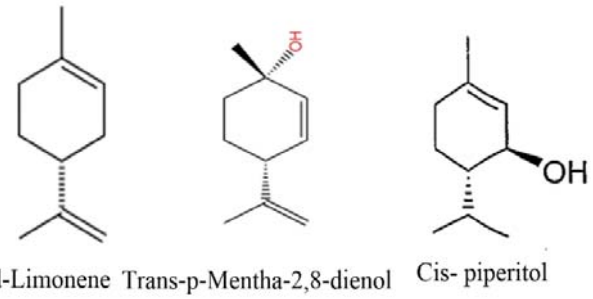
Investigation of *C. nervatus* essential oil using GC- MS showed that the oil is composed of twenty three compounds (Table 3).

It is noticed that the essential oil of *C. nervatus* inflorescence consisted mainly of oxygenated monoterpenes and no sesquiterpenes were detected, although presence of some sesquiterpenes were detected in essential oil from leaves of *C. nervatus* in a previous study [20]. The finding in this research is similar to those obtained by Heiba and Rizk [15] and El-Kamali [16].

**Table 3:** *C. nervatus* inflorescence essential oil constituents

| peak | R. T | Compound Name | Area, % |
|---|---|---|---|
| 1 | 5.958 | d-Limonene | 8.49 |
| 2 | 9.671 | Trans-p-Mentha-2,8-dienol | 14.14 |
| 3 | 10.057 | α-pinene oxide | 0.32 |
| 4 | 10.183 | Limonene oxide | 0.72 |
| 5 | 10.266 | Cyclo hexanol, 2-methylene -5-[1-methyle thenyl] | 8.32 |
| 6 | 11.958 | Trans-pinocarveol | 19.41 |
| 7 | 12.267 | 3,9-Epoxy-p-mentha-1,8(10)-diene | 1.58 |
| 8 | 12.479 | 2-cyclo pentyl cyclopentanone | 7.28 |
| 9 | 12.703 | Trans –Di hydrocarvone | 0.21 |
| 10 | 12.84 | Cyclohexanone, 2-methyl-5-(1-methyl | 0.47 |
| 11 | 12.911 | 5- cyclodiene,1,2- epoxy | 0.51 |
| 12 | 12.986 | 1-[2-ethyl-3-cycloehexa ethanol] | 1.14 |
| 13 | 13.106 | Cis- piperitol | 8.46 |
| 14 | 13.533 | α–verbenol | 20.53 |
| 15 | 13.705 | Trans-3-caren-2-ol | 0.4 |
| 16 | 13.964 | D-verbenone | 0.58 |
| 17 | 14.117 | Ethanone-1-(6,6-dimethyloiccyclo[3-1-hex-2-en-zyl] | 0.31 |
| 18 | 14.227 | 2- caren-l0-al | 0.58 |
| 19 | 14.672 | Carvone | 4.58 |
| 20 | 15.648 | Perillade hyde | 0.60 |
| 21 | 15.951 | trans-8-hydroxylinalool | 0.41 |
| 22 | 16.162 | Isopiperitone | 0.58 |
| 23 | 16.984 | Cis -2-pinanol | 0.39 |

The main constituents identified in *nervatus* inflorescence essential oil were α- verbenol (20%), trans-pincarveol (19.41%), trans- p- menthe-2,8- dien-ol (14.14), d-Limonene (8.49%), cis-piperitol (8.46%), cyclohexanol-2- methylene -5- (1-methyl ethenyl) (8.32%), 2-cyclo pentyl-cyclopentanone (7.28%) and carvone (4.58%) (Fig. 2).



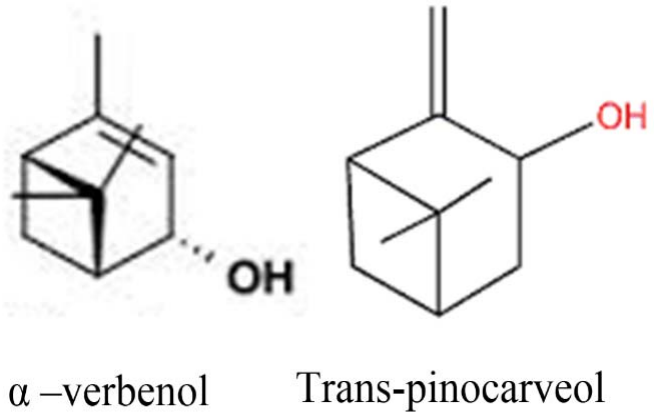



**Fig 2:** *C. nervatus* inflorescence essential oil main constituents

The chromatographic and mass spectrometry analysis of the *C. citratus* leaves volatile oil resulted in the identification of thirty seven compounds (Table).

**Table 4:** composition of *C. citratus* leaves essential oils

| Peak No. | RT | Compound | Area, % |
|---|---|---|---|
| 1 | 10.233 | α-pinene | 0.23 |
| 2 | 12.596 | Hepten-2-one,6-methyl | 3.65 |
| 3 | 12.736 | β-pinene | 9.36 |
| 4 | 13.906 | Limonene | 0.37 |
| 5 | 13.996 | 1,8-cineole | 0.79 |
| 6 | 14.221 | Cis-ocimene | 0.70 |
| 7 | 14.549 | β- ocimene | 0.43 |
| 8 | 15.981 | β- ocimene | 0.63 |
| 9 | 16.191 | Linalool | 1.44 |
| 10 | 17.820 | β-citronellal | 0.73 |
| 11 | 18.168 | Cis-verbenol | 0.84 |
| 12 | 18.502 | 4-(1,2-Dimethyl-cyclopent-2-enyl)-butan-2-one | 0.98 |
| 13 | 18.709 | Caren,4,5-epoxy trans | 1.39 |
| 14 | 19.106 | Borneol | 1.08 |
| 15 | 20.150 | β-citronellol | 1.95 |
| 16 | 20.599 | Neral (Citral b) | 26.23 |
| 17 | 20.930 | Geraniol | 4.47 |
| 18 | 21.489 | Geranial (Citral a) | 32.74 |
| 19 | 21.622 | Limonene oxide | 0.93 |
| 20 | 21.882 | Nonyl methyl ketone | 0.52 |
| 21 | 22.124 | Geranyl formate | 0.55 |
| 22 | 23.142 | 13-heptadecyn-1-ol | 0.55 |
| 23 | 23.669 | m-eugenol | 0.74 |
| 24 | 24.101 | 4-ethylcyclohexanol | 0.77 |
| 25 | 24.258 | Nerylacetate | 0.87 |
| 26 | 24.580 | Methl eugenol | 0.22 |
| 27 | 25.349 | Trans caryophyllene | 0.23 |
| 28 | 25.671 | Bergamotene | 0.27 |
| 29 | 27.072 | γ-cadinene | 0.47 |
| 30 | 27.385 | Δ-cadinene | 0.26 |
| 31 | 27.905 | Cupapene | 0.46 |
| 32 | 28.511 | Elemol | 1.76 |
| 33 | 29.395 | 5-epi-7-epi-α-eudesmol | 0.48 |
| 34 | 30.195 | α-cadinol | 1.13 |
| 35 | 30.471 | γ-cadinol | 0. 67 |
| 36 | 30.922 | β-eudesmol | 0.86 |
| 37 | 30.980 | 1(15) guaiene | 0.34 |

The monoterpenes constitute about (92.70%) in the investigated lemongrass essential oil, of which (80.89%) were oxygenated monoterpenes. The main identified constituent was citral, which is a mixture of the stereoisomer geranial (32.74%) and neral (26.23%). The oil also contained β-pinene (9.36%), Geraniol (4.47%) and other components present in lesser amounts (Fig. 3).

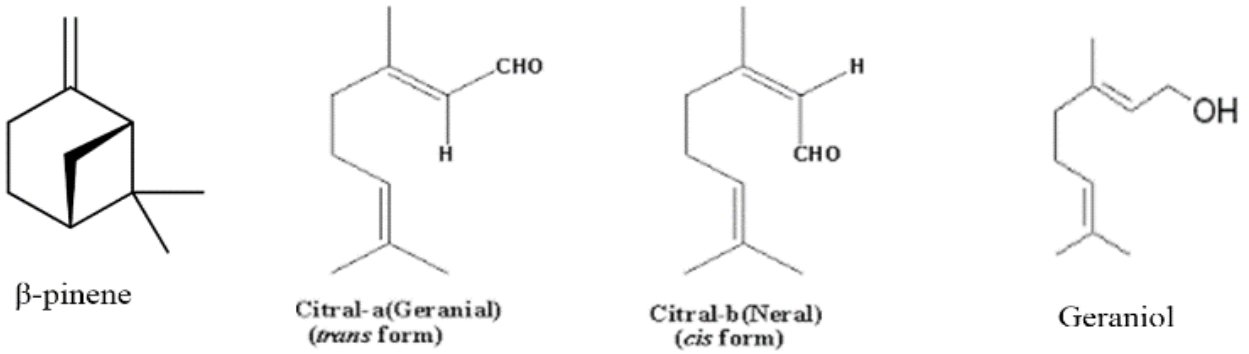



**Fig 3:** *C. citratus* leaves essential oil main constituents

The resulted variation in the physical properties as well as in the constituents and their percentages between the present findings and those in previous studies may be due to the variation in the factors which affect yield and quality of essential oil. These factors are variation in geographical location, climatic conditions and the seasonal and maturity variation, genetic variation, growth stages, part of plant utilized and post-harvest, drying and storage [21-28].

## 4. Conclusion

Although the essential oils distilled from *Cymbopogon* species, *C. citratus* leaves, *C. proximus* leaves, *C. proximus* inflorescences and C. *nervatus* inflorescences, revealed different chemical composition, but they shared a common feature which was that their major constituents were oxygenated monoterpenes among the other terpenoid classes.

## 5. References


1. Guenther E. The Essential Oils, Van Nostrand, London, 1950; IV:3-153.
2. Dutta SC. Cultivation of *Cymbopogon winterianus* Jowitt for production of citronella (Java) oil. In: Cultivation and Utilization of Aromatic Plants, Jammu-Tawi, India, 1982, 325-329.
3. Ganjewala D, Kumari Ambika, Khan KH. Ontogenic and developmental changes in essential oil content and compositions in Cymbopogon flexuosus cultivars. In: Recent Advance in Biotechnology, Excel India Publishers, New Delhi, 2008, 82-92.
4. Anand A. Essential oil Bearing Grasses, Medicinal and Aromatic Plants — Industrial Profiles, CRC Press, London, 2010, 46:25-106.
5. Chanthal S, Prachakoli S, Ruangviriyachai C. Influence of extraction methodologies on the analysis of five major volatile aromatic compounds of citronella grass and lemongrass grown in Thailand, Journal of AOAC International. 2012; 95:763-772.
6. Carlson LH, Machad CB, Pereira LK, Bolzan A. Extraction of lemongrass essential oil with dense carbon dioxide Journal of Supercritical Fluids. 2001; 21:33-39.
7. Paviani L, Pergher SB, Dariva C. Application of molecular sieves in the fractionation of lemongrass oil from high-pressure carbon dioxide extraction. Brazilian Journal of Chemical Engineering. 2006; 23:219-225.
8. Tajidin NE, Ahmad SH, Rosenani AB, Azimah H, Munirah M. Chemical Composition and Citral Content in Lemongrass (*Cymbopogon Citratus*) essential oil at three maturity stages, International Journal of Biotechnology. 2012; 11(11):2685-2693.
9. Al Yousef SA. Antifungal Activity of Volatiles from Lemongrass (*Cymbopogon citratus)* and Peppermint (*Mentha piperita*) Oils against Some Respiratory Pathogenic Species of Aspergillus. International Journal of Current Microbiology and Applied Science, 2013; 2(6):261-272.
10. Matasyoh JC, Wagara IN, Nakavuma JL, Kiburai AM. Chemical composition of *Cymbopogon citratus* essential oil and its effect on mycotoxigenic *Aspergillus* species. African Journal of Food Science. 2011; 5(3):138-142
11. Millezi AF, Caixeta DS, Rossoni DF. *In vitro* antimicrobial properties of plant essential oils *thymus vulgaris, cymbopogon citratus* and *laurus nobilis* against five important foodborne pathogens. Ciênc. Tecnol. Aliment., Campinas, 2012; 32(1):167-172.
12. Farhang V, Amini J, Javadi T, Nazemi1 J, Ebadollahi A. Chemical Composition and Antifungal Activity of Essential Oil of *Cymbopogon citratus* (DC.) Stapf. Against Three Phytophthora Species. Greener Journal of Biological Science. 2013; 3(8):292-298.
13. Worrall IG. The Butana Grass Patterns. Journal of Soil Science. 1959; 10(1):34-53
14. EL-Kamali HH. Assessing the Ecological Status of Prominent Medicinal Herbs in Central Sudan and the challenge of Sustainable Management. Bioresearch Bulletin, 2010; 1:10-15.
15. Heiba HI, Rizk AM. Constituents of Cymbopogon Species. Qatar Univ Sci Bull, 1986; 6:53075.
16. EL-Kamali HH. PhD thesis. University of Khartoum; Sudan, 2001.
17. Abushama MF, Hassan A, Abdelgadir H, Khalid H. The Chemical composition and antifungal activity of essential oil of *Cymbopogon nervatus* HOCHST. CHOIV. International Journal of Research in Pharmacy and Chemistry. 2013; 3(4):730-738.
18. Selim SA. Chemical composition, antioxidant and antimicrobial activity of the essential oil and methanol extract of the Egyptian lemongrass *Cymbopogon proximus* Stapf. grasasy aceites, 2011; 62(1):55-61.
19. British standards (BS 2073). Methods of tests for essential oils, London, 1976.
20. Modwi BM, Magar HR, Satti AM. Chemistry of Sudanese Flora: *Cymbopogon nervatus.* Journal of natural product, 1984; 47(1):167-169.
21. Pitarevic I, Kustrak D, Blazevic N. Influence of ecological factors on the content and composition of the essential oil: In proceeding of the 15th International Symposium on the Essential Oils, Boston, 1985, 19-21.

22. Marotti M, Piccaglia R, Giovanelli E. Effects of variety and ontogenic stage on the essential oil composition and biological activity of fennel (*Foeniculum vulgare* Mill.). Journal of Essential Oil Research. 1994; 6:57-62.
23. Telic I, Sahbaz N. Variation of Yield, Essential Oil and Carvone contents in Clones Selected from Carvone Scented Landraces of Turkish Mentha pecies. Journal of Agronomy. 2005; 4(2):96-102.
24. Orav A, Raal A, Arak E, M臬risepp M, Kailas T. Composition of the essential oil of Artemisia absinthium L. of different geographical origin, Proc. Estonian Acad. Sci. Chem, 2006; 55(3):155-65.
25. Bhattih HN, Iqbal, Shaid SA, Bukharii H. Variations in Oil Potential and Chemical Composition of Eucalyptus crebra Among Different Districts of Punjab, Pakistan. International Journal of Agriculture & Biology. 2007; 1:136-138.
26. Hussain AI, Anwar F, Sherazi ST, Przybylski R. Chemical composition. Antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chemistry, 2008; 108:986-995.
27. Anwar F, Hussain AI, Sherazi ST, Bhanger MI. Changes in composition and antioxidant and antimicrobial activities of essential oil of fennel *(Foeniculum vulgare* Mill.) fruit at different stages of maturity. Journal of Herbs, Spices and Medicinals Plants. 2009; 15:1-16.
28. Elhassan IA, Ayoub SM. The Effect of Geographical Location on Essential Oil Content and Composition of Xylopia aethiopica, American Journal of Research Communication. 2014; 2(1):251-261.