Spectroscopic studies on the essential oil of *Eucalyptus citriodora* L. from Kaduna, Nigeria

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Abstract: The simultaneous use of GC-MS and NMR provide valuable and complementary information on the molecular weight and chemical structure of a compound respectively. The aim of this study was to carry out a gas chromatography-mass spectrometry (GC-MS) and NMR spectroscopic studies on the essential oil obtained from the dried leaves of *Eucarlyptus citriodora*, collected from Kaduna, Nigeria. The dried leaves of *Eucarlyptus citriodora* were collected from several randomly selected trees in a farm site in Kaduna metropolis, Kaduna state and then processed into an extract. Hydrodistillation extraction (HDE) and GC-MS (HDE-GC-MS) were used to extract and analyse the volatile compounds, VOCs (essential oil) respectively from *Eucarlyptus citriodora* leaf and the percentage of each components were reported as raw area percentage based on the total ion current. The essential oil was obtained by hydrodistillation with a yield of 3.5% (v/w). Twenty-nine (29) different components accounting for 100% of the total volatile oils were identified. Citronellal (46.87%) was the predominant monoterpene constituent, followed by isopulegol (7.68%), citronellol (7.47%), 3-tetradecanol (4.98%) and citronellic acid (4.31%). 2D NMR analysis of the essential oil also identified citronellal as the major constituent. The other compounds present were citronellyl acetate (2.67%), *p*-methane-1,8-diol (1.78%), cyclohexylacetone (1.71), 5-caranol (1.7%), caryophyllene (1.41%), caryophyllene oxide (1.34%), β-pinene (1.25%), eucalyptol (0.96%), myrcenol (0.73%), menthone (0.59%), citronellol epoxide (0.54%), linalool (0.5%), citronellyl formate (0.47%), α-pinene (0.4%), methyleugenol (0.38%), geraniol (0.35 %), and geranyl acetate (0.35%) were identified as in minor quantities. This result is consistent with previous studies on the chemical composition of the leaf essential oils of *E. citriodora* samples from other parts of the world.

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1. Introduction

Essential oils are volatile constituents that give plants their characteristics odour (Mosad *et al.*, 2016). Essential oils are extracted from aromatic plants and are used as flavourings, fragrances and insecticides (Farkhanda *et al.*, 2013). Essential oils find application in pharmaceutical, food and perfumery industries (Noureddine *et al.*, 2013). In fact, they have a broad spectrum of biological and pharmacological activities- antioxidant, anticancer, antimicrobial and insecticidal properties (Mosad *et al.*, 2016). *Eucalyptus* genus is native to Australia, popularly referred to as gum tree, is rich in a large variety of volatile monoterpene oils which includes; citronellol, citronellal, cineole, limonene, linalool, among others (Mosad *et al.*, 2016). *Eucalyptus citriodora* Hook (syn. *Corymbia citriodora* Hook.), belongs to the family Myrtaceae, also known as “Lemon Scented Eucalyptus” or “Lemon-Scented Gum”, native of Queensland, Australia, highly cherished for the rich essential oil obtained from its leaves (Manika *et al.*, 2011). It is a tall, evergreen tree cultivated for the production of essential oil, wood fuel and timbers. The leaves are highly aromatic containing a large number of volatile terpenes which are released into the environment (Ali *et al.*, 2017).

Essential oil of the leaves possessed a strong antiseptic property, used frequently all over the world as a nasal decongestant, for symptomatic relief of cold, flu, cough, bronchitis, pneumonia. It also has a disinfectant effect, applied externally to treat wound, cut and skin infections. (Shahnaz and Mohammed, 2013). The flora of Nigeria comprises of so many species of eucalyptus ranging from *E. camaldulensis* (Sudan zone), *E. cloeziana, E. grandis, E. propinqua, E. saligna, E. tereticornis* grown in Sudan, northern and southern Guinea zones and the montane areas of the savannah zone in Nigeria (Adegbehin, 2012). –Essential oils are composed of molecules which are volatile in nature which could easily change to the gaseous phase, hence the use of gas chromatography (GC) for its separation. Gas chromatography has been designed for the analysis of volatiles compounds such as essential oil constituents (Sanpeng *et al.,* 2018).

Nuclear magnetic resonance (NMR) spectroscopy is an analytical technique for structure elucidation and identification of molecules. NMR spectroscopy can detect and measure components in plant extracts and other complex mixtures including essential oils without any need for additional purification and separation processes. The simultaneous use of GC-MS and NMR provide valuable complementary information on the molecular weight and chemical structure of a compound respectively (Wilczewska *et al*., 2013).

The aim of this research work was to carry out a gas chromatography-mass spectrometry (GC-MS) and NMR spectroscopic studies on the essential oil obtained from the dried leaves of *Eucarlyptus citriodora,* collected from Kaduna, Nigeria.

2. Materials and Methods

Plant Materials

The plant was collected in June 2017 from Kaduna, Nigeria. Identification and authentication of the plant material were done by a taxonomist at the Herbarium of the National Institute of Pharmaceutical Research and Development, Abuja, Nigeria, where the voucher specimen (NIPRD/H/7106) was deposited.

Essential oil isolation

The dried leaves (500g) were chopped into small pieces and hydrodistilled using a Clevenger-type apparatus. The extraction was carried out for 4hours. The colourless oil obtained with a yield of 3.5% (v/w) was dried over anhydrous sodium sulphate. The oil was filtered through 0.22 microns filter and kept at 4^{o}C in sealed vials in dark until analysis (Okhale *et al*., 2018).

Gas Chromatography-Mass Spectral analysis

The oil was analysed by GC-MS using Shimadzu QP-2010 GC with QP-2010 mass selective detector [MSD, operated in the EI mode (electron energy = 70eV), scan range = 45-400amu, and scan rate = 3.99 scans/sec], and Shimadzu GC-MS solution data system. The GC column was HP-5MS fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, length 30 m, internal diameter 0.25mm and film thickness 0.25µm. The carrier gas was helium with flow rate of 1.61 ml/min. The program used for GC oven temperature was isothermal at 60^{o}C, followed by 60-180^{o}C at a rate of 10^{o}C/min, then held at 180^{o}C for 2 minutes; followed by 180-280^{o}C at a rate of 15^{o}C/min, then again held at 280^{o}C for 4 minutes. The injection port temperature was 250^{o}C. The ionization of sample components was performed in the E.I. mode (70eV). Injector temperature was 250^{o}C while detector temperature was 280^{o}C. Helium was used as carrier gas at a flow rate of 1.61 ml/min. 1.0 µl of diluted sample (1/100 in hexane, v/v) was injected using autosampler and in the split mode. Split ratio was 10:90 (Okhale *et al*., 2018).

Identification of the compounds

The individual constituents of the essential oil were identified by comparing their mass spectra with known compounds in National Institute of Standards and Technology mass spectral library (NIST), and Flavour and Fragrance Natural and Synthetic Compounds mass spectral library database. The structure of citronellal being the main component of the oil was confirmed by 2D NMR analysis. Quantitatively, the area percentage of each component from the GC-MS analysis was reported as raw percentage based on the total ion current without standardization. Results are shown in Table 1.

NMR Spectroscopic analysis

The ^{1}H and ^{13}C NMR and 2D NMR spectra were obtained by a JEOL-LA 400 MHz NMR spectrometer system using deuterated

$CDCl_3$ ($CDCl_3$-d) as solvent (Ruwida *et al.*, 2017),

3. Results and analysis

Gas Chromatography-Mass Spectral analysis

The gas chromatography-mass spectral analysis of *E. citriodora* leaf essential oil, shown in Table 1, revealed twenty-nine different constituents accounting for 100% of the total volatile oils. Citronellal (46.87%) was the predominant monoterpene constituent, followed by isopulegol (7.68%), citronellol (7.47%), 3-tetradecanol (4.98%) and citronellic acid (4.31%). As shown in Figure 1, the total ion chromatogram showed peak 8 which is citronellal, as the most prominent constituent of *E. citriodora* leaf essential oil. The constituents were identified by comparing their mass spectra with known compounds in National Institute of Standards and Technology mass spectral library (NIST).

NMR Spectroscopic analysis

The nuclear magnetic resonance (NMR) analysis assignment shown in Table 2 indicated the characteristic citronellal proton NMR spectra comprising of one aldehydic proton 9.64 (1H, s, H-1), three methyl protons: 0.87 (3H, d, H-10), 1.50 (3H, s, H-8) and 1.58 (3H, s, H-9); three methylene protons: 1.22 (2H, m, H-4), 1.92 (2H, m, H-6) and 2.30 (2H, dd, H-2); and two methine protons: 1.96 (1H, d, H-3), 4.99 (1H, t, H-6). The ^{13}CNMR absorptions for citronellal (δ, $CDCl_3$) consisted of 203.3 (C-1), 51.2 (C-2), 27.7 (C-3), 37.0 (C-4), 25.5 (C-5), 124.1 (C-6), 131.7 (C-7), 17.6 (C-8), 25.6 (C-9) and 19.95 (C-10).

Table 1: Result of the GC-MS analysis of the essential oil of *E. citriodora* leaves

Peak No.	Name	Retention Time	% Composition
1	2-Methylpropyl-2-methylpropionate	3.195	0.45
2	α-Pinene	3.497	0.40
3	β-Pinene	4.033	1.25
4	Myrcenol	4.689	0.73
5	Eucalyptol	4.739	0.96
6	2,6-Dimethyl-5-heptenal	4.997	2.85
7	Linalool	5.646	0.50
8	Citronellal	6.563	46.87
9	Isopulegol	6.648	7.68
10	Menthone	6.724	0.59
11	5-Caranol	6.777	1.70
12	Dihydrocarveole	6.892	0.60
13	Cyclohexylacetone	7.330	1.71
14	Citronellol	7.530	7.47
15	7-Methyl-1,6-octadiene	7.822	1.02
16	Geraniol	7.898	0.35
17	3-Tetradecanol	7.991	4.98
18	Citronellyl formate	8.190	0.47
19	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethynona-2,6-dien-1-ol	8.725	0.91
20	Bicyclo[3.3.1]nonan-9-ol,9-methyl-	8.767	1.91
21	Citronellic acid	8.883	4.31
22	Citronellol epoxide	9.017	0.54
23	p-Methane-3,8-diol	9.113	3.83
24	Citronellyl acetae	9.283	2.67
25	p-Methane-1,8-diol	9.422	1.78
26	Geranyl acetate	9.699	0.35
27	Methyleugenol	10.006	0.38
28	Caryophyllene	10.379	1.41
29	Caryophyllene oxide	12.573	1.34

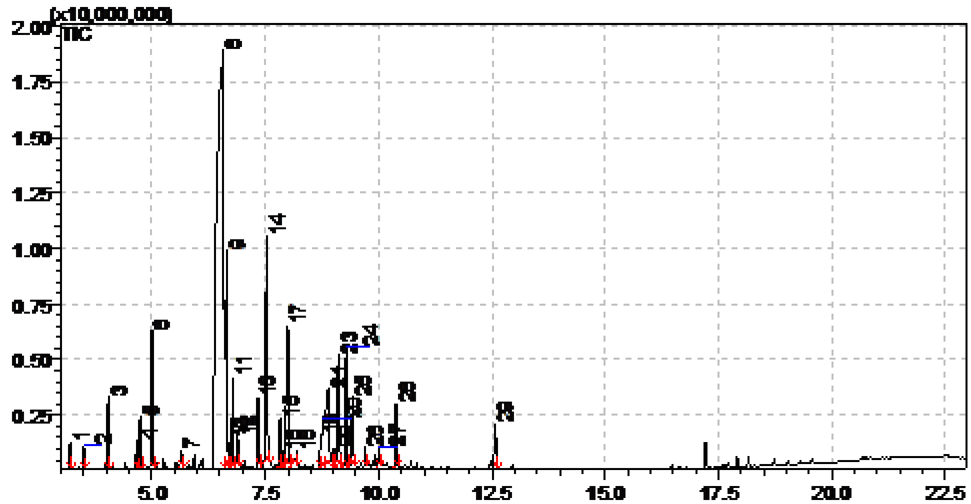

Figure 1: GC–MS chromatogram of *E. citriodora* essential oil analyzed on GC–MS (Shimadzu, Japan) using a capillary column (HP 5MS) coupled to mass selective detector. The chromatogram showed peaks representing chemical components found in the *E. citriodora*. Peak 8 is citronellal, the most dominant peak of the *E. citriodora* essential oil.

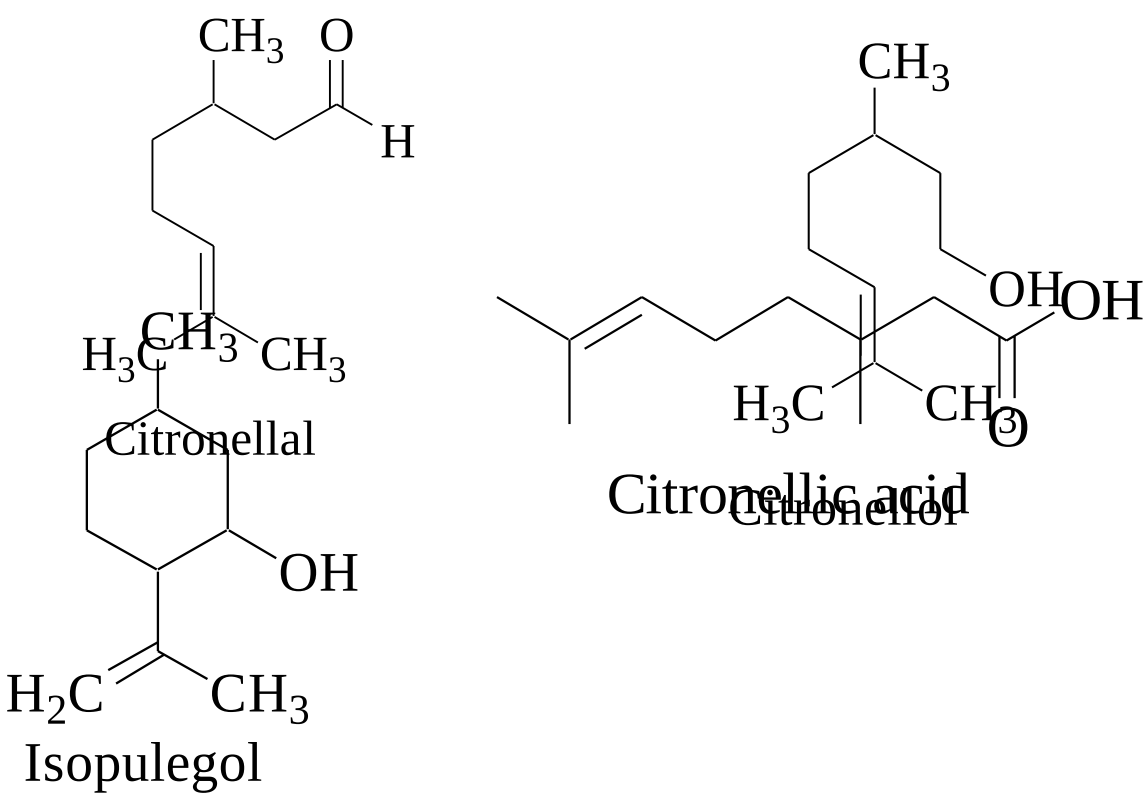

Figure 2: Structure of some major chemical constituents of *E. citriodora* leaf essential oil

Table 2: ^{13}C and ^{1}H NMR spectral assignment of citronellal ($C_{10}H_{18}O$)

Position	δ_C, type	δ_H (J in Hz)
1	203.3, CHO	9.64, s
2	51.2, CH_2	2.30, dd
3	27.7, CH	1.96
4	37.0, CH_2	1.22, m
5	25.5, CH_2	1.92, m
6	124.1, CH	4.99, t
7	131.7, C	
8	17.6, CH_3	1.50, s
9	25.6, CH_3	1.58, s
10	19.95, CH_3	0.87, d

4. Discussion

The GC-MS analysis of the essential oil of *Eucalyptus citriodora* revealed 29 different volatile components which are listed in Table 1, together with their retention times and percentage compositions. Citronellal (46.87%), isopulegol (7.68%), citronellol (7.47%), 3-tetradecanol (4.98%), and citronellic acid (4.31%) were the predominant monoterpene components of *E. citriodora* as observed from the result.The other minor constituents identified in the essential oilwere citronellyl acetate (2.67%), *p*-methane-1,8-diol (1.78%), cyclohexylacetone (1.71), 5-caranol (1.7%), caryophyllene (1.41%), caryophyllene oxide (1.34%), β-pinene (1.25%), eucalyptol (0.96%), myrcenol (0.73%), menthone (0.59%), citronellol epoxide (0.54%), linalool (0.5%), citronellyl formate (0.47%), α-pinene (0.4%), methyleugenol (0.38%), geraniol (0.35 %) and geranyl acetate (0.35%).

The mass spectrum of citronellal indicated the molecular composition $C_{10}H_{18}O$, as evidenced by a molecular ion peak at m/z 154. The mass spectrum showed significant ions at m/z 139 ($C_{10}H_{18}O$, M^+–CH_3) (15), 121 (30), 111 (25), 95 (50), 83 (18), 69 (95), 55 (45), 41 (100), 27 (20) (Figure 2).

In order to confirm the structure of the main component and to corroborate the GC-MS result, the essential oil was studied by 2D-NMR as shown in Figure 2. The ^{1}H NMR of the essential oil revealed the predominant constituents to becitronellal (δ, $CDCl_3$) contained characteristic signal for one aldehydic proton 9.64(1H, s, H-1), three methyl protons: 0.87 (3H, d, H-10), 1.50 (3H, s, H-8) and 1.58 (3H, s, H-9); three methylene protons: 1.22 (2H, m, H-4), 1.92 (2H, m, H-6) and 2.30 (2H, dd, H-2);and two methine protons: 1.96 (1H, d, H-3), 4.99 (1H, t, H-6).

The ^{13}C NMR absorptions for citronellal (δ, $CDCl_3$) consisted of 203.3 (C-1), 51.2 (C-2), 27.7 (C-3), 37.0 (C-4), 25.5 (C-5), 124.1 (C-6), 131.7 (C-7), 17.6 (C-8), 25.6 (C-9) and 19.95 (C-10). The dominant chemical composition of *E. citriodora* leaf essential oil obtained in this study being citronellal (46.87%), isopulegol (7.68%), citronellol (7.47%), 3-tetradecanol (4.98%) and citronellic acid (4.31%) is consistent with previous studies on samples from other parts of the world. *E. citriodora* leaf from Algeria yielded essential oil with dominant constituents being citronellal (64.7%) and citronellol (10.9%) (Benchaa *et al*., 2018). *E. citriodora* leaf harvested from elsewhere gave essential oil characterized by citronellal (29.31%), geraniol (27.63%), and β-citronellol (14.88%) (Costa *et al*., 2015). *E. citriodora* leaf from Thailand gave essential oil with major constituents as citronellal (60.55 ± 0.07%), isopulegol (10.57 ± 0.02%) and citronellol (9.04 ± 0.03%) (Insuan and Chahomchuen, 2020).

The leaf essential oil chemical composition of *E. citriodora* from Mali revealed the presence of citronellal (78%), and isopulegol (6%) as the dominant constituents (Chalchat *et al*., 2000). Similarly, Singh *et al*., 2012 reported the chemical composition of *E. citriodora* comprising of citronellal (60.66%), β-citronellol (12.58%), isopulegol (8.19%) as the major constituents with antioxidant activity**.**

Conclusion

The GC-MS and NMR study of the essential oil of *E. citriodora* showed that it consisted mainly of monoterpenes and the predominant constituent was citronellal (46.87%), and others including isopulegol (7.68%), citronellol (7.47%), 3-tetradecanol (4.98%), and citronellic acid (4.31%). This result is consistent with previous studies on the chemical composition of the leaf essential oils of *E. citriodora* samples from other parts of the world. The essential oil components are the characteristic odor active compounds in *Eucarlyptus citriodora* leaf.

Conflict of Interest

The authors declare no conflict of interest.

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