



CHARACTERIZATION AND PHYSICO-CHEMICAL ANALYSIS OF ESSENTIAL OIL OF *Cymbopogon citratus* LEAVES

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ABSTRACT

The genus Cymbopogon is important from the point of view of their essential oils. Essential oils from these species are widely used in flavours, fragrances, cosmetics, soaps, detergents and perfumery owing to their typical lemon-like aroma. Essential oil of Cymbopogon citratus was extracted by hydrodistillation and characterized using Gas chromatography/mass spectrometry (GC-MS) and Fourier Transform Infrared (FTIR). The oil was also subjected to physico-chemical analysis, the physical and chemical properties evaluated were Boiling point (74°C), Specific gravity (0.8960), Refractive index (1.4838) and pH (6.00) at 25°C. Saponification value (109.76) and Acid value (0.55) mg KOH/g oil. Iodine value (100g of I₂/g oil) 105, Ester value (189.21), Residue on evaporation (10%) at 100°C. The chemical composition of the essential oil analyzed by GC-MS showed citral (31.1%), β-Myrcene (14.2%), citronellal (9.8%), limonene oxide (7.7%), geraniol (7.3%) and linalool (6.2%) as the major compounds. The FTIR spectra revealed the presence of n-alkane, conjugated alkene, primary amide, amine, aldehyde, primary and secondary alcohols. The results obtained from the physico-chemical parameters, and functional groups identified by FTIR as well as the compounds identified by GC-MS revealed that the oil has medicinal and nutritive values as well as industrial applications in the pharmaceutical, perfumery and cosmetic industries. Key words: Characterization, Citral, Cymbopogon citratus, Essential oil, FTIR, GC-MS.

INTRODUCTION

Cymbopogon citratus is a perennial grass plant belonging to the family *Gramineae*, known as lemongrass, comprising approximately 500 genus and 8000 herb species (Barbosa *et al.*, 2008). The prefix 'lemon' owes to its typical lemon like odour, which is mainly due to the presence of citral, a cyclic monoterpene - a major constituent of essential oils of *Cymbopogon* species as well as citrus fruits, (Karkala and Bhushan, 2014). Lemon grass is a tufted perennial grass growing to a height of 1 meter with numerous stiff leafy stems arising from short rhizomatous roots (Carianne de Boer, 2005). The leaf-blade is linear, tapered at both ends and can grow to a length of 100 cm and width of 2 cm. Lemon grass is a perennial grass plant widely distributed worldwide and most especially in tropical and subtropical countries (Francisco *et al.*, 2011). Several reports have linked its origin to Asia, Africa and the Americas. When squeezed, the leaves usually produce yellow or amber coloured, aromatic, essential oil (Adejuwon and Esther, 2007). It is also grown as an ornamental plant and the essential oil of the plant is used in aromatherapy.

Lemongrass is a folk remedy for coughs, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmic, pneumonia and vascular disorders. Essential oils from the aerial parts of different species of *Cymbopogon* have folkloric medicinal use in fever, digestive disorders, diabetes, inflammation and nerve disorders (Joeng *et al.*, 2009). Mixed with pepper, it is a home therapy for menstrual troubles and nausea (Belew *et al.*, 2011). Traditionally, tea made from lemongrass leaves is popular among countries of South America, Asia and West Africa having been widely utilized as antiseptic, antifever, antidyspeptic, carminative and

anti-inflammatory effects. Others are febrifuge, analgesic, spasmolytic, antipyretic, diuretic, tranquilizer and stomachic agent (Negrelle and Gomes, 2007; Adejuwon and Esther, 2007; Tatiana and José 2011).

The study is aimed at determining the physico-chemical properties as well as the functional groups present in the essential oil of lemon grass obtained in Kaduna in order to ascertain its purity and suitability for nutritive, pharmaceutical and various industrial applications reviewed in the literature, and also deduce the components already established by GC-MS in the literature. All these will then confirm the medicinal values claimed by the herbal medicine practitioners in folk medicine.

MATERIALS AND METHODS

Plant material and sample preparations

Lemongrass was obtained from Federal College of Forestry, Afaka, Kaduna. After harvesting, the leaves were separated from stalks and then air-dried at room temperature for five days. After drying, the leaves were cut into small pieces at the length of 4 to 8 mm.

Extraction of essential oil

Dried leaves (500g) of *Cymbopogon citratus* were subjected to hydrodistillation for 3 hours in a Clevenger-type apparatus. Distillates of essential oil was dried over anhydrous sodium sulphate, filtered and stored at 4°C until analyzed (Yehouenou *et al.*, 2012).

Determination of Physico-chemical Properties of the oil

Boiling point, Refractive index, Specific gravity, pH, Iodine value, Saponification value, Acid value, Residue on evaporation, Ester value were determined using standard analytical techniques British Pharmacopoeia, (B P, 1980) and Association of Official Analytical Chemists (AOAC, 2005).

Iodine value and saponification number were determined according to (AOAC, 2005). Official Methods 920.158 (Hanus method) and 920.160, respectively. Specific gravity was determined at 25 °C using a 25 ml capacity pycnometer. Refractive index was measured with an Abbe refractometer (Bellin-ghan, and Stanley Ltd, UK).

Determination of Functional Groups

The Infrared spectroscopy was carried out Shimadzu FTIR 8400s.

Gas Chromatography

GC analysis were performed on an orion micromat 412 double focusing gas chromatography system fitted with two capillary columns coated with CP-Sil 5 and CP-Sil 19 (fused silica, 25m × 0.25mm, 0.15µm film thickness) and flame ionization detector (FID). The volume injected was 0.2µL and the split ratio was 1:30. Oven temperature was programmed from 50°C-230°C respectively. Qualitative data were obtained by electronic integration of FID area percents without the use of correction factors.

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 temperature 230°C. The GC was fitted with a 25m×0.25mm, fused silica capillary column coated with CP-Sil 5. The film thickness was 0.15 µm, 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 compositions of the oil were computed in each case from GC peak areas. The identification of the components was based on the retention indices (determined relative to the retention times of series of n-alkanes) and mass spectra with those of authentic samples and with data from Literature (Jennings and Shibamoto, 1980; Adams, 1995; Joulang and Konig, 1998).

Kovats Retention Index System

In the 1950s, the Kovats retention index system was introduced in gas chromatography (Kovats, 1958). The Kovats retention index KI, is defined and calculated by the following equation:

$$KI = 100N + 100n \frac{\text{Log } t^1_{R(A)} - \text{Log } t^1_{R(N)}}{\text{Log } t^1_{R(N+n)} - \text{Log } t^1_{R(N)}}$$

Where $t^1_{R(N)}$ and $t^1_{R(N+n)}$ are the adjusted retention times of n – paraffin hydrocarbon of carbon number N and (N + n) respectively, eluting before and after solute A with the adjusted retention time $t^1_{R(A)}$. Tables of retention indices can help identify constituents of the essential oil by comparing experimentally found retention indices with known values (Nic *et al.*, 2006).

RESULTS AND DISCUSSION

The extracted oil was pale yellow in colour with a yield of 1.03%. The physico-chemical parameters determined are listed in Table 1. The FTIR spectrum is shown in Fig 1, the functional groups associated with the absorption bands are listed in Table 2. Chemical composition of the oil determined using GC/MS are presented in Table 3.

Table 1:Physico-chemical Properties of Essential Oil of *Cymbopogon citratus* leaves

Parameter	Value
Acid value(mg KOH/g oil)	0.55
Ester value	189.21
Iodine value(100g of I ₂ / g oil)	105
pH at 25°C	6.0
Refractive index at 25°C	1.4838
Residue on evaporation at 100°C	10%
Saponification value (mg KOH/g oil)	189.76
Specific gravity at 25°C	0.8960

The acid value is the weight of potassium hydroxide in mg required to neutralize the organic acids present in 1 g of the substance. The acid value may be overestimated if other acid components are present in the system, *e.g.* amino acids or acid phosphates. The acid value is often a good measure of hydrolytic rancidity which has an adverse effect on the quality of many lipids. In general, it gives an indication about edibility and pharmaceutical applications of the lipid. The result obtained in Table 1 indicated acid value 0.55 which is <1. Codex standard (2001) gave a value ≤0.60. Therefore, the result obtained is within the range. A pH of 6.0 recorded implies that the oil is not strongly acidic. These imply that the oil is suitable for pharmaceutical uses.

The saponification value is the number of mg of potassium hydroxide required to neutralize the free acids and to saponify the esters in 1 g of the substance. The saponification number is a measure of

the average molecular weight of the triacylglycerols in a sample. The smaller the saponification number the larger the average molecular weight of the triacylglycerols present *i.e.* Saponification value is inversely proportional to the mean molecular weight of fatty acids (or chain length). Codex standard,(2001) gave a range of 186-195, and the result of 189.76 obtained is within the range.

Refractive index is used mainly to measure the change in unsaturation as the oil is hydrogenated. The refractive index of oils depends on their molecular weight, fatty acids chain length, degree of unsaturation and degree of conjugation. It is a measure of how fast light travels through a substance and it is used to identify, confirm purity and measure concentration of the substance. The value obtained is within the range of the literature (1.469-1.479)(Codex standard,2001).

The ester value is defined as the mg of KOH required to react with glycerin or glycerol after saponifying one gram of fat. It is calculated from the saponification value and the acid value: Ester value = saponification value – acid value.

The iodine value gives a measure of the average *degree of unsaturation* of a lipid: the higher the iodine value, the greater the number of C=C double bonds. By definition the iodine value is expressed as the grams of iodine absorbed per 100g of lipid. Iodine value is directly proportional to the degree of unsaturation (number of double bonds) and inversely proportional to the melting point of lipid. This value could be used to quantify the amount of double bonds present in the oil, which signifies the susceptibility of oil to oxidation. The value obtained was also within the range of established standard (104-120) (Codex standard, 2001).

Specific gravity is a ratio of densities which varies with temperature and pressure, therefore, reference and sample must be compared at the same temperature and pressure. Specific gravity is a parameter used to identify, measure concentration and confirm purity of substances. The value obtained 0.8960 is less than 1, indicating that the oil is less dense than water. This is further established by the boiling point of 74°C indicating that the oil is volatile and therefore composed of light molecular weight components. Evaporation residue, in which the percentage of the

oil that is not released at 100°C was determined. The result revealed 10% residue on evaporation implying that the oil is highly volatile as 90% of it was evaporated.

The FTIR spectra revealed the presence of alkene, primary amide, amine, aldehyde, alcohols, ketone, ester, carboxylic acid and ether. These functional groups identified are in agreement with compounds reported in the literature as well as the ones identified in the GC-MS analysis. For instance, citronellene, caryophyllene, α -pinene, β -myrcene and trans- α -bergamotene are alkenes. Geranial and citral are aldehydes, linalool, geraniol and terpeniol are alcohols, 6-Methyl-5-Hepten-2-one is a ketone, hexadecanoic acid and 9-octadecanoic acids are carboxylic acids.

Compounds such as hydrocarbon terpenes, alcohols, ketones, esters and mainly aldehydes have constantly been registered in the literature. Lemon grass contains active ingredients like myrcene, which is an alkene, citronellal, citral are aldehydes, citronellol and geraniol are alcohols. The biological activity of the essential oil obtained from *C. citratus* has been reported to be due to the presence of citronellal and citral (Joeng *et al.*, 2009). Geranial, neral, geraniol, limonene, β -myrcene, citral have been found as major compounds in many other *Cymbopogon citratus* (Huyhn *et al.*, 2008).

Table 2: Funtional Groups Identified in Essential Oil of *Cymbopogon citrates* leaves

Absorption wave number(cm^{-1})	Functional groups	Remark/inference
3486- 3445	N-H	Primary amide, amine
3026-2800	C-H	Alkene, aromatic ring
3000-2500	O-H	Carboxylic acid
1750-1650	C=O	Aldehyde, ketone, ester, carboxylic acid
1360-1180	C-N	Amine
1300-1000	C-O	Alcohols, ether, ester, Carboxylic acid
1000-675	C-H	Alkene
870-675	C-H	Aromatic ring

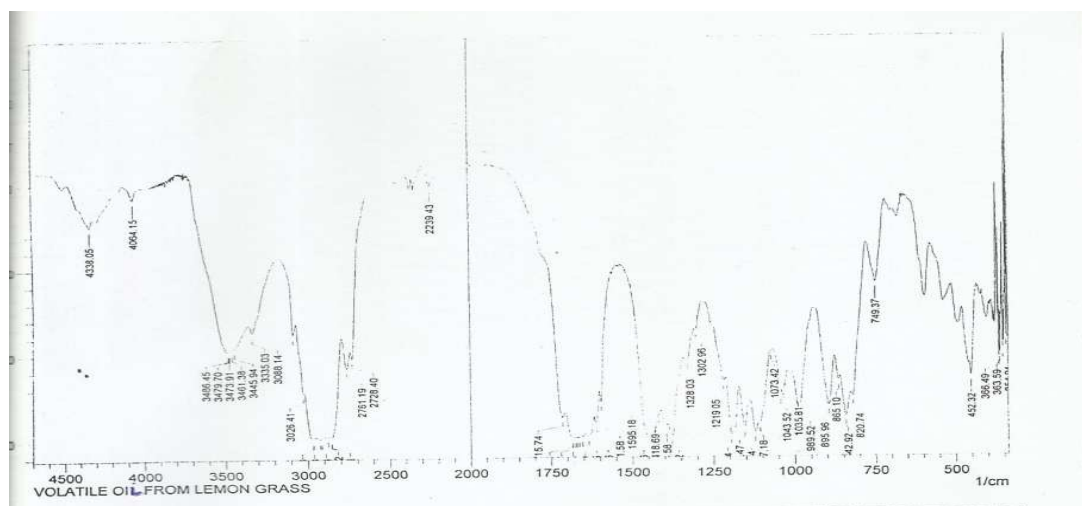


Fig 1: FTIR Spectra of *Cymbopogon citratus* leaf essential oil

It has been documented in various literatures that essential oils of plant origin exhibit a wide spectrum of biological activities (Sacchetti *et al.*, 2005). Biological activity of essential oils depends on their chemical composition (Chun *et al.*, 2005), which can vary dramatically, even within the same species (Zapataa and Smagghea, 2010). Sources of compositional variability can include geographical origin of the plant, the plant part extracted, phenological state of the plant, and time of year, as well as growth environmental conditions (Angioni *et al.*, 2006; Isman *et al.*, 2007). Biological activity of essential oils is also affected by interactions among their structural

components. Even minor compounds can have a critical function due to additive action between chemical classes and synergism or antagonism (Sampson *et al.*, 2005; Angioni *et al.*, 2006; Bakkali *et al.*, 2008).

The composition of *Cymbopogon citratus* essential oil is much more homogeneous, always with geranial and neral as the main constituents whatever the origin of the plant. The samples characterized in Burkina Faso (Menut *et al.*, 2000), Brazil and Portugal (Franz *et al.*, 2011), and Benin (Bossou *et al.*, 2013) confirm this observation

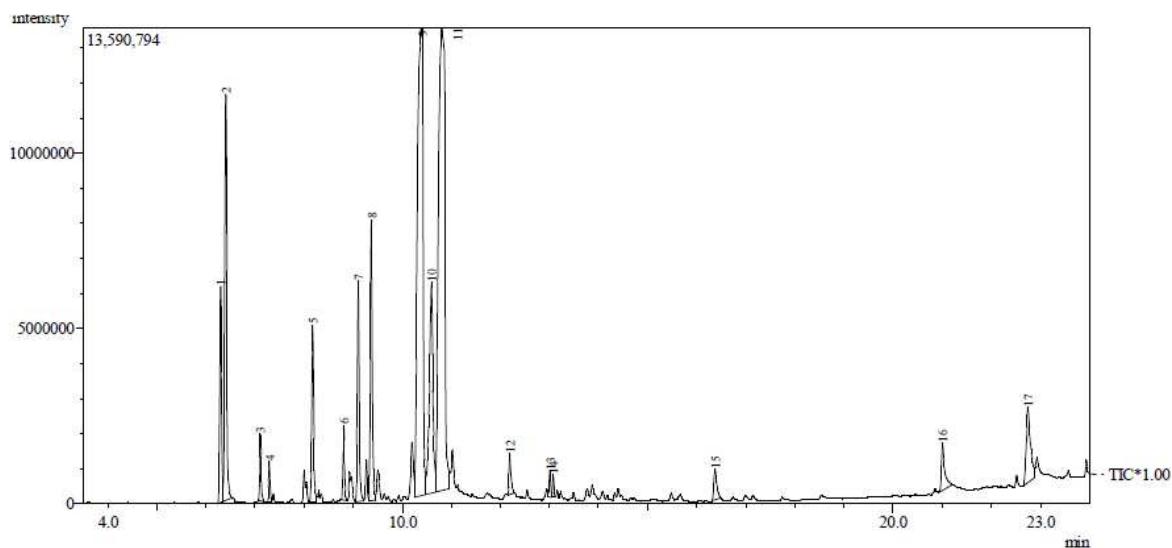


Fig 2 : Chromatogram of *Cymbopogon citratus* leaf essential oil

The chemical composition of the leaf essential oil of *Cymbopogon citratus* as listed in Table 3 revealed that geranial(18.8%), neral(16.3%), β -myrcene(14.2%), citronellal(9.8%), limonene oxide(7.7%),geraniol(7.3%) and linalool(7.3%) were the major compounds . A total of 16 compounds were identified representing 99% of the total oil. The oil was dominated by monoterpenes accounting for 88.9% of the total oil out of which monoterpene hydrocarbon was 20.3% whereas oxygenated monoterpene was 68.6%.Sesquiterpene hydrocarbon accounted for an insignificant amount of 1.7%, oxygenated sesquiterpene was absent whereas other non terpene components was 8.4%. This result is in agreement with previous report by Koba *et al.*(2009) where lemon grass leaf essential oil consisted of two monoterpene hydrocarbon (10.6%) and nine oxygenated monoterpenes (86.4%). Sessou *et al.*(2012) also reported 9.9% for hydrogenated monoterpenes, oxygenated monoterpenes 85.3%, hydrogenated sesquiterpens 2.2% and oxygenated sesquiterpens 0.1% representing a total of 97.5% of the oil. This is an indication that essential oils of *Cymbopogon* species mainly consist of the monoterpene fractions.

The chemical composition of essential oil of *Cymbopogon citratus* varies according to

geographical origin, as a function of genetic diversity, habitat and agronomic treatment of the culture(Ganjewela *et al.*,2008; Khanuja *et al.*,2005). The compounds such as hydrocarbon terpenes, alcohols, ketones, esters and mainly aldehydes have constantly been registered. Lemon grass contains active ingredients like myrcene, an antibacterial and pain reliever, citronellal, citronellol and geraniol.

Citral - an isomeric mixture of geranial and neral are major components in the essential oils of several species of *Cymbopogon* (Shah *et al.*, 2011; Huynh *et al.*, 2008). Both citral and geraniol have immense commercial significance due to their characteristic lemon and rose-like smell in the flavour, fragrance, cosmetics, perfumery and pharmaceutical industries(Ganjewela *et al.*,2008; Dubey *et al.*,2003). Citral is used in the manufacture of perfumes, colored soaps, synthesis of vitamin A and β -ionones and other specialty chemicals (Deepak *et al.*,2012; Mirghani *et al.*, 2012). The biological activity of the essential oil obtained from *C. citratus* is due to the presence of citronellal and citral (Joeng *et al.*,2009), which plays important detoxification and anti-cancer roles in the body by inducing glutathione-S-transferase (GST) activity (Hedges and Lister,2006; Puatanachokchai *et al.*, 2002).

Citral possesses many significant bioactivities of great medicinal and pharmaceutical significance. It has antimicrobial activity against many microorganism strains such as *Aspergillus flavus*, *Penicillium expansum*, *A. ochraceus*, *P. verrucosum*, *Listeria monocytogenes*, *S. aureus*, *E. coli*, *S. typhimurium* (Burt, 2004; Nguefack *et al.*, 2004, 2009, 2012). A number of reports published have substantiated citral for most of the bioactivities of lemongrass essential oils. Some of the activities of citral published recently are antimicrobial, antiparasitic, antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic and sedative (Ganjewala, 2009).

Furthermore, many studies have reported the antimicrobial activity of lemongrass oil originating from different parts of the globe against a diverse range of microorganisms comprising Gram positive and Gram negative bacteria, yeast and fungi namely *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* (Naik *et al.*, 2010), *Salmonella choleraesuis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (Falcão *et al.*, 2012) and *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Alternaria alternata*, *Penicillium citrinum*, *Curvularia lunata* and *Trichoderma harzianum* (Mahanta *et al.*, 2007). Also, (Shaaban, 2005) reported that the essential oil of *Cymbopogon citratus* can be used as a potential source of novel antimicrobial agent and can also be further exploited as an alternative food preservatives.

As can be seen in Table 3, a total of 16 compounds were identified in *Cymbopogon citratus* leaves representing 99.0 % of the total oil. The oil was rich in monoterpenoids (86.5%) dominated by oxygenated monoterpenes (58.8%) with hydrogenated monoterpenes representing 27.7% of the oil content. Hydrogenated sesquiterpenes accounted for only 1.7% and oxygenated sesquiterpenes were not detected at all. Furthermore three non-terpene compounds (one ketone and two carboxylic acids) representing 10.8% of the total oil were also identified.

Among the constituents occurring in higher proportions, geranial (18.8%), neral (16.3%), β -

myrcene (14.2%), citronellene (9.8%), limonene oxide (7.7%), geraniol (7.3%) and linalool (6.2%) were prominent, α -pinene(2.3%), cis- β -ocimene (1.4%), geranyl acetate (1.4%) and terpineol were detected in appreciable quantities .The minor constituents were trans- α -bergamotene(0.8%) and caryophyllene (0.9%). The abundance of citral (geranial+neral) accounting for 35.1% of the total oil signifies that the oil is of citral chemotype. This sample appeared similar to previous reports on oil chemotypes from other parts of the world.

Based on literature data, geranial, neral, geraniol, limonene and β -myrcene have been found as major compounds in many other *Cymbopogon citratus* species with citral as the main chemical component of lemongrass oil (Huynh *et al.*, 2008). In the data reported by Mirghani *et al.* (2012) for Malaysia specimen; they found a prominent composition in geranial (29.64%), neral (21.73%), geraniol (7.75%), limonene (5.92%) and β -myrcene (2.28%). This composition is also similar to the data provided by Sessou *et al.*,2012 where geranial(44.5%), neral(31.2%), myrcene(9.6%) and geraniol(7.9%) were the main components of lemon grass essential oil from Benin. Also, Koba *et al.* (2009) reported geranial (45.2%), neral (32.4%) and myrcene (10.2%) as the main components of essential oil of lemongrass specie from Togo. Matasyoh *et al.* (2011) found in lemongrass essential oil of Kenya, geranial (39.53%), neral (33.31%), and myrcene (11.41%) as major compounds. Geranial (40.82-44.44%), neral (33.56- 33.54%), and myrcene (13.60-14.78%) were the major compounds isolated from essential oil of lemongrass cultivated in Brazil as reported by Rocha *et al.* (2011).

Loumouamou *et al.* (2010) analyzed essential oil of lemongrass of Congo- Brazzaville and found geranial (48.88%) and neral (36.24%) in prominent concentration. Lemongrass essential oil from Malaysia studied by Tajidin *et al.* (2012) contained geranial (37.58%-45.95%) and neral (29.4%- 31.13 %) as main components. Similarly, Bharti *et al.*(2013) reported geranial (44.5%), neral (31.2%), myrcene (9.6%) and geraniol (7.9%) as main compounds.

TABLE 3: CHEMICAL COMPOSITION OF ESSENTIAL OIL OF CYMBOPOGON CITRATUS LEAVES.

S/N	COMPOUND	KI	PERCENTAGE COMPOSITION	MASS SPECTRA DATA
1	Citronellene	926	9.8	95 81 67 41
2	6-Methyl-5-Hepten-2-one	938	6.5	126 108 69 55
3	α -Pinene	948	2.3	121 105 93 77
4	β -Myrcene	958	14.2	121 93 79 69
5	Cis- β -Ocimene	976	1.4	121 105 93 79
6	Trans-Limonene oxide	1031	7.7	108 94 67 43
7	Linalool	1082	6.2	136 121 93 71
8	Terpineol	1137	1.1	136 121 81 43
9	E-Citral(Geranial)	1174	18.8	137 109 69 41
10	Z-Citral(Neral)	1174	16.3	134 109 84 69
11	Geraniol	1228	7.3	123 93 69 41
12	Geranyl acetate	1352	1.4	136 121 93 69
13	Trans- α -Bergamotene	1430	0.8	204 119 93 69
14	Caryophyllene	1494	0.9	204 161 93 69
15	Hexadecanoic acid	1968	1.6	256 129 73 60
16	9-Octadecanoic acid	2175	1.7	264 69 55 41
	Total (%)		99.0	
	Total number of compounds		16	

KI (Kovats Index) was calculated using a series of n-alkanes

The same data was reported by Bassole *et al.* (2011) who found geranial (48.1%), neral (34.6%) and myrcene (11.0%) as major constituents of *Cymbopogon citratus* essential oil from Burkina-Faso. Comparison of the chemical profile of the essential oil of Lemongrass (*Cymbopogon citratus*) from Nigeria with the reports of other scientist elsewhere, it can be established that there are significant quantitative differences between the chemical profiles of the major components of the oil. These variations may due to the diverse climatic and geographic differences and at the same time to different harvesting times and extraction procedures (Ashgari *et al.*, 2010; Ranitha *et al.*, 2014).

CONCLUSION

It is worthy of note that important pharmaceutical usage of lemon grass still remains under exploited especially in West Africa where these aromatic herbs are widely distributed. The study of the essential oil composition of various *Cymbopogon* species presented here led to the conclusion that the monoterpene compositions of the essential oils varied significantly among the species. Invariably, essential oil content and compositions are greatly influenced by climate, season and diurnal effects. All the physico-chemical properties determined in this study are important properties to determine the chemical state and quality of the extracted oil. The established constituents of the oil also suggested that the oil has medicinal and nutritive values as well as industrial application in the synthesis of ionones and vitamin A. Monoterpene constituents of the essential oil have been reported of useful biological activities.

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RECOMMENDATION

One of the major problems related to the use of synthetic drugs is the development of resistance by microorganisms and the adverse effects associated with their use. For these reasons, treatment using plant-based medicine appears to be an alternative approach. Essential oils classified as Generally Recognized As Safe (GRAS) and considered at low risk for developing resistance to pathogenic microorganisms could be a credible alternative. However, aromatic plants and their extracts should be standardized and properly controlled in their extraction and composition, in order for the study of these plants to yield meaningful data.

For the practical use of this oil, further research is needed on safety issues for human health and acceptability by consumers. Identifying the main components of the essential oil, testing their safety, and phytochemical study to uncover their medicinal value in disease treatment will provide an important means for the development of novel drugs.

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Supervision of the research.
Interpretation of IR spectrum.
Characterization of GC/MS results.
Writing and proof reading of manuscript.

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Determination of physico-chemical properties of the oil.
Writing and proof reading of manuscript.
Supervision of the research.

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Sample collection and preparation.
Extraction of essential oil.

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