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Comparative Study of Root, Stalk and Leaf Essential Oils of *Cymbopogon Citratus* (Lemon Grass)

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ABSTRACT

The root, stalk and leaf essential oils of *Cymbopogon citratus* grown in Kaduna, North Central Nigeria were extracted separately by hydrodistillation and characterized by GC-MS. The chemical composition analysis by GC-MS of the oils allowed the identification of 34, 26 and 16 compounds respectively. In the three oils, the main components were the isomers geranial and neral, which together form the compound citral. This corresponds to a total of 8.7, 27.8 and 35.1% of the compounds identified in the root, stalk and leaf oils respectively. The results obtained revealed a significant variability in the chemical composition and yield of the essential oils.

Keywords: Chemical composition, *Cymbopogon citratus*, Citral, Essential oil, GC/MS, Yield

INTRODUCTION

Cymbopogon citratus (Lemon grass) belonging to the family *Gramineae* (genus: *Cymbopogon*) is an aromatic perennial herb with rhizomes and densely tufted fibrous root (Adekomi *et al*.,2012). It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters(Omotade, 2009).It is a fast growing, aromatic grass native to South India and Sri Lanka, now widely cultivated in other tropical and subtropical countries of Africa, America and Asia(Chanthal, *et al*., 2012).Freshly cut and partially dried leaves are used extensively in Ayurvedic medicine. The grass is a folk remedy for coughs, elephantiasis, flu, gingivitis, headache, fever, hypertension, leprosy, malaria, ophthalmic pneumonia, nervous, gastrointestinal and vascular disorders(Karkala and Bhushan,2014). Studies indicate that *Cymbopogon citratus* possesses various pharmacological activities such as anti-bacterial(Danlami *et al*.,2011), anti-fungal(Nguefack *et al*.,2012), antioxidant(Hanisa *et al*., 2011; Garg *et al*.,2012) and anti-inflammatory(Figueirinha *et al*.,2010) properties.

Lemongrass is a plant in the grass family that contains 1 to 2% essential oil on a dry basis and its chemical composition varies widely as a function of genetic diversity, habitat and agronomic treatment of the culture (Ranitha *et al*., 2014). Compounds such as hydrocarbon terpenes, alcohols, ketones, esters and mainly aldehydes have constantly been registered. The leaf essential oil consists of, mainly, citral which is a mixture of two stereoisomeric monoterpene aldehydes; the trans isomer geranial dominates over the cis isomer neral and is used as raw material in the manufacture of perfumes, coloured soaps and synthesis of Vitamin A, ionones and β-carotene(Shah *et al*., 2011; Mirghani *et al*., 2012).

Essential oils are characterized by qualitative and quantitative differences depending on the part of the plant. The variability is especially related to the proportions of constituents and relatively to the presence of new compounds or the absence of particular ones. It has been suggested that the variation in essential oil yield and the composition could be due to the activity of enzymes responsible for the biosynthesis of volatile oils (Hendawy and Khaled, 2005; Bellili *et al*.,2016). This characteristic should contribute to the understanding of the pharmacological activities of the oil. Factors such as climatic, and geographic conditions, as well as ontogeny of collected plants can affect formation of essential oil, its composition and its biological activities.

There exist very little reports on the essential oils from the stalk (stem) and the root of lemon grass plant. It is for this reason that this study is aimed at investigating the composition of the essential oils from root, stalk and leaf of *Cymbopogon citrates* with a view to determining variations in their constituents. Furthermore, chemical variation in essential oil composition is a very important property for marketing and contributes to its commercialization as a main component in various industrial applications (Zouari, 2013).

MATERIALS AND METHODS

Plant Material

Lemon grass *(Cymbopogon citratus)* plant was obtained from Afaka, Kaduna, Nigeria. The plant was air-dried with ventilation for 5 consecutive days at 28±2ºC, after which it was separated into leaf, stalk and root parts. The plant parts (root, stalk and leaf) were cut into small pieces before extraction of their essential oils.

Oil isolation

250g each of the root, stalk and leaf parts of the plant were separately introduced into a 5 litre flask and water added until the sample was well immersed. Hydrodistillation was then carried out for 3 hours in an all glass clevenger-distillation apparatus designed according to British Pharmacoepoeia (BP, 1980) specifications. The oils were collected, preserved in a sealed sample bottle, and stored under refrigeration until analysis.

Gas Chromatography/ Mass Spectrometry (GC/MS) Analysis

Analysis of the oils were carried out using gas chromatograph (Agilent 19091S) coupled with a quadruple focusing mass spectrometer (433HP-5). Helium was the carrier gas at a flow rate of 1.5mL/min. The GC was fitted with a 30m×0.25mm, fused silica capillary column coated with phenyl methyl siloxane in split ratio of 1:50. The film thickness was 0.25 µm, oven temperature was initially kept at 100ºC for 5 min, then at 150ºC at a rate of 4ºC for 8 min and subsequently to 250ºC at a rate of 20ºC/min. The MS operating conditions were as follows: Transfer line temperature, 300ºC, ionization potential, 70eV. 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, 2007; Joulain and Koenig, 1998).

RESULTS AND DISCUSSION

The oil yields for the various parts of the plant were as follows: 0.9, 1.1 and 2.6% (w/w) for the root, stalk and leaf of *Cymbopogon citratus* respectively. The root oil had the lowest yield, whereas the highest yield was recorded for the leaf oil. It has been reported in the literature that plant organs influence the production of essential oil as well as its composition (Mubarak *et al*.,2015). The observed variations can therefore be explained by variation among different plant organs and their different stages of development.

Table 1 shows the identities, kovats indices and percentage composition of the constituents of essential oils obtained from various organs of *Cymbopogon citratus* plant. As can be seen from the table, a total of 34, 24 and 16 compounds were identified representing 99.2, 98.6 and 99% of the total oils in the root, stalk and leaf respectively. The leaf oil was yellow, whereas the stalk and root oils were light yellow with the root oil being lighter than the stalk oil.

In the root oil of *Cymbopogon citratus,* monoterpenoids represented 38.8% of which hydrocarbon monoterpenes accounted for 14.6% (six compounds) and 24.2% oxygenated derivatives (eight compounds). The oil was dominated by sesquiterpenoids (57.2%), among them, nine hydrocarbons (33.5%) and eight oxygenated derivatives (23.7%).Furthermore, three non-terpene compounds (one ketone and two carboxylic acids) were also identified. Among the constituents occurring in higher proportions, α-bulnesene (7.5%), β-ocimene (5.8%), germacrene-D (4.8%), trans-α-bergamotene (4.5%), neral (4.4%), linalool (4.4%), geranial (4.3%) and farnesol (4.1%) were prominent. β-myrcene (3.5%), α-cadinol (3.5%), β-elemene (3.4%), α-farnesene (3.3%), caryophyllene oxide (3.2%), geraniol(3.1%), γ-muurolene (3.1%), α-bisabolol (3.1%) , γ-elemene (3.0%), germacrene-D-4-ol (2.9%), ledol (2.6%), β-pinene (2.3%), limonene oxide (2.2%), terpinen-4-ol (2.0%), and β-cadinene (2.0%) were present in appreciable quantities. Also detected as minor constituents were α-amorphene (1.9%), camphene (1.4%), eudesm-4(14)-en-11-ol (1.4%), citronellene (1.3%), α-cadinol (1.1%), limonene diepoxide (0.8%) and α-pinene (0.3%).

The stalk oil largely consisted of monoterpenoids (75.3%) out of which four were hydrocarbons representing 22.3%, while oxygenated derivatives(seven compounds) accounted for more than half (53%) of the total oil. Hydrocarbon sesquiterpenes(six compounds) represented 11.7% of the total oil, while oxygenated sesquiterpenes(four compounds) accounted for only 6.1%. Similarly, three non-terpene compounds(one ketone and two carboxylic acids) were identified. The principal constituents of the oil were geranial (14.9%), neral (12.9%), β-myrcene (10.7%), limonene oxide (8.5%), citronellene (6.2%), geraniol (6.0%), terpineol (5.7%) and linalool (4.0%). Also detected in appreciable quantities were α-pinene (3.2%), ledol (2.5%), α-amorphene (2.4%), γ-muurolene (2.2%), caryophyllene (2.2%), β-ocimene (2.2%), and germacrene-D (2.1%). Furthermore τ-muurolol (1.9%), α-farnesene (1.7%), β-elemene (1.1%), α-cadinol (1.1), geranyl acetate (1.0%) and germacrene-D-4-ol (0.6%) were found in minor quantities.

Lastly the leaf oil was characterized by abundance of monoterpenoids (86.5%) with a preponderance of oxygenated compounds. Hydrocarbons (four compounds) accounted for 27.7% whereas oxygenated monoterpenes(seven compounds) constituted 58.8% of the oil. Sesquiterpenes were scarcely represented both as number of compounds and percentage composition

(7.8%). Among them two hydrocarbons (1.7%) and four oxygenated compounds (6.1%). Also, three non-terpene compounds(one ketone and two carboxylic acids) were identified. The major compounds in the oil were geranial (18.8%), neral (16.3%), β-myrcene (14.2%), citronellene (9.8%), limonene oxide (7.7%), geraniol (7.3%) and linalool (6.2%).α-pinene(2.3%) was found in sizeable quantity, β-ocimene (1.4%), geranyl acetate (1.4%), terpineol (1.1%), caryophyllene (0.9%), and trans-α-bergamotene (0.8%) were also present in minor quantities.

TABLE 1: CHEMICAL COMPOSITION OF ROOT, STALK AND LEAF ESSENTIAL OILS OF *CYMBOPOGON CITRATUS*(LEMON GRASS).

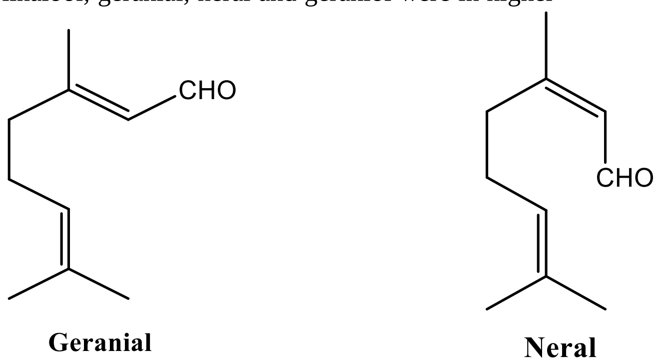
S/N	COMPOUND	% COMPOSITION				MASS SPECTRA DATA
		KI	ROOT	STALK	LEAF	
1	Citronellene	926	1.3	6.2	9.8	95 81 **67** 41
2	6-Methyl-5-Hepten-2-one	938	1.7	3.0	6.5	126 108 69 **55**
3	β-Pinene	943	2.3	-	-	136 121 **93** 69
4	α-Pinene	948	0.3	3.2	2.3	121 105 **93** 77
5	Camphene	953	1.4	-	-	136 121 107 **93**
6	β-Myrcene	958	3.5	10.7	14.2	121 **93** 79 69
7	β-Ocimene	976	5.8	2.2	1.4	121 105 **93** 79
8	Limonene oxide	1031	2.2	8.5	7.7	108 94 67 **43**
9	Linalool	1082	4.4	4.0	6.2	136 121 93 **71**
10	Limonene diepoxide	1128	0.8	-	-	107 79 67 **43**
11	Terpineol	1137	3.0	5.7	1.1	136 121 **81** 43
12	Geranial	1174	4.3	14.9	18.8	137 109 **69** 41
13	Neral	1174	4.4	12.9	16.3	134 109 84 **69**
14	Geraniol	1228	3.1	6.0	7.3	123 93 **69** 41
15	Terpinen-4-ol	1177	2.0	-	-	154 111 93 **71**
16	Geranyl acetate	1352	-	1.0	1.4	136 121 93 **69**
17	β-Elemene	1398	3.4	1.1	-	204 121 93 **81**
18	Trans-α-Bergamotene	1430	4.5	-	0.8	204 119 93 **69**
19	γ-Muurolene	1435	3.1	2.2	-	204 **161** 105 91
20	α-Farnesene	1458	3.3	1.7	-	119 107 93 **41**
21	γ-Elemene	1465	3.0	-	-	204 161 **121** 93

22	β-Cadinene	1472	2.0	-	-	204 189 **161** 105
23	α-Bulnesene	1490	7.5	-	-	204 107 **93** 79
24	Caryophyllene	1494	-	2.2	0.9	204 161 93 **69**
25	α-Amorphene	1506	1.9	2.4	-	204 162 119 **104**
26	Caryophyllene oxide	1507	3.2	-	-	177 109 93 **43**
27	Germacrene D	1515	4.8	2.1	-	204 **161** 119 105
28	Ledol	1530	2.6	2.5	-	204 161 69 **43**
29	α-Cadinol	1580	2.9	1.1	-	204 161 95 **43**
30	Eudesm-4(14)-en-11-ol	1593	1.4	-	-	204 119 69 **43**
31	τ-Muurolol	1608	-	1.9	-	222 204 **161** 95
32	α-Bisabolol	1625	3.1	-	-	204 119 69 **43**
33	δ-Cadinol	1636	3.5	-	-	204 **161** 119 105
34	Germacrene D-4-ol	1660	2.9	0.6	-	207 161 **81** 43
35	Farnesol	1710	4.1	-	-	161 121 69 **41**
36	Hexadecanoic acid	1968	0.6	1.3	1.6	256 129 **73** 60
37	9-Hexadecanoic acid	1976	0.9	1.2	-	97 83 69 **55**
38	9-Octadecanoic acid	2175	-	-	2.7	264 69 55 **41**
	Total (%)		**99.2**	**98.6**	**99.0**	
	Total Number of Compounds		**34**	**24**	**16**	

Qualitatively, there was a significant variation in the root, stalk and leaf essential oils of *Cymbopogon citratus*. For instance, β-pinene, camphene, limonene diepoxide, terpinen-4-ol, γ-elemene, β-cadinene, α-bulnesene, caryophyllene oxide, eudesm-4(14)-en-11-ol, α-bisabolol, δ-cadinol and farnesol were only detected in the root oil , but were not identified in the stalk and leaf oils. Geranyl acetate and caryophyllene were found in the stalk and leaf oils, but were not detected in the root oil. Trans-α-bergamotene was found in root and leaf oils, but was not detected in the stalk oil. Also, β-elemene, γ-muurolene, α-farnesene, α-amorphene, germacrene-D, ledol, α-cadinol and germacrene-D-4-ol were identified in the root and stalk oils, but were not found in the leaf oil. Only τ-muurolol was identified in the stalk oil, but not detected in the root and leaf oils. Absence of some compounds in different organs (root, stalk and leaf) may be due to the fact that the physiological conditions of the organs did not favour their biosynthesis(Usman *et al*.,2016).

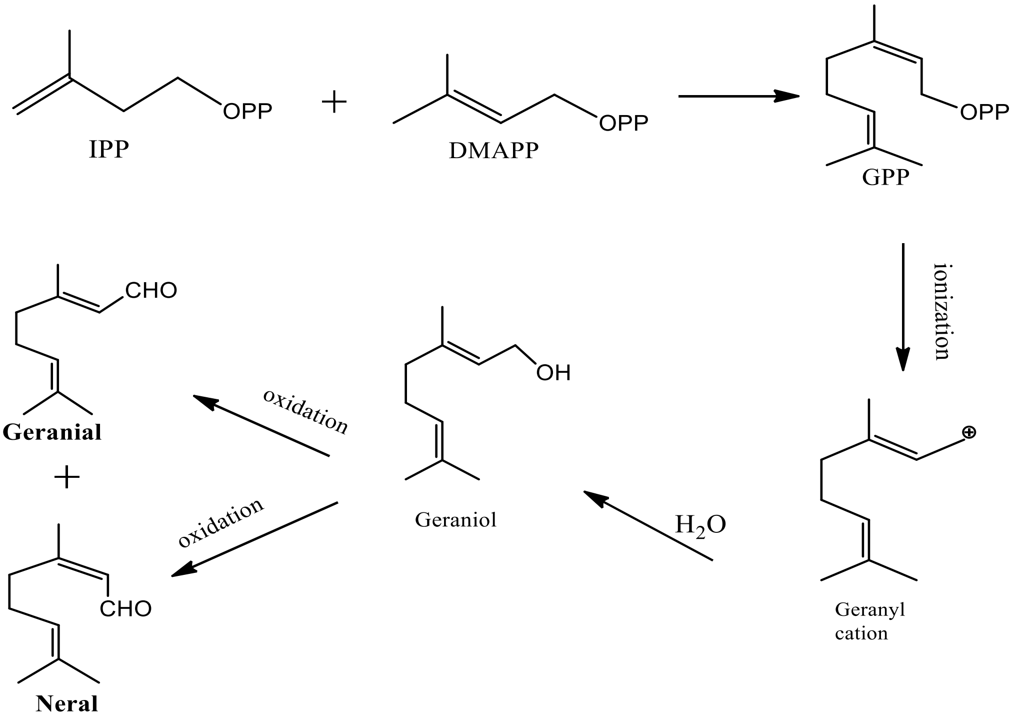
Quantitatively, α-pinene, β-myrcene, β-ocimene, limonene oxide, linalool, terpineol, geranial, neral and geraniol were detected in the three oils in varying quantities. However, the quantity of β-ocimene was higher in the root oil than in the other two (stalk and leaf) oils. Also, the quantities of α-pinene, limonene oxide and terpineol were higher in the stalk oil than in the root and leaf oils. Citronellene, β-myrcene, linalool, geranial, neral and geraniol were in higher quantities in the leaf oil than in root and stalk oils. The most abundant monoterpenoid in the stalk and leaf oils was citral (geranial+neral), β-ocimene was highest in quantity in the root oil. In the root oil which was dominated by sesquiterpenoids, α-bulnesene had the highest quantity, stalk and leaf oils were however dominated by monoterpenoids. The variations in the quantities of the constituents of the oils could be ascribed to differences in the activities of synthases that mediate the formation of the compounds from their respective precursors (Degenhardt *et al*., 2009).

It has been established that the enzyme of the most abundant mono- and/or sesquiterpenoids facilitate the transformation of their precursors (geranyl pyrophosphate (GPP) / farnesyl pyrophosphate (FPP) to various cationic intermediates in the presence of divalent metal ions (Degenhardt *et al*.,2009). The predominance of citral in the stalk and leaf oils implied that its synthase mediates the transformation of its precursor to all monoterpenoids. However, β-ocimene is the most abundant monoterpene in the root oil, hence its synthase catalyzed the formation of all monoterpenoids in the oil. Furthermore α-bulnesene was the most abundant sesquiterpene in the root oil, therefore its synthase also catalyzed the formation of all sesquiterpenoids in the oil. Sesquiterpenoids occurred in appreciable quantities in the stalk oil whereas they were negligible in the leaf oil.



Scheme 1: Chemical Structure of Geranial and Neral(3,7-dimethyl-2,6-octadienal).

The building units of terpenoids are biosynthetic units which are isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The biosynthetic isoprene units, IPP and DMAPP combine to form geranyl pyrophosphate(GPP). The biosynthesis of citral is initiated by ionization of GPP to the extended geranyl cation**.** Hydration of the cation gives geraniol which is subsequently oxidized to citral. (Degenhardt *et al*., 2009).



Scheme 2: Biosynthesis of Citral(geranial+neral)(Degenhardt *et al.*, 2009)

Biosynthesis of essential oils though determined genetically, is strongly influenced by ontogenetic and environmental factors (Priya *et al*., 2015). By considering the chemical profile of the essential oils of *Cymbopogon citratus* from Nigeria and comparing with the reports of other researchers from other parts of the world, it shows there are significant qualitative and quantitative differences between the chemical profiles of the major components of the oils. Thus variations in geographical conditions might be responsible for the compositional variations of essential oils observed. Based on literature data, geranial, neral, geraniol, limonene and β-myrcene have been found as major compounds in many other *Cymbopogon* species with the main chemical component of lemongrass oil being citral (Huynh, 2008; Loumouamou *et al*., 2010). The major components found in this research in the leaf oil are quite similar compared with data reported in the literature (Mirghani *et al*.,2012; Koba *et al*. 2009; Matasyoh *et al*. 2011; Tajidin *et al*.,2012; Rocha *et al*.,2011; Loumouamou *et al*.,2010; Bassole *et al*.,2011; Sessou *et al*.,2012).

According to Li *et al*.(2005), the essential oil obtained from the roots of *C. citratus* consisted of ten components including longifolene-(V4) that accounted for 56.67% of the oil; also present as a major constituent was selina-6-en-4-ol (20.03%).The chemical composition of the essential oil from the stalk of *C. citratus* was completely different from that of the root, with the former consisting of 12 components with citral(88%) as the major compound. This is quite different from the result obtained in this study where 34 and 24 compounds were identified in the root and stalk oils respectively. The major constituents of the root oil as reported by Li *et al*.(2005) were not identified in this study, also the citral content in the stalk they reported was by far higher than 28.8% recorded in this research. These variations may be due to the diverse seasonal, climatic and geographic differences and also as a result of different harvesting times, extraction and experimental procedures (Ranitha *et al*., 2014).

CONCLUSION

Factors that determine the composition and yield of essential oils are numerous, in some cases, it is difficult to segregate these factors from each other, since many of them are interdependent and influence one another. According to the results of this research, the study of the essential oil composition of various organs of *Cymbopogon citrates* plant led to the conclusion that yield and chemical compositions of the essential oils markedly varied among the samples. This characteristic should contribute to the understanding of the pharmacological activities of the herb. The results of the research revealed that plant organ was the main factor responsible for the observed variation because all the samples were subjected to similar environmental and experimental conditions.

RECOMMENDATION

The genus *Cymbopogon* is renowned for their essential oils and constituents of immense biological activities and therefore may be used in the treatment of several diseases. Lemongrass is of particular interest due to its commercially valuable essential oils widely used in foods, flavours, fragrances, cosmetics, soaps and detergents, perfumery and pharmaceuticals, as well as in traditional medicine. Monoterpenoid constituents of the essential oils have been established to display useful bioactivities (Karkala and Bhushan, 2014; Beier *et al*., 2014; Park *et al*., 2012; Ganjewala, 2009).Lemon grass is an underutilized plant as emphasis is placed on the leaves because it affords greater oil yield. This study revealed more constituents in the root (34) and stalk (24) oils than in the leaf (16) oil. These constituents are of immense biological importance, therefore detailed study of the essential oil constituents of the root and stalk will ensure full utilization of the plant's essential oil.

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