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Full Length Research Paper

Foliar Microscopy and GC-MS Analysis of the Volatile Oil Constituents of the Leaf of *Cymbopogon citratus* (DC.)Stapt. (Poaceae/Graminae)

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Abstract
The foliar epidermal microscopy and GC-MS analysis of the volatile oil constituents of the leaf of Cymbopogon citratus (DC) Stapf were carried out using standard methods. The leaf epidermal microscopy revealed parallel cell arrangement on both the lower and upper surfaces of the leaf, stomata were observed on both surfaces, oil globules were more abundant on the lower surface and the cells are rectangular or polygonal in shape. The GC-MS analysis of C. citratus essential oil of the leaf revealed a total of 30 volatile chemical components. Two (2) components constituting 100% (% peak area) were identified. The major components were β-citral (35.21%), β-myrcene (18.12%), thymol (15.42%), trans-4,5-epoxy-carane (6.51%), (S)-cis-verbenol (4.60%), 3,6-dimethyl-1,5-heptadiene (3.37%) and β-linalool (2.67%). About 90.68% were monoterpenoids while sesquiterpenoids constituted 4.5%. Also, 67.3% of the components were oxygenated compounds, which may be responsible for highly bioactive nature ofthe oilsince the presence of oxygen provides molecular binding sites.

Key words*: Cymbopogon citratus, Microscopy, Citral, Myrcene, Thymol.*

Introduction

Almost all plants are medicinal and the application of medicinal plants especially in traditional medicine is well acknowledged as a viable profession in Nigeria.*Cymbopogon citratus* (DC.) Stapt. Commonly known as lemon grass or oil grass, is a tropical plant from South Asia and Southeast Asia. Lemon grass belongs to the section of Andropogon called *Cymbopogon* of the family Poaceae/Graminae. It is a large genus of the family, including about 500 described species out of which six species occur in Nigeria namely: *C. citratus* (DC) Stapf.; *C. densiflora* (Steud.) Stapf*; C. gigantus* Chiov.;*C. nardus* (Linn.) Rendle; *C. schoenanthus* (Linn.) Sprang and *C. winterianus* Jowitt. Due to the production of lemon grass oil as major component, two of the species *Cymbopogon citratus* and *C. nardus* are generally called Lemon grass (Bonjar & Farrokhi, (2004). *Cymbopogon citratus* has slender sharp-edged green leaves with pointed apex. Common names in Nigeria include: *tsauri* (Hausa); *akwukwo, acharaehi* (Igbo) and *kookooba, korikooba* (Yoruba).

Medicinal use of lemon grass is known to mankind since antiquity. Its oil has been used to cure various ailments like cough, cold, spitting of blood, rheumatism, lumbago, digestive problems, bladder problems, leprosy, and as mouth wash for the toothache and swollen gums (Carter *et al.,* 2000). It has also been claimed to be stimulating, diuretic, anti-purgative and sudorific to reduce fever (Carter*et al.,* 2000). The leaves of *Cymbopogon citratus* (DC) Stapf. have been used in traditional medicine and are often found in herbal supplements and teas. Myriad of health benefits have been attributed to both their oral consumption and topical use, with modern research supporting many of their alleged benefits.

In the folk medicine of Brazil, it is believed to have anxiolytic, hypnotic, and anticonvulsant properties (Blanco *et al.,* 2009; Rodrigues *et al.,* 2006). In traditional medicine of India the leaves of the plant are used as stimulant, sudorific, antiperiodic, and anti-catarrhal, while the essential oil is used as carminative, depressant, analgesic, antipyretic, antibacterial, and antifungal agent (Wang *et al.,* 2014).

Laboratory studies have shown cyto-protective, antioxidant, and anti-inflammatory properties *in vitro* (Figueirinha *et al*., 2010; Lee *et al*., 2008; Tiwari *et al.,* 2010). Citronellol, which is thought to possess antihypertensive properties, is an essential oil constituent from *Cymbopogon citratus*, *Cymbopogon winterianus*, and *Lippia alba*. Citronellol has been shown to lower blood pressure in rats by a

direct effect on the vascular smooth muscle leading to vasodilation (Bastos *et al.,* 2010). In a small, randomized, controlled trial, an infusion made from *C. citratus* was used as an inexpensive remedy for the treatment of oral thrush in HIV/AIDS patients (Wright *et al*., 2009).

To cure cholera, colic and obstinate vomiting 3-6 drops of the oil was effective medicine of choice (Carter *et al*., 2000). The oil has been found to possess bactericidal and anti-fungal properties, which is comparable to penicillin in its effectiveness (Kolodziej & Kiderlen (2005). The oil also contains male sex hormone agent (Darten*et al.,* 2007). It is also reported to have strong activity against two dermatophytes, namely *Trichophyton rubrum* and *Microsporium gypsum* (International Union of Pure and Applied Chemistry-IUPAC (1995). Similarly, pharmacological investigation on the essential oil of *C. citratus* revealed that it has a depressant effect on the CNS (Robinson, 2006). It has analgesic and antipyretic properties. The extracted juice from the *C. citratus* contains inhibitor of the promotion stage of carcinogenesis induced by cotton oil. It is an oral anti-tumor drug for cancer and in combination with cyclodextrin lengthened the survival time [Sofowora (1984); Rios & Recio (2005)]. Gallstone dissolving preparations have been made of *C. citratus* oil (CDC, 2007). Lemon grass contains high percentage of Vitamin C, which is characteristic of plants used as drug such as Belladonna and Jaborandi. Lemon grass oils showed activity towards the phyto pathogenic fungi. A combination of lemon grass oil is given for use on human and domestic animal pathogens (Fair & Kormas 2008; Vandepette e*t al.*, 1991).

In the study by Mohd *et al*., 2010 tested organisms, particularly gram-negative organisms had shown high resistance towards different antibiotics whereas they were found to be inhibited by lemon grass oil even at lower concentration. Thus lemon grass oil is effective against drug resistant organisms. It can be suggested that use of lemongrass oil would be helpful in the treatment of infections caused by multidrug resistant organisms.

Leaf essential oil of *Cymbopogon citratus* has been studied extensively. However, the leaf essential oil of *C. citratus* collected from NIPRD garden has not been reported. The present study aims at sample authentication, foliar epidermal microscopy and the evaluation of the chemical constituents of *Cymbopogon citratus* leaf essential oil from NIPRD garden–Northern Nigeria.

Materials and methods

Chemicals

Hexane and anhydrous sodium sulphate are of analytical grade obtained from Sigma-Aldrich (Germany).

Plant Material

The leaves of *Cymbopogon citratus* were collected from NIPRD garden, Abuja, Nigeria on January2016 by Mr. John Atogwe. The identification and authentication of the plant was done at the Herbarium of the National Institute for Pharmaceutical Research and Development, Abuja, where the voucher specimen (NIPRD/H/) was deposited.

Leaf Microscopy

The method of Ugbabe and Ayodele (2008) was adopted. About $5mm^2$ - $1cm^2$ leaf fragments were obtained from the standard median portion of the leaf and macerated in concentrated Nitric acid in petri-dish for a period of 24 hrs. The appearance of bubbles on the surface of the leaf fragment indicated their suitability for separation. The fragments were transferred into water in a petri-dish with a pair of forceps. Both epidermises were carefully separated by teasing them apart and pulling each epidermis back at itself. The leaf epidermises were cleaned with the Carmel hair brush. These were rinsed in distilled water and later transferred into 50% ethanol to harden. They were then stained in Safranin O for 5 minutes and excess stain washed off in water. They were then mounted in glycerin on a slide with the edge of the cover slips ringed with nail vanish to prevent dehydration. The slides were labeled appropriately and examined under the light microscope while photographs were taken using NICON AFX-DX microscope with NICON FX-35DX camera attached at a magnification of x100 and x400.

Essential Oil Isolation

The dried leaves of *Cymbopogon citratus* were chopped into small pieces. 400g of the dried plant material was subjected to hydro-distillation using Clevenger type apparatus of 2 liter capacity. One liter of distilled water was added to the material. The mixture was heated on heating mantle at 100°C. The hydro-distillation was continued for four hours. The light yellow essential oil obtained was dried over anhydrous Sodium sulphate, stored in sealed vials and used within 30 minutes for the analysis.

Gas Chromatography- Mass Spectrometry (GC-MS) Analysis

The hydrodistilled leaf essential oil was analyzed on the GC-MS model QP 2010 SE (Japan) with QP-2010 Mass selective detector MSD, operated in the EI model (electron energy = 70eV), scan range = 45-600 amu and scan rate = 3.99 scan/sec. GC-MS analysis was carried out on optima 5ms, column of length 30m, internal diameter 0.25mm and film thickness 0.25 um, carrier gas was Helium, flow rate $6.2mL^{-1}$ and split 1.0. The condition for analysis was set as follows: column oven temperature was programmed from 60-280°C (i.e. temperature at 60°C was raised to 180°C at 10°C/min. and held for 2min. and then finally 280°C at 15°C/min and held for 4min.). The injector temperature was 250 and 280°C respectively. Sample dilution factor and injection volume were 1 and 1µL respectively. The MS parameters were: m/z range was 45-600Da and icon source temperature was 200°C. Essential oil compounds

were identified by matching their mass spectra with NIST 11 mass spectral library collection and other MS data of known compounds from literature search (Egharevba *et al*., 2016).

Results and Discussion

Leaf Microscopy

The leaf epidermal microscopy of *C. citratus* is shown in Plate 1A-D. The leaf epidermal microscopy revealed parallel cell arrangement on both the lower and upper surfaces of the leaf. This is typical of most monocotyledonous plants. Stomata were observed on both surfaces (a term known as amphistomatic). It is through the stomata that exchange of gases take place in plants. Oil globules were found more abundant on the lower surface of the leaf of *C. citratus* and the cells are rectangular or polygonal in shape (Plate 1A-D). No trichomes were observed on both the leaf surfaces.

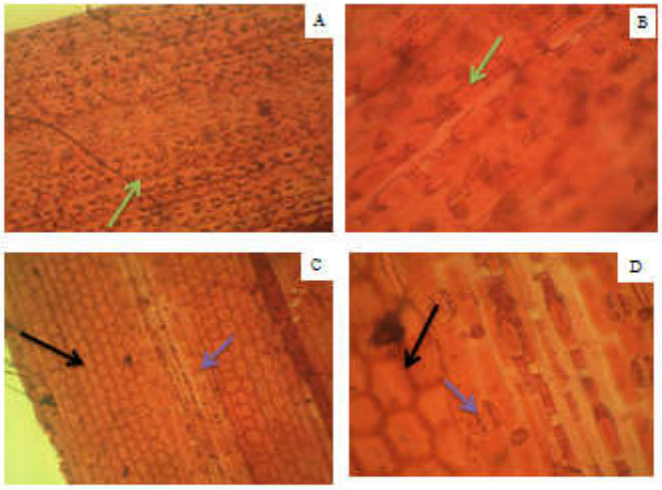

Plate 1: Leaf epidermal microscopy of *Cymbopogon citratus*
A. Abaxial/Lower surface x100: oil globules (green arrow) and parallel cell arrangement
B. Abaxial/Lower surface x400: oil globule (green arrow) and parallel cell arrangement
C. Adaxial/Upper surface x100: Stomata (purple arrow), polygonal cell shape (black arrow) and parallel cell arrangement
D. Adaxial/Lower surface x400: stomata (purple arrow), polygonal cell shape (black arrow) and parallel cell arrangement

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis of the essential oil of the leaf of *Cymbopogon citratus* is presented on Table 1.

Table 1: GC-MS analysis of *Cymbopogon citrates* leaf essential oil

S/N	RT (min)	% Composition (peak area)	Mwt (da)	Names of compounds
1	4.153	18.12	136	β-Myrcene
2	4.521	0.95	134	o-Cymene
3	4.655	0.87	136	trans-β-Ocimene
4	4.803	0.65	136	cis-β-Ocimene
5	4.985	0.57	136	γ-Terpinene
6	5.512	2.67	154	β-Linalool
7	6.134	3.37	124	3,6-dimethyl-1,5-Heptadiene
8	6.410	4.60	152	(S)-cis-Verbenol
9	6.669	6.51	152	trans 4,5-epoxy-Carane,
10	7.675	35.21	152	β-Citral (Neral)
11	8.281	15.42	150	Thymol
12	9.027	0.58	192	Thymol acetate
13	9.241	0.28	140	3-Nonyn-2-ol
14	9.375	0.73	196	Geranyl acetate
15	9.681	0.60	166	trans-octahydro-4a-methyl-1(2H)-Naphthalenone
16	9.870	0.15	150	2-ethylidene-6-methyl-3,5-Heptadienal
17	10.054	1.10	204	Caryophyllene
18	10.169	1.75	204	α-Bergamotene
19	10.366	0.72	204	cis-β-Farnesene
20	10.470	0.33	204	Humulene
21	10.721	1.64	184	2-Dodecanone

22	10.993	0.49	204	β-Bisabolene
23	11.199	0.46	222	Epiglobulol
24	11.549	0.15	264	Nerolidyl acetate
25	12.445	0.24	222	Selina-6-en-4-ol
26	13.247	0.15	168	α-Limonene diepoxide
27	17.945	0.25	168	4,8-dimethyl-3,7-Nonadien-2-ol
28	18.097	0.12	282	7-bromo-Cycloisolongifolene
29	19.574	0.18	166	1-Cyclohexyl-3-isopropylcarbodiimide
30	19.745	0.16	196	Ethyl geranate
	Monoterpenes	90.68		
	Sesquiterpenes	4.5		
	Oxygenated compounds	67.3		

The GC-MS analysis of *C. citratus* essential oil revealed a total of 30 volatile chemical components. The major components were β-citral or neral (35.21%), β-myrcene (18.12%), thymol (15.42%), *trans*-4, 5-epoxy-carane (6.51%), (S)-*cis*-verbenol (4.60%), 3,6-dimethyl-1,5-heptadiene (3.37%) and β-linalool (2.67%). About 90.68% of the components were monoterpenoids while sesquiterpenoids constituted 4.5%. Also, 67.3% of the components were oxygenated compounds, which may suggest the oil to be highly bioactive since the presence of oxygen may provide molecular binding sites. The result obtained in this study which gave neral (35.21%) and no geranial, was slightly different from that reported by Tajidin *et al*., 2012 who worked on the Malaysian species and reported higher (65-75%) citral (geranial and neral) composition at the three different stages of growth, and reported no thymol. The citral-rich Malaysian oil was similar to that reported from Malawi by Masamba *et al.,* 2003. However, the neral contents of the Malaysian and Malawian oils were about 40%, which compares favorably well with those obtained in this study. Citral had been reported to exhibit antiviral, antimicrobial, vasodilators, hypotensive, calming or sedative, antipyretic, and spasmolytic activities, while myrcene had been reported to possess stimulant, antiviral, antitumor, decongestant, antibacterial and hepato protective activities (Djilani and Dicko, 2012). Thymol exhibit antimicrobial, spasmolytic, anesthetic, irritant and immune stimulating activities (Djilani and Dicko, 2012).

Conclusion

The leaf epidermal microscopy of *C. citratus* revealed parallel cell arrangement on both the lower and upper surfaces of the leaf. It exhibited amphistomatic stomata. Oil globules were observed to be more abundant on the lower surface of the leaf. Trichomes were not observed on both the leaf surfaces. The wide range of pharmacological activities associated with essential oil of lemongrass may be due to the presence of citral, myrcene and thymol. This study identifies thymol as a new chemotype of *C. citratus* leaf essential oil.

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