STUDY OF ANTIOXIDANT AND LARVICIDAL ACTIVITY OF ESSENSTIAL OIL OBTAINED FROM LEMON GRASS LEAVES (*Cymbopogon citratus*)

Musa Runde

Department of Chemistry
Faculty of Science
National Open University of Nigeria

ABSTRACT

Essential oil was extracted from leaves of Lemon grass (*Cymbopogon citratus*) and analysed for composition, antioxidant and larvicidal activities. the GC-MS analysis of the essential oil reveals the major compounds as β.-Myrcene(18.39%),.3,7-di-methyl-2,6-octadienal(16.28%),3,7-di-methyl-2,6-octadienal(citral)(15.12%),Bicyclo[3.1.1],4,6,6-trimethylhept-3-en-2-ol(5.19%),1,4-heptadiene(3.15%). The *Cymbopogon citratus* essential oil exhibited high larvicidal activity with 100% mortality and LC 50 at 37 and 19.0 ml respectively, after 24 hours. The strongest free radical scavenging activity (97.43%) was exhibited by 50 µL/ml *Cymbopogon citratus* essential oil whereas, free radical scavenging potentials of the standard (ascorbic acid) was determined as 98.74% at the same concentration of 50 µL/ml. xxxx

Keywords. Essential oils; phytochemicals; larvicidal; antioxidants; medicinal plant

INTRODUCTION

Medicinal plants are used by many tribal people as folk medicine to treat diseases and some metabolic disorders such as diabetes mellitus, rheumatism, bowel disorders and inflammation. Some are used for ameliorating insect poisoning. Many metabolites have been purely obtained from medicinal plants, such as saponins, flavonoids, terpenes and glycosides. Essential oils, Phenolic compounds and these compounds are reported to exhibit protective effects due to their chemical properties. (Manjamalai *et al*., 2012).

Cymbopogan citratus (commonly named, lemongrass) is an odoriferous shrub belonging to Phocaea family. It is an average heigh, clumped perennial grass growing to a height of 1 m. The leaf-blade is linear, tapered at both ends and can grow to a length of 50 cm and width of 1.5 cm (Mirghani *et al*., 2010). The tubular shaped leaf-sheath serve as frame for which the plant grows and acts as a pseudostem. The commercial value and its application in food industry of lemongrass essential oils confer on the plants great value and at the same, reason why the plant is of great interest (Payne*et et al*., 1991). Diseases such as diabetes mellitus and gout are among the chronic diseases affecting worldwide population. In-depth study is required in order to discover some alternative remedies for these diseases. Tea prepared from lemon grass leaves has served as antiseptic anti-fever, anti-dyspeptic, carminative and tranquilizer (Negrelle *et al.,* 2007).

Essential oils are liquid, generally colorless to slightly yellowish substances obtained from odoriferous plants. They are slightly soluble in water and dissolve fairly well in ethanol and other organic solvents, and can mixed very well with vegetable oils, fats and waxes. The odour of essential oil is similar to that of the portion of plant from which they are extracted from and may be relatively more intensive while their specific gravity varies from 0.84 to 1.18 (Carter *et al.,*2000).

Free radicals are not required because of their unwanted roles in human ageing process and in diseases progression. On the other hand, antioxidants provide major defence against free radicals' invasions, and are required for maintaining good health and wellbeing. The desire for antioxidant becomes even more important with increased risks for coming in contact with free radicals. Environmental pollutants such as cigarette smoke and other factors like drugs, illness, stress and even exercise can increase risk of free radical exposure (Mark, 1998).

Substances which target insect larvae in the breeding arears before they can mature into adult are called Larvicides. Application of Larvicides to mosquito breeding arears help in reducing the adult mosquito population around the environment. Substances which have larvicidal property can be in liquid, tablet, pellet, granular and briquette. Each formulation has a particular method of application (Das *et al*., 2007).

MATERIALS AND METHODS

The materials requirement for this study includes; Fresh leaves of *Cymbopogan citratus*, GC-MS Machine, Steam distillation apparatus, Heat source, Laboratory glass wares, reagents of analytical grade.xxx

SAMPLE COLLECTION, AUTHENTICATION AND PREPARATION

Fresh leaves of *Cymbopogon citratus* (lemon grass) were collected at American University of Nigeria Yola and were air dried.

SAMPLE EXTRACTION

Screening the extracts for bioactive components

Phytochemical screening for major constituents of the plant extracts was carried out using standard qualitative methods as described by various authors (Odebiyi & Sofowora, 1990 Fadeyi *et al*., 1989, Kubmarawa *et al*., 2007, Runde, *et al,* 2015) as follows:-

Test for Saponins

5ml of the extract was vigorously shaken for 2 minutes with 10 ml of water in a test tube. Frothing which persisted on warming was taken as an evidence for the presence of Saponins.

Test for Tannins

To a small quantity of the plant extract, 10ml of water was added followed by a

drop of ferric chloride. Green precipitate indicates the presence of Tannin.

Test for Flavanoids
To a small quantity of the extract, a small quantity of magnesium chips was added and a few drops of concentrated H_2SO_4 down the side of the test-tube. Reddish coloration indicates the presence of Flavanoids.

Test for Alkaloids
Picric acid was added to small quantity of the extract. Orange colouration was taken as an evidence for the presence of Alkaloids.

Test for Essential oils
Small quantity of the extract was dissolved in 90% alcohol and drop of ferric chloride added. Green colouration indicates the presence of essential oils.

Test for Glycosides
To 5ml of the extract, 25ml of dilute H_2SO_4 was added in a test-tube and boiled for 15 minutes. It was cooled and neutralized with 10 % NaOH and then Fehling's solution A and B was added. A brick red precipitate indicates the presence of Glycosides.

Test for Phenols
An equal volume of the extract was added to equal volume of ferric chloride, a deep bluish solution indicates the presence of phenols.

EXTRACTION OF ESSENTIAL OILS
500g of the pulverized form of each of the fresh samples was subjected to steam distillation in a modified steam distiller (as modified by Runde *et al.,* 2015) while other conditions were adhered to according to the British Pharmacopoeia (BP) method. The time taken for the isolation of the oil was 4 hours (Kubmarawa *et al*., 2013).

GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC/MS) ANALYSIS
GC-MS analysis was performed as adopted by Runde *et al.* (2015) on a J and W Scientific gas chromatography directly couple to the mass spectrometer system (model GC Agilent S/N 20102969, polarise Q S/N 210729) HP 5ms 5% pheny! Methyl] silox: 469.56. Capillary Colum (30M x 250m) was used under the following condition: ovum temperature 50^0 C for 1 min, then 10 and 20 min to 300^0C for 2 min and the descriptions is as follows.

Injector temperature 230^0C carrier gas He, flow rate 1m/min; the volume of the injected sample was 0.2L of diluted oil in hexane. Split less injection techniques, ionization energy 70ev. In the electron ionization (EI) mode, ion source temperature 230^0 C scan mass range of M/Z 60-335; the constituents of the essential oils were identified base on comparison of the retention indices and mass spectra of most of the compound with data generated under identical experimental conditions by applying a two dimensional search algorithm considering the retention index as well as mass spectra similar with those of authentic compounds available in NBS75K Library.

The retention indices (RI) are in relation to a homologues series of n-alkanes on the GC column under the same chromatographic condition components relative concentration will be obtained by peak area normalization (Ramzi *et al*., 2013).

DPP FREE RADICALS SCAVENGING ASSAY OF ESSENTIAL OIL
The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out for the evaluation of the antioxidant activity of the various essential oils. The purple color, typical for free DPPH radical fades, and the change in absorbency at λ = 517 nm is measured spectrophotometrically.

The method was carried out as described previously by Kubmarawa *et al*. (2013) and adopted by Runde *et al.* (2015). The essential oil was dissolved in methanol, and various concentrations (2, 6, 12, 24, and 50µL/mL) was used. The assay mixture contained in a total volume of 1 mL, 500 µL of the oil, 125 µL prepared DPPH (1mM in methanol), and 375 µL solvent (methanol). After 30 min incubation at 25°C, the decrease in absorbance was measured at λ = 517 nm. The radical scavenging activity was calculated from the equation below: % or radical scavenging = [(Abs control – Abs Sample) ÷ Abscontrol] x 100 (Runde *et al.,* 2015).

LARVA SUSCEPTIBILITY TEST

The assay for larvicidal activity of the essential oils of *Cymbopogan citratus* was done according to the standard WHO protocol for larval susceptibility test. A stock solution was prepared by adding 525 micro litre of the essential oil in 100ml of Normal saline using DMSO as the emulsifier and final concentration of 38.0ml, 28.5ml, 19.0ml, 9.0ml and 4.0ml of the essential oil was tested. The chamber containing the control larvae received 1ml of DMSO served as the negative control and the positive control containing the control larvae also received 1ml of commercial pesticide in 100ml of deionized water. Four replicates were carried out for each dilution and for the control.

After 24hrs of contact, living and dead larvae were counted. The results of susceptibility testing were expressed in percentage of mortality versus concentrations of essential oils used. The percentage of mortality were calculated using Abbott's formula (% test mortality - %control mortality observed) / (100-%control mortality) x 100 (Kihampa *et al.*, 2009).

RESULTS

Table 1: Phytochemical screening of *Cymbopogan citratus* (Lemon grass)

Alkaloid	Essential Oil	Flavonoid	Glycoside	Phenols	Saponnins	Tannins	Terpenoids
+	+	+	+	-	-	+	+

Key += Present, **-** = Absent

The phytochemical screening of *Cymbopogon citratus* reveals the presence of Alkaloid, Essential oil, Flavonoids, Glycoside, Tannins and Terpenoids whereas, Phenols and Saponnins were absence.

Table 2: Chemical Composition of Essential oil of *Cymbopogon citratus* (Lemon **grass)**

Constituent	RT	Area %	MW
β.-Myrcene	2.779	18.39%	140
Cyclohexene	3.154	0.94%	82
Bicyclo[3.1.1],4,6,6-trimethyl- hept-3-en-2-ol	3.254	1.10%	150
.3,7-di-methyl-2,6-octadienal (citral)	5.372	15.42%	151
.3,7-di-methyl-2,6-octadienal	5.879	29.28%	152
2,6-octadien-1-ol.3,7-di-methylacetate(E)	4.317	0.60%	`54
3, 7, 11-trimethyl Dodecatriene.	6.906	0.13%	204
2,6-octadien-3-ol	3.914	0.65%	154
1,6-octadiene	4.317	0.60%	138
1,4-heptadiene	4.481	3.15%	96
,Caryophyllene	6.342	0.05%	204
1,6-octadien-3-ol	3.930	0.61%	140
Bicyclo[3.1.1],4,6,6-trimethyl- hept-3-en-2-ol	5.532	5.29%	151

The GC-MS analysis of the essential oil obtained from the leaves of *Cymbopogon citratus* shows the presence of the following compounds shown in Table 1. However, the major components were β.-Myrcene (18.39%),.3,7-di-methyl-2,6-octadienal(16.28%),3,7-di-methyl-2,6-octadienal(citral)(15.12%), Bicyclo[3.1.1],4,6,6-trimethylhept-3-en-2-ol(5.19%),1,4-heptadiene(3.15%), Bicyclo[3.1.1],4,6,6-trimethyl- hept-3-en-2-ol(1.10%).

Table 3: DPPH Radical scavenging activity

Sample	% Scanverging property/µL				
	2	6	12	24	50
Cymbopogon citratus	85.51	85.83	86.29	86.83	97.43

Ascorbic acid	80.22	83.06	85.01	92.44	98.74

From the result, the scavenging ability of the essential oils showed a concentration-dependent activity profile (Table 3). The strongest free radical scavenging activity was exhibited by 50 µL/ml *Cymbopogon citratus* essential oil (97.43%). Free radical scavenging potentials of the standard (ascorbic acid) was determined as 98.74% at the same concentration of 50 µL/ml.

Table 4: Result larva Susceptibility Testing using Dimethyl sulfoxide (DMSO)

Extracted Essential oil	Concentration(ml)	%mortility	LC50
Cymbopogon citratus	37.0	100	
	28.5	80	
	19.0	50	19.0ml
	9.0	10	
	4.0	0	

The larvicidal susceptibility of all the plants essential oils tested at various concentration were found to be effective against the larvae of Anophele mosquito. At lowest concentration of 9.0 ml, 10% of the larva were inactive whereas, at the concentration of 37 ml, the larva motility reached 100 %. The Lethal Concentration at 50 (LC 50) was observed when the concentration of the essential oil was increase to 19 ml. No larva motility was observed in the negative control (DMSO) but motility was observed in the positive control. (insecticide). The results on the use of different concentrations of the plant essential oils were recorded in term of motility against the larvae of Anophele mosquitos under laboratory condition were shown in table 4 above.

DISCUSSION

Phytochemical screening of *Cymbopogan citratus* (Lemon grass)

The phytochemical screening of *Cymbopogon citratus* is presented in table 1 above. Other researchers revealed that phenolic compounds such as phenolic acid, flavonoids, tannins, stilbenes, quinines and others, have anticarcinogenic and antioxidant activities (Huang and Cai, 2010) whereas, saponin are reported to have anti-imflamatory, hypocholesterolemic and immune-stimulating properties (Yukuyoshi *et al.*, 2012).

Phytochemical screening of *Cymbopogon citratus* conducted by Ewansiha, *et al*, 2012, also revealed the presence of essential among other metabolites. This essential oil is responsible for specific aroma in lemon grass as confirmed by Bonjar and Farrokhi, 2004. The pleasant aroma in lemon grass also qualifies the plant for application in cosmetics, food and beverages and pharmaceutical industries (Seenivasan, *et a*l, 2006).

Chemical Composition of Essential oil of *Cymbopogon citratus* (Lemon grass)

The GC/MS analysis of essential oils of *Cymbopogon citratus* revealed the presents of various compounds as shown in table 2. Another author revealed that lemongrass' stalk essential oils consist of geraniol (5.40% and 7.75%), limonene (5.71% and 5.92 oil while the essential oil of lemongrass' leaves revealed that geranial (32.10% and 29.64%), neral (22.36% and 21.73%), %) and β-myrcene (2.20% and 2.28%), were the major constituents of the stalks and leaves' lemongrass essential oil respectively (Mirghani *et al.,*2012). From the GCMS analysis on the standard essential oil lemon grass, it revealed that the major constituents of the essential oil are higher compared to the essential oil extracted in this study, with the total of geranial (44.29%),neral (31.36), geraniol (10.01%), limonene (6.09%) and β-myrcene (3.56%), comprising 95.31% of the total oil. Most of the studies and literature found on lemongrass were focused only on the leaves part. Based on literature data, it appears that geranial, neral, geraniol, limonene and β-myrcene have been found as major compounds in many other Cymbopogan species with the main chemical component of lemongrass oil is citral (Luiz *et al*., 2001; Huynh, 2008). Citral or 3, 7-dimethyl-2, 6-octadienal is the name given to a natural mixture of two isomeric acyclic monoterpene aldehydes: geranial (*trans*citral, citral A) and neral (*cis*-citral, citral B) (Huynh, 2008).

DPPH Radical scavenging activity

Similar work shows that the antioxidant activities of the essential oils of *cympobogon citratus* examined using DPPH scavenging test and the highest inhibition was obtained from the essential oil extracted from the stalk (89.5%)and the major components are geranial (32.10% and 29.64%),neral (22.36% and 21.73%), geraniol (5.40% and 7.75%), limonene (5.71% and 5.92%) and β-myrcene (2.20% and 2.28%), were the major constituents of the stalks and leaves' lemongrass essential oil respectively, (Mirghani *et al*,. 2012). A plot of % inhibition against concentration (µL/ml) is shown in the figure below.

Larva Susceptibility Testing using Dimethyl sulphur (1V) oxide (DMSO).

.*Cymbopogon citratus* exihibited high larvicidal activity with 100% mortility and LC50 at 19.0ml concentration after 24 hours. This result is comparable with the earlier reports of the work x Beena (2013), who observe the larvicidal activity of the essential oils of *cymbopogon flexeous* and *Tagetes erecta* against Aedes aegypti larvae which showed that the LC50 of value of *cymbopogon flexeous* are 136.8, 52.7 and 24.056 ppm after 12, 24 and 48hrs of exposure and that of *tagetes erecta* were 81.765, 48.951 and 17.729 ppm after 12, 24 and 48 hrs of exposure respectively

(Beena, 2013). A similar research also reported that LC50 of *Citrus sinensis* and *Citrus paradise* against Anopheles gambiae are 73ppm and 76 ppm respectively (Okunowo *et al*., 2015).

CONCLUSION

From the results obtained, it shows that the essential oil of *Cymbopogon citrates* contains compounds which have strong antioxidant activity and is comparable with the antioxidant activity of ascorbic acid available in the market. The larvicidal activity of the oil shows that it can be harnessed for use as agent for control of mosquitoes in the environment. Further research to establish the toxicity of this essential oil is encouraged and its effects on all the stages in the lifecycle of mosquito be analysed.

REFERENCES

Beena Joshi Bhatt, (2013).Comparative analysis of larvicidal activity of essential oils of *Cybopogon flexeous* and *Tagetes erecta* against *Aedes aegypti* larvae.*Euro.J.Exp.Boi*.3(5):422-427.

Bonjar, G., H., S and Farrokhi, P., R. (2004). Anti-bacillus activity of some plant used in traditional medicine of Iran. *Nigerian Journal on Natural Product Medicine*. (8), 34-39.

Carter, A.P., Clemons, W.M., Brodersen, D.E., Morgan-Warren, R.I., Wimberly, B.T. and Ramakrishnan, V. (2000). "Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics". *Nature* 407 (6802): 340-8.

Das, N.G., Goswami, D. and Rabha, B. (2007). Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *J Vector Borne Dis.*; 44:145–148.

Eansiha J. U., Garba S. A., Mawak J. D. Oyewole O. A. (2012). Antimicrobial Activity of *Cymbopogon Citratus* (LemonGrass) and It's Phytochemical Properties. *Frontiers in Science*, 2(6). 214-220

Fadeyi, N.G., Adeoye A.E and Olowokodejo, J.D. (1989); Epidermal and phytochemical studies with genus of Boerhavia (Nyetanginaceae) crude Drug Res. 29, 178-184

Huang W. and Cai Y. (2010); Natural phenolic compounds from medicinal Herbs and Dietry plant: Potential use for Cancer prevention. *Taylor and Francis Nutrition and Cancer*. 1(62): 1-20

Huynh, K. P. H, Maridable, J., Gaspillo, P., Hasika, M. Malaluan, R. and Kawasaki, J. 2008. Essential Oil from lemon grass extracted by supercritical carbon dioxide and steam distillation. *The PhilippineAgricultural Scientist* 91(1): 36 -41.

Kihampa, C., Joseph, C.C., Nkunya, M.H., Magesa, S.M., Hassanali, A. and Heydenreich, (2009). Larvicidal and IGR activity of extract of Tanzanian plants against malaria vector mosquitoes. *J Vector Borne Dis*. 46:145–52

Kubmarawa, D., Ajoku, G.A., Enwerem, N.M. and Okorie, D.A. (2007); Preliminary Phytochemical and Antimicrobial screening of 50 Medicinal plants from Nigeria. *African Journal of Biotechnology*. 6(14), 1690-1696

Kubmarawa, D., Akiniyi, J.A. and Okorie, D.A. (2013). Ethnomedicinal survey of traditional medicine of Lala people of Nigeria. *International Journal of Medicinal plant and Alternative Medicine*, 1(3), 39-57.

Luiz, H. C. C., Ricardo, A. F. M., Cinthia, B. S., Lia, K.P. and Ariovaldo, B. (2001). Extraction of lemongrass essential oil with dense carbon Dioxide. *Journal of Supercritical Fluids* 21: 33–39.

Manjamalai, A., Jiflin, G.J. and Grace, V.M. (2012). Study on the effect of essential oil of *Wedelia chinensis (Osbeck)* against microbes and inflammation. *Asian J Pharmaceutical and Clinical Res*, **5**, 155-63.

Mark, P. **(1998)**. Antioxidant Clinical Nutrition Insight AdvancedNutrition, marschaliana Sprengel and its secretory elements. *Flavour Fragr J*.17, 141-143.

Mirghani, M.E., Parveen, J. and Liyana, Y. (2010). Comparison studies of chemical composition analysis from different parts of lemon grass (*Cymbopogon citratus*) essential oil. In: Proceeding of the 9th International annual symposium on sustainability science and management. 232-237

Odebiyi, A. and Sofowora A.E. (1990); Phytochemical screening of Nigerian medicinal plants part III Lloydia 41: 234-246

Negrelle, R.R.B. and Gomes, E.C. (2007). *Cymbopogon citratus* (DC.) Stapf: chemical composition and biological activities. *Rev. Bras. Pl. Med.*, *9*, 80-92.

Okunowo,W.O,Oyedeji,O.,Afolabi,L,O.,Matanmi,E and Awodele,O.(2015).Chemical composition of essential oils of sweet orange (*Citratus sinensis*) peels and its antimicrobial activity against some clinical isolates.*Americal Journal of Plant Sciences*.4(7A2),1-9.

Ramzi, A. M., Mansour, S. A., Mohammed, A. A., Adnan, J. A. and Jamal, M. K. (2013). GC and GC/MS Analysis of Essential Oil Composition of the *Endemic Soqotraen Leucasvirgata* and Its Antimicrobial and Antioxidant Activities. *Int. J. Mol. Sci.* 14**(11),** 23129-23139

Runde, M., Kubmarawa, D. and Maina, H. (2015) Compositional analysis and Antimycobaterium Tuberculosis Activity of Essential oil of *Hyptis Suaveolens LAMICEAE*. Res. *J. of Chem. Sci*. 5**(7),** 1- 4

Seenivasan, P.,Manickkam, J., Savarimuthu,I. (2006). *In-vitro* antibacterial activity of some plant essential oils. *BMC Complement journal of Alternative Medicine*. 6, 39.

Yukuyoshi T., Masazumi M. and Masaji Y. (2012). Application of Saponin containing plants in foods and cosmetics. *Intech*. 86-101