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GC-FID and FT-IR Characterization of Lemongrass Oil Extracted With SOXHLET Extraction Apparatus Using Ethanol as Solvent

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Abstract: The extraction of lemongrass oil was performed with Soxhlet extractor apparatus using ethanol as solvents. The extraction was carried out with lemongrass sample with particle size 0.5cm, 1.0cm, 1.5cm, 2.0cm and 2.5cm and the respective oil yields were noted.The characterization of the oil sample was donewith Fourier's Transformed-Infrared Spectroscopy (FTIR) and Gas Chromatography-Flame Ionization Detector (GC-FID) analytical techniques. The FTIR result showed ten (10) functional groups that include halo-compound, alkene, alkane, sulphate, aldehyde, thiol,isothiocyanate, carboxylic acid, alcohol, and aromatic compound. Furthermore, the GC-FID analyses indicated the occurrence of nineteen (19) different components in the lemongrass extract which includeα-pinene (12.9%), 2-nonanone(10.3%), acetonitrile(7.5%), β-cedrenes(6.6%), camphor(6.4%), citronellal(6.0%), 4-nonanone(5.9%), terpinolene(5.4%), decanal(5.3%), α-cedrol(5.3%), citral A(5.0%), β-pineane(4.4%), atlantone(3.6%), pentane(3.3%), p-cymene(3.1%), γ-terpinene(2.8%), α-cedrenes(2.6%), thujopsene(2.0%) and genaniol(1.7%). In this research work, the highest constituent obtained was α-pinene making up of 12.9% of the oil sample and the citral content was 5.0%. Itis concluded that lemongrass oil is made up of different bioactive chemicals which sometimes varies according to geographical origin, biodiversityand culture.
Keywords: Essential Oil, Ethanol, Lemongrass, Modelling, Physicochemical, Soxhlet Extraction

I. INTRODUCTION

Essential oils of plant origin are natural occurring volatile and odoriferous aromatic extracts of plants. These plant essences are mostly located in cells, ducts and glands of leaves, barks, roots, buds, flowers and fruits of most plant matrix. Lemongrass (*Cymbopogon citratus*) is a rich source of lemon-scented essential oil. Lemongrass last all season of the year (perennial plant). The plant has long, thin leaves, and is largely cultivated medicinal plants in parts of tropical and subtropical areas of Asia, Africa and America (Suryawanshi*et al.*, 2016; Chantal*et al.*,2012). The chemical compositions of lemongrass (Cymbopogon citratus) essential oil may vary widely due to geographical locations and agronomic treatment of the culture. The leaves of lemongrass *(Cymbopogon citratus)* possess lemon like odour characteristic aromatic flavours due to its citral contents and this made itof great importance to the industry.Lemongrass essential oil found broad application in food, pharmaceutical, perfumery and cosmetics industries (Ganjewala and Luthra, 2010). Lemongrass oil has a pleasant, refreshing aroma, antifungal and antibacterial properties (Ukpong*et al.*, 2016; Anaruma*et al.*, 2010). Many researchers have reported that plants contain valuable chemicals (Morrison and Boyd, 1987). These natural chemicals and their synthetic components have continued to serve as feedstock or intermediate feedstock in most industries. While some are used in pharmaceutical and chemical allied industries, others are applied as food flavours, fragrances, sweeteners, pesticides etc. Many researches carried out on lemongrass extraction and characterizations are whollycentered on steam distillation technique. Moreover, there are no reports on characterization of lemongrass oil extracted with Soxhlet extraction apparatus using ethanol as solvent especially from lemongrass sample from Ozoro and its environ- Delta State Nigeria. Therefore, the present study is aimed to characterize bioactive chemical constituentlemongrass essential oil with the aid of FTIR and GC-FID analytical methods.

II. MATERIALS AND METHODS

Materials collection and preparation

Fresh lemongrass leaves used for this research work were harvested from a private garden in Ozoro located at 5° 32′ 18″ N, 6° 12′ 58″ E, Delta State, Nigeria.Sample were washed and dried for eight (8) hours in an oven to reduce the moisture content. The dried lemongrass leaves were kept in sealed bag to avoid direct

sunlight. Thereafter, the dried lemongrass leaves were cut with a knife into various sizes of 0.5cm, 1.0cm, 1.5cm, 2.0cm and 2.5cm so as to increased contact area of the plant matrix.

Reagents

Ethanol used as solvent for the extraction process. Reagent is of analytical grade purchased by the technologist attached to Chemical Engineering Department, University of Port Harcourt, River state.

Equipment

The equipment used for the study include; 250ml Shuniu GG-17 Soxhlet extractor, Setra analytical weighing balance BL-410s, Buck scientific M530 USA Fourier Transform-Infrared Spectroscopy (FT-IR) machine and Buck scientific M910 USA Gas Chromatography-Flame Ionization Detector (GC-FID) machine.

III. METHOD:

100g of 0.5cm particle size lemongrass samples was measured using weighing balance. The weighed sample was put into extractor thimble and 300ml of ethanol were added into the flask. The heating mantle was set at a pre-determine temperature according to the boiling point of the solvent use for the experiment and the extraction was conducted for the set time (say 1 hour). Thereafter, experimental set up was dismantled and mixture of solvent and extracted oil obtained was placed on rotary evaporator set at initial speed 110rpm. This is done to enable ethanol recovery from extracted oil by evaporation under reduced pressure. Thereafter, the oil was kept in desiccator for about 25 minutes and the mass in grams of oil extract was determined with a digital electronic weighing balance. The above steps were repeated for otherparticle size 1.0 cm, 1.5cm, 2.0 cm and 2.5cm. The results were recorded accordingly. The percentage oil yield was estimated accordingly using Equation 1;

$$\text{Oil yield} = \frac{\text{Weight of extracted oil}}{\text{Weight of lemongrass sample}} \times \frac{100\%}{1} \qquad 1$$

Determination of lemongrass oil composition using GC-FID analysis

GC-FID analysis was performed using a Buck M910 scientific gas chromatography instrument equipped with a capillary column. Helium was applied as carrier gas at constant (steady) flow rate of 1.2ml/min and split ratio 1:30. Oven temperature was initially held at 50°C for 1 minute, and later programmed to be 280°C at a rate of 5°C/min. Helium flux is 30ml/min and air flux is 300ml/min. The injector temperature was 280^{o}C and that of detector (FID) temperature was 300°C. Injection volume is 1µl. The percent (%) compositions of various compounds were obtained by GC-FID analysis.Identification of lemongrass oilcomposition was based on retention index (RI) in co-injection with standards (Sigma Aldrich and standard isolates), MS Library search (NIST 98 and WILEY), the same timecomparing with the MS literature data (Adams, 2007; Adams, 2001; Shibamoto, 1987; Jennings and Shibamoto, 1980).And finally, the percentage (%) composition of individual constituent of the sample lemongrass oil was determined from GC-FID peak areas without using any correction factors.

Determination of functional groups using FT-IR analysis

The determination of functional groups in the lemongrass oil was done with Buck scientific M530 USA FTIR Spectrometer at SpringLab Awka Anambra state Nigeria. The instrument was fitted with deuterated triglycine sulphate detector and potassium bromide beam splitter. Gram A1 software was used to obtain spectra. About 1.0g of sample was properly placed on the salt pellet. Thereafter, the FT-IR spectrogram were obtained at frequency range between 4,000 – 600cm^{-1} and co-added at 32 scans and at 4cm^{-1} resolution. The functional groups were numerated with the assistance of IR correlation charts.

IV. RESULTS AND DISCUSSION

FT-IR analysis

FT-IR's absorption spectrum of lemongrass oil extract using ethanol is given in Figure 1. TheFT-IR's absorption spectra result ispresented in Table 1. FT-IR spectra confirmed seven (7) functional groups. The FT-IR spectra established peak values were 803, 1387, 1860, 2059, 2554, 3291, and 3430 as presented in the chromatogram in Figure 1.

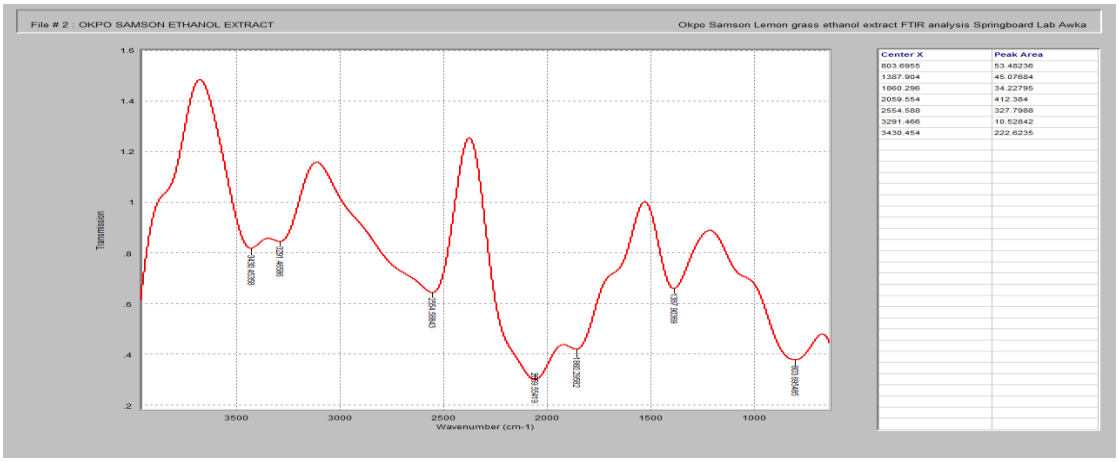


Figure 1:FT-IR Spectra of lemongrass oil extracted with ethanol

Table 1: FT-IR spectroscopy analysis for ethanol extract

Peak (Absorption) cm^{-1}	Intensity	Assignment	Functional group
803.6955	Strong	C-Cl stretching	Halo compound
	Medium	C=C bending	Alkene
		C=C	Alkane
1387.904	Strong	S=O stretching	Sulphate
	Medium	C=H bending	Aldehyde
1860.296	Weak	C-H bending	Aromatic compound
2059.554	Strong	N=C=S stretching	Isothiocyanate
2554.588	Weak	S-H stretching	Thiol
	Strong, broad	O-H stretching	Carboxylic acid
3291.466	Strong, broad	O-H stretching	Carboxylic acid
	Strong, broad	O-H stretching	Alcohols
3430.454	Strong, broad	O-H stretching	Alcohols

The FTIR spectra analysis of lemongrass oil is shown in Table 1. The corresponding absorptions were compared with standard IR spectrum table and chart using frequency range, the lemongrass oil spectra affirmed the presence halo compound, alkene, alkane, sulphate, aldehyde, aromatic compound, isothiocyanate, thiol, carboxylic acid, alcohols as functional groups. These identified functional groups were in agreement with compounds reported in the work of Olayemi*et al.* (2018). In addition, the functional groups identified confirmed GC-FID analysis. For instance, camphor, allantone, 2- nonanone, 4-nonanone are ketone, beta-pineane, pentane are alkanes. Beta-cedrenes, terpinolene, alpha-pinene, gamma terpinene are alkene. Citrals A, citronellal, decanal are aldehydes while p-cymene is aromatic compound, alpha- cedrol, gananiol are alcohol and thujopsene belong to amines.

GC-FID analysis

The GC-FID's analysis is presented in Table 2 and the chromatogram of the lemongrass oil extracts is presented in Figure2. The peaks in chromatogram were compared and integrated with database spectrum of known components stored in GC-FID library. The GC-FID analysis confirmed that lemongrass oil contains nineteen (19) compounds.

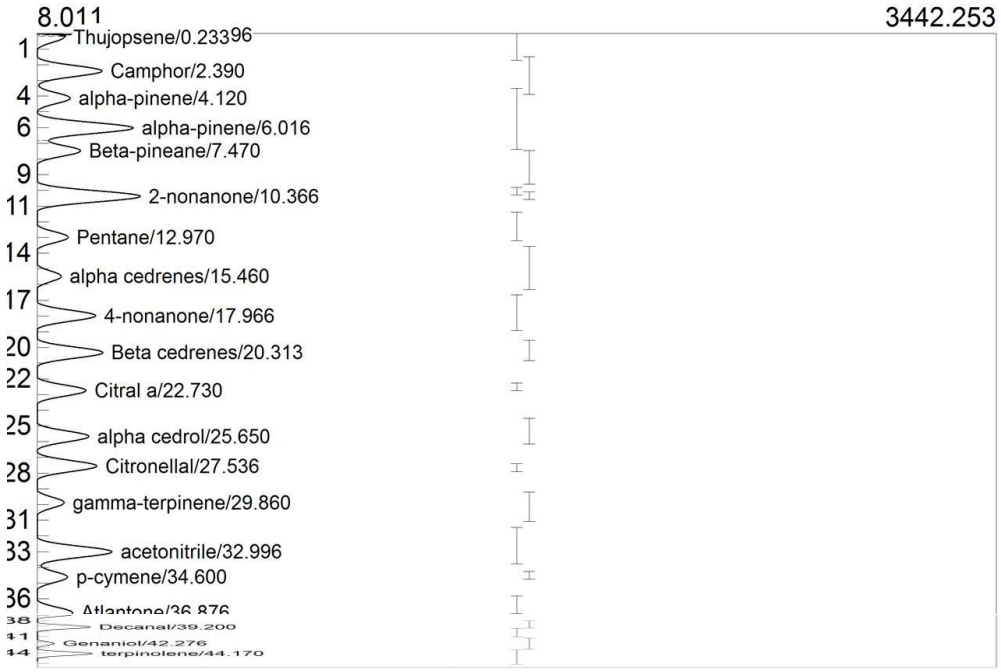
Lab name: Springboard Lab Awka
Client: Okpo samson
Collected: 7/11/19
Method: Syringe Injection
Lab ID: FID
Description: FID
Column: RESTEK 15METER MXT-1
Carrier: HELIUM AT 5 PSI
Data file: Okpo samson Ethanol extract lemon grass essential oil.CHR ()
Sample: Essential
Operator: David
Comments: TYPE YOUR COMMENTS HERE

Temperature program:

Init temp	Hold	Ramp	Final temp
80.00	5.000	5.000	220.00
220.00	2.000	7.000	280.00

Events:

Time	Event



Component	Retention	Area	Height	External	Units
Thujopsene	0.096	390.2835	187.824	0.1541	ppm
Thujopsene	0.166	596.8881	110.899	0.2356	ppm
Thujopsene	0.233	2786.6938	105.676	1.1000	ppm
Camphor	2.390	12226.5320	238.756	19.9044	
alpha-pinene	4.120	6406.9956	125.544	0.0810	ppm
alpha-pinene	6.016	18241.2318	351.971	0.2307	ppm
Beta-pineane	7.470	8378.2564	163.967	11.7914	ppm
2-nonanone	10.366	19567.5278	378.811	18.7428	ppm
Pentane	12.970	6200.4714	120.729	1.5687	ppm
alpha cedrenes	15.460	4927.4465	96.039	0.8865	ppm
4-nonanone	17.966	11229.8418	218.863	10.7566	ppm
Beta cedrenes	20.313	12615.9522	244.427	8.5812	ppm
Citral a	22.730	9509.6778	184.853	9.1970	ppm
alpha cedrol	25.650	10042.2102	194.954	1.0521	ppm
Citronellal	27.536	11449.1788	232.627	2.4687	
gamma-terpinene	29.860	5413.8648	105.734	0.0000	ppm
acetonitrile	32.996	14322.7033	277.023	2.1934	ppm
p-cymene	34.600	5959.5226	116.801	4.1100	ppm
Atlantone	36.876	6866.2699	134.457	2.8276	ppm
Decanal	39.200	10162.0437	197.604	0.6984	
Genaniol	42.276	3192.6428	65.133	0.2183	ppm
terpinolene	44.170	10204.4334	201.491	2.5817	ppm
		190690.6682		99.3804	

Figure2: Chromatogram for lemongrass oil using ethanol

Table 2: GC-FID analysis

Component	Retention time	Area	Height	External (ppm)	% Composition
α-pinene	10.136	24648.2274	477.515	0.3117	12.9
2- nonanone	10.366	19567.5278	378.811	18.7428	10.3
Acetonitrile	32.996	14322.7033	277.023	2.1934	7.5
β-cedrenes	20.313	12615.9522	244.427	8.5812	6.6
Camphor	2.39	12226.532	238.796	19.9044	6.4
Citronellal	27.536	11449.1788	222.627	2.4687	6.0
4- nonanone	17.966	11229.8418	218.863	10.7566	5.9
Terpinolene	44.17	10204.4334	201.491	2.5817	5.4
Decanal	39.2	10162.0437	197.604	0.6984	5.3
α-cedrol	25.65	10042.2102	194.954	1.0521	5.3
Citral A	22.73	9509.6778	184.883	9.197	5.0
β-pineane	7.47	8378.2564	163.997	11.7914	4.4
Atlantone	36.876	6866.2699	134.457	2.8276	3.6
Pentane	12.97	6200.4714	120.729	1.5687	3.3
p-cymene	34.6	5959.5226	116.801	4.11	3.1
γ-terpinene	29.86	5413.8648	105.734	0	2.8
α-cedrenes	15.46	4927.4465	96.039	0.8865	2.6
Thujopsene	0.495	3773.8654	404.399	1.4897	2.0
Genaniol	42.276	3192.6428	65.133	0.2183	1.7
	Total	**190690.6682**		**99.3804**	100.0

The observed chemical composition (constituent) of lemongrass oil is as presented in Table2 in their descending order. The sample oil is dominated with α-pinene, 2-nonanone, acetonitrile, β-cedrenes, camphor, citronellal, 4-nonanone, terpinolene, decanal, α-cedrol, citral A for both extract. The minor constituents were β-pineane, atlantone, pentane, p-cymene, γ-terpinene, α-cedrenes, thujopsene and genaniol. It has been proved that the composition of lemongrass oil varies according to geographical origin, biodiversityand culture (Ganjewela, *et al.,* 2008; Khanuja*et al.,*2005). In this research work, the highest constituent obtained was α-pinene making up of 12.9% of oil. This result is at variance with literature reports where citral has always been reported to be highest constituent of lemongrass oil. Joy *et al.,* (2001) have reported 74-76% citral. In this work the citral content was 5.0%. Bleasel*et al.,* (2002) reported that dry lemongrass oil yields of 0.4% contain 72.3% citral. Citral has immensecommercial significance due to its characteristiclemon like odour in flavor, cosmetics, perfumery and pharmaceuticalindustries (Ganjewela*et al.,* 2008).Citral is use in the formulations of perfumes, coloured soaps and synthesis of vitamin-A (Deepak *et al.,* 2012). Ganjewala (2009) also report antimicrobial, antiparasitic, antispasmodic, analgesic, anti-inflammatory activities of citral. On the other hand, camphor is used to grow hair loss and treat cold, sores, poor blood recirculation, anxiety, heart disease symptoms, ear aches, acnes, flatulence, depression, muscle spasms, low libido, hemorrhoids etc. α-pinene is used as ingredient in food flavoring and it has anti-depressant, anticonvulsant, antioxidant effects.α-pinene has a physiological effect on human. Finally, comparing chemical compositions of lemongrass oil from Nigeria with results of other researchersshows that there are variations in chemical composition of lemongrass oilgotten from different location. These variation in composition could be traced to diverse climatic, geographical differences, varied harvesting time and extraction technique adopted (Ashgari*et al.,* 2010).

V. CONCLUSION AND RECOMMENDATIONS

The FT-IR and GC-FID analytical techniques are important methods of characterizing plant extracts.The FTIR analysis confirmed ten(10) functional groups that include halo-compound, alkene, alkane, sulphate, aldehyde, thiol, isothiocyanate, carboxylic acid, alcohol, and aromatic compound.GC-FID analysis shows the sample lemongrass oil is made up of nineteen(19) bioactive chemicals which include α-pinene, 2-nonanone, acetonitrile, β-cedrenes, camphor, citronellal, 4-nonanone, terpinolene, decanal, α-cedrol, citral A, β-pineane, atlantone, pentane, p-cymene, γ-terpinene, α-cedrenes, thujopsene and genaniol. The major constituent was α-pinene making up of 12.9% of oil for ethanol extract. From this study, it can be affirmed that composition

of lemongrass oil varies according to diverse climatic conditions, geographical differences, varied harvesting time and may be extraction technique adopted. It is therefore recommended that further study be carried out using Gas Chromatography Mass Spectrometry (GC-MS) technique for the characterization. In addition, furtherresearchshould be carried out on lemongrass obtained from different locations of the worldin order to compare chemical composition of their essential oils.

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