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EXTRACTION, GC-MS ANALYSIS AND PRODUCTION OF PERFUME FROM AROMATIC PLANT (LEMON GRASS)

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ABSTRACT
Perfume is a substance, extract or preparation for diffusing or imparting an agreeable or attractive smell, especially a fluid containing fragrant natural oils extracted from flower, wood etc. Lemon grass was collected from the garden of Federal Polytechnic Ede Osun State and was extracted for its oil using Soxhlet extraction method which yielded 8.78%. The extracted oil was analysed by GC-MS and chemical constituents in the oil were identified. 25 components were found in the oil with four major peaks comprising of 9-octadecanoic acid, methyl ester (32.64%), pentadecanoic acid, 4 methyl ester (23.82)%, 9,12 octadecanoic acid methyl ester (7.57%) and dodecanoic acid methyl ester (4.69%). Antibacterial activities of the oil were tested, the tested microorganism were sensitive and no resistance was not observed, all growth inhibition zone occurred in the high activity level at different concentration (>5mm) at 10 concentration. The extracted oil was formulated into perfume using a fixative and methanol. The result obtained from the antimicrobial susceptibility test and compounds identified by the GC-MS revealed that the oil can be used in industrial applications in the pharmaceutical, perfumery and cosmetic industries.

Keywords: *Anti-bacterial activity, GCM, Lemon grass, Percentage yield.*

INTRODUCTION
Perfume is a fragrant liquid made from an extract that has been distilled in alcohol and water. Since the beginning of recorded history, humans have

attempted to mask or enhance their own odor by using perfume, which emulates nature's pleasant smells (Gadea et al., 2017). Many natural and man-made materials have been used to make perfume to apply to the skin and clothing, to put in cleaners and cosmetics, or to scent the air. A perfume is composed of three notes. The base note is what a fragrance will smell like after it has dried. The smell that develops after the perfume has mixed with unique body chemistry is referred to as the middle note. And the top note is 13 the first smell experienced in an aroma. Each perfumery has a preferred perfume manufacturing process, but there are some basic steps. The notes unfold over time, with the immediate impression of the top note leading to the deeper middle notes, and the base notes gradually appearing as the final stage. Most modern perfumes are alcohol-based and contain synthetic scents. While the term 'perfume' usually refers to fragrances in general, in the more technical language of the perfumer, a perfume must contain over 15% of fragrance oils in alcohol. The preferred fragrances for perfumes are by no means universal, but differ according to cultural dictates and fashions. Techniques involved in perfume extraction from plants include; solvent extraction, distillation and effleurage method. These methods to a certain extent, distort the odor of the aromatic compounds that are obtained from the raw materials. Important thing in relation to perfume making is that there are three key ingredients you will need to produce perfume; Essential Oils (these have been extracted from various plants (organic or nonorganic) and when combined give the smell of the perfume you are trying to produce, pure Grain Oil and water.

Plants have long been used in perfumery as a source of essential oils and aroma compounds. These aromatics are usually secondary metabolites produced by plants as protection against herbivores, infections, as well as to attract pollinators. Plants are by far the largest source of fragrant compounds used in perfumery. The sources of these compounds may be derived from various parts of a plant. A plant can offer more than one source of aromatics, for instance the aerial portions and seeds of coriander have remarkably different odors from each other. Orange leaves, blossoms, and fruit zest are the respective sources of petitgrain neroli, and orange oils.

Commercially, C. citratus is cultivated mostly for its essential oil which is biosynthesized mainly in leaves of the plant [Avila et al., 2016]. Lemongrass essential oil is of a considerable commercial importance because it is used in

the manufacture of fragrances, flavours, perfumery, cosmetics, detergents, and pharmaceuticals.

Lemongrass essential oil may be extracted by many different methods like solvent extraction, steam distillation, hydrodistillation (HD), microwave-assisted hydrodistillation (MAHD), and supercritical fluid extraction (SFE) with CO_2. A number of studies have proved that the quality of essential oils depends mainly on its constituents, which is significantly influenced by the extraction techniques [Schaneberg & Khan, 2002; Desai & Parikh, 2015; Wu et al., 2019].

Lemongrass essential oil is mainly produced by leaves which contain about 1-2% of essential oil in a dry matter [Skaria et al., 2012]. The essential oil is accumulated by plant in specific oil cells of the parenchyma tissues [Ganjewala & Luthra 2010]. The characteristic feature of lemongrass essential oil is a sherry colour, pungent taste, and lemon like odour. The chemical composition of the essential oil obtained from leaves of C. citratus has been extensively studied using GC and GC/MS methods. It varies according to the geographical origin, geobotanical conditions of the environment, farming practices, plant age, photoperiod, harvest period, genetic differences, and the extraction methods. Despite these differences, such compounds as hydrocarbon terpenes, alcohols, ketones, esters and mainly aldehydes have always been detected.

A variety of essential oils have been screened for their antimicrobial activity (Cantrell et al., 1998; Cowan, 1999). The antimicrobial activity of plant-derived essential oils is the basis of many applications, especially in food preservation, aromatherapy and medicine. The main demonstrated bioactivities are antimicrobial, anti-inflammatory, anticancer, antimutagenic and antidiabetic activities. The antimicrobial activity is the most explained. Lemongrass essential oil has a nonselective activity against both Gram-negative *(Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris*) and Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*), yeasts and fungi.

There is a high demand of essential oils for various purposes such as medicinal, perfumery, soap making, insecticides to mention but a few have opened up wide opportunities for global warming. Imported essential oils are very expensive to meet the demand of our local consumer industries, therefore it becomes necessary to source and extract these oils from local source.

The aim of the study is to produce perfume from lemon grass (*Cymbopogon citrasus*) using Soxhlet extraction.

MATERIALS AND METHODS

Sample Source and Preparation

Fresh lemongrass sample was collected from the garden of Federal Polytechnic Ede Osun state and it was air dry for about 10 to 15 day in the laboratory. The leaves were later cut into slices and blend with a mechanical blender until it became powdered.

Apparatus and Solvents

Soxhlet apparatus, Retort stand, Weighing balance, 250ml and 100ml beaker, 500ml separating funnel, knife, Aluminium, N hexane, Methanol, Coconut oil,

Solvent Extraction Method

A medium size Soxhlet apparatus was set up with 150g of powdered sample in a thimble and 2400ml of N hexane solvent in a round bottom flask. The solvent was heated at 70^{o}C gently with the aid of heating mantle. The vapour passing through the vapour tube was condensed by condenser and the condensed hot solvent dropped into the thimble containing the sample. As it reaches the top of the tube, it siphoned over into the flask. The process continued until complete extract was obtained. It was decanted into a beaker and covered with aluminium foil and left for about 48hrs for the N hexane to evaporate, Raphael et al (1994).

Formulation of Perfumes

Apparatus and Reagents

Perfume bottle, Funnel, 50ml and 120mlbeakers, Methanol, Distilled water, Lemongrass oil, Preservative (camphor), Coconut oil.

Production of Perfume and Packaging

7g of the extracted lemon grass oil was dissolved in 1000ml of methanol in a clean container. 100ml of coconut oil was added to it, 5ml of the fixative (camphor) was added (to improve the longevity of the perfume) and was allowed to settled then it was filtered with white handkerchief and packaged, Raphael et al., (1994).

Percentage Yield

The lemon grass powdered sample and the extracted essential oil was weighed and recorded. The weight of the extracted oil was divided by the weight of lemon grass powdered sample and multiplied by 100 and the result was recorded.

$$\text{Percentage Yield} = \frac{\text{Weight of extracted oil}}{\text{Weight of lemongrass sample}} \times 100$$

Antimicrobial susceptibility test

The antimicrobial screening method carried out was the agar well diffusion method as described by Lino and Deogracious (2006). The stored essential oil extracted from *Cymbopogon citatus* was taken from the laboratory refrigerator. Prior to this, 3 sub cultured 18 hours test organisms were spread on the labelled and separated nutrient filled petri plates in duplicates appropriately using cotton swabs. Holes were made on the solidified nutrient plates containing the test organisms using a 6mm cork borer which was repeatedly flamed and sterilized in ethanol after each hole was made. The negative control, methanol and DMSO (dimethylsulfoxide) were dispensed into their appropriate holes and the positive control ampicillin was also dispensed into its hole. The essential oil was tested at 15%, 10% and 5% concentrations. This procedure was carried out on n-hexane extract. The inoculated plates were incubated at 38 ^{o}C for 24 hours. And was observed.

Determination of Fatty Acid Profile in Lemongrass Oil Using GC-MS**.** The analysis was carried out in Shimadzu Training Centre for Analytical Instrument at FIIRO (Federal Institute of Research Oshodi, Lagos.

Results

Table 1: Soxhlet extraction of the lemon grass indicating percentage yield

Weight of lemongrass sample(g)	Weight of oil (g)	Time (mins)
5	0.26	120
15	0.91	360
15	0.9	360
15	1.0	360

15	0.9	360
10	0.56	240
20	1.1	480
20	1.15	480
15	0.9	360
20	1.1	480

The amount of essential oil obtained by Soxhlet extraction method was 8.78g of essential oil per 150g of dry lemongrass sample. This gave 5.85% yield of essential oil per 150g of dry lemongrass.

Plate 1: Plate showing zone of Inhibition of Lemon grass oil By E. coli

Plate 2: Plate showing zone of Inhibition of Lemon grass oil by S. aureus

Plate 3: showing zone of Inhibition of Lemon grass oil By B. subtilis

Table 2: Test showing the antimicrobial effects (zones of inhibition Z.I) of lemongrass oil extracted using N hexane

Bacteria	5% Conc. (mm)	10% Conc . (mm)	15% Conc . (mm)	Ampicillin (mm)
E.coli	2.5	3.0	1.0	2.0
S. aureus	2.0	5.0	2.0	3.0
B. subtilis	3.0	7.1	3.2	2.0

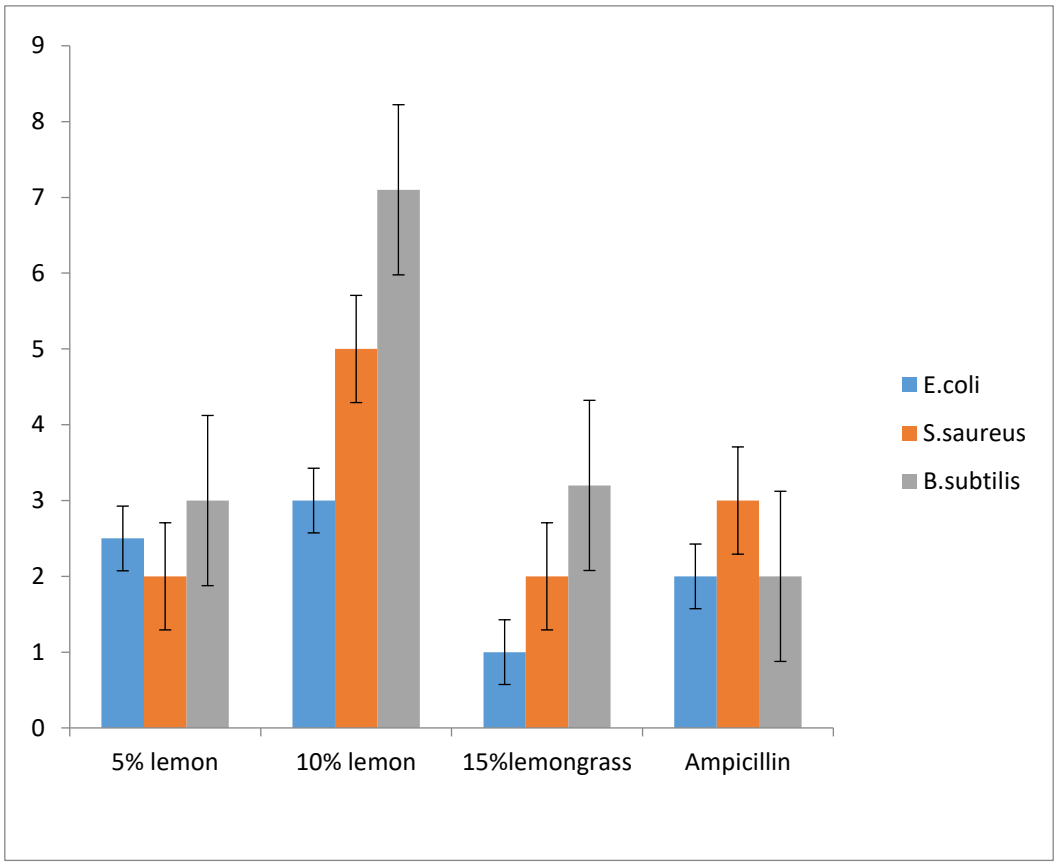

Figure 1. Antimicrobial effect of lemongrass oil extracted using N hexane

The antimicrobial effect of the N hexane extract of *Cymbopogon citratus* revealed that the largest zone of inhibition was 7.1 recorded against *Bacillus subtilis* at 10% concentration and the least zone of inhibition was 1.0 recorded against E.coli at 15% concentration of the oil. The N hexane extract showed very good activity against the bacterial species when compared with the standard antibiotics ampicillin.

The GCMS analysis of the leaf oil of lemon grass was performed using a gas chromatography mass spectrophotometer (QP2010SE) equipped with non-polar and polar double capillary columns (25.0m×0.25µm 1.d., 0.25µm), column oven temperature of 100oC. High helium was used as a carrier gas at a constant flow rate of 0.99ml/min. 1 µl sample was injected into GCMS auto injector for analysis. The identification of the various components was based on comparisons of their mass spectra with those of NIST library software in Mass Spectra Data Base.

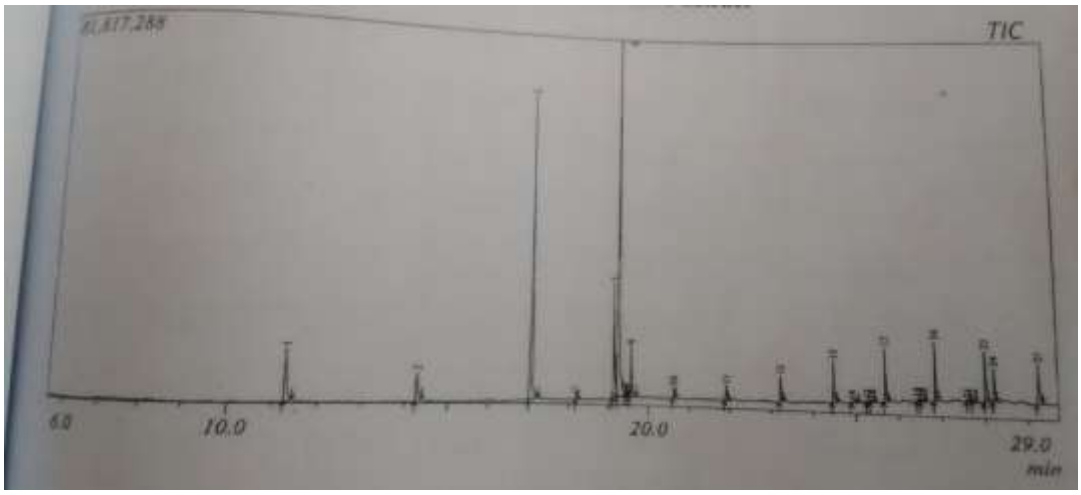

Fig 2. GC-MS Analysis of *Cymbopogon citratus* (lemon grass) leaf extract

Table 3: the chemical composition of N-hexane leaf extraction of *Cymbopogon citratus*

S/N	RETENTION TIME	PEAK AREA%	MOLECULAR FORMULAR	NAME OF COMPOUND
1	11.278	4.69	$C_{13}H_{26}O_2$	Dodecanoic acid methyl ester
2	14.276	2.55	$C_{15}H_{30}O_2$	Methyl tetradecanoate
3	17.060	23.82	$C_{17}H_{34}O_2$	Pentadecanoic acid, 14-methyl methyl ester
4	18.201	0.26	$C_{18}H_{38}$	Octadecane
5	19.142	7.57	$C_{19}H_{34}O_2$	9,12-octadecanoic acid (ZZ) methyl ester
6	19.270	32.64	$C_{19}H_{36}O_2$	9-octadecanoic acid methyl ester (E)
7	19.436	0.37	$C_{21}H_{44}$	Heneicosane
8	19.483	0.33	$C_{20}H_{40}O$	Phytol
9	19.571	3.50	$C_{19}H_{38}O_2$	Methyl stearate
10	20.637	0.72	$C_{28}H_{58}$	Octacosane
11	21.915	1.08	$C_{28}H_{58}$	Octacosane
12	23.227	1.77	$C_{28}H_{58}$	Octacosane

13	24.490	2.64	$C_{28}H_{58}$	Octacosane
14	24.915	0.42	$C_{26}H_{54}$	Hexacosane
15	25.273	0.29	$C_{28}H_{58}$	Octacosane
16	25.391	0.25	$C_{34}H_{70}$	Tetracosane, 11-decyl
17	25.707	3.40	$C_{24}H_{50}$	Tetracosane
18	26.453	0.34	$C_{28}H_{58}$	Octacosane
19	26.575	0.33	$C_{29}H_{60}$	2-methyloctacosane
20	26.866	3.78	$C_{24}H_{50}$	Tetracosane
21	27.595	0.23	$C_{28}H_{58}$	Octacosane
22	27.722	0.20	$C_{29}H_{60}$	2-methyloctacosane
23	28.026	3.42	$C_{29}H_{60}$	nonacosane
24	28.229	2.36	$C_{30}H_{50}$	Squalene
25	29.260	3.06	$C_{29}H_{60}$	Nonacosane

Discussion

In this study, the extraction yield of lemongrass was 8.78%. Sass- kiss et al. (1998) reported a 35% yield for onion powder treated with ethanol. The essential oil is pale yellow and completely insoluble in water, it has a clear lemongrass fragrance and it is volatile and has a cooling effect in the skin, (Atal et al., 1976).

The results of the GCMS analysis of the N-hexane extract of the lemon grass are shown in Fig 1, while the constituents are listed on Table 3. The GC-MS spectrum confirmed the presence of various compounds with different retention times ass illustrated in table 3. GC-MS analysis revealed the presence of 25 compounds but 8 compounds present in the essential oil were selected with the highest and lowest percentage range of values. It is well-known that grass plants produce terpenoidal hydrocarbon and essential oils that can be grouped as medicinal, industrial and perfumery depending on their chemical composition. The major components acid, were 9-Octadecenoic acid methyl ester (32.64%), Pentadecanoic acid, 14methyl methyl ester (23.82%), 9, 12 –Octadecanoic acid, (Z,Z) methyl ester(7.57%), Dodecanoic acid, methyl ester (4.69%). Hexacosane, 2-methyloctacosane, Tetracosane 11decyl, Octacosane were minor but significant components. Tetracosane 11decyl, 2 methyloctacosane and Hexacosane has the lowest height of 0.25%, 0.23% and 0.21% respectively.

9-Octadeeenoic acid methyl ester, Pentadecanoic acid, 14methyl methyl ester and Dodecanoic acid, methyl ester has the highest area of 32.64%, 23.82% and 4.69% respectively. Tetracosane 11decyl, Octacosane, and 2 methyloctacosane has the lowest area of 0.25%, 0.23% and 0.20% respectively. In this study, there is presence of dodecanoic acid methyl ester which is a white crystalline solid with rancid odour, soluble in most organic solvents and in dilute nitric acid, non-toxic. It is used to make esters for perfumes and fruit flavours, while 9-octadecanoic acid methyl ester is a fatty ester derived from natural oils that has excellent solvency roperties, is readily biodegradable and has low toxicity characteristics. It is a pale yellow oily liquid with a pleasant fatty ester odour. It is used as raw materials for emulsifiers or oiling agents for spin finishes, surfactant and base materials for perfumes and solvents or co-solvents. This result is in agreement with previous report by Koba et.al (2009) where lemon grass leaf essential consist of two monoterpene hydrocarbon (10.6%) and nine oxygenated monoterpenes (86.4%). Sessou et al. (2012) also reported 9.9% for hydrogenated monoterpenes 85.3%, hydrogenated sesquiterpenes 2.2% and oxygenated sesquiterpens 0.1% representing a total of 97.5% of the oil. This is an indication that essential oils of cymbopoqon species mainly consist of the monoterpenes fractions. The chemical composition of essential oil of cymbopoqon citnatus 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). Lemon grass contains active ingredients like myrcene, an antibacterial and pain reliver, citronellal, citronellol and geraniol. Both citral and geraniol have immense commercial significance due to their characteristics lemon and rose-like smell in the flavor, fragrance, cosmetics, perfumery and pharmaceutical industries (Ganjewela et al., 2008; Dubey etal., 2003).

The extracted oil using N-hexane from *Cymbopogon citratus* were tested against microorganisms like *Bacillus subtilis, Escherichia coli,* and *Staphylococcus aureus* (Cantrell et al., 1998). The tested organisms showed a clear zone of inhibition. They inhibitory effect of the essential oil at different percentages (15%, 10% and 5%) showed relatively high zone. In accordance with the results of Tortorano et al., (1998) the test organisms were found inhibited by lemongrass oil at very low concentration in both dilution method as compared to agar diffusion method.

Conclusion

In this study, an attempt was made to produce a perfume from local plant (lemon grass) using Soxhlet extraction method. Imported essential oils are very expensive to meet the demand, therefore it becomes necessary to source and synthesis this oil from local sources, in particular lemongrass.

Recommendation

There is urgent need for perfume production and from local raw materials in order to supplement the existing ones. I recommend more research to be carried out on extraction of essential oil and its formulation from vast variety of oil bearing plants in our ecosystem.

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APPENDIX

Pictures of packaged perfume