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Comparative analysis of phytochemicals and fatty acids from lemon peel and lemongrass essential oils by GC-FID technique

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Abstract
In this study, the concentrations of fatty acids and phytochemicals present in the essential oils extracted from *Citrus limon* peel and *Cymbopogon citrates* were studied. The essential oils were extracted and then characterized quantitatively using Gas Chromatography-Flame Ionization Detector (GC-FID) technique. The results showed that the essential oil from both lemon peel and lemongrass contained lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, α-linoleic acid, arachidonic acid, and eicosapentaenoic acid. The essential oil from *C. limon* peel showed higher concentrations for lauric acid (76.461 mg/g), myristic acid (32.792 mg/g), and stearic acid (110.410 mg/g) compared to *C. citrates* (14.507, 20.490, and 60.182 mg/g respectively). The lemongrass essential oil showed a higher concentration of palmitic acid (72.194 mg/g) than the lemon peel (31.349 mg/g). The distributions of unsaturated fatty acids in *C. limon* peel essential oils were α-linoleic acid (49 %) > oleic acid (36 %) > linoleic acid (13 %) > arachidonic acid (2 %) > eicosapentaenoic acid. A similar trend was showed by *C. citrates* essential oil except that oleic acid was not detected. A total of 20 and 18 different phytochemical compounds were identified in the *C. limon* peel, and *C. citrates* essential oils. Among the phytochemicals, spartein was not detected in the lemon peel oil, while quinine, kaempferol, and oxalate were absent in lemongrass oil. Anthocyanin (27 %), quinine (11 %), and phenol (10 %) were the most significant phytochemicals in the peel oil while sapogenin (17 %), catechin (16 %), and phenol (15 %) were the most prominent phytochemicals in the lemongrass oil. The essential oil from these plants can be a source of fatty acids and phytochemicals with important medicinal roles in man and animals.

Keywords: *Citrus limon*, *Cymbopogon citrates*, antioxidant, essential oil, fatty acid, phytochemicals

Introduction
Lemon (*Citrus limon*) is one of the most commonly utilized fruits in Nigeria due to its sweet taste and nutritional values [1, 2]. Currently, Nigeria is the ninth global producer of this fruit and the largest in Africa [3]. As of 2008, the Food and Agriculture Organization placed annual local production to be about 3.4 million metric tonnes from an estimated 3 million hectares of land [4]. Production has grown ever since at an average rate of 3.21 %, reaching 4.09 million metric tonnes in 2017 [5] due to increasing demand, mainly from the composition and many usefulness of its different parts. Because of the vast consumption of lemon, a large amount of wet solid waste is produced. This waste mainly includes lemon peels that contain numerous oil bearing glands that enclose significant amounts of citrus oil [2, 3]. Lemongrass (*Cymbopogon citratus*) is a tropical perennial herb belonging to the family Poaceae (true grasses) [6, 7]. It is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root used in folk medicine to treat nervous and gastrointestinal disturbances, fever, and hypertension [6, 8]. The essential oils extracted from lemon peels and lemongrass can also be used as a green insecticide as well in medicine to cure different ailments [6, 9, 10]. Furthermore, they are also used to flavour distasteful drugs in pharmaceutical industries to make them easier to consume. The oil also finds application in extraction processes as an eco-friendly solvent as the principal constituent of the oil is limonene [6]. Many studies have already been conducted to extract the essential oil from lemon and lemongrass peels through steam distillation and microwave-assisted extraction [9-11]. These studies have characterized the essential oils for phytochemicals, and meaningful results have been obtained. However, variations in the phytochemical composition of essential oils from lemon peel and lemongrass [12-14] are strictly correlated with the geographical origin [6]. While studies on essential oils from lemon peel and lemongrass have focused on phytochemical composition, fatty acid compositions have been largely unstudied.

Furthermore, data for essential oils of lemon peel and lemongrass from this region was not found. This study, therefore, was set to fill this knowledge gap.

This study aimed to extract and quantify the concentrations of fatty acids and phytochemicals in *C. limon* peel and *C. citrates' essential oils*. To achieve this, the following objectives were set: (1) extract essential oil from *Citrus limon* peel and lemongrass by hydrodistillation (2) identify fatty acids and phytochemicals in the extracted oil using gas chromatography-flame ionizing detection technique (GC-FID) and (3) compare the results for the lemon peel and lemongrass. The results from this study would reveal the potentiality of using essential oils as medicine and food. It would also contribute to the data concerning extracted oils' characteristics from lemon peel and lemongrass in southeastern Nigeria. GC-FID technique for essential oil characterization used in this study is considered significant as it offers high sensitivity, excellent stability, and an exceptionally high linear dynamic range that allows the analysis of volatile components of the essential oils at very low concentrations or trace levels [15, 16].

Methodology

Collection of plant materials and essential oil extraction

The lemon peel and lemongrass used for this study were collected from household gardens within the vicinity. They were properly washed with tap water and the peel of the lemon fruit removed with a penknife. The leaves and peels were sliced to small pieces and subjected to extraction immediately. Hydrodistillation extraction of the essential oil was employed using a hot plate and Clevenger system as condenser and oil collector. 200 g of chopped freshly lemon peels were immersed in 500 mL distilled water in a 1 L flat bottom flask with Clevenger system. The extraction was carried out for 2 h at 100 °C, and the essential oil was collected, dried over anhydrous sodium sulphate, and filtered. The same procedure was used to extract the essential oil from *Cymbopogon citrates* leaves. The essential oil samples were stored at -20 ^{o}C in a glass amber vial.

Phytochemical analysis of essential oils

The phytochemicals in the essential oils were analyzed using a Buck 530 gas chromatograph equipped with an on-column, automatic injector, Electron capture detector, HP 88 capillary column (100m × 0.25 µm film thickness) CA, USA. A RESTEK 15 m MXT-1 column (15 m ×250 µm ×0.15 µm) was used, and the injector temperature was kept at 280 °C with splitless injection of 2 µL of sample and a linear velocity of 30 cms^{-1}. Helium 5.0 Pa.s was the carrier gas with a flow rate of 40 mL/min. The oven was operated initially at 180 °C while the GC was allowed to warm up, and then heated to 330 °C at a rate of 5 °C/min, and kept at this temperature for 5 min. The detector was operated at a temperature of 300 °C. The ratio between the area and mass of internal standard and the area of the phytochemicals detected were used to identify the phytochemicals [17, 18].

Fatty acid analysis of essential oils

The fatty acids in the essential oils were converted into FAME by transferring 0.1 g of each sample into 40 mL glass vial and then mixing the oil with 5 mL of 0.5 M methanolic NaOH. The mixture was heated for 3 min at 60 °C and allowed to cool. 6 mL of 14 % BF_3 solution was added to the mixture and the solution heated for further 3 min at 60 °C. The mixture was again cooled, and then 10 mL isooctane was added, and the mixture was stirred and then allowed to settle. The upper layer of the mixture was transferred into a beaker containing anhydrous sodium sulphate to remove moisture. The extract was analyzed with GC-FID, and the identification and quantity of fatty acids in the oil were determined through comparison with standard supelco 37 FAME components.

Results and Discussion

The chromatogram of the fatty acids in the essential oils from lemon peel and lemongrass is shown in Figure 1. The essential oils from the lemon peel and the lemongrass showed similarities in the fatty acids identified. The types of fatty acids present in the essential oils are presented in Table 1. Four unsaturated fatty acids, including lauric acid, myristic acid, palmitic acid and stearic acid were identified in the oils, while oleic acid, linoleic acid, α-linoleic acid, arachidonic acid and eicosapentaenoic acid, were the unsaturated fatty acids in the essential oils

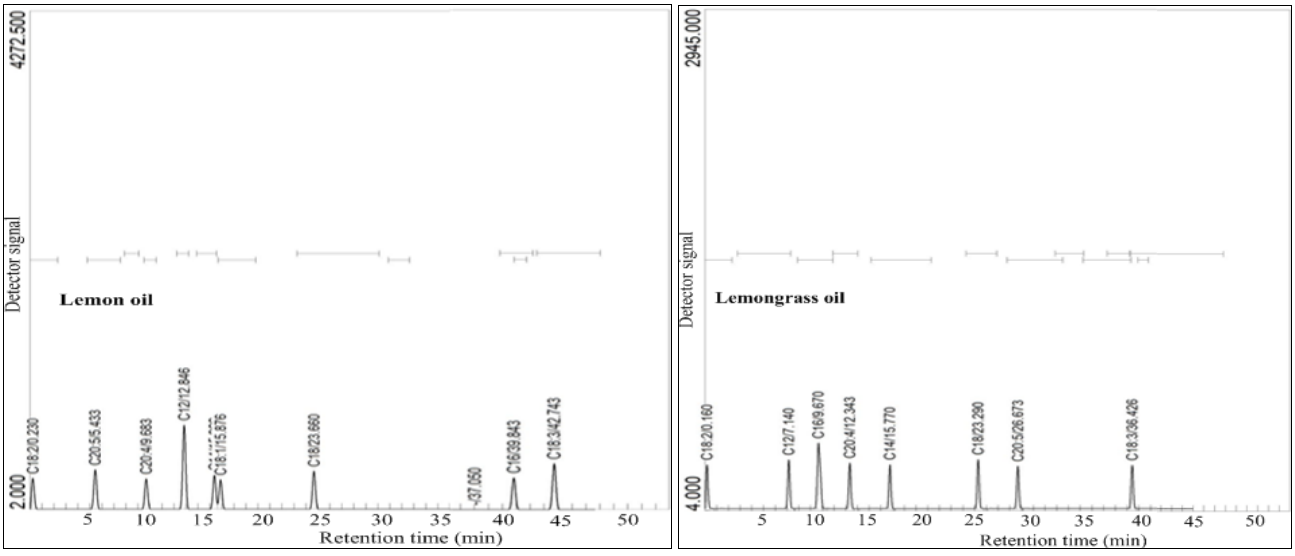

Fig 1: Chromatogram of fatty acids in the essential oils

Oils from the lemon peel showed higher concentrations of lauric acid (76.461 mg/g), myristic acid (32.792 mg/g), and stearic acid (110.410 mg/g) compared to the oil from lemongrass (14.507, 20.490, and 60.182 mg/g respectively).

However, the lemongrass oil showed a higher concentration of palmitic acid (72.194 mg/g) than lemon peel (31.349 mg/g). Stearic acid was the highest FA (44 %) in the lemon peel, while palmitic acid with 43 % was the most abundant FA in the lemongrass oil. Generally, the uses of stearic and palmitic acid endeavor its bifunctional character, useful in the industry, food, and medicine [6,20-22]. The data on the fatty acid composition in the present study are in contrast to those reported by [23] for essential oils from lemon seed sample collected in Kerman, Iran, with regard to the major constituents [Linoleic acid (33.2 % to 36.3 %), oleic (24.8 % to 29.3 %) and palmitic acids (23.5 % to 29.4m%)]. Myristic acid was only reported in essential oil from lemon peels by Ganiyu and co workers in 2014 [24]. Studies on the fatty acid composition of essential oil from lemongrass were not found.

Table 1: Saturated and unsaturated fatty acids in lemon peel and lemongrass essential oils

Saturated fatty acids				
Lipid number	**Common name**	**Systematic name**	**Concentration (mg/g)**	
			Lemon	**Lemongrass**
C12	Lauric acid	Dodecanoic acid	76.461	14.507
C14	Myristic acid	Tetradecanoic acid	32.792	20.490
C16	Palmitic acid	Hexadecanoic acid	31.349	72.194
C18	Stearic acid	Octadecanoic acid	110.410	60.182
Unsaturated fatty acids				
Lipid number	**Ω-n**	**Common name**	**Concentration (mg/g)**	
			Lemon	**Lemongrass**
C18:1	9	Oleic acid	52.128	–
C18:2	6	Linoleic acid	18.457	19.059
C18:3	6	α-linoleic acid	71.907	63.769
C20:4	6	Arachidonic acid	3.430	9.288
C20:5	3	Eicosapentaenoic acid	0.029	0.015

For the unsaturated fatty acids, oils from the peel generally showed higher fatty acid concentrations except linoleic and arachidonic acids (Table 1). Oleic acid was only detected in the peel oil. The distributions of unsaturated fatty acids in the peel oil were α-linoleic acid (49 %) > oleic acid (36 %) > linoleic acid (13 %) > arachidonic acid (2 %) > eicosapentaenoic acid. At the same time, similar distributions were also obtained for the lemongrass oil. α-linoleic acid is popular for preventing and treating diseases of the heart and blood vessels. It is used to prevent heart attacks, lower high blood pressure, lower cholesterol, and reverse atherosclerosis. However, higher consumption is associated with a moderately lower risk of cardiovascular disease. Still, a wide variation in results across multiple studies highlights the need for additional research before drawing firm conclusions [25,26].

The chromatograms of the phytochemicals in the essential oils are shown in Figure 2, and their quantities presented in Table 2

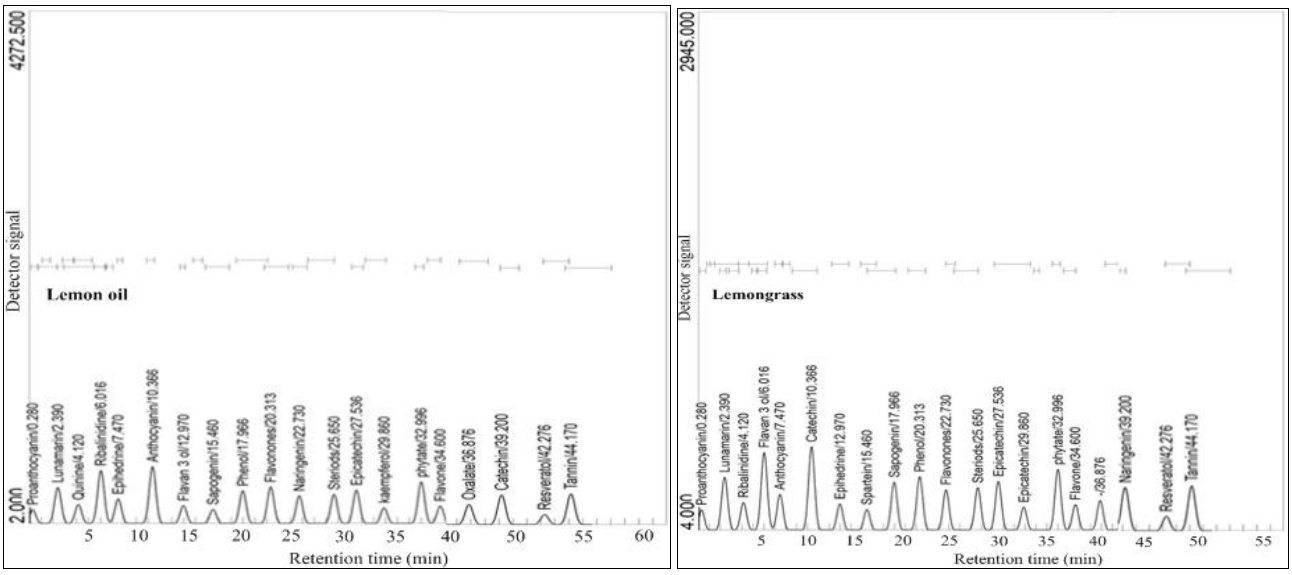

Fig 2: Chromatogram of Phytochemicals in the essential oils

Spartein was not detected in the lemon peel oil, while quinine, kaempferol, and oxalate were absent in lemongrass oil. Similar results have been reported in essential oils from lemon peel [12, 13] and lemongrass [7, 27] collected elsewhere. Anthocyanin (27 %), quinine (11 %), and phenol (10 %) were the most significant phytochemicals in the peel oil, while sapogenin (17 %), catechin (16 %), and phenol (15 %) were more prevalent in the lemongrass essential oil. The presence of these compounds in high abundance suggests that the oils can be used for diverse biological functions, including anti-malarial, anti-inflammatory, and antimicrobial activities [6, 14, 30]. Specifically, catechin and phenol are well-known anti-oxidant with further uses as anti-cancer, cardioprotective agents, immune system promoting, and skin protective effect from UV radiation [14, 28-30].

Table 2: Phytochemical concentrations in the lemon peel and lemongrass oil

Phytochemical	Concentration (µg/g)	
	Lemon	Lemongrass
Proanthocyanin	11.4335	20.9895
Lunamarin	21.1353	33.7911
Quinine	50.6287	–
Ribalinidine	7.1201	2.4884
Epihedrine	1.4473	1.0694
Anthocyanin	125.1318	6.4689
Flavan-3-ol	7.6543	0.4605
Sapogenin	34.4369	60.1313
Phenol	46.9029	52.7650
Flavanones	17.7356	13.3098
Naringenin	27.1063	28.9792
Steroids	19.8069	17.8163
Epicatechin	3.5833	5.2966
Kaempferol	0.4100	–
Phytate	6.9286	6.9286
Flavone	4.6003	4.6003
Oxalate	2.5472	–
Catechin	36.6305	55.3242
Resveratol	12.6255	17.8604
Tannin	26.1309	21.3508
Spartein	–	3.6077

In Figure 3, the comparison between the phytochemicals in essential oils from lemon peel and lemongrass is presented. The essential oil from lemon peel showed higher concentrations for the following phytochemicals; tannin, steroids, flavanones, flavan-3-ol, anthocyanin, ephedrine, and ribalinidine, while the remaining phytochemicals were higher in the lemongrass essential oils.

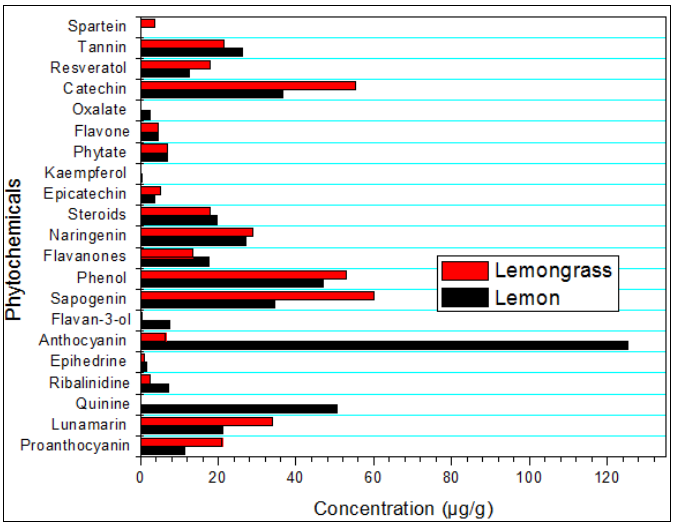

Fig 3: Comparative plot for phytochemicals in essential oils from lemon peel and grass

Conclusions

This study evaluated the fatty acid and phytochemical composition of essential oils from lemon peel and lemongrass. The essential oils showed good content of fatty acids that are of medicinal importance. Four unsaturated fatty acids, including lauric acid, myristic acid, palmitic acid, and stearic acid, were identified in the oils, while the five unsaturated fatty acids identified were oleic acid, linoleic acid, α-linoleic acid, arachidonic acid, and eicosapentaenoic acid. Stearic and palmitic acids were the most abundant in the lemon peel and lemongrass oils. α-linoleic acid was the most abundant unsaturated fatty acid in both the peel and grass oil. The essential oils further contained twenty and eighteen phytochemicals, which are of pharmacologically important. Anthocyanin, quinine, sapogenin, catechin, and phenol were the most abundant phytochemicals. The presence of these compounds with biological activities in the essential oils indicates that they could be used as a contemporary medicine to treat malaria and other diseases. Therefore, the essential oils could represent potential sources of lead molecules with pharmacological activities for the development of new novel pharmaceutical products for the treatment of malaria and other diseases.

Conflict of interest

The authors declare no conflict of interest.

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