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Chemical composition of *Citrus limon* (L.) Osbeck growing in southwestern Nigeria: Essential oil chemo types of both peel and leaf of lemon

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Abstract
The leaf and peel essential oils of *Citrus limon*, collected from the Agboku area of Ado-Odo Local government Area in Ogun state, Nigeria, were obtained by hydro distillation and analysed by GC-FID and GC-MS. The leaf oil was rich in limonene (31.5%), sabinene (15.9%), citronellal (11.6%), linalool (4.6%), neral (4.5%), and geranial (4.5%), while the peel essential oil was dominated by limonene (85.9%). A numerical cluster analysis of leaf and peel essential oils reported in the literature revealed three identifiable clusters of peel oil (a limonene chemotype and two mixed chemotypes, limonene/citral/β-pinene and limonene/linalool/linalyl acetate). The leaf essential oils fell into four clusters (limonene/citral/β-pinene, β-pinene/γ-terpenene, limonene/sabinene, and linalool/linalyl acetate). The leaf and peel essential oils from Agboku, Nigeria occupied the limonene/sabinene and the limonene chemotypes, respectively.

Keywords: Lemon, essential oil composition, limonene, hierarchical cluster analysis

1. Introduction
The genus *Citrus* (Rutaceae) is believed to have originated on the Asian continent and comprises of 140 genera and about 1300 flowering species commonly found in the tropical, semi-tropical, and warm temperate countries, including the Mediterranean region [1–3]. The genus is represented by *Citrus sinensis* (orange), *Citrus aurantium* (sour orange), *Citrus* × *paradisi* (grapefruit), *Citrus limon* (lemon), *Citrus aurantifolia* (lime), *Citrus reticulata* (tangerine), *Citrus bergamia* (bergamot), *Citrus medica* (citron), and *Citrus junos* (yuzu) are some important fruits of genus *Citrus* [4, 5]. *Citrus* species are known as one of the major fruit crops world's fruit crops produced in countries like Brazil, USA, Japan, China, Mexico, and some tropical and Mediterranean countries [3].
Citrus limon (L.) Osbeck, lemon, is an evergreen flowering tree or aromatic shrub growing up to 6 m tall, with stout spines. The leaves are dark green, leathery with oval shape of about 14 cm (4 in) long. The flower buds are purplish color with 5 white petals of 5 cm in diameter. The fruits are oblong in shape between 7.5 to 12.5 cm long yellow when ripe with smooth dotted oil glands [6, 7]. *C. limon* fruits are high in citric acid and vitamin C. The flavor is commonly use in beverages, ice creams, salad dressing and vegetable dishes [8, 9].
The phytochemical components in *Citrus* species have been reported to be responsible for their antiseptic, antioxidant, sedative, anti-inflammatory, anticancer activities and their use in soaps, beverages, cosmetic and pharmaceutical industries [10–15]. Bioactive compounds, including coumarins, flavonoids, carotenes, terpenes and linalool have been reported as a major constituents present in *Citrus* peel essential oil [5, 16].
Recent studies revealed that peel and leaf essential oils of *C. limon* under varying conditions have significantly affected chemical profile and biological activities of some insects and microorganisms [17–21]. Previous studies have shown that essential oil composition of *C. limon* from some locations were characterized by high monoterpene content, predominantly limonene [19, 22–26], γ-terpinene [19, 23, 24], β-pinene [17, 19, 22, 23, 26], 1,8-cineole [23], and *cis*-sabinene hydrate [22]. The purpose of the present study was to investigate the chemical composition of *Citrus limon* growing in southwestern Nigeria and the need to characterized the chemotypes of *C. limon* based on its volatile chemical constituents present on both the peel and leaf oils and

to compare and contrast the Nigerian *C. limon* samples with compositions previously reported from other geographical locations.

2. Materials and Methods

2.1 Plant Material

Fresh fruits and leaves of *Citrus limon* were collected from Agboku area of Ado-Odo Local government Area in Ogun state Nigeria (6° 41.19′ N, 3° 13.66′ E). The plants were taxonomically identified and authenticated at the Herbarium of the Department of Botany of the University of Lagos with a voucher number (LUH 7811). The leaves and fruit peels were manually removed, air-dried in the laboratory for 7 and 10 days, respectively, and then pulverized using electric blender. Samples (450 g) of leaves and peels of *C. limon* were each subjected to hydro distillation in a Clevenger-type apparatus for 4 h. The yields of the oils were 0.85% and 1.45%, respectively, on a dry weight basis respectively. The oils were dried over anhydrous sodium sulfate and stored in a sealed vial under refrigeration prior to analysis.

2.2 Gas Chromatography (GC) Analyses

GC analysis of the oils were carried out on a Hewlett Packard HP 6820 Gas Chromatograph equipped with a FID detector and DB-5 column (60 m × 0.25 mm id, film thickness was 0.25 μm) and the split ratio was 1:25. The oven temperature was programmed from 50 °C (after 2 min) to 240 °C at 5 °C/min and the final temperature was held for 10 min. Injection and detector temperatures were maintained at 200 °C and 240 °C, respectively. Hydrogen was the carrier gas at a flow rate of 2 mL/min. An aliquot (0.5 μL of the diluted oil) was injected into the GC. Peaks were measured by electronic integration. A homologous series of *n*-alkanes were run under the same conditions for determination of retention indices.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Analyses

GC-MS analyses of the oil were performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with a Hewlett Packard 5973 mass spectrometer system equipped with a DB-5 capillary column (30 m × 0.25 mm id, film thickness 0.25 μm). The oven temperature was programmed from 70-240 ºC at the rate of 5 °C/min. The ion source was set at 240 °C and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 mL/min. The scanning mass range was 35 to 425 amu. Diluted oil in *n*-hexane (1.0 μL) was injected into the GC/MS. Identification of the constituents of the volatile oil was achieved based on their retention data (retention indices) determined with reference to C10-C40 *n*-alkane homologous series, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [27] and stored on the MS library [NIST database (G1036A, revision D.01.00) / Chem Station data system (G1701CA, version C.00.01.08)]. The chemical compositions of the essential oils of the leaves and peels of *C. limon* are summarized in Table 1.

2.4 Hierarchical Cluster Analysis

The chemical compositions of both the leaf and peel *Citrus limon* essential oils, obtained from the literature [19, 22, 33–40, 23–25, 28–32] and this present work, were used in the cluster analysis. The 30 peel essential oil compositions and 38 leaf essential oil compositions were treated as operational taxonomic units (OTUs), and the concentrations (percentages) of 23 major essential oil components (α-pinene, sabinene, β-pinene, myrcene, *p*-cymene, limonene, 1,8-cineole, (*E*)-β-ocimene, γ-terpinene, linalool, citronellal, 4-terpineol, α-terpineol, nerol, neral, linalyl acetate, geraniol, geranial, neryl acetate, geranyl acetate, β-caryophyllene, *trans*-α-bergamotene, and β-bisabolene) were used to determine the chemical relationship between the different *C. limon* essential oil samples using agglomerative hierarchical cluster (AHC) analysis using XLSTAT Premium, version 19.5.47159 (Addinsoft, Paris, France). Dissimilarity was determined using Euclidean distance, and clustering was defined using Ward's method.

3. Results and Discussion

Analysis of the chemical composition of both the leaf and peel essential oil of *C. limon* resulted in the identification of 41 and 22 components, representing 100.0% and 99.8 % of the total volatile oils, respectively (Table 1). The leaf essential oil was dominated by monoterpene hydrocarbon (57.2%), namely limonene (31.5%) and sabinene (15.9%), and oxygenated monoterpenoids citronellal (11.6%), linalool (4.6 %) neral (4.5%), geranial (4.5%) and geranyl acetate (3.4 %). The peel essential oil was dominated by the monoterpene hydrocarbons limonene (85.9%), sabinene (3.9%), and myrcene (3.1%).

Table 1: Chemical compositions of essential oils of dry peel and leaves of *Citrus limon*

Constituents	RI	Relative abundance (%)	
		***Citrus limon* peel EO**	***Citrus limon* leaf EO**
α-pinene	941	1.1	1.2
sabinene	976	3.9	15.9
myrcene	993	3.1	2.9
octanal	1001	0.1	-
α-terpinene	1018	-	0.2
limonene	1032	85.9	31.5
sylvestrene	1033	-	0.6
(*E*)-β-ocimene	1052	0.4	3.9
γ-terpinene	1062	0.2	0.9
cis-sabinene hydrate	1070	-	0.6
terpinolene	1088	0.1	0.2
linalool	1101	0.5	4.6
nonanal	1102	0.1	-
cis-p-menth-2-en-1-ol	1124	-	0.1
cis-limonene oxide	1134	-	0.1
isopulegol	1146	-	0.2

citronellal	1155	0.5	11.6
isoneral	1171	-	0.1
4-terpineol	1178	0.9	1.4
isogeranial	1184	-	0.4
α-terpineol	1191	0.6	0.6
γ-terpineol	1195	-	0.2
decanal	1204	-	0.2
citronellol	1230	0.3	2.3
neral	1240	0.4	4.5
geraniol	1257	0.2	1.3
geranial	1271	0.4	4.5
δ-elemene	1340	-	0.2
citronellyl acetate	1350	-	0.3
linalool isobutyrate	1373	0.2	1.9
geranyl acetate	1385	0.1	3.4
β-caryophyllene	1420	0.2	1.7
trans-α-bergamotene	1438	0.3	0.1
α-humulene	1456	-	0.2
γ-muurolene	1477	-	0.1
bicyclogermacrene	1495	-	0.3
β-bisabolene	1509	0.5	0.3
germacrene B	1556	-	0.3
spathulenol	1576	-	0.1
caryophyllene oxide	1581	-	0.3
isospathulenol	1639	-	0.3
α-cadinol	1654	-	0.2
epi-α-bisabolol	1686	-	0.2
Monoterpene hydrocarbons		94.7	57.2
Oxygenated monoterpenes		4.0	38.2
Sesquiterpene hydrocarbons		1.0	3.2
Oxygenated sesquiterpenes		-	1.1
Non-terpene derivatives		0.2	0.2
Total identified (%)		100.0	99.8

There are three chemotypes of *C. limon* peel essential oil: (1) a limonene/β-pinene cluster, (2) a limonene/linalool/linalyl acetate cluster, and (3) a limonene cluster (see Figure 1). The peel oil sample in this work falls into the limonene cluster, 3. Also, there are at least four chemtypes of *C. limon* leaf essential oils: (1) limonene/citral/β-pinene, (2) β-pinene/γ-terpinene, (3) limonene/sabinene, and (4) linalool/linalyl acetate. The leaf oil sample in this work falls into chemotype 3. Variation in chemical compositions due to genetic, climatic, and seasonal factors may be considered as an important factor for their biological activities and their use in folk medicine.

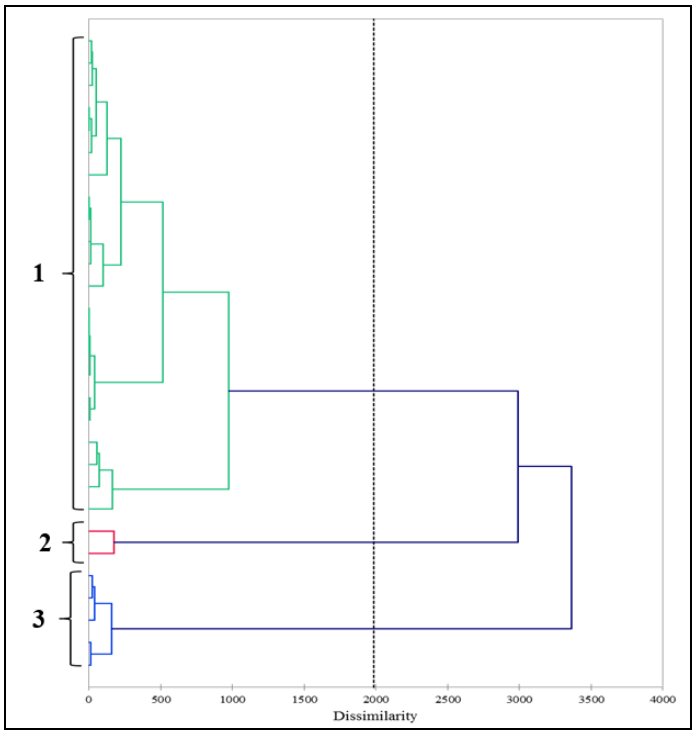

Fig 1: Dendrogram obtained from the agglomerative hierarchical cluster analysis of 30 *Citrus limon* peel essential oil compositions. Cluster 1: Limonene/β-pinene. Cluster 2: Limonene/linalool/linalyl acetate. Cluster 3: Limonene.

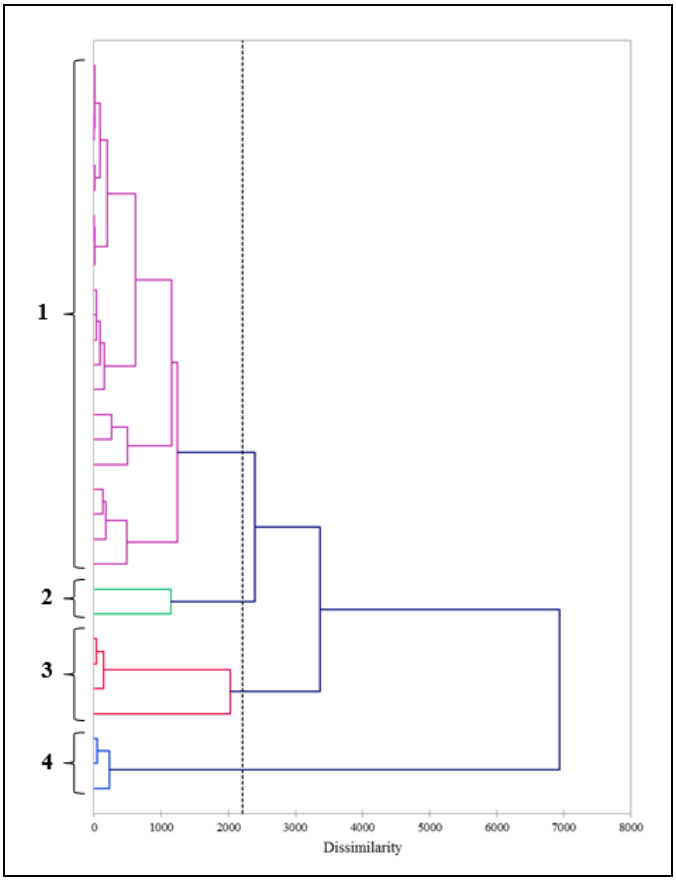

Fig 2: Dendrogram obtained from the agglomerative hierarchical cluster analysis of 38 *Citrus limon* leaf essential oil compositions. Cluster 1: Limonene/citral/β-pinene. Cluster 2: β-Pinene/γ-terpinene. Cluster 3: Limonene/sabinene. Cluster 4: Linalool/linalyl acetate.

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5. References

1. Burkill HM. The Useful Plants of West Tropical Africa, 2nd ed. Kew, UK: Royal Botanic Gardens, 2000, 5.
2. Brown D. The Royal Horticultural Society New Encyclopedia of Herbs and Their Uses. London, UK: Dorling Kindersley, 2002.
3. Mabberley DJ. Mabberley's Plant-Book. 3rd ed. Cambridge, UK: Cambridge University Press, 2008.
4. Anwar F, Naseer R, Bhanger MI, Ashraf S, Talpur FN, Aladedunye FA. Physico-chemical characteristics of citrus seeds and seed oils from Pakistan. J Am Oil Chem Soc. 2008; 85(4):321-30.
5. Dosoky N, Setzer W. Biological activities and safety of *Citrus* spp. essential oils. Int. J Mol Sci [Internet]. 2018; 19(7):1966. Available from: http://www.mdpi.com/1422-0067/19/7/1966
6. Gmitter FG, Hu X. The possible role of Yunnan, China, in the origin of contemporary *Citrus* species (Rutaceae). Econ Bot. 1990; 44(2):267-77.
7. Lorenzi H, Bacher L, Lacerda M, Sartori S. Frutas Brasileiras e Exóticas Cultivadas: De Consumo in Natura. São Paulo, Brazil: Instituto Plantarum de Estudos da Flora, 2006, 672.
8. Nguyen H, Campi EM, Jackson WR, Patti AF. Effect of oxidative deterioration on flavour and aroma components of lemon oil. Food Chem. 2009; 112(2):388-93.
9. Steuer B, Schulz H, Läger E. Classification and analysis of citrus oils by NIR spectroscopy. Food Chem. 2001; 72(1):113-7.
10. Awad AB, Fink CS. Phytosterols as anticancer dietary components: Evidence and mechanism of action. J Nutr [Internet]. 2000; 130(9):2127-2130. Available from: https://doi.org/10.1093/jn/130.9.2127
11. Benavente-García O, Castillo J. Update on uses and properties of *Citrus* flavonoids: New findings in anticancer, cardiovascular, and anti-inflammatory activity. J Agric Food Chem. 2008; 56:6185-205.
12. Dharmawan J, Kasapis S, Curran P, Johnson JR. Characterization of volatile compounds in selected citrus fruits from Asia. Part I: freshly-squeezed juice. Flavour Fragr J. 2007; 22:206-13.
13. Ju-Ichi M. [Chemical study of citrus plants in the search for cancer chemopreventive agents]. Yakugaku Zasshi J Pharm Soc Japan [Internet]. 2005; 125(3):231-54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15738623%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/15738623?dopt=AbstractPlus
14. Ladanyla M. *Citrus* Fruit: Biology, Technology and Evaluation. Amsterdam, Netherlands: Elsevier; 2008, 576.
15. Tanaka T, Maeda M, Kohno H, Murakami M, Kagami S, Miyake M *et al*. Inhibition of azoxymethane-induced colon carcinogenesis in male F344 rats by the citrus limonoids obacunone and limonin. Carcinogenesis. 2001; 22(1):193-8.
16. Mondello L, Casilli A, Tranchida PQ, Dugo P, Dugo G. Comprehensive two-dimensional GC for the analysis of citrus essential oils. Flavour Fragr J. 2005; 20(2):136-40.
17. AL-Jabri NN, Hossain MA. Comparative chemical composition and antimicrobial activity study of essential oils from two imported lemon fruits samples against pathogenic bacteria. Beni-Suef Univ J Basic Appl Sci [Internet]. 2014; 3(4):247-53. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2314853514000626
18. Bourgou S, Rahali FZ, Ourghemmi I, Tounsi MS. Changes of peel essential oil composition of four Tunisian *Citrus* during fruit maturation. Sci World J. 2012; 2012:ID 528593.
19. Djenane D. Chemical profile, antibacterial and antioxidant activity of Algerian *Citrus* essential oils and their application in *Sardina pilchardus*. Foods [Internet]. 2015; 4(2):208-28. Available from: http://www.mdpi.com/2304-8158/4/2/208/
20. Isman MB. Plant essential oils for pest and disease management. Crop Prot. 2000; 19:603-8.
21. Saeidi M, Moharramipour S, Sefidkon F, Aghajanzadeh S. Insecticidal and repellent activities of *Citrus reticulata*, *Citrus limon* and *Citrus aurantium* essential oils on *Callosobruchus maculatus*. Integr Prot Stored Prod. 2011; 69:289-93.
22. Ayedoun AM, Sossou PV, Mardarowicz M, Leclercq PA. Volatile constituents of the peel and leaf oils of *Citrus limon* L. Burm. F. from Benin. J Essent Oil Res. 1996; 8(4):441-4.
23. Gomes M, de S, Cardoso M, das G, de Souza PE, Machado SMF *et al*. Multivariate analysis of the essential oil components of the genus *Citrus* and their antifungal activity. Cient Jaboticabal. 2013; 41(2):111-21.

24. Lota ML, de Rocca Serra D, Tomi F, Jacquemond C, Casanova J. Volatile components of peel and leaf oils of lemon and lime species. J Agric Food Chem. 2002; 50(4):796-805.
25. Ojeda de Rodriguez G, Morales de Godoy V, Gonzalez de Colmenares N, Cabrera Salas L, Sulbaran de Ferrer B. Composition of Venezuelan lemon essential oil *Citrus limon* (L.) Burm.f. Rev la Fac. Agron Univ del Zulia. 1998; 15(4):343-9.
26. Wu PS, Kuo YT, Chen SM, Li Y, Lou BS. Gas chromatography-mass spectrometry analysis of photosensitive characteristics in *Citrus* and herb essential oils. J Chromatogr Sep Tech [Internet]. 2014; 6(1):1000261. Available from: http://omicsonline.org/open-access/gas-chromatographymass-spectrometry-analysis-of-photosensitive-characteristics-in-citrus-and-herb-essential-oils-2157-7064.1000261.php?aid=36481
27. Adams RP. Identification of Essential Oil Components by Gas Chromatography / Mass Spectrometry. 4th ed. Carol Stream, Illinois: Allured Publishing, 2007.
28. Ahmed M, Arpaia ML, Scora RW. Seasonal variation in lemon (*Citrus limon* L. Burm. F) Leaf and rind oil composition. J Essent Oil Res. 2001; 13(3):149-53.
29. Azam M, Jiang Q, Zhang B, Xu C, Chen K. *Citrus* leaf volatiles as affected by developmental stage and genetic type. Int. J Mol Sci. 2013; 14(9):17744-66.
30. Bousbia N, Vian MA, Ferhat MA, Meklati BY, Chemat F. A new process for extraction of essential oil from *Citrus* peels: Microwave hydro diffusion and gravity. J Food Eng. 2009; 90(3):409-13.
31. Campêlo LML, de Lima SG, Feitosa CM, de Freitas RM. Evaluation of central nervous system effects of *Citrus limon* essential oil in mice. Brazilian J Pharmacogn. 2011; 21(4):668-73.
32. Ekundayo O, Bakare O, Adesomoju A, Stahl-Biskup E. The composition of lemon petitgrain oil (*Citrus limon* (L.) N.L. Burm.). J Essent Oil Res. 1990; 2(5):269-70.
33. Fancello F, Petretto GL, Zara S, Sanna ML, Addis R, Maldini M *et al*. Chemical characterization, antioxidant capacity and antimicrobial activity against food related microorganisms of *Citrus limon* var. *pompia* leaf essential oil. LWT - Food Sci Technol [Internet]. 2016; 69:579-85. Available from: http://dx.doi.org/10.1016/j.lwt.2016.02.018
34. Fatta Del Bosco S, Abbate L, Tusa N, Strano T, Renda A, Ruberto G. Genetic improvement of *Citrus* fruits: The essential oil profiles in a *Citrus limon* backcross progeny derived from somatic hybridization. Food Res Int. 2013; 50(1):344-50.
35. Ferhat MA, Meklati BY, Chemat F. Comparison of different isolation methods of essential oil from *Citrus* fruits: Cold pressing, hydro distillation and microwave "dry" distillation. Flavour Fragr J. 2007; 22:206-13.
36. Hojjati M, Barzegar H. Chemical composition and biological activities of lemon (*Citrus limon*) leaf essential oil. Nutr Food Sci Res. 2017; 4(4):15-24.
37. Kirbaslar G, Kirbaslar SI. Composition of Turkish bitter orange and lemon leaf oils. J Essent Oil Res. 2004; 16(2):105-8.
38. Mondello L, Cotroneo A, Dugo G, Dugo P. Italian *Citrus* petitgrain oils. Part IV. Composition of lemon petitgrain oil. J Essent Oil Res. 1997; 9(5):495-508.
39. Öntaş C, Baba E, Kaplaner E, Küçükaydin S, Öztürk M, Ercan MD. Antibacterial activity of *Citrus limon* peel essential oil and *Argania spinosa* oil against fish pathogenic bacteria. Kafkas Univ Vet Fak Derg [Internet]. 2016; 22(5):741-9. Available from: http://vetdergi.kafkas.edu.tr/extdocs/2016_5_1/741-749.pdf
40. Vekiari SA, Protopapadakis EE, Papadopoulou P, Papanicolaou D, Panou C, Vamvakias M. Composition and seasonal variation of the essential oil from leaves and peel of a Cretan lemon variety. J Agric Food Chem. 2002; 50(1):147-53.