

How to Cite:

Al-Sharifi, H. F. H., & Alnomani, R. M. H. (2022). GC-MS profiling of the chemical compounds in the for the oily extract of Citrus sinensis and Citrus Limon peels. *International Journal of Health Sciences*, 6(S3), 7939–7947. <https://doi.org/10.53730/ijhs.v6nS3.7886>

GC-MS profiling of the chemical compounds in the for the oily extract of Citrus sinensis and Citrus Limon peels

Hussein Fouad Hamza Al-Sharifi

Faculty of Education for girls, University of Kufa, Najaf, Iraq

Email: hussen07806059920@gmail.com

Ruqayah Manoon Hasan Alnomani

Faculty of Education for girls, University of Kufa, Najaf, Iraq

Email: ruqayah.alnomani@uokufa.edu.iq

Abstract--Citrus fruits are used for the prevention of many chronic diseases such as cancer, diabetes, and cardiovascular diseases. The leaves and fruits of the citrus genus contain antioxidants. The oil of the family plants has uses in folk medicine , This article outlines the differences in phytochemical constitutions of two species of the genus citrus in Iraq by the GC-MS technique , nine different compounds are recorded in Citrus sinensis whereas 21 compounds in Citrus limon , The present study proved its assistance in the classification of species studied using chemotaxonomic techniques, especially the GC-MS technique, which represents an easy and direct analytical approach for the identification of phytoconstituents.

Keywords--Citrus, D-limonene, GC-MS technique.

Introduction

Citrus fruit peels which are considered as agro industrial waste are potential source of plant secondary metabolites in the form of essential oils (Andrea *et al.*, 2003). Citrus peel essential oils have a wide range of potential activities in food, perfumery, sanitary, cosmetics and pharmaceuticals (Mondello *et al.*, 2005).The most important applications of citrus peel essential oils is the presence of some bioactive compounds in them which serve as alternatives to the synthetic antioxidants (Tepe *et al.*, 2006; ViudaMartos *et al.*, 2008; Choi *et al.*, 2000). Citrus essential oils have been classified as generally recognised as safe (GRAS) due to their wide spectrum of biological activities such as antimicrobial (Fisher and Phillips, 2008), antifungal (Chutia, *et al.* 2009), antioxidant (Rehman, 2006), anti-inflammatory and anxiolytic (De Moraes *et al.* 2006).

Previous studies regarding the essential oil of *C. sinensis* from different locations were focused on the peel oil composition of VAL cultivar. For example, limonene (94.7%) and myrcene (2%) were reported as the main components of the Vietnamese VAL cultivar (Minh Tu *et al.*, 2002). Four years later, a study from Algeria reported that limonene (78.5%), myrcene (5.3%) and β -pinene (2.7%) were the typical components of the VAL cultivar peel oil (Ferhat *et al.*, 2006). Limonene (94.95%) and myrcene (1.87%) were reported as the basic components of the Italian VAL cultivar (Caccioni *et al.*, 1998). In an earlier study from California, Scora and Newman (1967) reported that the limonene content ranged from 34.74% to 58.54% in the same cultivar. In a study of four types of *Citrus* [*C. sinensis* (Malta), *C. sinensis* (Mousami), *C. paradise* and *C. limon*] Pakistani researchers, they found that the percentage of limonene extracted from the peels of the fruit (61.08, 76.28, 86.27 and 53.61) respectively (Ahmad *et al.*, 2006).

D-limonene is a colorless liquid at room temperature. The structural {1-methyl-4-(1-methylethenyl)cyclohexane} is a natural compound monocyclic monoterpene (Figure 1) and the main component of the oil extracted from Citrus peels (Sun, 2007) with chemo preventive and anti-tumor activities with odor lemon like and is a major constituent in several citrus oils (orange, grapefruit, lemon and lime etc.). due to its pleasant citrus aroma, D-limonene is used widely as a flavor, fragrance additive in perfumes, foods, beverages, chewing gum and soaps. Being a good solvent of cholesterol, d-limonene is used clinically to dissolve cholesterol-containing gallstones. Citrus fruit peels have volatile oil composed of d-limonene, gamma-aminobutyric acid, d-linalool and N-acetyl octopamine. Limonene contains sedative effects and anti-anxiety (Carvalho Freitas and Costa, 2002).

Materials and Methods

Extraction of volatile oils

The fruits peel of *C. sinensis* and *C. limon* were collected from the farms of Babylon Governorate in Iraq. Fresh fruits were peeled with a razor carefully to prevent damage to the oil glands. 100 g of peels were put in 200 ml distilled water into a Clevenger apparatus. The temperature of the device was set at 95°C for 3 hours. (Davidowski and Di Marco, 2009). After obtaining the volatile oils, they were sent to the Al-Razi Center for a medical examination GC/MS.

Gas Chromatography/ Mass Spectrophotometer (GC/MS)

The chemical analysis of plant extracts was carried out at the Iraqi Ministry of Industry's Ibn Al-Bitar Research Center, with an Agilent Technologies 7820A GC System and a Mass Spectrometer Analytical column for the same company's model 5977E. The operating conditions of the device were as follows:

- Separation column type HP-5ms ultra Inert By dimensions (30m length 250µm diameter 0.25mm internal diameter) that works in Electron Impact Mode 70-EV (Electron fixed Detector).
- As a carrier gas, helium gas (99.9% purity) was used

- The injected fluid volume is 1µl with a split ratio of 1.2 minutes.
- The injector temperature is 250 degrees Celsius, and the scan range is 50 to 500 m/z.
- The device pressure is 11.93 psi, and the average flow rate is 1.20 ml/min.
- The oven temperature starts at 80 degrees Celsius, then rises at a rate of 10 degrees per minute for 10 minutes to reach 180 degrees Celsius in ramp 1, and then rises at a rate of 10 degrees per minute for 10 minutes to reach 280 degrees Celsius in ramp 2. The temperature then rises at a rate of 5 degrees per minute to 300 degrees Celsius in ramp 3.
- The total time taken for sample analysis was 24 min.

Results and Discussions

The results of the study showed GC-MS analysis of the extract from the peels fruits the presence of 9 active compounds in type *C. sinensis* show in table (1) and Figure (1), as the results showed the main compounds in the orange type, which are (D-Limonene 90.41% , Geranyl isobutyrate 5.56% , Decanal 2.40%) , These results are slightly different from the results you get Ahmad *et al.* (2006) and Kamol *et al.* (2013) The slight difference is due to genetic differences, environmental conditions, soil type, and the amount of mineral elements in the soil .As for the *C. limon* Contains 21 active ingredients show in table (2) and Figure (2), the results showed the main compounds were (D-Limonene 28.97% , beta.- Bisabolene 11.53% , Geranyl acetate 10.68% , Citral 9.44 , Bicyclo(3.1.1)hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)- 9.40% and 2,6-Octadienal, 3,7-dimethyl-, (Z) 7.42%) , These results are slightly different from the results you get Ahmad *et al.* (2006) and Kamol *et al.* (2013) The slight difference is due to genetic differences, environmental conditions, soil type, and the amount of mineral elements in the soil .

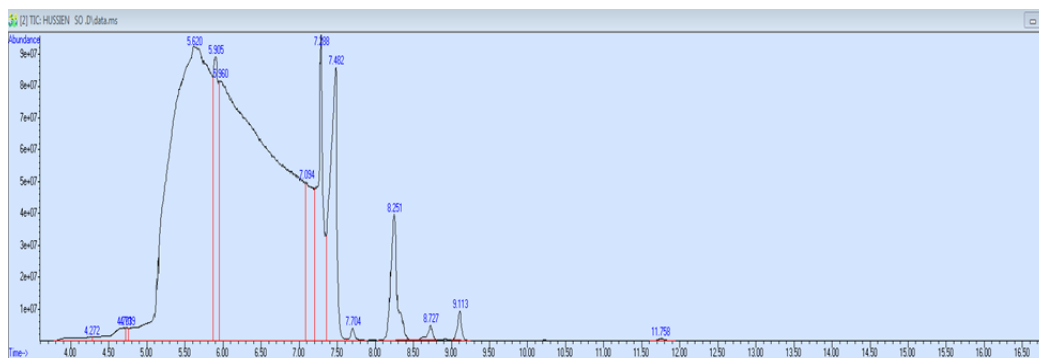

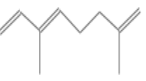
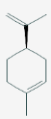

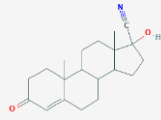


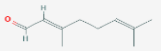


Figure 1. An outline of chemical compounds in Peel fruit of *C. sinensis*

Table 1
Components of Volatile Oil Extract of Peel *C. sinensis* of Using GC-MS

No	Chemical Names	Peak	R. time	Area %	Molecular Formula	Molecular Weight	Structure
1	(1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	1	4.270	0.24	C ₁₀ H ₁₆	136.234	
2	1,3,7-Octatriene, 3,7-dimethyl-(alpha-Ocimene)	2 3	4.699 4.741	0.57 0.08 0.65	C ₁₀ H ₁₆	136.234	
3	D-Limonene	4 5 6 7 8	5.620 5.906 5.959 7.092 7.288	34.13 4.30 43.49 3.52 4.97 90.41	C ₁₀ H ₁₆	136.234	
4	Geranyl isobutyrate	9	7.484	5.56	<u>C₁₄H₂₄O₂</u>	224.34	
5	Preg-4-en-3-one, 17.alpha.-hydroxy-17.beta.-cyano	10	7.701	0.15	<u>C₂₀H₂₇NO₂</u>	313.4	
6	Decanal	11	8.252	2.40	<u>C₁₀H₂₀O</u>	156.26	
7	Longipinene epoxide	12	8.707	0.15	<u>C₁₅H₂₄O</u>	220.35	
8	2,6-Octadienal, 3,7-dimethyl-, (E) (Citral)	13	9.115	0.33	<u>C₁₀H₁₆O</u>	152.23	

9	Trichloroacetic acid, 2-methyloct-5-yn-4-yl ester	14	11.758	0.04	$C_{11}H_{15}Cl_3O_2$	285.6	
---	---	----	--------	------	-----------------------	-------	---

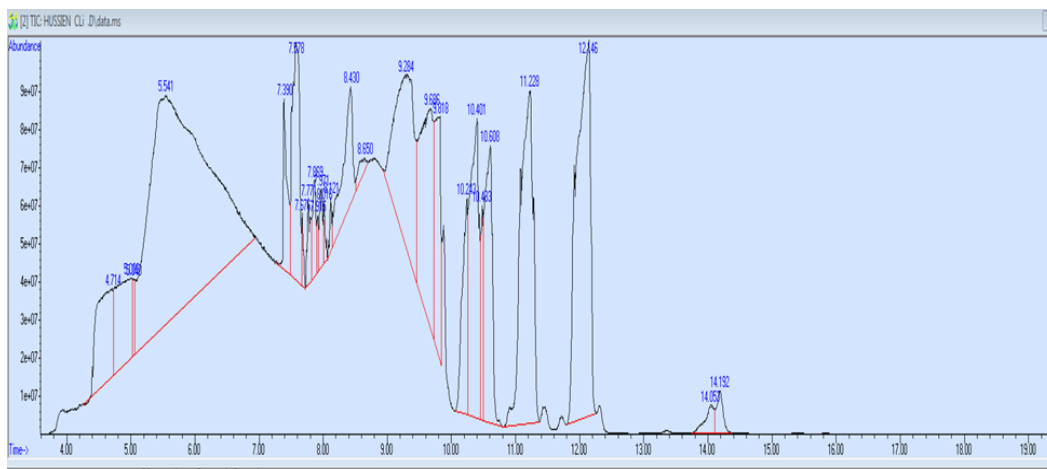







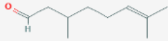



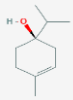
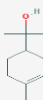
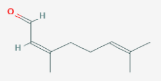
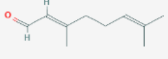
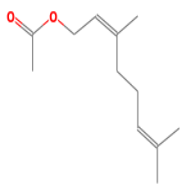
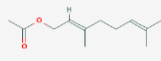
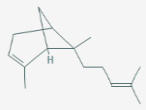
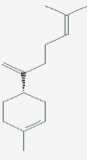



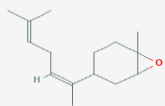
Figure 2. An outline of chemical compounds in the peel fruit of *C. limonimedica*

Table 2
Components of volatile oil extract of the Peel fruit of *C. limonimedica* of Using GC-MS

No	Chemical Names	Peak	R. time	Area %	* Molecular Formula	Molecular Weight	Structure
1	beta-Pinene	1 8	4.715 7.770	3.38 0.59 3.97	$C_{10}H_{16}$	136.234	
2	3-Carene	2	5.001	3.06	$C_{10}H_{16}$	136.234	
3	beta-Phellandrene	3	5.038	0.32	$C_{10}H_{16}$	136.234	
4	D-Limonene	4	5.541	28.97	$C_{10}H_{16}$	136.234	

5	Cyclohexene, 3-methyl-6-(1-methylethylidene)-	5	7.389	1.48	$C_{10}H_{16}$	136.234	
6	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-	6	7.579	3.93	C₁₀H₁₈O	154.25	
7	Bicyclo[2.2.1]hept-2-ene, 2,7,7-trimethyl-	7	7.675	0.27	C₁₀H₁₆	136.234	
8	Citronellal	9 10	7.871 7.913	0.77 0.19 0.96	C₁₀H₁₈O	154.25	
9	Thujone	11	7.971	0.56	C₁₀H₁₆O	152.23	
10	Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-, [1S-(1.alpha.,4.alpha.,5.alpha.)]	12	8.019	0.24		152.23	
11	9-Octadecyne	13	8.119	0.22	$C_{18}H_{34}$	250.5	
12	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (1R)-	14	8.432	2.55	C₁₀H₁₈O	154.25	

13	alpha.- Terpineol	15	8.649	0.30	C₁₀H₁₈O	154.25	
14	2,6- Octadienal, 3,7-dimethyl-, (Z)	16	9.284	7.42	C₁₀H₁₆O	152.23	
15	Citral	17 18	9.687 9.819	6.20 3.24 9.44	C₁₀H₁₆O	152.23	
16	-2,6- Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)	19 21	10.243 10.481	2.50 1.04 3.54	C ₁₂ H ₂₀ O ₂	196.286	
17	Geranyl acetate	20 22	10.402 10.608	5.87 4.81 10.68	C ₁₂ H ₂₀ O ₂	196.286	
18	Bicyclo(3.1.1) hept-2-ene, 2,6-dimethyl- 6-(4-methyl- 3-pentenyl)-	23	11.228	9.40	C₁₅H₂₄	204.35	
19	.beta.- Bisabolene	24	12.144	11.53	C₁₅H₂₄	204.35	
20	2- Pentadecyn- 1-ol	25	14.056	0.55	C₁₅H₂₈O	224.38	

21	trans-Z- .alpha.- Bisabolene epoxide	26	14.193	0.61	C₁₅H₂₄O	220.35	
----	---	----	--------	------	---	--------	---

Conclusions

The present study proved Citrus peels contain a large proportion of limonene acid, this percentage decreases or increases according to the type, and thus knowing the most appropriate type for extracting limonene as well as its assistance in the classification of species studied using chemotaxonomic techniques, especially the GC-MS technique, which represents an easy and direct analytical approach for the identification of phytoconstituents.

References

- Ahmad, M.M., S. Rehman, Z. Iqbal, F.M. Anjum and J.I. Sultan. (2006). Genetic variability to essential oil composition in four citrus fruit species. *Pak. J. Bot.*, 38: 319-324.
- Andrea, V., N. Nadia, R.M. Teresa and A. Andrea. 2003. Analysis of some Italian lemon liquors (Limoncello). *J. Agric. Food Chem.*, 51(17): 4978-4983
- Caccioni, D. R. L., Guizzardi, M., Biondi, D. M., Renda, A., & Ruberto, G. (1998). Relationship between volatile components of citrus fruit essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*. *International Journal of Food Microbiology*, 43, 73–79.
- Carvalho Freitas ,M. I. R., and Mirtes C. (2002). Anxiolytic and Sedative Effects of Extracts and Essential Oil from *Citrus aurantium* L., Pharmaceutical Society of Japan, *Biol. Pharm. Bull.* 25(12) 1629—1633.
- Choi, H.S., H.S. Song, H. Ukeda and M. Sawamura. 2000. Radical scavenging activities of citrus essential oils and their components: Detection Using 1,1-Diphenyl-2-picrylhydrazyl. *J. Agric. Food Chem.*, 48: 4156-4161.
- Chutia, M., Bhuyan, P. D., Pathak, M. G., Sarma, T. C., & Boruah, P. (2009). Antifungal activity and chemical composition of *Citrus reticulata* Blanco essential oil against phytopathogens from North East India. *LWT – Food Science and Technology*, 42, 777–780.
- Davidowski, S. ; Dimarco, B. (2009) The Extraction and Quantification of Limonene from *Citrus Rinds* Using GC/MS. PerkinElmer, Inc. Shelton, CT 06484 USA .
- De Moraes Pultrini, A., Galindo, L. A. I., & Costa, M. (2006). Effects of the essential oil from *Citrus aurantium* L. in experimental anxiety models in mice. *Life Sciences*, 78, 1720–1725.
- Ferhat, M. A., Meklati, B. Y., Smadja, J., & Chemat, F. (2006). An improved microwave Clevenger apparatus for distillation of essential oils from orange peel. *Journal of Chromatography A*, 1112, 121–126.
- Fisher, K., and Phillips, C. (2008). Potential antimicrobial uses of essential oils in food: Is citrus the answer? *Trends in Food Science & Technology*, 19, 156–164
- Kamal, G.M. , Ashraf, M. Y., Hussain, I.H. and Shahzadi, N. (2013). Antioxidant potential of peel essential oils of three Pakistani citrus species: Citrus

- reticulata, *Citrus sinensis* and *Citrus paradisi*. *Pakistan Journal of Botany*, Pak. J. Bot., 45(4): 1449-1454.
- Minh Tu, N. T., Thanh, L. X., Une, A., Ukeda, H., & Sawamura, M. (2002). Volatile constituents of Vietnamese pummelo, orange, tangerine and lime peel oils. *Flavour and Fragrance Journal*, 17, 169–174.
- Mondello, L., A. Casilli, P.Q. Tranchida, P. Dugo and G. Dugo. 2005. Comprehensive two-dimensional GC for the analysis of citrus essential oils. *Flavour Frag. J.*, 20: 136-140.
- Rehman, Z. (2006). Citrus peel extract – A natural source of antioxidant. *Food Chemistry*, 99, 450–454.
- Scora, R. W., & Newman, J. E. (1967). A phonological study of the essential oils of the peel of Valencia oranges. *Agricultural Meteorology*, 4, 11–26.
- Sun , J. (2007). D-Limonene: Safety and Clinical Applications, *Alternative Medicine Review Volume 12*, Number 3 , Page 259-264.
- Tepe, B., H.A. Akpulat, M. Sokmen, D. Daferera, O. Yumrutas, E. Aydin, M. Polissiou and A. Sokmen. 2006. Screening of the antioxidant and antimicrobial properties of the essential oils of *Pimpinella anisetum* and *Pimpinella flabellifolia* from Turkey. *Food Chem.*, 97: 719-724.
- Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernánde z-Lópe z and J. Pe´rez A´lvarez. 2008. Antifungal activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils. *Food Control*, 19 (12): 1130-1138.