BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, August 2014. Vol. **11**(2), 895-899

Analysis of Aroma Active Constituents, Antioxidant and Antimicrobial activity of *C. sinensis,* Citrus *limetta* and *C. limon* Fruit Peel Oil by GC-MS

Monika Gupta[1]*, Pankaj Gularia[1], Devi Singh[1] and Sudhakar Gupta[2]

[1]Department of Chemistry, [2]Department of Zoology,
Lovely Professional University, Phagwara - 144402, India

doi: http://dx.doi.org/10.13005/bbra/1355

(Received: 10 April 2014; accepted: 22 May 2014)

Citrus **peel essential oils have an impressive range of food and medicinal uses. In the present study we investigated the variation in the yield and chemical composition of the essential oils isolated from fresh peels of three *Citrus* species namely *Citrus sinensis, C. limetta* and *C. limon.* The chemical composition of the volatile fraction obtained from the peels of *C. sinensis* by gas chromatography–mass spectrometry (GC—MS). The main components were limonene (96.05%) followed by Phenol,2-methoxy-3(2-propenyl) (11.79%) and α-Terpineol (50.25) whereas β sinesol(0.01%), n- tetradecane (0.01%), 3Allyl 6 methoxyphenol (1.0%) and Phenol-2ethyl4,5-dimethyl (3.30%) were as minor compounds, respectively obtained by hot extraction. Variation is also found in the free radical activity and microbial activity of citrus *sps*. peel oil.**

Key word : GCMS analysis, citrus peel oil, free radical activity and microbial activity.

The genus *Citrus* (Rutaceae) is represented by the species *Citrus sinensis, C. limetta* and *C. limon*. Citrus fruits are the most common subtropical crops in the world. The essential oils of *Citrus sps*. are placed within the glands in the outer layer of the fruit skin. This oil is composed of many constituents, including monoterpenes, sesquiterpenes, alcohols, esters and aldehydes. Among the given three species most valuable oils are those of orange and lemon. Several studies were performed on the composition of the essential oils from leaves and peel of *C. sinensis* and its combination and on their biological activities[1-9]. The GCMS is the key tool for identification of secondary metabolites of volatile oil separated by a variety of isolation techniques[10-12].

Citrus peel essential oils is one of the rich sources of bioactive compounds namely coumarins, flavonoids, carotenes, terpenes and linalool etc.13. Essential oils of *Citrus* peels are medicinally very important and show variety of biological effects because they

are rich in flavonoids (flavone, flavonol and flavanone), terpenes, carotenes and coumarines which are responsible for antimicrobial activity[14]. A lot of studies have been reported in the literature showing the quality and characteristics of essential oils from aromatic plants[15-16] however, to the best of our understanding, no such comparative study investigating the yield and chemical composition of essential oils, isolated from peels of different *Citrus* species. The main objective of the present study was to observe the changes in the yield and chemical composition of essential oils of three *Citrus* species from himachal region.

* To whom all correspondence should be addressed.
E-mail: dr.guptmonika@gmail.com

EXPERIMENTAL

Plant material

The fruits of *Citrus sinensis, C. limetta* and *C. limon* were collected from Hoshiyarpur, Province of Himachal Pradesh in January 2014. The material plant was identified and a voucher specimen was deposited at the IHBT Palampur.

Isolation of the essential oil

The samples of fresh *Citrus* peels were subjected to hydro-distillation for 3 hour using a Clevenger- apparatus. Distillates of essential oils were dried over anhydrous sodium sulfate, filtered and stored at -4°C until analysed[17].

Gas chromatography–mass spectrometry analysis

The GC–MS analyses were performed on a Thermo Scientific TSQ 8000 Gas Chromatograph - Mass Spectrometer. This mass spectrometer comes paired with the TRACE 1300 GC along with auto-sampler for automated sample handling.

The analytes were separated in RT: 3.00 30.10 SM: 15G. The split/ splitless injector temperature was set at 400 oC and volume was 1.0 µl.

Antioxidant activity of *Citrus sinensis, C. limetta* and *C. limon* peels oil

Antiradical activity was evaluated by measuring the scavenging activity of the examined *Citrus sinensis, C. limetta* and *C. limon* peel oil on the 2, 2- diphenyl-1-picrylhydrazil (DPPH) radical. The DPPH assay was performed as described by Epsin 19. The samples (100 µl each) were mixed with 3 ml of DPPH solution. The absorbance of the resulting solutions and the blank (with only DPPH and no sample) were recorded after an incubation time of 30 min at room temperature against ascorbic acid as a positive control. For each sample, three replicates were recorded. The disappearance of DPPH was measured spectrophotometrically at 517 nm. The percentage of radical scavenging activity was calculated using the following equation.

DPPH scavenging effect (%) = (A0- A1)/A0 ×100

Where, A0 is the absorbance of the control at 30 min and A1 is the absorbance of the sample at 30 min.

Antibacterial and antifungal activities of *Citrus sinensis,* C. limetta and *C. limon peel oil*

The agar disc diffusion method was employed for the determination of antibacterial and antifungal activity of *Citrus sinensis, C. limetta* and *C. limon* peel oil applying the procedure of Baydar[18]. The growth medium were prepared, autoclaved and transferred aseptically to sterilized petri plates. Microbial cultures collected from the biotech lab with in the campus, in sterile condition were transferred to the petri plates. Sterile and dried 6 mm paper discs were soaked with sterilized freshly extracted *Citrus sinensis, C. limetta* and *C. limon* peel oil. These oil- soaked discs were dried under laminar flow cabinet. The discs were placed on freshly seeded microbial lawns (3 discs in each plate) with a control. All experiments were conducted in triplicate. The petri plates were incubated at their respective temperatures and zones of inhibition thus developed against tested microorganisms were measured in millimeters after a period of bacteria and 48 h for other fungi and similarly at 48 and 96 h. The results of antimicrobial activity of peel oil against different micro-organisms were expressed as resistant, intermediate and sensitive.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The volatile components of fruit peel of *Citrus sinensis, C. limetta* and *C. limon* that were isolated are listed in Table 1, 2 and 3.

Table 1. Compounds in GCMS *Citrus sinensis*

S. No	Name of coompound	RTmin	% Area
1	Octane	3.85	0.04
2	Nonane	4.80	0.03
3	Limonene	5.51	96.05
4	Sabinene	6.65	0.65
5	γ-Terpinene	8.21	0.04
6	3cyclohexene 1-methanol	9.52	0.20
7	Geraniol	11.29	0.64
8	Ho-trienol	12.66	0.35
9	Germacrene B	14.26	0.31
10	Caryophyllene oxide	15.50	0.52
11	Beta -Eudesmol	17.20	0.01
12	α- humulene	18.35	0.02
13	n-iridecane	19.44	0.01
14	Phytol	20.55	0.03
15	β sinesol	21.61	0.01
16	unknown	23.02	0.01
17	n- tetradecane	24.14	0.01
18	n- hexadecane	25.48	0.02
19	n-tridecane	27.23	0.03
20	n- pentadecane	29.21	0.02

Antioxidant activity of *Citrus sinensis, C. limetta* and *C. limon* peel oil

The ability of essential oils to act as a donor for hydrogen atoms or electrons in the transformation of DPPH radical into its reduced form DPPH-H (which is measured spectrophotometrically) gives them antioxidant activity characteristic. The results of DPPH scavenging activity of *Citrus sinensis,* C. limetta and *C. limon* peel oil compared with ascorbic acid as a reference standard are shown in Figure 1, Table-4 indicating that it has slightly lower antioxidant activity comparative to reference standard, ascorbic acid, being a strong anti- oxidant reagent. The essential peel oil of *Citrus sinensis,* C. limetta and *C. limon* peel oil was able to reduce the stable radical DPPH to yellow-colored DPPH-H reaching 87.77% of DPPH scavenging effect at its 100% concentration whereas the reference standard, ascorbic acid, gave a 99.67% DPPH scavenging effect at its 100% concentration[23].

Table 2. Compounds in GCMS of *C. limetta*

S. No	Name of compound	RT min	Area%
1	α- Pinene	4.82	1.99
2	Cyclohexene, 1-methyl 5 (1-methyl ethenyl)(R)	5.53	74.19
3	linalyl anthranilate	6.68	7.50
4	Terpinen4-ol	8.02	1.85
5	α- Terpineol	8.23	2.31
6	Phenol, 2 methoxy3 (2 propenyl)	10.70	11.16
7	3Allyl 6 methoxyphenol	12.86	1.00

Table 3. Compounds in GCMS of peel oil *C. limon*

S. No	Name of compound	RT (min)	Area%
1	Cyclohexene 1methyl 4(1methylethenyl) S	5.50	10.47
2	1,6 Octadien3ol,3,7dimethyl	6.65	3.27
3	1,4Cyclohexadiene,1methyl	7.83	3.86
4	Terpinen 4-ol	8.01	8.61
5	Phenol, 2ethyl 4,5 dimethyl	8.11	3.30
6	α-Terpineol	8.21	50.25
7	1,4dihydroxy p-menth 2 ene	9.44	4.69
8	3Cyclohexene1methanol,2 hydroxy α, α, 4 trimethyl	9.65	6.23
9	Cyclohexene, 3acetoxy4(1hydroxy1 –methylethyl)-1 methyl	9.94	9.31

Table 4. Antioxidant activities of *Citrus sinensis, C. limetta* and *C. limon*

S. No.	Sample O	Absorbance	λ max
1	DPPH (A blank)	0.744	514nm
2	5 mg C.sinensis	0.011	514 nm
3	10 mg C.sinensis	0.014	514 nm
4.	15 mg C.sinensis	0.015	514 nm
5.	5 mg C. limetta	0.002	514 nm
6.	1o mg C. limetta	0.005	514 nm
7.	15 mg C. limetta	0.007	514 nm
8.	5 mg *C. limon*	0.016	514 nm
9.	10 mg *C. limon*	0.017	514 nm
10	15 mg *C. limon*	0.034	514 nm

Antibacterial and antifungal activities of *Citrus sinensis, C. limetta* and *C. limon peel oil*

The results of antibacterial and antifungal activity of *Citrus sinensis, C. limetta* and *C. limon* peel oil, observed against different *Salmonella typhimurium, E-coli* , *Aspergillus fumigates* and *Penicillium chrysogenum* Many other studies also revealed that α-pinene, limo-nene and linalool have a strong antibacterial activity20-22. Essential oils usually occur as complex mixtures and their activity can generally be accounted for in terms of their major monoterpenoid components. In addition, some com-ponents that occur in lesser amount may also contribute to the antimicrobial

Table 5. Antimicrobial Activity of *Citrus sinensis, C. limetta* and *C. limon* peel oil

S.No.	Peel Oil	Bacteria (mic)	Bacteria (mic)	Fungi (mic)	Fungi (mic)
1.	*C. sinensis*	*S. typhimurium (9*mm)	*E. coli* (5 mm)	*A. fumigates* (4mm)	*P. chrysogenum* (7mm)
2.	*C. limon*	*S. typhimurium* (10mm)	*E. coli* (4mm)	*A. fumigates* (2)	*P. chrysogenum* (1mm)
3.	*C. limon*	*S. typhimurium (6mm)*	*E. coli* (7mm)	*A. fumigates* (4)	*P. chrysogenum* (2)

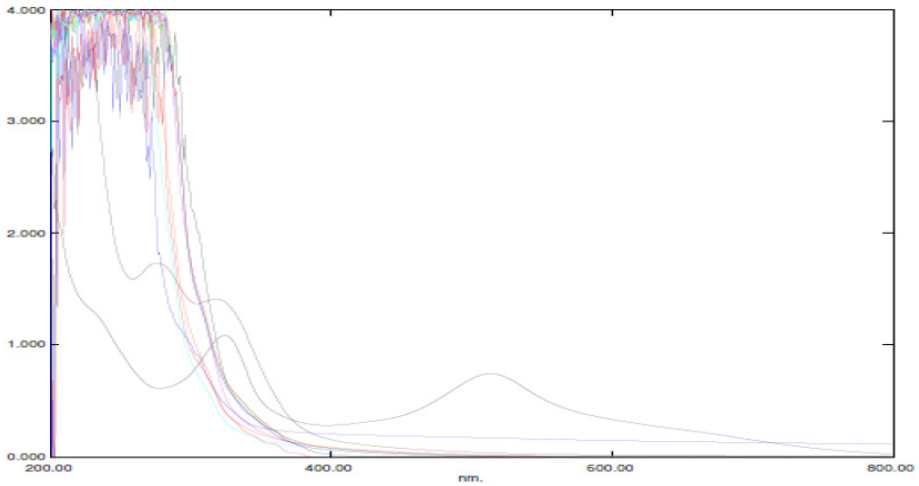

Fig. 1. UV Analysis for the Antioxidant properties of *Citrus sinensis, C. limetta* and *C. limon*

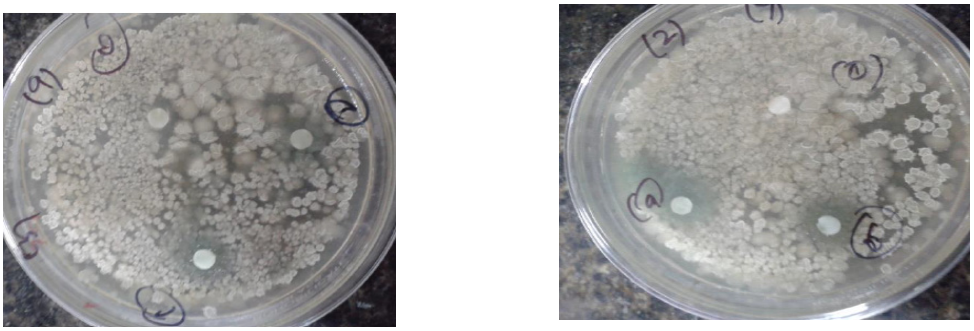

Fig. 2. Antibacterial activity of *Salmonella typhimurium and E-coli* against n-hexane extract of *Citrus sinensis, C. limetta* and *C. limon*

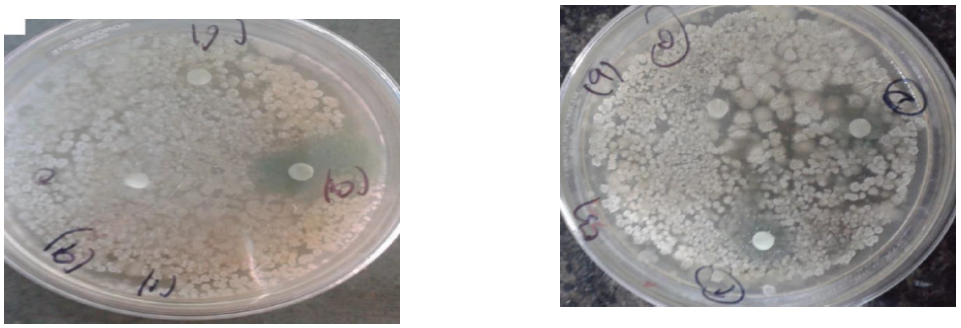

Fig. 3. Antifungal activity of *Aspergillus fumigates* and *Penicillium chrysogenum* against n-hexane extract of *Citrus sinensis, C. limetta* and *C. limon*

activity of the oil Table 5, figure 2 and 3.

CONCLUSION

In conclusion, the Chemical analysis of essential oil extracted from *Citrus sinensis, C. limetta* and *C. limon* showed that limonene (96.05%) was found as major component followed by Phenol,2methoxy3(2 propenyl) (11.79%) and α-Terpineol (50.25) whereas β sinesol(0.01%), n- tetradecane (0.01%), 3Allyl 6 methoxyphenol (1.0%) and Phenol, 2ethyl 4,5 dimethyl (3.30%) were as minor compounds. The results of our study showed that *Citrus sinensis, C. limetta* and *C. limon* peel oil have the probability to be applied as a natural constituent of food preservations, cosmetics and medi-cines as they exhibit a strong antioxidant, antibacterial and antifungal activity against food borne pathogens.

REFERENCES

1. Mozaffarian, V., A dictionary of Iranian plants names, Farhang Mo'aser Publishers, Tehran, Iran, 1998
2. Singh, P., Shukla, R., Prakash, B., Kumar, A., Singh, S., Mishra, P. K., Dubey, N. K., *Food Chem. Toxicol.,* 2010; **48**: 1734.
3. To, N. G., Liu, Y. J., Zhang, M. L., *Int. J. Food Sci. Technol.,* 2009; **44** : 1281.
4. Njoroge, S. M., Phi, N. T., Sawamura, M., *J. Essential Oil Bearing Plants.,* 2009; **12**: 26.
5. Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J., Pérez-Álvarez, J., *Food Control.,* 2008; **19** : 1130.
6. Araújo-Junior, C. P., Dacamara, C. A. G., Ribeiro, N. C., Gomes, C. A., Moraes, M. M. de, Botelho, P. S., *Nat. Prod. Commun.,* 2010; **5**: 471.
7. Ribeiro, N. C., Dacamara, C. A. G., Born, F. S., Siqueira, H. A. A.,*Nat. Prod. Commun.* 2010; **5**: 1819.
8. Njoroge, S. M., Koaze, H., Karanja, P.N., Sawamura, M., *Flavour Fragr. J.,*2005; **20**: 80.
9. Trozzi, A., Verzera, A., Lamonica, G., *J. Essent. Oil Res*., 1999; **11**: 482.
10. Adams, R. P. Identification of essential oil components by gas chromatography / quadrupole mass spectroscopy, Allured Publishing Corp., Carol Stream, IL, USA, 2004.
11. Gupta, M., Gupta. A and Gupta, S. Phytochemical Analysis of Hot Petroleum ether Extracts of *Piper nigrum. Current World Environment*. 2013; **8**(1): 151-152.
12. Gupta, M.,Gupta. A and Gupta, S. Phytochemical Analysis of Cold Toluene Extracts of *Piper nigrum* and its Antibacterial and Antifungal Activity. *Oriental Journal of Chemistry.* 2013; **29**(2): 559-563.
13. Mondello, L., Casilli, A., Tranchida, P. Q., Dugo, P., Dugo, G. Comprehensive two- dimensional GC for the analysis of *Citrus* essential oils. *Flavour and Fragrance Journal.* 2005; **20**: 136-140.
14. Tepe, B., Daferera, D., Sokmen, A., Sokmen, M., & Polissiou, M..Antimicrobial and antioxidant activities of the essential oils and various extracts of Salvia tomentosa Miller (Lamiaceae). *Food Chemistry.* 2005; **90**:333-340.
15. Asekun, O. T., Grierson, D. S. and Afolayan, A. J. Effect of drying methods on the quality and quantity of the essential oil of *Mentha longifolia* L. Subsp. *Capansis*. *Journal of Essential Oil Research*. 2007a; **101**(3): 995-998.
16. Asekun, O. T., Grierson, D. S. and Afolayan, A. J. Characterization of essential oils from *Helichrysum odoratissimum* using different drying methods. *Journal of Applied Science.* 2007b; **7**: 1005-1008.
17. Hussain, A.I., Anwar, F., Sherazi, S.T.H., Przybylski, R.Chemical composition, antioxidant and antimicrobial activeities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food Chemistry*. 2008; **108**: 986-995.
18. Baydar H, Sagdic O, Ozkan G, Karadogan T. Antibacterial activity and composition of essential oil from *Origanam*, *Thymbra* and *Satureja* species with commercial importance in Turkey. *Food Conf.* 2004; **15**: 169-172.
19. Epsin JC.,Soler-Rivas C., Wichers HJ. Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2, 2-diphenyll-1-picrylhydrazyl radical. *J. Agri. Food Chem.* 2000; **48**: 648-56.
20. Mecciaa G, Luis B, Judith V, Tulia D, Alfredo U.. Composition and antibacterial screening of the essential oils of Leaves and Roots of *Espeletiopsis angustifolia* Cuatrec. *Nat. Product Comm*. 2007; **2**: 1221-1224.
21. Filipowicz N, Kamiñski M, Kurlenda J, Asztemborska M. Antibacterial and antifungal activity of juniper berry oil and its selected components. *Phyt. Res*. 2003; **17**: 227-231.
22. Koji YK, Yamamoto T, Kawai Y, Inoue N. Enhancement of antilisterial activity of essentials oil constituents by nisin and diglycerol fatty acid ester. *Food Micro.*2004;**21**: 283-289.
23. Mahmud S, Saleem M, Siddiqui S, Ahmad R, Khanum R, Parveen Z. Volatile components, antioxidant and antimicrobial activity of Citrus acida var. sour lime peel oil. *J. Saudi Chem. Soc.* 2009; **12**: 195-198.