RESEARCH ARTICLE

Hydrodistillation and Soxhlet Method of Extraction and Analyses of Chemical Composition of Citrus Peel Essential Oil

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Abstract

The peel which is the primary waste of citrus fruit processing is usually discarded. But these peels are good source of pectin, phenols, flavonoids, and limonene. The peel can be used to extract essential oil that can be used in food system. The present study aimed to extraction and analyse the chemical composition of citrus peel essential oil by hydrodistillation and soxhlet methodThe optimum condition for essential oil extraction by hydrodistillation method was standardized and the oil extraction was efficient at 95 °C. The extraction time was 3 hours with solid solvent ratio of 1:10 (g sample/mL of water). d-Limonene is the major compound present in all three citrus peel essential oils extracted by both the methods followed by β myrcene, α pinene, β pinene, and γ terpinene. Essential oil from fresh citrus peel contains many other compounds such as neral, caryophyllene, β bisbolene, etc. These bioactive volatile compounds are responsible for the antioxidant and antimicrobial properties of the essential oil.

Keywords: *Citrus peel; Essential oil; Hydrodistillation; Soxhlet extraction; Limonene*

INTRODUCTION

Citrus is one of the major horticulture crops produced and traded all over the world. China is the world's top producer of citrus fruits with about 44.6 million tonnes that accounting for 28.21 % of the world's citrus fruit production. Brazil, India, Mexico, and the United States of America are the other major citrus-producing countries that contribute 59.45 % of the total production. The world's total citrus fruit production in the year 2020 was 158 million tonnes.

Citrus fruits contribute about 14 % of total fruit production in India with 33 %, banana 21 % mango and 6% papaya production (Chavan *et al*., 2018). Citrus belongs to the family Rutaceae and comprises 17 species which include (sweet oranges (*C. sinensis*), mandarins (*C. unshi, C. tangerine, C. reticulate, C. clementine*), sour/bitter oranges (*C. seville, C. aurantium*), lemons (*C. limon*), limes *(C. aurantifolia and C. latifolia)*, grapefruit (*C. paradisi*), and pummelos (*C. grandis*)). Three kinds of citrus produced in India (Mandarin (Kinnow, Nagpur, Coorg, and Khasi), Sweet orange (Mosambi, Jaffa, Malta, and Satgudi), and lime/lemon) have gained focus for cultivation and commercialization (Turner and Burri, 2013). The average production of mandarin is 6.4 million metric tons, lemon is 3.7 million metric tons and sweet orange is 3.53 million metric tons in India during the year 2021 (Statista, 2022).

These fruits are the source of many beneficial nutrients such as vitamin C, folic acid, potassium, and pectin which are beneficial to human beings. Citrus fruits can be consumed as such or as juices. The peel which are the primary wastes that are discarded after processing or consumption. This peel contains a wide variety of secondary components with substantial antioxidant and antimicrobial activity when compared with other parts of the fruit (Kodagoda and Marapana, 2017). It is a good source of phenols, flavonoids, and limonene (Rafiq *et al*., 2018). The peel can be used to extract the essential oil.

Citrus essential oil is mainly present in the flavado portion of the peel and present in lower quantities in leaves, flowers, fruits, and seeds. The essential oil possesses germicidal, antioxidant, antimicrobial, and anticarcinogenic properties (Kademi & Garba, 2017 and Pathak *et al*., 2017).

Essential oils are natural extract that contains volatile and complex compounds characterized by a highly stimulating odour. These are produced as a secondary metabolite of aromatic plants and can be extracted from various parts of the plants such as leaves, seeds, or fruit peel. Essential oils are a rich source of flavonoids, coumarins, limonoids, carotenoids, phenolic acid, and many polymethoxylated flavones (Bakkali *et al*., 2008). This essential oil can be extracted either by conventional (hydrodistillation, steam distillation, hydro diffusion, solvent extraction) or advanced (supercritical fluid extraction, subcritical extraction liquid, solvent-free microwave extraction) methods. The advantages of using an advanced method for essential oil extraction than conventional methods are less extraction time, low energy consumption, low solvent used, and less carbon dioxide emission (Aziz *et al*., 2018).

For this study, three different citrus fruits ((Lemon (*Citrus limon*), Mosambi (*Citrus limetta*), and Mandarin orange (*Citrus reticulata*) were selected and the essential oils were extracted by conventional soxhlet method and hydrodistillation method and the chemical compounds were analyzed.

Materials and methods

Procurement and processing of citrus peel

Orange, mosambi, and lemon peel were procured from the local juice shops of Madurai. The citrus peels were separated from the endocarp and cut into small pieces (approximately 1×1 cm).

Sun drying

Samples were spread over a clean white cloth and kept under the direct sun for 48 hours until the moisture content reached 10 %. Drying occurs by the radiation of the sun transmitted through the surface of the material under temperature ranges of 15 to 37 °C (15 to 20 °C at night and 30 to 37 °C during day). The dried samples were powdered and sieved to get uniform size particles.

Shade drying

Samples were kept in a dark and dry room (25±5 °C) with no direct sunlight for about 60 hours until the moisture content reached 10 %. These dried samples were powdered and sieved to get uniform size particle.

Cabinet drying

Samples were kept in a cabinet drier for about 8hrs at 60 °C until the final moisture content reached 10 %. The dried sample was powdered using a blender and sieved to get the uniform size particles.

These powdered samples were taken for essential oil extraction.

Extraction of citrus essential oil

Hydro distillation method

Hydro distillation was carried out in Clevenger apparatus. The sample material was directly immersed in water. The solid-liquid mixture was heated until boiling under atmospheric pressure. The volatile substance present in the sample material evaporated along with the steam generated by the water. This azeotropic mixture would be condensed and separated by its density difference and immiscibility (Li *et al*., 2014).

Optimum condition was standardized using time (60, 120, 180 and 240 minutes), temperature (75, 80, 85, 90, 95 and 100 °C) and solid-solvent ratio (1:5, 1:10, 1:15 and 1:20 g sample/ mL of water). The extraction was carried out for 3 hours from the first drop of distillate until the amount of essential oils stabilized. The essential oils were dried with anhydrous sodium sulfate and stored at a low temperature (<5 °C) until gas chromatography coupled to mass spectrometry (GC-MS) analysis.

Calculation of oil yield

The yield of the extracted oil was calculated using the given formula (Eqn.1).

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

(Equation 1)

Sensory analysis of the essential oil

Sensory properties such as color, odour, consistency, and clarity of the essential oil were analyzed by semi-trained panel members of Community Science College and Research Institute, Madurai.

Soxhlet Extraction Method

Solvent extract of the citrus peel was obtained from an Automated Soxhlet extractor with a circulating refrigerator water bath. 75 grams of dry citrus peel powder was directly taken into the fat thimble and 200 mL of the solvent (hexane- HPLC grade) was added into the flask and heated to boiling point. The extraction was carried out for 6 hours at 8 °C. Excess solvent was evaporated using a vacuum rotary evaporator to get the citrus peel oil. Essential oil extract was stored under refrigeration conditions (+4 °C) until gas chromatography coupled to mass spectrometry (GC-MS) analysis (Hasibuan and Gultom, 2021).

Volatile compounds analysis- GC-MS

Gas Chromatography- Mass Spectrometry analysis was carried out to analyze the volatile compounds present in the citrus peel essential oil. The analysis was carried out in Shimadzu QP 2020 GC - MS System, fitted with Rxi - 5 Sil MS fused silica column cross bonded with 1, 4 - bis (dimethylsiloxy) phenylene dimethyl polysiloxane. The column length and inner diameter is 30 meters and 0.25 µm. The film thickness was 0.25. The maximum temperature limit for the column was 320 °C. Ultra-high purity helium (99.99%) was used as carrier gas at a constant flow rate of 1.0 mL/ min. The injection, transfer line and ion source temperatures were 260 °C, 290 °C, and 230 °C. The ionizing energy was 70 eV. Electron multiplier voltage was obtained from auto-tune. The oven temperature was programmed from 60 °C (hold for 2 min) to 290 °C at a rate of 5 °C / min. 1.0 µL of the sample was injected into the system in split mode and the split ratio of 30: 1. The solvent cut-off time is 4 min. All data were obtained by collecting the full-scan mass spectra within the scan range 45-550 amu. The identification and characterization of chemical compounds in citrus peel essential oil were based on GC retention time. The obtained mass spectra were computer matched with those of standards available in NIST mass spectrum libraries.

Result and Discussion

Citrus peel essential oil was extracted by hydro distillation method by keeping time, temperature, and solid solvent ratio as treatment variables and the essential oil obtained as treatment effect. The volatile compounds present in the essential oil by hydrodistillation method and soxhlet method were analyzed by GC-MS.

Oil recovery from different drying methods of sample

The citrus essential oil was extracted from sun-dried, shade dried and cabinet-dried samples and the results were given in Figure 1. It was revealed that the oil recovery from shade-dried samples was higher than that of sun-dried and cabinet-dried samples. The oil recovery was 2 mL/100 g for orange peel and mosambi peel and 1.5 mL/100 g of lemon peel. This result was in agreement with the study of Farahmandfar *et al.,* (2020). It was reported that an increase in oil yield in bitter orange peel by shade drying method when compared with other drying methods. Khalid *et al*., 2008 also reported that the essential oil recovery was higher in the shade drying (0.29 %) method than in the sun drying (0.13 %) and oven drying (0.18 %) method. This may be due to easy evaporation of volatile compounds of essential oil at higher temperatures (30-90 °C) and higher oxidation of other compounds which leads to a reduction in essential oil yield in plant species.

Standardization of extraction temperature, time and solid-solvent ratio

Essential oil from citrus peels was extracted by hydro distillation method. The extraction condition was standardized using time, temperature, and solid-solvent ratio (g/mL).

Effect of temperature in hydro distillation of citrus peel for essential oil extraction

In the first phase, time (3hr) and solid-solvent solvent ratio (1:10) were kept constant whereas temperature was gradually increased by 5 °C from 75-100 °C. Figure 2 represents the oil recovery at varying temperature. Oil recovery was higher at 95 °C, after that the yield decreases. Likewise, Sikdar *et al.,* in 2016 reported a higher yield of sweet lime by steam distillation at 98 °C. Automatik and Minyak (2013) also stated that the oil recovery was higher at higher temperatures (4.26 % w/w at 90 ^{0}C) than at lower temperatures (1.87 % at 80 ^{0}C, 2.19 % at 85 ^{0}C).

Effect of time in hydro distillation of citrus peel for essential oil extraction

In the second phase, temperature (95 °C) and solid-solvent ratio (1:10) was kept constant whereas time was taken at 1 hour interval (60, 120, 180, and 240 minutes). Figure 3 denotes that the oil recovery was higher at 180 minutes and after that, there was no increase or decrease in the oil yield. Kamal *et al.,* 2013 and Javed *et al.*, 2014 also found a similar result of a higher yield of citrus peel essential oil during 3 hours of extraction by hydrodistillation method.

Effect of solid-solvent ratio in the hydro distillation of citrus peel for essential oil extraction

In the final phase of extraction, time (180 minutes) and temperature (95 °C) are kept constant, whereas the solid solvent ratio was taken as 1:5, 1:10, 1:15 and 1:20 (g sample/mL of water). Figure 4 represents the oil yield at varying solid-solvent ratios. The oil recovery was higher at 1:10 solid-solvent ratios. This result was in agreement with Ferhat *et al.*, 2007. 100 g of sample was extracted with 1000 mL of water for about 3hr to obtain maximum essential oil from citrus fruits.

Sensory parameters of citrus essential oils

Sensory parameters such as color, odor, consistency a clarity were evaluated and the result was given in Table 1. Extraction of orange peel and mosambi peel gave a clear colourless oil whereas the lemon peel oil was clear pale yellowish in color. There was a peculiar lemony odor in the lemon peel oil. The consistency of citrus peel essential oil was like that of water as most of essential oil has a consistency like water.

Volatile compounds of citrus peel essential oils (GC-MS analysis)

Volatile components present in fresh and dried citrus peel essential oil (by hydrodistillation method and soxhlet extraction method) were analyzed by GC-MS. GC-MS results revealed that the essential oil had a mixture of monoterpene hydrocarbon, sesquiterpene hydrocarbon, and oxygenated monoterpene. The major compounds present in the essential oils is given in Table 2.

GC-MS peak of orange peel oil was denoted in Figure 5. d-Limonene was the major compound present in all three essential oils. The limonene content of the orange peel oil extracted by hydro distillation method was 90.33 %, Linalool (1.96 %) was the second major compound present in orange peel oil followed by β bisbolene (1.77 %), γ terpinene (1.60 %), α pinene (1.59 %), β pinene (1.48 %) and β myrcene (1.16 %). The result was in agreement with Njoroge *et al.*, 2006. It was reported that the limonene content of the Mandarin (Citrus reticulata Blanco) Peel Oil from Burundi was 84.8%. Other major compounds were γ-terpinene (5.4 %), β myrcene (2.2 %), and α-pinene (1.1 %). Minh Tu *et al.*, 2002 also reported that the limonene content of Citrus reticulata Blanco var. tangerine was 95.1 %. Hosni *et al.*, 2010 studied the composition of peel essential oils from four selected Tunisian citrus species. It was mentioned that the major components of the essential oil from Citrus reticulata were limonene (92.6 %), γ-terpinene (3.39 %), β-pinene (1.55 %), and α-pinene (0.61 %).

Figure 6 indicated the volatile compounds present in the mosambi peel oil. d-Limonene content of mosambi peel oil was 88.57 %, α pinene was 3.14 %, linalool was 2.37 % and β pinene was 2.16 %. Khan *et al.*, 2012 reported that the limonene content of Citrus limetta was about 69.1%. Colecio-Juárez *et al*., 2012 also evaluated the chemical composition of essential oil from sweet lime (Citrus limetta Risso) and mentioned that the limonene content of the oil was 77.7 % followed by β pinene 6.48 %, linalool 1.98 % and β myrcene 1.68 %

Volatile compounds of lemon peel oil analysed by GC-MS were given in Figure 7. Lemon peel oil contained 54.39 % of d-Limonene. γ terpinene was the second major compound present in the essential oil at about 13.39 %. α pinene, β pinene, and β myrcene were the other major compounds that occupy about 6.78, 5.28, and 2.23 % of the peak area, respectively. Other than these compounds, lemon peel oil contained many other active compounds such α farnesene (1.25 %), β bisbolene (1.09 %), α caryophyllene (1.16 %), α bergamotene (1.66 %), α terpineol (1.44 %) and neral (1.58 %). This result was in agreement with Kirbaşlar *et al.,* 2009. The major components of Turkish lemon peel oil were limonene (61.8 %), γ-terpinene (10.6 %), and βpinene (8.1 %) and they also had other components such as β-bisabolene (1.6 %), α bergamotene (1.0 %) and β-caryophyllene (0.7 %). Caputo *et al.,* 2020 evaluated the volatile components of lemon (Citrus limon) peel essential oil. It was indicated that the limonene content of the oil was 57.65 %, γ terpinene was 10.45 % and β pinene was 9.31 %. Paw *et al.*, 2020 reported that the limonene content of Citrus *limon* L. Burmf peel essential oil was 55.40 %.

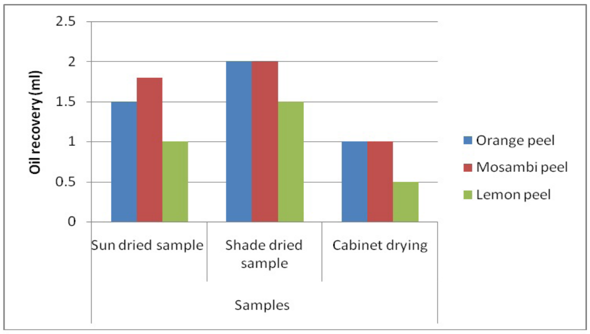

Figure 1. Oil recovery from different drying methods of sample

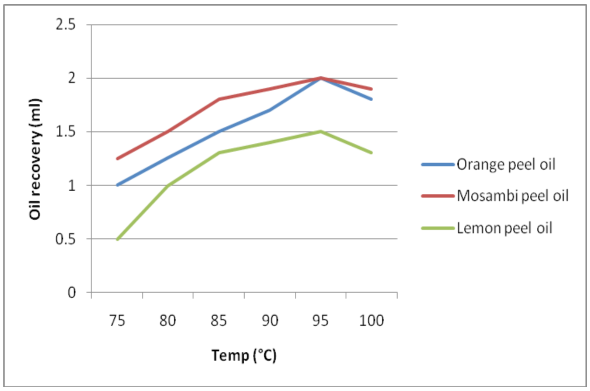

Figure 2. Effect of temperature on oil recovery

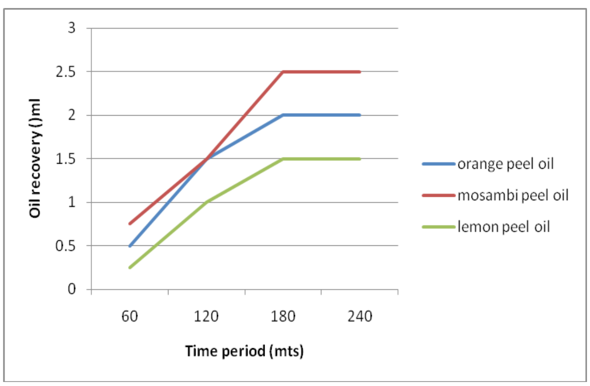

Figure 3. Effect of time on oil recovery

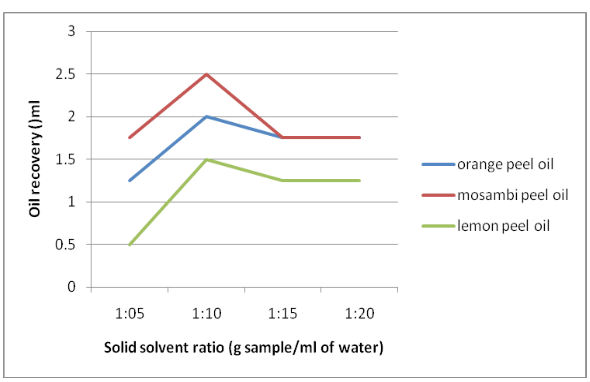

Figure 4. Effect of solid-solvent ratio on oil recovery

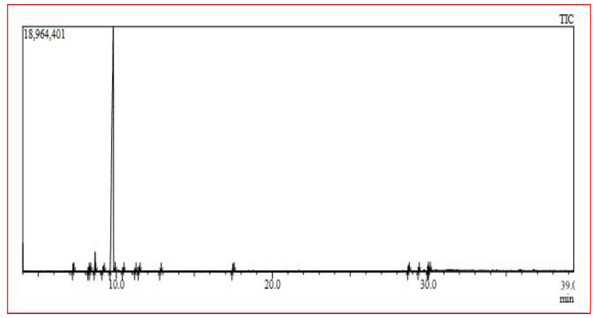

Figure 5. Volatile components of orange peel oil

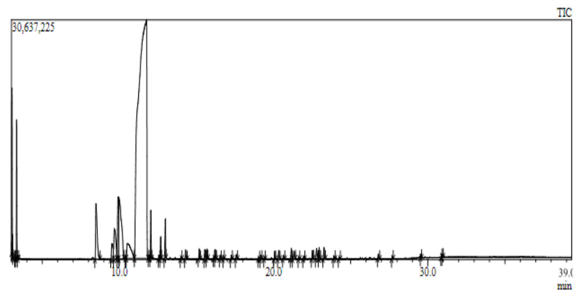

Figure 6. Volatile components of Mosambi peel oil

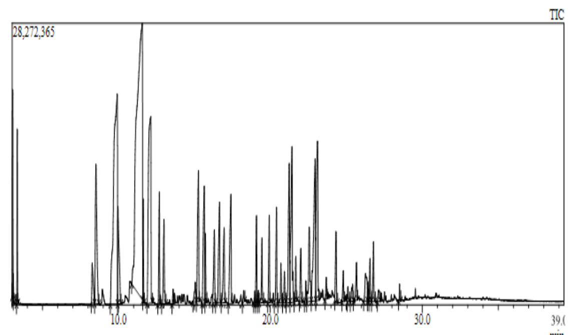

Figure 7. Volatile components of Lemon peel oil

Table 1. Sensory parameters of citrus essential oils

S. No	Sample	Colour	Odour	Touch/ Consistency	Clarity
1.	Orange peel oil	Colourless	Terpenic aroma	Thinner	Clear
2.	Mosambi peel oil	Colourless	Sweet pungent	Thinner	Clear
3.	Lemon peel oil	Pale yellowish	Lemony	Thinner	Clear

Compounds	Chemical profile	Orange peel oil			Mosambi peel oil			Lemon peel oil		
		Fresh	Hydro	Soxhlet	Fresh	Hydro	Soxhlet	Fresh	Hydro	Soxhlet
d-Limonene	Monoterpene hydrocarbons	50.34	90.33	66.16	74.09	88.57	75.52	42.63	54.39	53.82
β- Myrcene	Monoterpene hydrocarbons	2.16	1.16	1.41	1.35	0.89	2.22	1.42	2.23	1.51
α-Pinene	Monoterpene hydrocarbons	2.30	1.59	0.90	1.20	3.14	0.87	5.34	6.78	1.83
β-pinene	Monoterpene hydrocarbons	2.36	1.48	2.20	1.50	2.16	3.24	5.85	5.28	3.60
γ-terpinene	Monoterpene hydrocarbons	2.00	1.60	0.36	0.29	0.86	0.67	14.63	13.39	5.87
Linalool	Oxygenated monoterpene	2.07	1.96	0.56	1.95	2.37	1.04	1.43	1.04	0.82
alpha.-Farnesene	Sesquiterpene hydrocarbon	0.10	0.65	0.90	0.20	0.40	1.21	2.03	1.25	1.99
beta.-Bisabolene	Sesquiterpene hydrocarbon	1.24	1.77	2.36	0.10	0.28	0.54	1.91	1.09	1.86
α-Caryophyllene	Sesquiterpene hydrocarbon	-	-	-	0.10	0.26	0.62	1.09	1.16	1.16
cis-.alpha.-Berga-motene	Sesquiterpene hydrocarbon	-	-	-	-	-	-	1.89	1.66	1.24
L-alpha Terpineol	Oxygenated monoterpene	1.02	1.06	2.75	-	-	-	1.31	1.44	1.65
Neral	Monoterpene aldehyde	-	-	-	0.20	0.22	0.32	1.62	1.58	1.45

Conclusion

Citrus peel can be used to extract essential oil as a waste management technique. The oil can be extracted by hydro distillation method at optimum conditions (temperature 95 °C, time 3hr, and solid solvent ratio 1:10 g sample/mL of water). D-Limonene is the major compound present in all three citrus fruit peel oil. α pinene, β pinene, γ terpinene, β myrcene and linalool were some of the other compounds present in all three essential oils. This citrus peel essential oil can be used in the food system as a flavoring agent or as coating material.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

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