e-ISSN: 2687-6698 **Research Article**

Turkish Journal of

Analytical Chemistry

TurkJAC

https://dergipark.org.tr/tr/pub/turkjac

Microwave hydrodistillation of *Poncirus trifoliata* (L.) Raf. peels and essential oil profile: Greenness assessment

Yusuf Can Gerçek

Istanbul University, Faculty of Science, Department of Biology, 3116 Beyazıt, Istanbul, Türkiye

Abstract

The aim of the study was to determine the profile of essential oils obtained from *Poncirus trifoliata* (L.) Raf. fruit peels by hydrodistillation (MHD) and classical steam distillation (SD) methods and the bioactive potential of the wastewater from this process. The chemical composition of the essential oils was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS), while the total phenolic content (TPC), total flavonoid content (TFC), total proanthocyanidin (TPA), and total antioxidant capacity of the wastewater (aqueous phase) after distillation were determined by spectrophotometric methods. GC-MS analysis revealed that the main component of the essential oils obtained by both methods was limonene, but there were significant differences in the relative proportions of the components. The SD method yielded a higher proportion of monoterpene hydrocarbons (70.27%) and esters (8.59%), while the MHD method was more efficient in sesquiterpene hydrocarbons (14.06%) and oxygenated monoterpenes (2.10%). In wastewater analysis, the wastewater obtained by the SD method showed higher antioxidant capacity (with CUPRAC and CERAC) and higher TPC and TFC values compared to MHD. In addition, MHD (0.53 points) was found to be slightly more environmentally friendly than SD (0.49 points) in terms of energy consumption and sample size in the greenness assessment using the AGREEprep tool. In conclusion, it can be concluded that post-distillation wastewater is also a valuable source of bioactive compounds, and the choice of method should be based on the targeted compound profile and the requirements of the application.

Keywords: *P. trifoliata*, wastewater, antioxidant, essential oil

1. Introduction

Poncirus trifoliata (L.) Raf., also known as three-leaved orange, is a deciduous shrub or small tree belonging to the family Rutaceae, widely distributed in East Asia. This plant has historically been used in traditional Chinese and Korean medicine for the treatment of gastrointestinal disorders, inflammation, and allergic reactions due to its rich phytochemical composition [1]. Recently, there has been increased interest in the potential of *P. trifoliata* as a source of high-value bioactive compounds, especially in fruit peels containing essential oils, flavonoids, limonoids, coumarins, and phenolic acids [2].

Essential oils from *P. trifoliata* exhibit various biological activities, such as anti-inflammatory, antimicrobial, and antioxidant properties. These bioactivities are primarily attributed to the volatile terpenes, aldehydes, ketones and oxygenated compounds present in high concentrations in the peel [3]. However, efficient recovery of these essential oils requires careful consideration of extraction techniques that can preserve the chemical integrity of the sensitive compounds. The first step of extraction is to extract bioactive materials from the plant, and various methods have been used to extract these compounds from peel residues. These methods include conventional solvent extraction, alkaline extraction [4], microwave-assisted extraction [5], resin-based extraction [6], enzyme-assisted extraction [7], subcritical water extraction [8], and supercritical fluid extraction [9]. Extraction techniques primarily target plant-derived compounds. These plants are rich in bioactive substances, including a variety of lipids, fragrances, flavors, phytochemicals, and pigments, which are extensively utilized in the pharmaceutical, food, and cosmetic sectors [10]. The growing interest in these compounds has spurred a demand for improved extraction methods that can yield a higher quantity of bioactive ingredients in less time and at a reduced cost [11].

Citation: Yusuf Can Gerçek, Microwave hydrodistillation of *Poncirus trifoliata* (L.) Raf. peels and essential oil profile: Greenness assessment, Turk J Anal Chem, 7(2), 2025, 154–161.

****Author of correspondence:** yusuf.gercek@istanbul.edu.tr
Tel: +90 (212) 440 00 00 → 20343 **Fax:** N/A

Received: April 14, 2025 **Accepted:** April 24, 2025
https://doi.org/10.51435/turkjac.1675694

With the development of the concept of "Green Chemistry" in recent years, environmentally friendly techniques have become increasingly attractive. In this context, researchers aim to optimize the most environmentally friendly extraction method [12]. Microwave-assisted extraction (MHD) has emerged as a sustainable and innovative alternative to traditional hydro-distillation and solvent-based methods. Utilizing microwave energy to rapidly heat the moisture in plant cells, MHD facilitates cell disruption and improves the release of essential oils while significantly reducing extraction time, solvent use, and energy consumption [13]. Furthermore, MHD has been shown to enhance the recovery of thermolabile and low abundance components, making it a highly suitable method for essential oil extraction from citrus and Rutaceae family plants [14]. However, the aqueous phase remaining after MHD, which is usually discarded as waste, contains a significant amount of water-soluble bioactive compounds, including phenolics and flavonoids. These compounds play a key role in scavenging free radicals and protecting biological systems from oxidative stress [15].

The primary objective of this study was to characterize the essential oil profile of *Poncirus trifoliata* (L.) Raf. fruit peels using two distinct extraction techniques: microwave hydrodistillation (MHD) and conventional steam distillation (SD). Additionally, the study aimed to evaluate the bioactive potential of the residual aqueous phase (wastewater) generated from both extraction methods. The essential oils obtained were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) to identify and quantify their chemical constituents, with particular emphasis on variations in the levels of limonene—the major component—as well as other monoterpene and sesquiterpene hydrocarbons. Furthermore, the total phenolic content (TPC), total flavonoid content (TFC), total proanthocyanidin content (TPA), and total antioxidant capacity of the wastewater were determined using spectrophotometric assays. The environmental sustainability (greenness) of the MHD and SD techniques was also assessed using the AGREEprep tool, which considers criteria such as energy efficiency and sample throughput. This comparative evaluation aimed to identify the more environmentally sustainable extraction method.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu phenol reagent, sodium carbonate, Trolox, gallic acid, 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), quercetin, and gallic acid were obtained from Sigma-Aldrich. Cu(II) chloride, neocuproin, ammonium acetate, aluminum chloride, cerium sulfate, sodium hydroxide, sodium nitrite, ethanol, methanol, hexane were obtained from Merck. All chemicals used in the experiments were of analytical purity.

2.2. Plant material

Poncirus trifoliata was obtained from Istanbul University Faculty of Science Botanical Garden. In this study, approximately 500 g of *P. trifoliata* fruits were collected, and the skins were removed. Fresh plant material was used in the study, and an experimental design with three replications was applied.

2.3. Microwave hydrodistillation (MHD) and steam distillation (SD) of essential oils

Microwave hydrodistillation (MHD) and steam distillation (SD) were performed using Milestone Ethos X (Bergamo, Italy) and ISOLAB brand steam distillation systems, respectively. This system is a 2.45 GHz multimode microwave reactor providing variable maximum power up to 1000 W in 10 W increments. The temperature was monitored with an external infrared (IR) sensor. In a typical MHD procedure performed under atmospheric pressure, 150 g of fresh plant material was heated with constant power application for 15 min with the addition of 100 mL of distilled water. In the steam distillation procedure, 1000 mL of distilled water was added to 200 g of fresh plant material, and essential oil separation was carried out using a Clevenger apparatus with a heater at 110°C for 4 h.

2.4. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of essential oils

The analysis of essential oil composition was performed using gas chromatography-mass spectrometry (GC-MS), following a modified procedure by Fan et al. [16]. An Agilent 7890A gas chromatograph coupled to a 5975C mass spectrometer was used for the analysis. A fused silica HP-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) facilitated the separation of volatile compounds. Injection and detector temperatures were both set at 210 °C. The temperature gradient initiated at 40 °C (held for 4 min), increased at 4 °C/min to 90 °C (held for 4 min), then at 3 °C/min to 115 °C (held for 8 min), followed by a ramp of 2 °C/min to 140 °C (held for 10 min), and finally increased to 220 °C at 3 °C/min (held for 10 min). Helium, at a constant flow rate, was employed as the carrier gas. Mass spectrometric detection was operated in electron ionization mode at 70 eV, scanning a mass range between 45 and 550 atomic mass units (AMU) with a scan interval of 0.3 seconds. Compound identification was carried out by matching

mass spectra with those in the WILEY and NIST spectral libraries. The relative abundance of each component was quantified using peak area normalization.

2.5. Total phenolic content (TPC)

The total phenolic content of the samples was determined through the Folin–Ciocalteu colorimetric assay, using gallic acid as a calibration standard, in accordance with the method described by Magalhaes et al. [17], with slight modifications. Briefly, 50 µL of the wastewater sample was mixed with 50 µL of Folin–Ciocalteu reagent, followed by the addition of 100 µL of 0.35 M sodium hydroxide solution, resulting in a final reaction volume of 200 µL per well. After an incubation period of three minutes, the absorbance of the solution was read at 760 nm. The phenolic content was quantified and expressed as milligrams of gallic acid equivalents (mg GAE/g) based on a gallic acid calibration curve.

2.6. Total flavonoid content (TFC)

The quantification of total flavonoid content in the wastewater samples was performed according to a modified colorimetric method based on the procedure described by Zhishen et al. [18]. The reaction mixture, with a final volume of 6 mL, was prepared in glass tubes by sequentially adding 1 mL of the sample, 0.3 mL of 5% sodium nitrite ($NaNO_2$), 0.3 mL of 10% aluminum chloride hexahydrate ($AlCl_3{\cdot}6H_2O$), 2 mL of 1 M sodium hydroxide (NaOH), and finally 2.4 mL of distilled water. After thorough mixing, the absorbance of the mixture was measured at 510 nm using a microplate reader (BioTek Instruments, Inc., P). Flavonoid concentration was calculated and expressed as milligrams of quercetin equivalents per gram of sample (mg QE/g).

2.7. Total proanthocyanidin assay (TPA)

Total proanthocyanidin levels were determined using the vanillin-hydrochloric acid (vanillin-HCl) assay, based on the method outlined by Zurita et al. [19], with slight adaptations. For each measurement, 200 µL of the wastewater sample was mixed with 800 µL of freshly prepared vanillin reagent to achieve a final volume of 1 mL. The resulting mixture was incubated, and its absorbance was recorded at 500 nm using a microplate reader. The concentration of proanthocyanidins was calculated and presented as milligrams of catechin equivalents per gram of dry weight (mg CAE/g DW).

2.8. Cupric reducing antioxidant capacity (CUPRAC) assay

To determine antioxidant capacity, a reaction system was prepared in a test tube by sequentially combining 1 mL of copper (II) chloride solution, 1 mL of neocuproine reagent, and 1 mL of ammonium acetate buffer. Following this, 1.1 mL of the wastewater sample was added, yielding a final volume of 4.1 mL. The resulting solution was incubated at ambient temperature for 30 minutes. Absorbance readings were then recorded at 450 nm using a spectrophotometer. Results were expressed in terms of Trolox equivalents (mg TE/g) [20].

2.9. Cerium (IV)-based antioxidant capacity (CERAC) assay

The Cerium (IV) assay, as outlined by Özyurt et al. [21], was employed to assess the total antioxidant capacity (TAC) of the samples. A mixture was prepared by combining wastewater, diluted with distilled water to reach a final volume of 9 ml, with 1 ml of Ce(SO4)2, resulting in a total volume of 10 ml. This reaction mixture was then left to incubate at room temperature for 30 minutes. The absorbance was recorded at 320 nm, and the findings were reported in terms of trolox equivalents (mg TE/g).

2.10. 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

A reaction system with a total volume of 4 mL was prepared by mixing 1 mL of wastewater, 1 mL of ABTS solution, and 2 mL of methanol in a reaction tube. The tubes were then sealed and allowed to stand at room temperature for six hours. Following incubation, absorbance was recorded at 734 nm using a spectrophotometric method as described in a previous study [22]. Antioxidant capacity was expressed as milligrams of Trolox equivalents per gram (mg TE/g).

2.11. Statistical analysis

All experiments were conducted in triplicate. The results are expressed as mean±standard deviation (SD). Data analysis was performed using GraphPad Prism version 8 (San Diego, CA, USA).

3. Results and discussion

The fruits of Poncirus trifoliata cultivated in Alfred Heilbronn Botanical Garden of Istanbul University Faculty of Science were collected, washed with pure water, and then peeled with a knife (Fig 1).

In this study, the chemical compositions of the essential oils obtained from *P. trifoliata* fruit peel using two different extraction methods, MHD and SD, were determined, and the results obtained were evaluated comparatively. Compound classification based on GC-MS analysis revealed that there were significant quantitative differences between the essential oils obtained using different extraction methods (Table 1, Table 2).

Figure 1. *Poncirus trifoliata* fruits

Monoterpene hydrocarbons were the most dominant class among the essential oils isolated by both methods. While 70.27% monoterpene hydrocarbons were obtained by the SD method, this rate was 58.39% by MHD. Especially D-limonene was isolated by SD with a rate of 36.66%, while this rate was 30.07% by the MHD method. This may be attributed to the high temperature and prolonged distillation conditions of SD, which increase the release of volatile monoterpenes [23,24]. Similarly, other volatile hydrocarbons such as β-myrcene, β-pinene, and α-pinene were obtained with similar profiles in both methods, but method-induced variations in concentration levels were observed.

Table 1. Profile of the essential oil obtained by steam distillation

Peak number	Retention time	Compound	Retention index	Relative amount (%)
1	7.925	Alpha-pinene, (-)-	939	1.07
2	9.643	Sabinene	975	1.11
3	9.781	Beta-pinene	979	4.64
4	10.636	Beta-myrcene	991	16.46
5	10.823	Butanoic acid, butyl ester	985	3.25
6	11.032	Hexanoic acid, ethyl ester	1010	3.53
7	11.168	l-Phellandrene	1005	3.36
8	11.337	3-Hexen-1-ol, acetate, (Z)	1006	0.13
9	11.696	Acetic acid, hexyl ester	1015	1.28
10	12.630	D-Limonene	1031	36.66
11	13.175	Benzeneacetaldehyde	1049	0.08
12	13.606	Beta-ocimene	1049	6.35
13	14.029	Gamma-Terpinene	1059	0.37
14	14.762	1-Octanol	1080	0.19
15	15.683	Alpha-terpinolene	1089	0.12
16	16.474	Linalool	1100	1.09
21	21.494	Benzeneacetonitrile	1172	0.2
22	21.570	Cryptone	1240	0.17
25	24.525	Beta-citronellol	1238	0.58
26	27.157	Phellandral	1259	0.1
27	27.327	1-Decanol	1275	0.31
28	31.251	Alpha-terpinene	1015	0.14
29	33.190	Neryl acetate	1370	0.13
30	34.416	Geranyl acetate	1388	0.13
31	34.653	Beta elemene	1390	0.46
32	35.198	Decanoic acid, ethyl ester	1400	0.14
33	35.861	Dodecanal	1200	0.05
34	36.237	Caryophyllene	1418	5.58
35	37.164	Gamma-elemene	1430	0.21
36	38.197	Alpha-humulene	1455	0.3
37	38.809	trans-beta-farnesene	1456	0.82
38	39.934	Germacrene D	1480	1.78
39	40.816	bicyclogermacrene	1502	0.24
40	41.892	E,E-.alpha-farnesene	1456	1.77
41	42.589	beta-sesquiphellandrene	1505	0.11
42	42.849	Oxacyclotridec-10-en-2-one	1975	0.45
43	44.276	Germacrene B	1560	1.34
44	45.696	Caryophyllene oxide	1418	0.31
45	80.578	Tricosane	2300	0.1
46	88.462	Eicosane	2000	0.13
47	95.783	Docosane	2200	0.15
		Alcohols		***0.5***
		Aldehydes		***0.13***
		Esters		***8.59***
		Monoterpene hydrocarbons		***70.27***
		Oxygenated monoterpenes		***1.42***
		Sesquiterpene hydrocarbons		***12.81***
		Others		***1.42***
		Total		**95.14**

Table 2. Profile of essential oil obtained by microwave distillation

Peak number	Retention time	Compound	Retention index	Relative amount (%)
1	7.943	Alpha-pinene, (-)-	939	1.39
2	9.881	Beta-pinene	979	6.56
3	11.005	Beta-myrcene	991	16.86
4	11.341	Hexanoic acid, ethyl ester	1010	4.06
5	11.478	l-Phellandrene	1005	2.73
6	11.903	Acetic acid, hexyl ester	1015	1.68
7	13.220	D-Limonene	1031	30.07
8	13.317	trans-beta-ocimene	1049	0.16
9	13.475	Benzeneacetaldehyde	1049	0.22
10	14.004	1,3,7-Octatriene, 3,7-dimethyl-	1054	6.7
11	14.311	Gamma-terpinene	1059	0.44
12	14.934	1-Octanol	1080	0.22
13	15.797	Alpha-terpinolene	1089	0.15
14	16.681	Linalool	1100	1.35
18	19.754	Citronella	1228	0.05
19	21.282	Terpinen-4-ol	1177	1.58
20	21.620	Benzeneacetonitrile	1172	0.3
21	21.717	Cryptone	1240	0.15
24	24.679	Beta-citronellol	1238	0.65
25	27.202	Phellandral	1259	0.14
26	27.438	1-Decanol	1275	0.35
27	31.285	Delta-elemene	1389	0.2
28	32.526	Alpha-Terpinene	1015	0.05
29	33.236	Neryl acetate	1370	0.14
30	34.512	Geranyl acetate	1388	0.19
31	34.696	Beta-elemene	1390	0.35
32	34.817	Butyl caprylate	1260	0.18
33	35.334	Decanoic acid, ethyl ester	1200	0.16
34	35.982	Dodecanal	1200	0.06
35	36.467	Caryophyllene	1418	5.92
36	37.221	Gamma-elemene	1430	0.24
37	38.265	Alpha-humulene	1455	0.34
38	38.654	trans-beta-farnesene	1456	0.97
39	40.111	Germacrene D	1480	1.97
40	40.890	bicyclogermacrene	1502	0.3
41	42.081	E,E-.alpha-farnesene	1456	1.97
42	42.681	Beta-sesquiphellandrene	1505	0.13
43	42.962	Oxacyclotridec-10-en-2-one	1975	0.48
44	44.448	Germacrene B	1560	1.45
45	45.016	d-Nerolidol	1545	0.1
46	45.784	Caryophyllene oxide	1418	0.35
47	80.596	Tricosane	2300	0.12
48	88.490	Heneicosane	2100	0.15
49	95.816	Tetracosane	2400	0.18
		Alcohols		***0.57***
		Aldehydes		***0.28***
		Esters		***6.22***
		Monoterpene hydrocarbons		***58.39***
		Oxygenated monoterpenes		***2.1***
		Sesquiterpene hydrocarbons		***14.06***
		Others		***10.18***
		Total		**91.8**

β-myrcene was detected at 16.46% in SD and 16.86% in MHD. Sesquiterpene hydrocarbons were 14.06% in the essential oil obtained with MHD and 12.81% in the essential oil obtained with SD. Among the prominent compounds, caryophyllene was 5.92% in MHD and 5.58% in SD; germacrene D was 1.97% in MHD and 1.78% in SD. These results indicate that the MHD method provides higher efficiency on sesquiterpene structures. In terms of oxygenated monoterpenes, the MHD method (2.10%) provided higher yields compared to SD (1.67%). In particular, linalool was determined to as 1.35% in MHD and 1.09% in SD. This result can be attributed to the fact that MHD is a method with shorter duration, intrinsic moisture, and rapid heat transfer [25,26].

In addition, microwave energy increases the release of compounds by breaking down cell walls, contributing to the preservation of a wider phytochemical diversity [27,28].

Table 3. Phytochemical analysis from Poncirus trifoliata peels wastewater

	CUPRAC (mg TE/g)	CERAC (mg TE/g)	ABTS (mg TE/g)	TF (mg QE/g)	TP (mg GAE/g)	TPA (mg CAE/g)
MHD	11.06±0.98	15.37±1.1	13.24±9.87	2.63±0.04	7.26±0.42	1.02±0.08
SD	13.27±1.24	19.98±1.84	13.50±1.43	2.84±0.21	7.67±0.65	0.99±0.08

Ester compounds were obtained at a significantly higher rate (8.59% vs. 6.23%) in the SD method compared to MHD. In particular, ethyl hexanoate was 3.53% by SD and 4.06% by MHD; acetic acid, hexyl ester was 1.28% by SD and 1.68% by MHD. Excluding the alcohol (0.50-0.57%) and aldehyde (0.13-0.28%) classes, which were obtained in lower proportions, the differences in the basic structure groups reveal the method-specific chemical selectivity effect. Sesquiterpene hydrocarbons such as germacrene B/D, caryophyllene, farnesene, and their oxygenated derivatives were more stable in oils obtained by MHD. These compounds are important phytochemicals, especially for their antimicrobial, anti-inflammatory, and aromatic functions [29]. As a matter of fact, in some studies, it was reported that *P. trifoliata* essential oils showed inhibitory effects on foodborne pathogens, and this was especially associated with sesquiterpenes [29]. Again, in essential oils obtained by MHD, the possibility of detecting rare aromatic compounds containing sulfur, such as 3-sulfanylbutyl alkanoates increases, which can provide differentiation, especially for the aroma industry [30]. MHD offers advantages not only in terms of chemical profile but also in environmental and economic aspects. Compared to conventional distillation techniques: energy consumption is lower, time saving, reduced water use, low risk of thermal degradation, high purity, and biologically active fractions are obtained [31,32]. In these aspects, it offers a more sustainable alternative in the production of functional food

additives, natural preservatives, cosmetic formulations, and pharmaceutical ingredients.

In this study, in addition to the chemical profile of the essential oil obtained from *P. trifoliata* fruit peels by MHD and SD methods, the antioxidant capacity and phenolic compound contents of the wastewater obtained during the processing process were compared. The antioxidant capacity of the wastewater was evaluated by various spectrophotometric methods such as CUPRAC, CERAC, and ABTS, and the TFC, TPC, and TPA contents were also analyzed (Table 3).

When evaluated in terms of antioxidant capacity, the antioxidant activity values of the wastewater obtained with SD (in all three antioxidant methods) were higher than MHD. While the antioxidant activity value of MHD wastewater was determined as 11.06 ± 0.98 mg TE/g by CUPRAC method, this value increased to 13.27 ± 1.24 mg TE/g in SD. Similarly, in CERAC analysis, 15.37 ± 1.1 mg TE/g for MHD and 19.98 ± 1.84 mg TE/g for SD. In the ABTS radical scavenging capacity test, similar results were obtained in both methods (MHD: 13.24 ± 9.87 mg TE/g, SD: 13.50 ± 1.43 mg TE/g). When the antioxidant activity results obtained are evaluated, especially the methods based on metal ion reduction such as CERAC and CUPRAC , they reveal a stronger electron transfer capacity in the wastewater obtained with SD.

When the data on the phenolic compound content of the wastewater obtained during different processes were evaluated, the TFC was determined as 2.84 ± 0.21 mg QE/g for the wastewater obtained during the SD process and 2.63 ± 0.04 mg QE/g for the wastewater obtained during the MHD process. Furthermore, the TPC of the wastewater was determined as 7.67 ± 0.65 mg GAE/g in the SD process and 7.26 ± 0.42 mg GAE/g in the MHD process. In addition, the TPA of wastewater was found to be quite similar in both methods (MHD: 1.02 ± 0.08 mg CAE/g, SD: 0.99 ± 0.08 mg CAE/g). The findings indicate that the SD method promotes the transition of hydrophilic and heat-stable compounds, especially polyphenols, to the wastewater more. Similarly, in different studies reported in the literature, it has been reported that wastewater obtained during distillation processes contains significant amounts of soluble phenolic compounds and has antioxidant activity [23,24]. When these results obtained for different extraction methods are evaluated, this study once again supports that the wastewater obtained after distillation is not waste but a potential source of antioxidants and phenolic compounds.

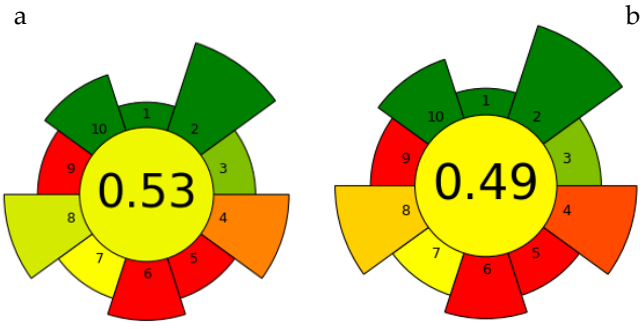

Figure 2. AGREEprep assessment scores a) for MHD method b) for SD method

Finally, the greenness assessment of the sample preparation procedure for essential oil extraction was carried out in this study. At this stage, in recent years, several tools have been developed to assess the greenness of analytical procedures, and one of the most widely used in sample preparation is the AGREEprep

tool [33]. The assessment criteria are grounded in ten principles of green sample preparation, including the selection of solvents, materials, and reagents; the volume of waste produced; energy usage; and the quantity and yield of samples. AGREEprep pictograms illustrate varying degrees of environmental sustainability. The central score of these pictograms is 0.53 in the microwave-assisted technique (Fig. 2a) and 0.49 in the steam distillation method (Fig. 2b), this discrepancy is attributed to the differences in energy consumption and sample amount.

4. Conclusion

This study compares MHD and SD methods to extract essential oil from fruit peels of *P. trifoliata* (three-leaved orange). When the chemical composition of the obtained essential oils was compared by GC-MS, it was observed that the essential oils isolated by both methods contained similar major components, but there were marked differences in the relative abundance and distribution of these components. While the MHD method offered advantages in terms of speed, energy efficiency, and certain compound groups (oxygenated monoterpenes and sesquiterpenes), the SD method provided higher yields for monoterpene hydrocarbons and esters. However, it was determined that MHD has the potential to diversify the composition of essential oils obtained from *P. trifoliata* peels and increase some bioactive components. Therefore, the MHD method can be recommended as a more efficient and environmentally friendly alternative in the production of essential oils to be used for food, cosmetic, or pharmaceutical purposes. In addition, the composition of the wastewater obtained during the essential oil extraction reveals that this product should be evaluated in terms of functional content and may serve as an additional source of bioactive components in food, cosmetic, or pharmaceutical products.

Acknowledgements

The author wishes to acknowledge the technical support of Istanbul University Science Faculty Alfred Heilbronn Botanical Garden and Food, Environmental and Instrumental Analysis Laboratory for the sample's analysis (https://cealab-fen.istanbul.edu.tr).

Funding

No funding was provided.

References

[1] H.Y. Lee, S. W. Kim, Y.K. Kim, S.Y. Lee, M.Y. Yoon, Protective effect of Poncirus trifoliata fruit extracts against gastric ulcer in rats, Food Sci Biotechnol, *20*, 2011 1097–1102.

[2] Y. Zhang, Y. Li, Y. Wang, X. Zhang, H. Zhang, Chemical constituents and pharmacological activities of Poncirus trifoliata (L.) Raf.: A review. Phytochem. Rev., 19, 2020, 97–119.

[3] H.J. Kim, J.H. Kim, H.Y. Park, J.Y. Kim, H.J. Lee, Antioxidant and antimicrobial activities of extracts from Poncirus trifoliata fruit, Korean J Food Sci Technol, 46, 2014, 351–357.

[4] A. Bocco, M. E. Cuvelier, H. Richard, C.Berset, Antioxidant activity and phenolic composition of citrus peel and seed extracts. J Agric Food Chem, 46, 1998, 2123-2129.

[5] Z. Wang, Q. Shang, W. Wang, X. Feng, Microwave-assisted extraction and liquid chromatography/mass spectrometry anaysis of flavonoids from grapefruit peel. J Food Process Eng, 34, 2011, 844–859.

[6] A. Di Mauro, B. Fallico, A. Passerini, E.Maccarone, Waste water from citrus processing as a source of hesperidin by concentration on styrene– divinylbenzene resin. J Agric Food Chem, 48, 2000, 2291-2295.

[7] B.B. Li, B. Smith, M.M Hossain, Extraction of phenolics from citrus peels: I. Solvent extraction method, Sep Purif Technol, 48, 2006, 182-188.

[8] M. J. Ko, C. I. Cheigh, M. S. Chung, Relationship analysis between flavonoids structure and subcritical water extraction (SWE). Food Chem, 143, 2014, 147-155.

[9] A.N. Giannuzzo, H. J. Boggetti, M. A. Nazareno, H. T. Mishima, Supercritical fluid extraction of naringin from the peel of Citrus paradisi. Phytochem Anal, 14, 2003, 221-223.

[10] P.A. Uwineza, A. Waśkiewicz, Recent advances in supercritical fluid extraction of natural bioactive compounds from natural plant materials, Molecules 2020, *25*(17), 3847.

[11] A. Krakowska-Sieprawska, A. Kiełbasa, K. Rafińska, M. Ligor, B. Buszewski, Modern methods of pre-treatment of plant material for the extraction of bioactive compounds, Molecules, 27, 2022, 730.

[12] M. Boukroufa, C. Boutekedjiret, L. Petigny, N. Rakotomanomana, F. Chemat, Bio-refinery of orange peels waste: A new concept based on integrated green and solvent free extraction processes using ultrasound and microwave techniques to obtain essential oil, polyphenols and pectin. Ultrason. Sonochem., 24, 2015, 72-79.

[13] W. Routray, V. Orsat, Microwave-assisted extraction of flavonoids: A review. Food Bioproc. Tech., 5, 2012,409–424.

[14] F. Chemat, N. Rombaut, A. G. Sicaire, A. Meullemiestre, A. S. Fabiano-Tixier, M. Abert-Vian, Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrason. Sonochem., *34*, 2017, 540–560.

[15] J. Dai, R. J. Mumper, Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules, *15*, 2010, 7313–7352.

[16] S. Fan, J. Chang, Y. Zong, G. Hu, J. Jia, GC-MS analysis of the composition of the essential oil from Dendranthema indicum Var. Aromaticum using three extraction methods and two columns. Molecules, 23, 2018, 576.

[17] L.M. Magalhães, F. Santos, M. A. Segundo, S. Reis, J.L. Lima, Rapid microplate high-throughput methodology for assessment of Folin-Ciocalteu reducing capacity. Talanta, 83, 2010, 441-447.

[18] J. Zhishen, T. Mengcheng, W. Jianming, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem, 64, 1999, 555-559.

[19] J. Zurita, M. E. Díaz-Rubio, F. Saura-Calixto, Improved procedure to determine non-extractable polymeric proanthocyanidins in plant foods. Int J Food Sci Nutr 63, 2012, 936–939.

[20] R. Apak, K. Güçlü, B. Demirata, M. Özyürek, S. Çelik, B. Bektaşoğlu, D. Özyurt, Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules, 12, 2007, 1496-1547.
[21] D. Ozyurt, B. Demirata, R. Apak, Modified cerium (IV)-based antioxidant capacity (CERAC) assay with selectivity over citric acid and simple sugars. J. Food Compos. Anal., 23, 2010, 282-288.
[22] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 26, 1999, 1231-1237.
[23] Y. Zhang, X. Zhao, Q. Yang, Li, Y., X. Liu, M. Huang, Recovery and utilization of phenolic compounds from distillation wastewater of essential oil plants: A review. Ind Crops Prod, 170, 2021,113753.
[24] Y.J. Fu, Y.G. Zu, L.Y. Chen, X.H. Shi, Z. Wang, S. Sun, T. Efferth, (2010). Antioxidant properties of waste residues from vine tea (*Ampelopsis grossedentata*) extraction. Afr. J. Biotechnol., 9, 2010, 7248–7253.
[25] Y. Liu, Z. Liu, C. Wang, Q. Zha, C. Lu, Z. Song,... A. Lu, Study on essential oils from four species of Zhishi with gas chromatography–mass spectrometry. Chem. Cent. J., 8, 2014, 22.
[26] F. Papa, F. Maggi, K. Cianfaglione, G. Sagratini, G. Caprioli, S. Vittori, Volatile profiles of flavedo, pulp and seeds in *Poncirus trifoliata* fruits. J Sci Food Agric 94, 2014, 2874–2887.
[27] M. E. Lucchesi, F. Chemat, J. Smadja, Solvent-free microwave extraction of essential oil from aromatic herbs: Comparison with conventional hydro-distillation. J. Chromatogr. A, 1043, 2004, 323–327. H
[28] V. Mandal, Y. Mohan, S.Hemalatha, Microwave assisted extraction – an innovative and promising extraction tool for medicinal plant research. Phcog Rev, 5, 2011, 7.
[29] A. Rahman, S. M. Al-Reza, J. I. Yoon, S. C. Kang, In vitro inhibition of foodborne pathogens by volatile oil and organic extracts of *Poncirus trifoliata* seeds, J Sci Food Agric, *89*, 2009, 876–881.
[30] C. Starkenmann, Y. Niclass, S. Escher, Volatile organic sulfur-containing constituents in *Poncirus trifoliata* fruit peel. J Agric Food Chem, *55*, 2007, 4511–4517.
[31] K. Boztaş, Y. C. Gercek, G. Türer, D. Canlı, S. Bayram, S. Çelik,... N. Ecem Bayram, Microwave hydrodistillation of Pelargonium graveolens L'Her leaves: Essential oil profile, phytochemical composition of wastewater, histo-anatomical structure. J. Essent. Oil-Bear. Plants, 27, 2024, 659-677.
[32] L. Zhang, W. Bai, L. Zhou, C. Li, Comparative evaluation of extraction technologies on the chemical composition and antioxidant activity of essential oils from citrus peels. Ind Crops Prod, 191, 2023, 116066.
[33] W. Wojnowski, M. Tobiszewski, F. Pena-Pereira, E. Psillakis, AGREEprep–analytical greenness metric for sample preparation. TrAC Trends Anal. Chem. 149, 2022, 116553