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Extraction of d-limonene from pomelo (*Citrus maxima*) peel waste using solvent-free microwave process and antioxidant activity analysis

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ABSTRACT

Solvent-free microwave assisted extraction has been performed as an efficient green technology for the extraction of d-limonene from pomelo peel waste. This environmentally friendly method does not require solvents or water and can be operated at atmospheric pressure and room temperature. The extraction results were identified by gas chromatography–mass spectrometry (GC-MS). It was found that d-limonene was the most abundant compound in the extracted essential oils, comprising 95% of the total, with an additional 17 compounds detected. For 100 grams of pomelo peel, the energy requirement was 336.0 kJ to achieve the highest yield of 1.2% essential oil by weight of the pomelo peel plant material. The essential oil showed the antioxidant activities of IC_{50} at 4.87 ± 0.06 mg/mL using ABTS radical scavenging assay.

Keywords: limonene; extraction; pomelo; solventless; microwave

1. INTRODUCTION

The scent and usefulness of essential oils, due to their composition of several bioactive components, have increased interest in the extraction of essential oils from plants in recent years (Ni et al., 2021). Essential oils are obtained from the volatile molecules in plant material. d-limonene is a natural essential oil that has been extensively studied for its anti-inflammatory, antioxidant, and anti-tumor properties (Ghadiri et al., 2020; d' Alessio et al., 2022; Akhavan-Mahdavi et al., 2022).

Nowadays, d-limonene is often used as a natural treatment for a variety of health issues and is a popular ingredient in household items. Additionally, many other essential oils possess antibacterial activity, making them useful in treating diseases caused by bacteria, viruses, and fungi (Vieira et al., 2018). d-limonene is the main component naturally produced by citrus fruits such as

Citrus maxima (pomelo), *Citrus aurantifolia* (lime), *Citrus reticulata* (mandarin orange), *Citrus limon* (lemon), and *Citrus paradisi* (grapefruit) (Eddin et al., 2021). d-limonene has been extracted from citrus fruits for decades. Pomelo, the largest citrus fruit in the world, has a peel that is a rich source of d-limonene. In Thailand, particularly in Nakhon Pathom province, pomelo is a common economic fruit crop that can be produced year-round.

Despite the high d-limonene content in its peel, pomelo is a very wasteful fruit, with approximately 50% of it becoming waste; a significant amount of pomelo peel is discarded. Therefore, the extraction of d-limonene (and other essential oils) from pomelo peel presents an interesting and challenging opportunity.

Numerous methods can be employed for the extraction of d-limonene from pomelo and other citrus fruits, including hydrodistillation, steam distillation, and Soxhlet extraction. However, these conventional methods come with several drawbacks, such as low extraction efficiency, loss of volatile compounds, and reaction between the solvents and products at high temperatures. Steam distillation and hydrodistillation, for instance, require large amounts of water (Kusuma & Mahfud, 2015) and longtime extraction times can lead to loss of volatile molecules. Solvent extraction methods, while commonly used for essential oil extraction (Attard et al., 2014), pose environmental and health risks due to the toxicity of solvents such as hexane, heptane, and benzene. Furthermore, obtaining a solvent-free product is challenging, and this process often results in the loss of highly volatile components.

To address these challenges, Felicia et al. (2024) explored supercritical fluid extraction as a sustainable alternative for extracting orange peel essential oil, yielding high amounts of main ingredients such as d-limonene, α-pinene, β-myrcene, and γ-terpinene. However, the apparatus required for supercritical fluid extraction is complex, operating costs are high, and the process is slow. These limitations have prompted consideration of new green methods for d-limonene extraction that offer convenience, low energy consumption, reduced operating costs, and shorter processing times.

Microwave-assisted distillation appears to be an effective method for d-limonene extraction. Attard et al. (2014) and Ferhat et al. (2006) have demonstrated its utility due to its short processing time, limited compound participation, and production of uncontaminated products. However, separating the solvent from the desired product can be inconvenient and may result in the loss of highly volatile components. Therefore, in this study, solvent-free microwave extraction (SFME) was employed to extract essential oil, which contains d-limonene from pomelo peel waste.

The SFME method eliminates the need for organic solvents. Pomelo peel waste material was placed in a spherical flask and subjected to microwave heating without any solvent or water. Rapid changes in molecular direction occurred as polar molecules rearranged in a redirected electromagnetic field, leading to collisions and heat generation that affected plant cell tissues, causing them to break down. This process facilitated the extraction of d-limonene, essential oil, and evaporated water from inside the plant material. SFME methods can reduce the operating costs associated with essential oil extraction and lower carbon dioxide emissions due to decreased energy consumption (Kusuma et al., 2017). The study also investigated the effects of processing time and microwave radiation power. The antioxidant activity of the extracted essential oil was evaluated by ABTS radical scavenging assay.

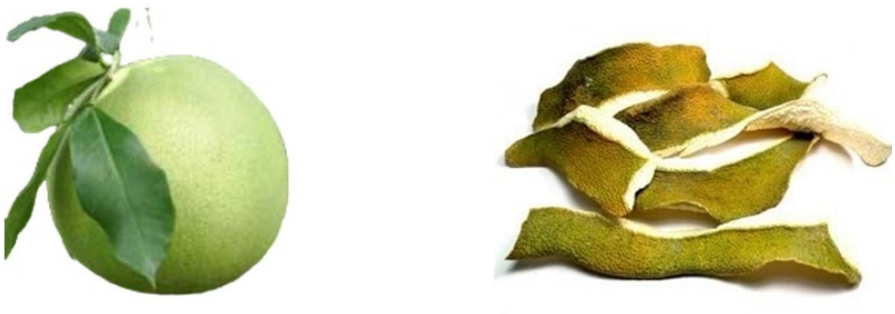
2. MATERIALS AND METHODS

2.1 Materials

Pomelo (*Citrus maxima*) peel waste was sourced from discarded fruit residues at local markets in Nakhon Pathom, Thailand. Only fresh outer green peel was used in all experiments as shown in Figure 1. The peels were cut into small, equal-sized pieces measuring approximately 5 x 5 ±0.5 cm.

2.2 Apparatus

A microwave oven with a maximum delivered power of 700 Watts and a wave frequency of 2.45 GHz was utilized. The microwave oven was modified by drilling a small hole at the top. A 500 ml round bottom flask was placed inside the microwave oven, and the gap between the hole and flask was sealed with silicone to prevent any wave and heat loss from the oven. The flask was connected to the condenser, as illustrated in Figure 2.



(a) (b)

Figure 1. (a) pomelo (*Citrus maxima*) and (b) pomelo peel

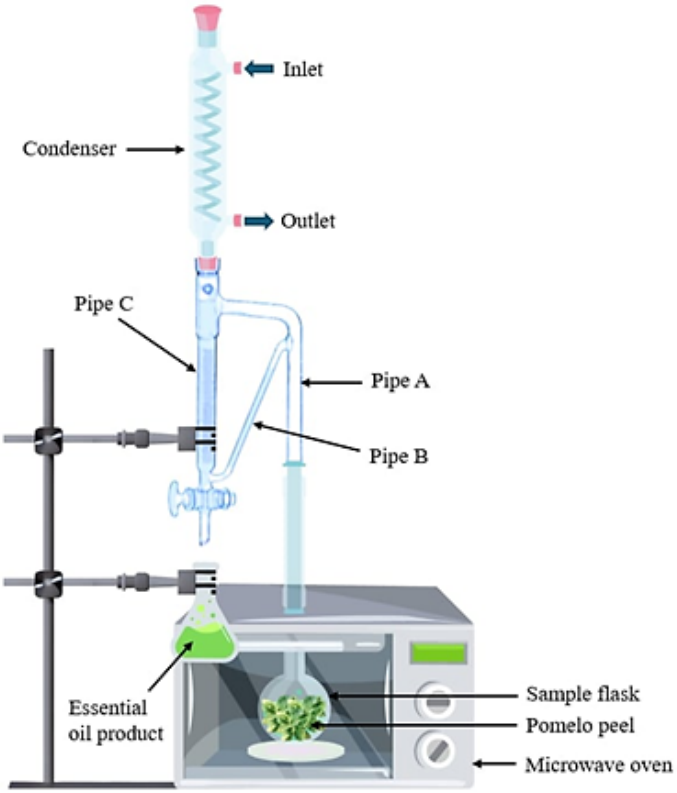


Figure 2. Schematic representation of the solvent-free microwave extraction apparatus

2.3 Analytical instrument

The contents of d-limonene and other essential oils obtained from pomelo peel were analyzed using gas chromatography-mass spectrometry (GC-MS). The analysis was conducted with a Trace 131 gas chromatograph and a TQS 9000 mass spectrometric detector. A TG-5SILMS chromatography column was utilized. The injection port and interface temperatures were maintained at 220°C and 280°C, respectively. The temperature range was set between 80°C and 280°C, with an increasing rate of 5°C/min. Helium was used as the carrier gas. The percentage of peak area relative to the total peak areas of all substances was determined and displayed as the relative amount of each compound.

2.4 Procedure

A 100-g sample of pomelo peel waste was placed into 1000 mL round bottom flask. The flask was then subjected to microwave radiation ranging from 120 to 700 watts for different durations without the addition of water or solvent. Initially, the microwave radiation heated the pomelo peel, causing the essential oil to evaporate along with the water present inside the plant material. The vapor mixture ascended through pipe A, the condenser. During the process, the condensate, composed of water and oil, was formed in the condenser and flowed down through pipe C. The excess water, which settled in the bottom layer, was removed before returning to the round-bottom flask through pipe B, while the essential oil was collected from the upper layer in pipe C (Figure 2).

In the funnel, the extracted d-limonene and other essential oils, being immiscible with water, formed a distinct upper layer. Samples of the product were extracted from the apparatus and analyzed using GC-MS, as shown in Table 3. The extraction yield of essential oil was calculated using Equation 1. The essential oil and water from the pomelo itself were subsequently separated from each other using a separating funnel.

$$Extraction\ yield\ (\%) = \frac{Mass\ of\ essential\ oil}{Mass\ of\ pomelo\ peel} \text{ x } 100 \qquad (1)$$

2.5 ABTS radical scavenging assay

The antioxidant activity of the essential oil was evaluated by ABTS radical scavenging assay. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 16 h at room temperature in the dark. The solution was then 100-fold diluted. Fresh ABTS solution was prepared for each assay. The essential oil was diluted in ethanol, and the standard rutin solution was used as positive control. Volumes at 10 µL of the essential oil solution and rutin standard solution were allowed to react with 190 µL of the ABTS solution, and the absorbance was taken at 734 nm after 5 min using a microplate reader (Clariostar Plus, BMG Labtech, Ortenberg, Germany). Triplicate determinations were performed and the percentage inhibitions at 734 nm were plotted against the concentrations to determine the IC_{50}.

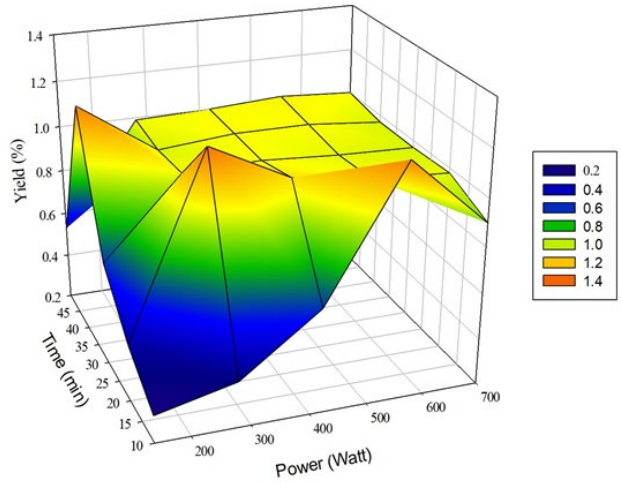
3. RESULTS AND DISCUSSION

3.1 Effect of microwave power and operating time on the essential oil yield

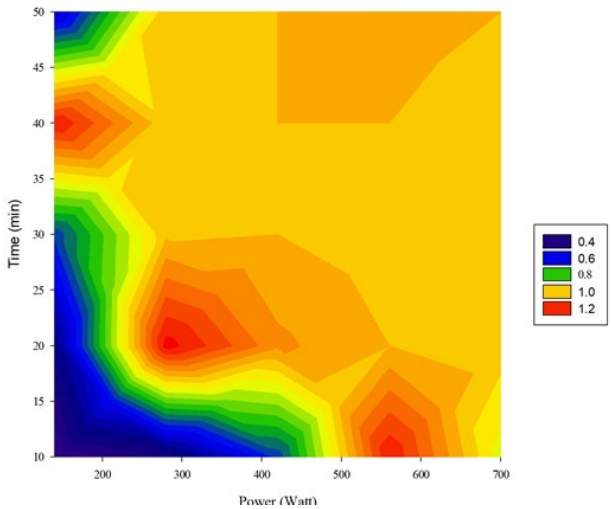
The effect of power, defined as the rate of transferred energy per unit time, on the yield was investigated. Microwave power varied from 140 to 700 watts, and operating times ranged from 10 to 60 min. The energy received by the plant (pomelo peel) and the microwave oven played a crucial role in this process. Initially, the energy received by the plant was limited, and then it converted to heat. Subsequently, the disruption of plant cells occurred, releasing d-limonene and essential oil (Putri et al., 2019; Kusuma et al., 2016). As depicted in Figure 3, the yields exhibited a trend of increasing and decreasing with changes in microwave power. Similarly, variations in operating time resulted in fluctuations in yield, reaching a peak and then declining. These observations suggest that the energy requirement for the separation method had occurred.

3.2 Energy requirement determination

Table 1 shows the effect of microwave power and operating time on energy consumption, the extraction temperatures and the resulting yields of the essential oil. The highest yield was achieved when the microwave energy reached approximately 336.0 kJ with the temperature of 150°C (highlighted in gray). Interestingly, higher energy levels did not increase the yield. Therefore, it can be concluded that the optimal energy required to extract d-limonene from 100 g of pomelo peel is 336 kJ, and any excess energy beyond this threshold is unnecessary, as the yield of d-limonene decreases due to thermal degradation.



(a)



(b)

Figure 3. (a) 3D plot and (b) contour plot for the effect of microwave power and operating time on the yield of essential oil

Table 1. The demonstration of the effect of power, time and energy on the yield of d-limonene

Ratio	Power (W) (a)	Time (min) (b)	Time (s) (c)	Energy (kJ) (d)	Temperature (°C)	Yield (%)
0.1	140	10	600	84.0	90	0.32
0.1	140	20	1200	168.0	110	0.400
0.1	140	30	1800	252.0	135	0.65
0.1	140	40	2400	336.0	150	1.20
0.1	140	50	3000	420.0	165	0.90
0.1	140	60	3600	504.0	180	0.96
0.1	280	10	600	168.0	110	0.45
0.1	280	20	1200	336.0	150	1.20
0.1	280	30	1800	504.0	180	1.05
0.1	280	40	2400	672.0	190	1.00
0.1	280	50	3000	840.0	200	0.98
0.1	280	60	3600	1008.0	205	0.94
0.1	420	10	600	252.0	135	0.65
0.1	420	20	1200	504.0	180	0.99
0.1	420	30	1800	756.0	195	1.00
0.1	420	40	2400	1008.0	205	0.98
0.1	420	50	3000	1260.0	210	0.97
0.1	420	60	3600	1512.0	220	0.97
0.1	560	10	600	336.0	150	1.20
0.1	560	20	1200	672.0	190	0.97
0.1	560	30	1800	1008.0	205	1.00
0.1	560	40	2400	1344.0	220	1.0
0.1	560	50	3000	1680.0	225	0.97
0.1	560	60	3600	2016.0	230	0.96
0.1	700	10	600	420.0	165	0.53
0.1	700	20	1200	840.0	200	0.99
0.1	700	30	1800	1260.0	210	1.00
0.1	700	40	2400	1680.0	225	1.02
0.1	700	50	3000	2100.0	230	1.00
0.1	700	60	3600	2520.0	240	0.95

* Example: when the power of 140 watts was operated for 20 min, the energy that fed to the pomelo peel was $140^{(a)} \times 20^{(b)} \times 60 = 168000$ J = 168.0 kJ$^{(d)}$

3.3 Effect of mass-volume ratio on the d-limonene yield

The effect of mass-volume ratio, which defined as the ratio between the weight of the pomelo peel material and the volume of the round-bottom flask, was studied. The pomelo peel masses were 100, 200, and 300 g, and the round-bottom flask volume was 1000 mL, resulting in mass-volume ratios of 0.1, 0.2, and 0.3 g/mL, respectively. As shown in Table 2, at a fixed operating energy. From the results, the energy requirement also increases proportionally with the mass-volume ratio. This increase in ratio makes it more challenging for the microwave power to dissipate evenly throughout the plant material (Putri et al., 2019).

3.4 Chemical analysis of essential oil constituents

The results obtained from GC-MS analysis illustrate the composition of the essential oil extracted from pomelo peel, as depicted in Figure 4 and summarized in Table 3. d-limonene emerged as the predominant compound, constituting 95% of the total extract, alongside 17 additional identified compounds. Normally, the essential oil that is in the pomelo peel is about 0.8–1.3% by weight. Therefore, it means that the initial amount of d-limonene in pomelo peel is about 0.76–1.23% by weight.

The results from solvent-free microwave extraction (SFME) were compared with those from water solvent microwave extraction (WSME). The data showed that the solvent-free microwave extraction method was more effective than WSME in extracting various volatile essential oils, including α-pinene, β-myrcene, neral, geranial, β-caryophyllene, α-humulene, and carvone. Additionally, two compounds, caryophyllene and germacrene D, were extracted using SFME but not by WSME.

3.5 ABTS radical scavenging assay

In this study, the antioxidant activity of the extracted essential oil was evaluated using the ABTS radical scavenging assay (Figure 5). The essential oil exhibited measurable antioxidant capacity, with average IC_{50} values of 6.98 ± 0.26 μg/mL for standard rutin and 4.87 ± 0.06 mg/mL for the extracted pomelo peel oil. The substantial

difference in the tested concentrations is attributable to the nature of the samples: rutin is a purified reference standard with a well-defined molecular weight and high intrinsic antioxidant activity, thus requiring only microgram-per-milliliter levels to exert its effect. In contrast, pomelo peel oil is a crude extract containing a complex mixture of compounds, necessitating higher concentrations (in the milligram-per-milliliter range) to achieve comparable activity. A previous study reported IC_{50} values for pomelo peel oil of 4.83 ± 1.00 mg/mL as determined by the ABTS assay (Yang & Park, 2025). Variations in antioxidant activity may be influenced by the botanical source and extraction method. The IC_{50} value obtained in this study using solvent-free microwave extraction is comparable to that reported for oil obtained via hydrodistillation (Yang & Park, 2025).

Table 2. The effect of mass-volume ratio, power, and time on energy requirement to maintain the highest yield

Ratio	Power (W)	Time (min)	Time (s)	Energy (kJ)	Yield (%)
0.1	140	40	2400	336.0	1.2
0.1	280	20	1200	336.0	1.2
0.1	560	10	600	336.0	1.2
0.2	140	80	4800	672.0	1.2
0.2	280	40	2400	672.0	1.2
0.2	560	20	1200	672.0	1.2
0.3	140	120	7200	1008.0	1.2
0.3	280	60	3600	1008.0	1.2
0.3	560	30	1800	1008.0	1.2

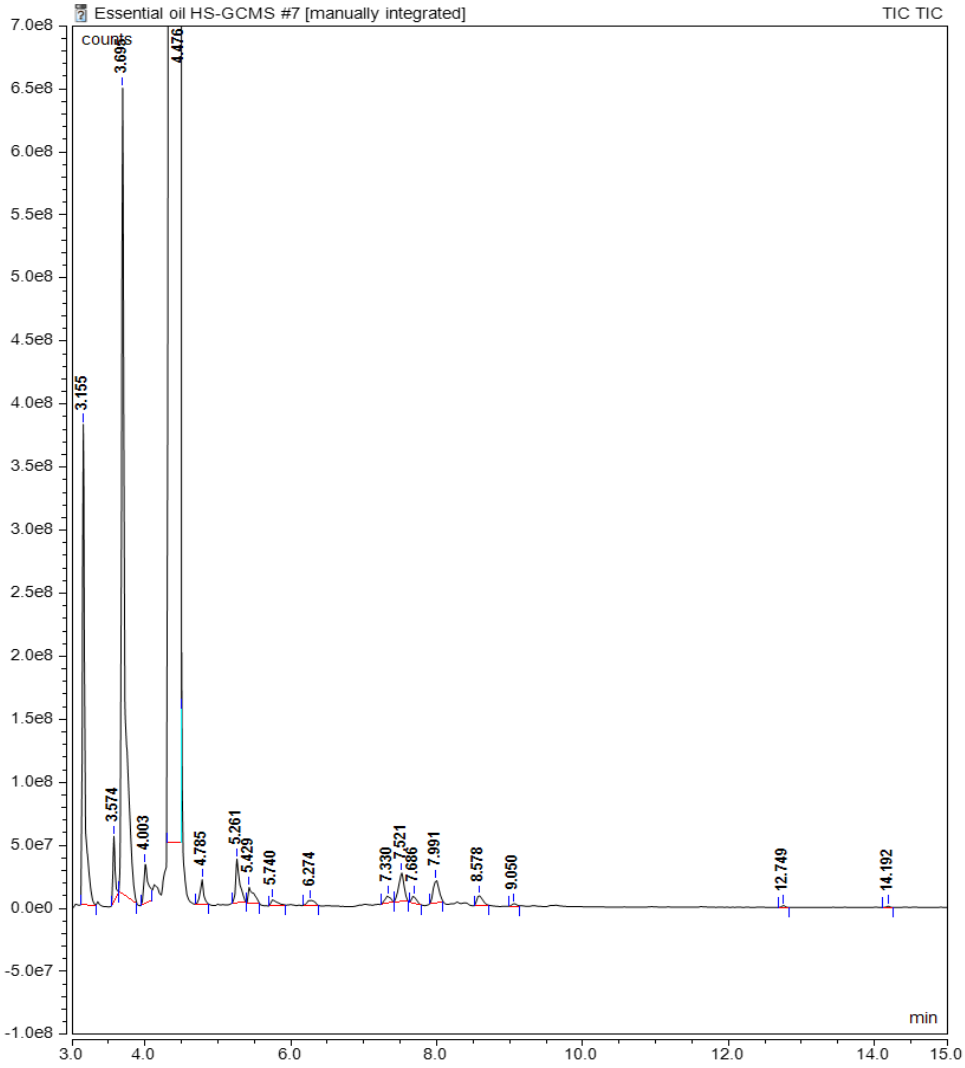
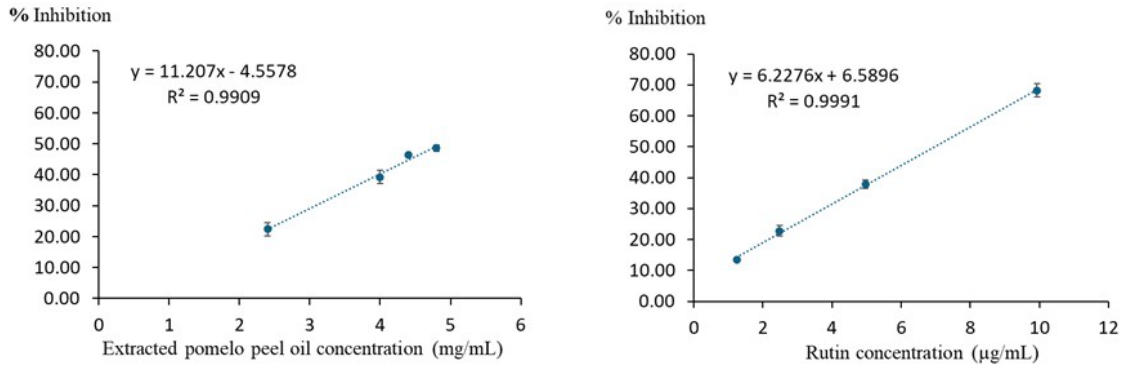


Figure 4. Chromatogram of essential oil extracted by solvent-free microwave extraction from pomelo (*Citrus maxima*) peel

Table 3. Essential oil compounds extracted by SFME determined using GC-MS analysis

Peak no.	Library compound	Retention time (min)		% Relative area	
		SFME	WSME	SFME	WSME
1	α-pinene	3.15	3.15	1.15	0.89
2	β-phellandrene	3.57	3.56	0.139	0.14
3	β-myrcene	3.69	3.68	2.613	2.51
4	α-phellandrene	4.00	4.00	0.173	0.42
5	d-limonene	4.48	4.49	95.035	95.30
6	γ-terpinene	4.78	4.78	0.087	0.06
7	cyclohexene, 1-methyl-4-(1-methylethylidene)-	5.26	5.25	0.181	0.16
8	linalool	5.43	5.42	0.086	0.21
9	3-cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl-	5.74	5.77	0.041	0.01
10	p-mentha-1,8-dien-7-ol	6.27	6.27	0.04	0.01
11	cis-p-mentha-1(7),8-dien-2-ol	7.33	7.49	0.041	0.14
12	cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans	7.52	7.67	0.158	0.02
13	(E)-3(10)-caren-4-ol	7.69	7.98	0.037	0.04
14	cis-verbenol	7.99	7.98	0.037	0.04
15	carvone	8.58	8.58	0.059	0.02
16	neral	9.05	9.05	0.014	0.03
17	caryophyllene	12.75	12.74	0.004	0.00
18	germacrene D	14.19	14.17	0.005	0.00



(a) (b)

Figure 5. ABTS radical scavenging assay of (a) the extracted pomelo peel oil and (b) rutin as the positive control

4. CONCLUSION

The solvent-free microwave extraction method has proven highly effective in extracting d-limonene in essential oil from pomelo peel waste. GC-MS analysis revealed the extraction of d-limonene and 17 other components, with a remarkable 95% yield of essential oil achieved. The energy of 336.0 kJ for 100 g of pomelo peel material was required. This method demonstrates both efficiency and environmental consciousness. The extracted essential oils showed the antioxidant activity at IC_{50} of 4.87 mg/mL by ABTS radical scavenging assay.

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