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Comparison of properties of spearmint (*Mentha spicata* L.) essential oil from microwave and hydrodistillation extraction

Princess Monica Shankar and Venkatachalapathy Natarajan

Abstract
A comparison was done between microwave-assisted extraction (MAE) and hydrodistillation (HD) for the extraction of essential oil (EO) from the dried *Mentha spicata* leaves. This study analyzed the oil yield, antioxidant activity, and chemical composition of MAE-spearmint essential oil (SEO) and HD (control sample). Microwaves obtained maximum oil yield due to their mass and heat transfer acting similarly. Gas chromatography-mass spectrometry (GC-MS) analysis showed the differences between the chemical compounds of essential oils extracted by MAE in comparison with HD. DPPH radical scavenging activity of the MAE-SEO showed that microwave irradiation has stronger antioxidant activity than the control sample. The results obtained from this study indicate that the MAE technique can be mentioned as a green technology due to its minimal energy consumption for the extraction of EO.

Keywords: Spearmint, microwave-assisted extraction, oil yield, antioxidant activity, GC-MS

1. Introduction
Traditionally, hydrodistillation (HD) and steam distillation (SD) have been used for essential oil extraction from medicinal herbs or different plant parts (Golmakani & Rezaei, 2008) [11]. These traditional methods have many drawbacks, such as minimum extraction efficiency, consumption of longer time duration, and loss of volatile aromatic compounds (B. Liu *et al*., 2018) [16]. To overcome these disadvantages, novel methods such as the microwave-assisted extraction (MAE) method have been used, which have high extraction efficiency, minimum extraction time, and minimum loss of volatile compounds (Chemat *et al*., 2013) [7]. The novel method commonly used was pressurized solvent extraction (PSE), supercritical fluid extraction (SFE), and microwave-assisted extractions (MAE). Particularly MAE has been limelight by various researchers in different fields, mainly in medicinal herbs research, because of its moderate cost, better performance under atmospheric conditions, and heating mechanism (Zhang *et al*., 2003) [23].

A microwave is an electromagnetic wave with a frequency range from 0.3 to 300 GHz. It consists of an electric and magnetic field that perpendicularly oscillates with each other. Microwaves produce heat by penetrating and interacting with polar compounds. Microwave heating is caused by dipole rotation and ionic conduction, and depending on their dielectric constant only selective substances can get heated (Chan *et al*., 2011) [6]. Many studies have reported the application of this novel method for the extraction of bioactive compounds and essential oils. The study reported by (Liazid *et al*., 2007) [15] stated that the MAE extraction with methanol for red and white hot pepper varieties showed a minimum loss of 22 phenolic substances such as benzoic aldehydes, acids, coumarins, flavonols, catechins, stilbene and cinnamic acids and their stability even at 100 °C temperature for 20 min was noted.

The *Mentha* genus is an aromatic herb commonly called mint, where the genus consists of about 25–30 species, mainly cultivated in temperate areas of Australia, South Africa, and Europe (Mohammadhosseini *et al*., 2021) [20]. All aerial parts of *Mentha spicata*, also known as spearmint, are brewed as a medicinal tea. The mint varieties have important applications in both the medicinal and commercial fields. *Mentha* flowers, leaves, and stems are regularly added to commercial spice blends as aromatic and flavor compounds (Mahendran *et al*., 2021) [18]. Due to the presence of anticatarrhal, carminative, diaphoretic, anti-inflammatory, antiemetic, stimulant, and antispasmodic properties, *Mentha spp.* has been applied in ancient medicinal treatments for curing ulcerative colitis, asthma, nausea, flatulence, liver problems, bronchitis, and anorexia (Boukhebti *et al*., 2011) [3].

EOs are natural compounds used in many commercial fields (Zhao *et al*., 2013) [24]. The EO from spearmint was mostly utilized by industries such as spice, pharmaceuticals, and food (Asekun *et al*., 2007) [2]. Also, due to high-level flavors, SEO was used as an aromatic compound in bakery products, candies, soft drinks, chewing gums, and cosmetics products such as facial creams (Mkaddem *et al.*, 2009) [19]. The antimicrobial effect of SEOs and their components are used as tooth root canal fillers, feed supplements, and antiseptics (Mahendran *et al*., 2021) [18]. The chemical composition of SEO was analyzed by gas chromatography with mass spectrometry. The main compound in SEO, carvone, is the phenolic compound responsible for its strong aroma and antimicrobial properties (Guliani *et al*., 2021) [12]. This study focused on the MAE of SEO in comparison with the HD to determine their differences in oil yield efficiency, DPPH radical scavenging activity and identification of their chemical composition

2. Materials and methods

2.1 Sample collection and processing

Mentha spicata from the Lamiaceae family were collected from Thanjavur, Tamil Nadu. The fresh leaves were picked from the mint stem. The impurities like dust and other debris were removed and cleaned. Then the leaves were dried in a Low Humidity Adsorption dryer at 30 °C with 30% RH, and the dried leaves were changed into homogeneous powder using an electric blender and stored in Ziplock covers for further analysis.

2.2 Isolation of spearmint essential oil

2.2.1 Extraction by hydrodistillation

About 20g of the dried and powdered mint was taken in a round bottom flask with 200mL of water connected with Clevenger-type apparatus for hydrodistillation extraction of EO. In this type of extraction, the contents inside the round flask were heated at 100 °C using a heating mantle for about 3 hours.

2.2.2 Extraction by Microwave-assisted extraction

Microwave-assisted hydrodistillation was employed using lab scale microwave apparatus (IFB Industries Limited), model (30SC2, frequency 2450 MHz). The mint powder of about 20 g was taken in a round flask and exposed for microwave treatment at three different power levels (450W, 630W, and 810W) for three different time durations (3min, 6min, and 9min) in a 1:2 (powder: water) ratio. Immediately the hydrodistillation extraction procedure was carried out using Clevenger-type apparatus. The percentage yield was calculated as (mL/g) using equation (1). The extracted EOs were dried over anhydrous sodium sulfate and stored at 4 °C in amber vials until further analysis.

$$Percentage\ of\ yield = \frac{Volume\ of\ essential\ oil\ (mL)}{Weight\ of\ sample(g)} \times 100 \qquad (1)$$

2.2.3 Essential oil antioxidant activity by DPPH analysis

Based on the study explained by (Morshedloo *et al*., 2018) [21] antioxidant activity of EO was analyzed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity with minor modifications. This method was based on the capability of the antioxidant to scavenge the DPPH radical. Briefly, a series of SEO (0.5lL/mL) were taken in the 96-well microplate and diluted with methanol to 100 µL. Then 200 mM of about 100 µL DPPH solution was added to each well in the microplate and vortexed in SpectroMax iD3 (LLC, USA) microplate reader. The sample mixture in the microplate was incubated in a dark place at room temperature for about 30 min, and the absorbance was measured at 517 nm using the SpectroMax iD3 (LLC, USA) microplate reader. The DPPH radical scavenging activity was calculated by using the formula:

$$\%\ scavenging\ activity = \frac{(A_{initial\ DPPH} - A_{final\ DPH})}{A_{initial\ DPPH}} \times 100 \qquad (2)$$

2.3 Identification of chemical compounds by Gas chromatography-mass spectrometry analysis (GC/MS)

The SEOs composition was analyzed by gas chromatography-mass spectrometry (Shimadzu, China) Agilent 8890GC/5977B/MSD chromatograph, with column Rtx -5MS (5% Diphenyl / 95% Dimethyl poly siloxane, 30m x 0.25mm ID x 0.25µm film thickness). The flow rate of Helium carrier gas was 1.0 mL/min. At the onset, the oven temperature was controlled at 110 °C for 2 min, and then it was slowly risen to 280 °C at a rate of 5 °C/min, followed by 250 °C. (Casuga *et al*., 2016) [5].

2.4 Statistical analysis

Statistical analysis was performed to determine the significant difference between the obtained data. All the experiments, such as oil yield and antioxidant activity, were done for MAE-SEO in triplicates. The chemical characteristics were analyzed statistically using SPSS statistics 20.0 (International Business Machines Corp., USA) for analysis of variance (One-Way ANOVA). The values were considered statistically significant at a 95% confidence level *($p>0.05$)*. Data were updated as mean with their standard deviation (Mean ± std. deviation). For the graphical representations, excel charts (MS Excel 2017, Microsoft) were used.

3. Result and discussion

3.1 Impact of MAE on SEO content

The SEO (%v/w) content differed based on the different microwave power levels and times are shown in Fig 1. The oil yield obtained from the microwave-treated spearmint leaves was from 1.0% v/w to 2.5% v/w. The MAE-EO from spearmint leaves showed a higher oil yield than the control sample (untreated microwave sample) (0.3% v/w). This current study showed differences in the EO yield, chemical composition, and antioxidant activity in spearmint leaves based on the different microwave power levels and times. The microwave treatment of spearmint leaves at 630W power level for 3min showed the essential oil with a high amount of about (2.533±0.057 % v/w). The spearmint leaves microwave treated at 450W power level for 9 min, and 3 min showed essential oil yield of about (2.0067±0.1155) and (1.7433±0.1155).

The microwave power level of 450W, 630W for about 6 min, and 810W power level for 3 min and 9 min showed (2.033±0.577 %v/w) essential oil content. The SEO treated at a microwave power level of about 630W for 9 min and 810W for 6 min showed an oil yield of (1.0000±0.0000) and (1.0667±0.11547) is the minimum amount of SEO obtained. Statistical analysis showed that the oil content was significantly higher for microwave treatments with a 630W power level for 3min relative to the control sample. The study explained by (Tran *et al*., 2018) [22] showed that 0.6% (v/w) of

EO from ground Vietnamese Basil (*Ocimum basilicum* L.) leaves was obtained for microwave treated at a power of 430W for an extraction time of about 97 min which was higher than the control sample. Fig. 1 demonstrates that the EO yield increases and decreases with different microwave power levels and times.

The increase in microwave time and power level with smaller particle sizes of the samples affects the EO yield obtained. Compared with hydrodistillation, microwave-assisted extraction of SEO was found to have a higher essential oil yield, shown in Fig. 1. The study (Z. Liu *et al*., 2018) [17] investigated that the optimal conditions for the microwave-assisted extraction of EO from *Cinnamomum camphora* leaves showed maximum oil yield of (3.51 ± 0.12%) at power level 580W for 23 min.

Therefore, the increase in oil efficiency was found to be 12 times higher at 630W power level for 3 min when compared with the control sample. Due to the maximum interaction time between the spearmint powder and the water and the extended high-temperature thermal treatment, the degradation of heat-sensitive compounds in the EO was observed in the control sample with a minimum oil yield of about (). The impact of the high-power level of the microwave showed better extraction, but this was achieved up to a certain extent; after that, a decrease in oil yield was found.

Therefore, the efficiency of oil was increased (0.5 to 2.5%) when the microwave was applied, and the movement of the molecules was induced, which affected the EO yield at increased temperatures. First and foremost, the water molecules inside the cells were heated by the magnetic wave, which causes internal pressure and leads to cell disruption. Next, due to the high temperature, the surface tension and water viscosity get reduced, leading to the maximum heat transfer from the outer environment into the solid materials. However, few heat liable compounds present in the EO lead to disintegration at a very high temperature, affecting the EO yield and quality (Tran *et al*., 2018) [22]. Therefore, the extraction time and microwave power level for SEO were found to be 3 min and 450 W.

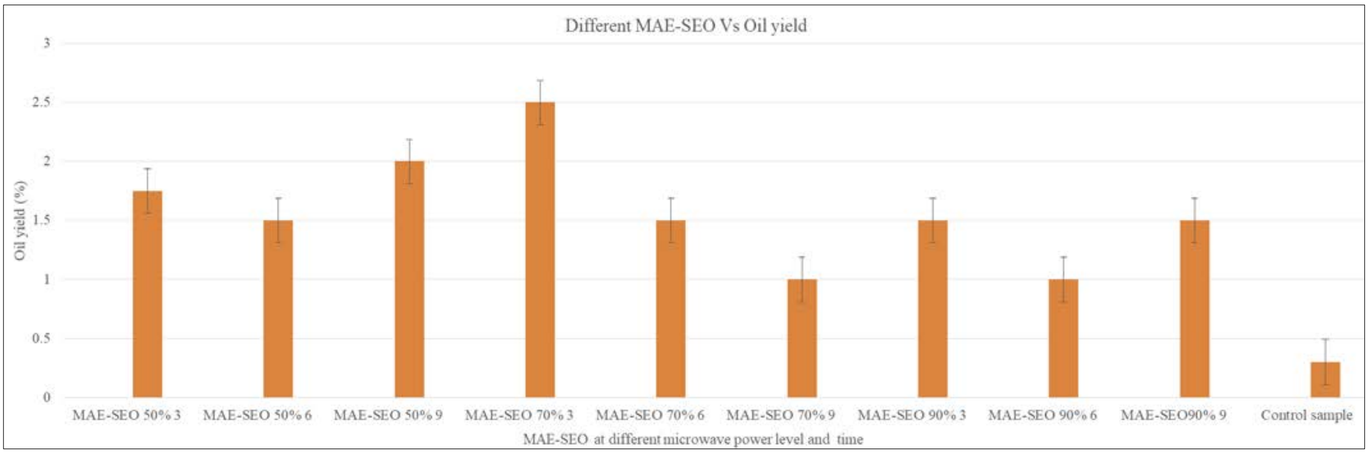


Fig 1: The plot between different MAE-SEO samples and their oil yield (%)

3.2 Impact of MAE-SEO on DPPH radical scavenging activity

Fig 2 indicates the radical scavenging activities of EO extracted by MAE from spearmint leaves, with different power levels and times. The DPPH radical scavenging activity increased as the microwave's power level and time increased. In this work, the SEO extracted from MAE at 810W power level for 6 min treated sample showed the maximum antioxidant activity of EC505 1.159±0.50 mg/mL, which leads to less disintegration of the main components as carvone and cis-carveol, which are the responsible for the DPPH radical scavenging activity. The power level at 450W for 9 min treated SEO showed the minimum radical scavenging activity of about EC505 0.0445±0.005 mg/mL. The study investigated previously by (Golmakani & Moayyedi, 2015) [10] stated that the EO extraction from citrus peel showed the highest antioxidant activity in microwave-assisted hydrodistillation were IC_{50} 42.03 mg/mL, and hydrodistillation IC_{50} 44.06 mg/mL when compared with the solvent-free microwave-assisted hydrodistillation IC_{50} 97.23 mg/mL. The study by (Dorman *et al*., 2003) [9] stated that Mentha varieties like *M. spicata*, *M. longifolia*, and *M. piperita* essential *oil* (Desam *et al*., 2019) [8] showed radical scavenging activity. (Hussain *et al*., 2010) [14] in their study, the SEO was found to have 61.5% inhibition for the oxidation reaction. Another study (Cai *et al*., 2013) [4] stated that the microwave-assisted extraction with ethanol (MAEE) of star anise oil from *Illicium verum Hook. f* with a 1000 W power level of about 45 min have stronger radical scavenging activities than the steam distillation. This study by (Abedi *et al*., 2017) [1] found that the EO extracted from the microwave-assisted treatment of *Nigella sativa* L. seeds at 450 W power level for 30 min showed stronger DPPH radical scavenging activity of about IC_{50}28.10 µg/ml than hydrodistillation IC_{50}36.90 µg/ml where low IC_{50} indicates stronger antioxidant activity. The observations from this study showed similar radical scavenging activity based on their different microwave power level and time and obtained maximum DPPH radical scavenging activity than the control sample.

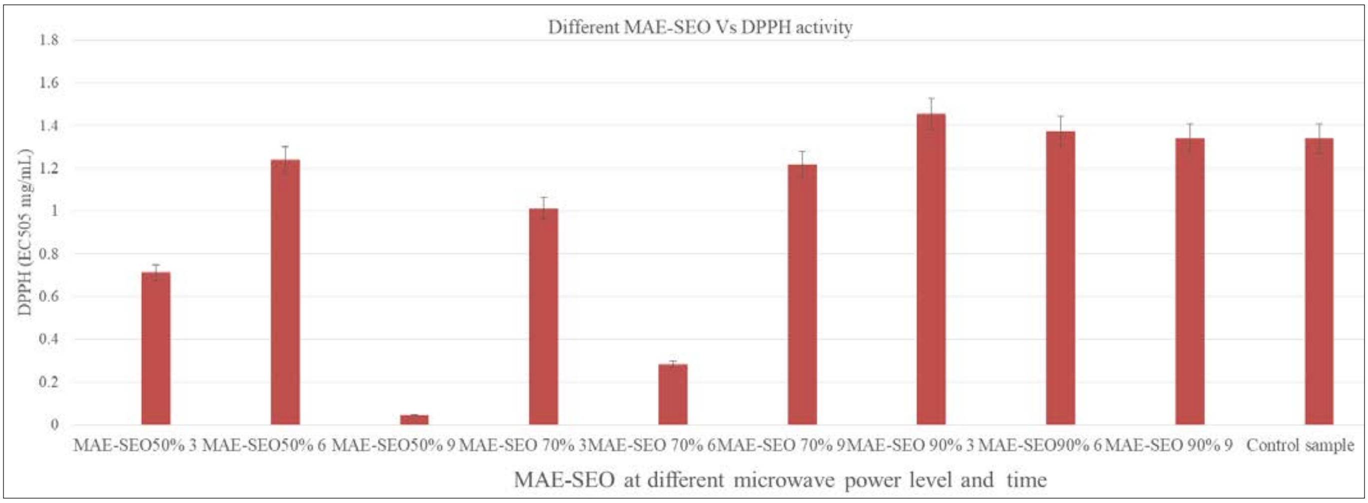
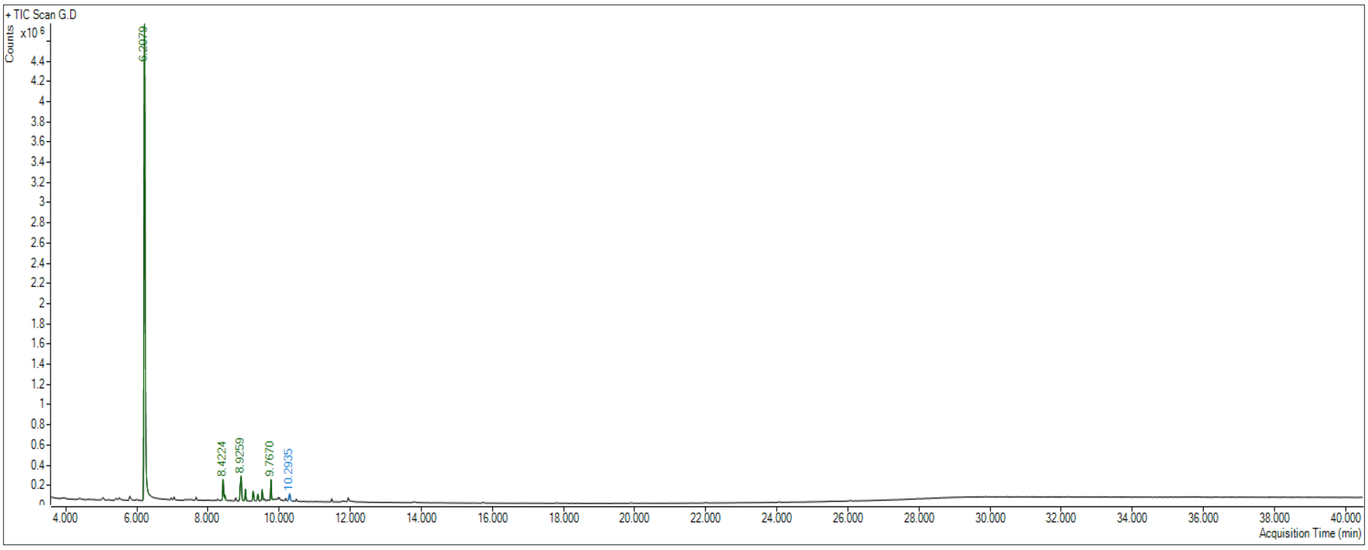


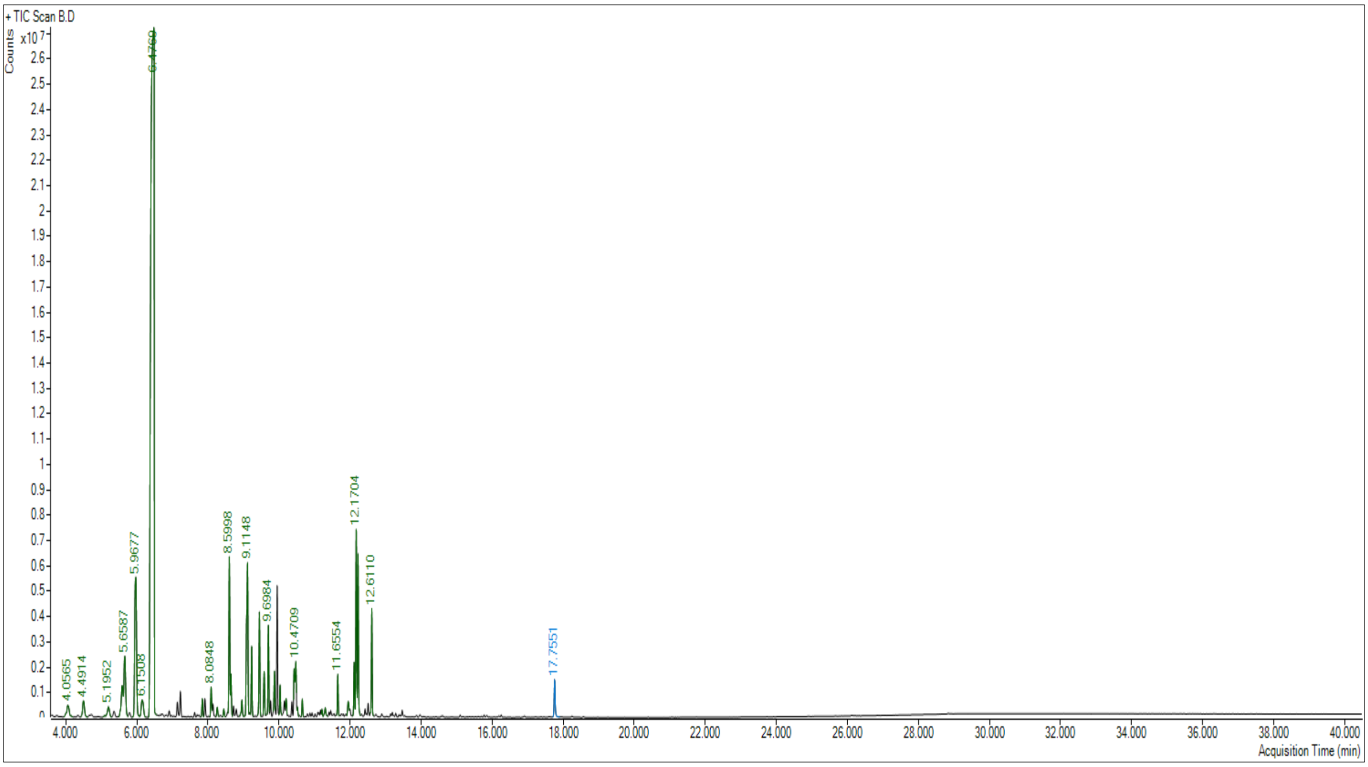
Fig 2: The graph between different microwave-treated samples and their DPPH activity

3.3 GC-MS analysis for the identification of chemical compounds

Table 1 indicates the chemical compounds and the relative component concentrations (calculated as GC peak area percentages) identified by GC-MS analysis. Carvone was the compound present in the largest amount in microwave treated at 630W power level for 9 min SEO. (-)-Carvone (83.10%), caryophyllene (4.83%) and (-)-beta. -Bourbonene (2.70%) is the main important compound present in MAE-SEO. alpha. -Cubebene (2.49%), (+)-epi-Bicyclosesquiphellandrene (1.25%), Copaene (1.24%), Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1- methylethenyl)-, [1S-(1. alpha.,2. beta.,4. beta.)] (0.78%), and gamma. -Muurolene (0.77%) was the other component in the microwave treated at 630W power level for 9 min SEO. The maximum (-)-Carvone compound was found in microwave power level at treated 630W for 9 min SEO sample (83.10%) than the control sample SEO (54.01%). (Hawrył *et al*., 2015) [13] their study observed that hydro-distillation-treated SEO showed a maximum carvone level of (15.76 %). β -Bourbonene of about (2.70%) was found in the MAE-SEO sample (630W microwave power level for 9 min), where similar results were reported in the (Hawrył *et al*., 2015) [13] study with (0.41%). gamma. -Muurolene (0.77%) was found to be high in microwave treated at 630W power level for 9 min SEO than the control sample (0.23%). Caryophyllene, an oxygenated terpenoid with antibacterial activity, was observed in both control (4.01%) and 630W power level for 9 min treated SEO (4.83%). The obtained data stated the MAE-SEO and control sample (SEO) composition with some changes and similarities, and fingerprinting these changes and similarities would be useful to identify the *Mentha* species.



(a)



(b)

Fig 3: The GC-MS chromatogram of (a) MAE-SEO 630W power level for 9 min and (b) Control sample (SEO)**.**

Table 1: Chemical composition of MAE-SEO and control SEO by GC-MS

S.no	Compound	Library molecular weight	RT		Area %	
			MAE-SEO	Control SEO	MAE-SEO	Control SEO
1	Cyclohexane, 1-ethenyl-1-methyl-2, 4-bis (1-methylethenyl)-, [1S-(1. alpha., 2. beta.,4. beta.)]	152.1	8.4681	8.6456	0.78	0.83
2	(-)-Carvone	150.1	6.2079	6.4769	83.10	54.01
3	Gamma.-Muurolene	204.2	9.4008	10.1962	0.77	0.23
4	Copaene	204.2	9.2692	8.4453	1.24	0.17
5	(-)-. beta. -Bourbonene	204.2	8.4224	8.5998	2.70	3.39
6	Caryophyllene	204.2	8.9259	9.1148	4.83	4.01
7	(+)-epi-Bicyclosesquiphellandrene	204.2	9.5210	9.6984	1.25	1.71
8	Alpha.-Cubebene	202.2	9.7670	-	2.49	-

4. Conclusion

This study's main aim was to examine the MAE of EO from spearmint leaves and to investigate their EO efficiency and antioxidant activity without any considerable difference in the chemical composition of SEO. The optimum parameters for microwave-treated extraction for spearmint leaves were 9 min at microwave power 630 W. Carvone content in SEO extracted by HD was decreased substantially due to the prolonged extraction time of HD, which leads to the degradation of the compound. The DPPH radical scavenging activity of EO obtained by MAE showed stronger antioxidant activity when compared with the control sample. According to the results obtained, we may conclude that for the extraction of EO from spearmint leaves, the MAE technique is a suitable substitute method for the conventional HD method leading to better oil efficiency, stronger antioxidant activity, and a higher amount of carvone content in the SEO. The future scope for this study is to determine the antibacterial activity from MAE of spearmint EO compared with other extraction techniques.

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