# COMPARATIVE STUDY ON THE CHARACTERISTICS OF ESSENTIAL OILS FROM SPEARMINT PLANTS (*MENTHA SPICATA* L.) BY MICROWAVE-ASSISTED HYDRODISTILLATION AND CONVENTIONAL METHODS

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## Abstract

Essential oils from plant species have played an important role in the fields of food industry, drugs, mosquito repellent, perfumes and other chemicals of important economic values. In this research work, essential oils from spearmint plants (*Mentha spicata* L.) were extracted using a method of microwave-assisted hydrodistillation (MHD) and conventional methods such as steam distillation (SD) and hydrodistillation (HD). Microwave-assisted hydrodistillation is an advanced and innovative hydrodistillation technique, in which a microwave oven (250v-50Hz power source; 2450MHz, output power 800Watt) was used as the heating source. The effects of extraction time (15 min, 30 min, 45 min, 60 min and 75 min) for microwave-assisted hydrodistillation and (30 min, 60 min, 90 min, 120 min, 150 min and 180 min) for steam distillation and hydrodistillation on the yield of essential oils were investigated. It was found that the highest yield percent of spearmint essential oils obtained by steam distillation and hydrodistillation were 0.07 % w/w and 0.05 % w/w while that of 0.09 % w/w for microwave-assisted hydrodistillation. The physico-chemical properties of essential oils such as color, specific gravity, refractive index, acid value and solubility in ethanol were analyzed. The extracted essential oils were also identified by Gas Chromatography-Mass Spectrometry (GC-MS). It was observed that carvone in spearmint essential oils was the major key component, with the highest concentration in 58.63 % by steam distillation, 56.91 % by hydrodistillation and 59.49 % by microwave-assisted hydrodistillation.

**Keywords:** essential oils, spearmint plants, microwave-assisted hydrodistillation, steam distillation, hydrodistillation

## Introduction

Essential oils are botanical extracts of various plant materials and they are extracted not only from flowers but from herbs, trees and various other plant materials. These essential oils are commercially used in different industries including food, beverages, pharmaceutical and cosmetics industries due to the multifunctional properties such as antiviral, antibacterial, insecticidal and antioxidant properties. However, essential oils are obtained very low yield, making it fragile substances (Teixeira *et al.,* 2013). The use of medicinal plants extracts including for spearmint (*Mentha spicata* L.) is a part of competitive market, which includes pharmaceuticals, food, cosmetics, and perfumery markets, mainly to use their active substance (Husnu, 2010). The essential oils extracted from spearmint plants containing mainly carvone 50-70 % w/w and menthone, have shown strong insecticidal and mutagenic activity (Hussain, 2009).

Essential oils can be isolated using several extraction methods that differ from one another by the time required for extraction, performance and energy consumption, etc. (Romdhane *et al.,* 2011). The conventional hydrodistillation and steam distillation remain the most common method used for the extraction of essential oils. However, these extraction methods have several disadvantages such as loss of the ability of some compounds of the extract due to thermal degradation, a long extraction time and the considerable consumption of energy (Wenqiang *et al.,* 2007). As a means to overcome this sort of drawbacks, an advanced and improved method such as microwave-assisted extraction, subcritical water extraction and ultrasound-assisted extraction have

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been applied for the extraction of essential oils in order to shorten the extraction time, improve the extraction yield and reduce the operational costs.

Recently, microwave-assisted hydrodistillation (MHD) procedures for extracting essential oils have become attractive for use in laboratories and industries due to its effective heating, fast extraction process, high yield of oil, good quality of the extracts and also environmental friendly extraction technique (Wenqiang *et al.*, 2007). The aim of this research is to investigate the applicability of microwave-assisted hydrodistillation method as an alternative and effective technique over steam distillation and hydrodistillation in the extraction of essential oils based on the extraction yield and chemical compositions of essential oils obtained from native plant species, spearmint.

## Materials and Methods

### Materials

Fresh spearmint plants were collected from Hlegu Township, Yangon Region. Air-dried leaves were used as raw materials in this research work. Ethanol (95 %) and sodium sulfate were purchased from Empire Chemical Shop, 27$^{th}$ street, Pabedan Township, Yangon.

### Preparation of Sample

The collected plant materials were thoroughly washed with tap water to remove the dirt. These plant materials were dried at room temperature for one day and cut into small pieces. Then, the small pieces of sample were ground using a grinder (Super Blender, Panasonic, Tokyo, Japan).

### Extraction of Essential Oil from Spearmint Plants

### Steam Distillation (SD)

The ground plant material, 400 g, was placed in a 1 L round bottom flask. This flask was then connected to a steam generating equipment (Clevenger-type apparatus). 1 L of water was boiled in a flat bottom flask. The generated steam was passed through the dried sample and the volatilized oil which passed through the condenser was collected in a receiver. Steam distillation was conducted for 3 hours. The distillate from the receiver was transferred into a separating funnel and left quiescently overnight for layer separation. The bottom layer, which is water, was withdrawn. The extracted essential oil was retained in the upper layer. The residual moisture in the extracted oil was removed using anhydrous sodium sulfate. Then, the resultant oil was put in an air tight amber glass bottle and stored in a cool and dry place.

### Hydrodistillation (HD)

400 g of ground plant material was placed in a 1 L round bottom flask containing 400 mL of distilled water and hydrodistilled for 30 min, 60 min, 90 min, 120 min and 150 min using a Clevenger-type apparatus. The system was operated at a fixed power of 500 W and under the atmospheric pressure. The oil and water layers separation was conducted according to the above steam distillation method.

### Microwave-assisted Hydrodistillation (MHD)

A modified domestic microwave oven model Electrolux EMM2331MK connected to the Clevenger apparatus was modified for microwave-assisted hydrodistillation operation as shown in Figure (1). The Electrolux EMM2331MK has 1150 W power consumption, 800 W output power with 250v-50Hz power source; 2450MHz. The cavity dimensions of the microwave oven were 220 × 340 × 320 mm.

The microwave-assisted hydrodistillation was conducted at microwave power 800 W for durations of 15 min, 30 min, 45 min, 60 min and 75 min. Firstly, 1 L of flat bottom flask containing 400 g of plant material with 400 mL of distilled water was put into the microwave oven cavity. A condenser which has been set on the top, outside the oven, was used to collect the extracted essential oils. Microwave-assisted hydrodistillation was conducted for 75 min.

The extracted essential oils were dehydrated using anhydrous sodium sulphate. The pure extracted essential oil was placed in an air tight amber glass bottle and stored in a clean and dry place.

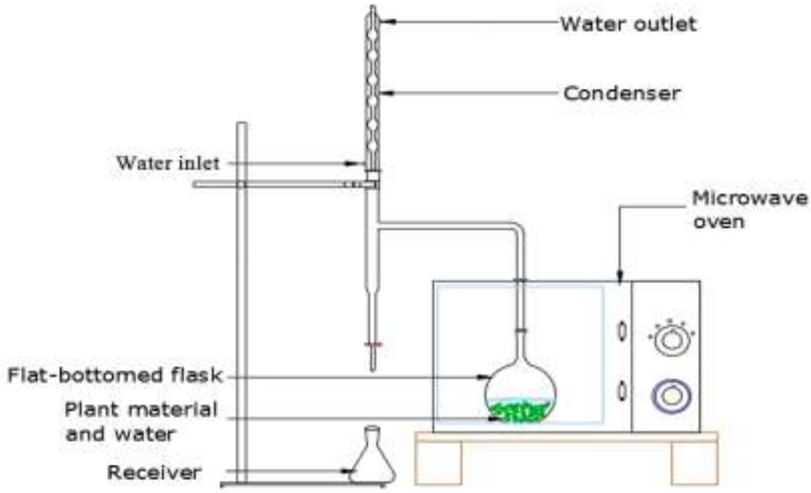


**Figure 1** Microwave-Assisted Hydrodistillation Apparatus

**Determination of Physico-chemical Properties of Extracted Essential Oils**

The physico-chemical characteristics such as color, specific gravity, refractive index, acid value and solubility of the extracted essential oils from spearmint plants were analysed at room temperature. Lovibond Tintometer (Model No. E, England Made) was used to determine the color of essential oil. The specific gravity of extracted essential oil was determined using density bottle AOAC-2000(9.009). The refractive index was measured with an Abbe '60' type refractometer. The acid value was determined by AOAC-2000 (969.33) method. The solubility in alcohol was determined by AOAC-2000(2021.25) method.

**Identification of Extracted Essential Oils by Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis**

The constituents in extracted essential oils were identified by GC-MS (PerkinElmer, Clarus 680 GC coupled to PerkinElmer, Clarus 600 MS Detector equipped with an Elite 5 MS capillary non polar column - 30.0 m length x 0.25 mm ID x 0.25 μm film thickness). The components of essential oils were identified based on their retention time and mass spectra, matching with National Institute of Standards and Technology (NIST05) libraries provided with computer controlling of the GC-MS system.

The GC-MS analysis of the extracted essential oils was conducted at the National Analytical Laboratory, Department of Research and Innovation, Ministry of Education, Yangon Region.

## Results and Discussion

In this research work, essential oils were extracted from spearmint plants by steam distillation, hydrodistillation and microwave-assisted hydrodistillation methods. Figure (2) shows that the effect of extraction time on yield percent of extracted spearmint essential oil was graphically compared with steam distillation and hydrodistillation methods. According to the results of the research work, the amounts of essential oils extracted by both methods did not change significantly after 150 min. The extraction rate was high at the beginning of the extraction but it can be seen that no significant improvement in yield of oil after a certain period. From the point of view of distillation methods, the yield of essential oils from spearmint plants extracted by steam distillation was higher than that of hydrodistillation. Guenther, 1948 stated that the distillation of plant materials with high water content usually provides the problems to completely extract the essential oil. It was found that fresh spearmint herb, like most plants or plant parts with a high moisture content, cannot be exhausted completely by distillation, or only with great difficulty, and after long hours of distillation. It was also observed that yield of essential oils by hydrodistillation were slightly lower than that of steam distillation method.

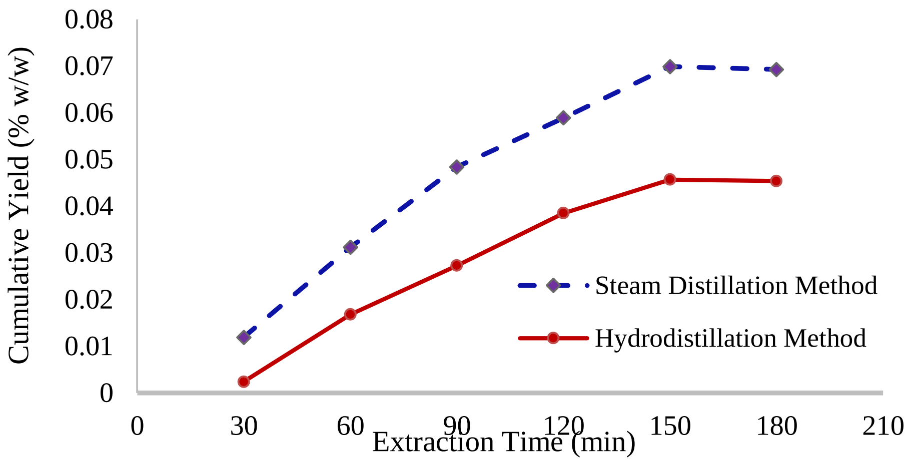


**Figure 2** Effect of Extraction Time on the Yield Percent of Spearmint Essential Oils by Steam Distillation and Hydrodistillation

Figure (3) shows the effect of extraction time on the yield of oil from the spearmint plants by microwave-assisted hydrodistillation method. It can be seen that the extraction time of 45 min was found to obtain the highest yield of spearmint essential oil. When compared to the yields of essential oils obtained by steam distillation and hydrodistillation methods, the extraction time for microwave-assisted hydrodistillation was only 15 min whereas for steam distillation and hydrodistillation needed 30 min. This result shows that nearly 30 % w/w of the total yield of oil could be extracted using the microwave-assisted hydrodistillation within a short extraction time of 15 min.

The amount of yield resulted by microwave-assisted hydrodistillation after 45 min was almost similar to the oil obtained after 150 min by steam distillation and hydrodistillation methods. It can also be seen that the yield of essential oil extracted by microwave-assisted hydrodistillation was slightly higher than that of steam distillation and hydrodistillation methods.

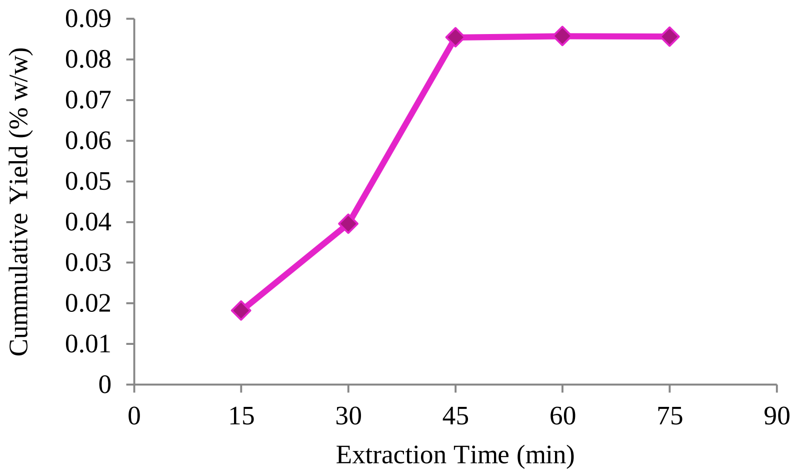


**Figure 3** Effect of Extraction Time on the Yield Percent of Spearmint Essential Oil by Microwave-assisted Hydrodistillation

The results in Table (1) show that physico-chemical properties of essential oils from spearmint plants. Physico-chemical properties of essential oils are an assessment of the purity and quality of the volatile oil as well as for identification. Therefore, the specific gravity, refractive index, acid value and solubility were determined for the isolated essential oils from spearmint plants by steam distillation, hydrodistillation and microwave-assisted hydrodistillation methods. According to the results, it can be seen that specific gravity of extracted essential oil is less than 1 for all samples.

Barkatullah *et al*., (2012) stated that specific gravity of essential oils is less than one for all the oils except some plant oil like eugenol and cinnamon oils. The value of refractive index of essential oil extracted by steam distillation was 1.47, 1.48 for hydrodistillation but 1.45 for microwave-assisted hydrodistillation. Duarte *et al*., (2018) reported that the essential oils which have high refractive index, are sparingly soluble in water, and less dense than water and liquid at room temperature, but exception for trans-anethole (anise camphor) from the oil of anise (*Pimpinella anisum* L.). Guenther, 1948 stated that essential oils contain several volatile aroma compounds; often these are free fatty acid. Free fatty acids are considered as defect in oils/fats because these are degraded or become rancid. Hassan, (2019) reported that spearmint essential oil contains acid value 1.1 mg, specific gravity 0.81 and refractive index 1.49. Acid values of extracted essential oils are 1.35, 1.50 and 1.30 for steam distillation, hydrodistillation and microwave-assisted hydrodistillation, respectively. It can also be found that all the samples were miscible in ethanol. According to the literature study of essential oils, Gamarra *et al*., 2000 stated that physico-chemical properties of the plant oils can vary depending on the chemotype and biotype of the plant, the soil condition as well as the extractive process.

**Table 1 Physico-chemical Properties of Spearmint Plants Essential Oils**

| Sr. No. | Parameters | Experimental Values | | |
|---|---|---|---|---|
| | | SD | HD | MHD |
| 1 | Color | Yellow 0.1, Blue 0.1 | Yellow 0.1 | Yellow 0.1, Blue 0.2 |
| 2 | Refractive Index | 1.47 | 1.48 | 1.45 |
| 3 | Specific Gravity | 0.84 | 0.90 | 0.89 |
| 4 | Acid Value (mg KOH/g) | 1.35 | 1.50 | 1.30 |
| 5 | Solubility in ethanol | Soluble | Soluble | Soluble |

SD – Steam Distillation HD – Hydrodistillation MHD – Microwave-assisted Hydrodistillation

From the results of the Table (2), carvone was found as the most dominant compound and followed by D-lemnene, Beta-phellandrene and Alpha-cardinol. It was also observed that concentrations of key component, carvone, found in the spearmint (*Mentha spicata* L.) essential oil, shown in Figure (4), were nearly the same in all methods. This finding is in accordance with the literature values. According to the literature review, Bayan *et al.*, (2018) reported that the Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the essential oil from *Mentha spicata* L. contained the main component of carvone 56.94 % w/w, followed by limonene 11.63 % w/w, sabinene hydrate 7.04 % w/w and caryophyllene 4.06 % w/w. Moreover, Hassan, (2019) stated that D-carvone 51.91 % w/w, D-limonene 24.64 % w/w and eucalyptol 2.81 % w/w were found as major compounds of *Mentha viridis* leaves oil. GC-MS Chromatogram of extracted essential oil by steam distillation, hydrodistillation and microwave-assisted hydrodistillation are shown in Figures (5), (6) and (7).

However, some oxygenated monoterpenes compounds such as Beta-Ocimene, and Terpenen-4-ol cannot be found in the extracted essential oils by microwave-assisted hydrodistillaton and hydrodistillation methods. Handa *et al.*, (2008) stated that if refluxing was controlled like hydrodistillation, there will be losses of some oxygenated compounds and these could be dissolved into distilled water to some extent. Ranitha *et al*., (2014) reported that there can be a difference for chemical compositions in extracted essential oil by microwave distillation. Microwave radiation can penetrate into biological materials and creates heat by interaction with polar molecules such as water. Therefore, water soluble constituents cannot be completely extracted by microwave-assisted hydrodistillation.

**Figure 4** Spearmint Plants Essential Oil Extracted by Microwave-assisted Hydrodistillation Method

**Table 2 Dominant Compounds in Extracted Essential Oils by Steam Distillation, Hydrodistillation and Microwave-assisted Hydrodistillation**

| Sr. No. | Compound Name | Retention Time (min) | | | Molecular Weight | Formula | Related Amount (% w/w) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | SD | HD | MHD | | | SD | HD | MHD |
| 1 | 3- Carene | 3.08 | 3.38 | 3.24 | 136 | $C_{10}H_{16}$ | 1.14 | 1.18 | 1.34 |
| 2 | Beta-Phellandrene | 3.81 | 3.84 | 3.64 | 136 | $C_{10}H_{16}$ | 4.38 | 1.17 | 1.66 |
| 3 | Gamma- Terpinene | 3.94 | 3.68 | 3.75 | 136 | $C_{10}H_{16}$ | 1.38 | 0.08 | 0.15 |
| 4 | Beta-Pinene | 3.99 | - | 3.76 | 134 | $C_{10}H_{14}$ | 0.77 | - | 0.84 |
| 5 | D-Limonene | 4.67 | 4.05 | 4.31 | 154 | $C_{10}H_{18}O$ | 19.64 | 24.30 | 22.19 |
| 6 | Beta-Ocimene | 4.79 | - | - | 154 | $C_{10}H_{18}O$ | 0.27 | - | - |
| 7 | Trans-Carveol | 7.84 | 6.72 | 6.90 | 154 | $C_{10}H_{18}O$ | 2.64 | 1.65 | 2.02 |
| 8 | Terpenen-4-ol | - | 6.18 | 6.72 | 154 | $C_{10}H_{18}O$ | - | 8.91 | 3.65 |
| 9 | Carvone | 7.95 | 7.01 | 7.24 | 152 | $C_{10}H_{16}O$ | 58.63 | 56.91 | 59.49 |
| 10 | Beta-Bourbonene | 9.73 | - | 9.04 | 154 | $C_{10}H_{18}O$ | 1.12 | - | 2.92 |
| 11 | Azulene,1,2,3,3A,4, 5,6,7-Octahydro-1,4-Dimethyl-7-(1-Methylethenyl) | 8.19 | - | - | 204 | $C_{15}H_{24}$ | 2.13 | - | - |
| 12 | Caryophyllene | 10.21 | 9.12 | 9.51 | 154 | $C_{10}H_{18}O$ | 0.89 | 0.37 | 0.23 |
| 13 | Dihydro-Cis-Alpha-Copaene-8-ol | 10.25 | 10.25 | 10.28 | 222 | $C_{15}H_{26}O$ | 2.40 | 2.86 | 2.10 |
| 14 | Alpha-Cardinol | 13.10 | 11.98 | 12.37 | 222 | $C_{15}H_{26}O$ | 2.27 | 0.19 | 0.63 |

SD – Steam Distillation HD – Hydrodistillation MHD – Microwave-assisted Hydrodistillation

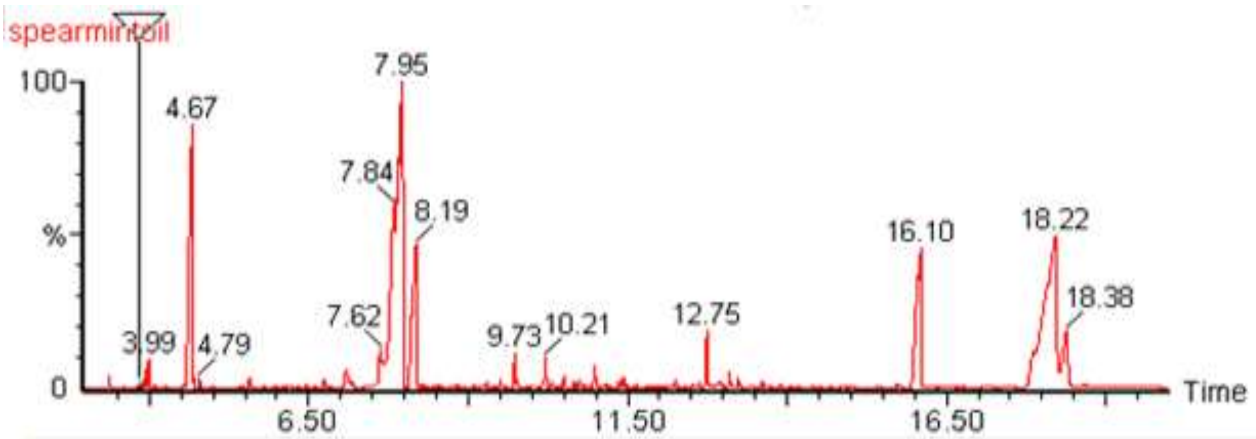


**Figure 5** GC-MS Chromatogram of Extracted Spearmint Essential Oil by Steam Distillation

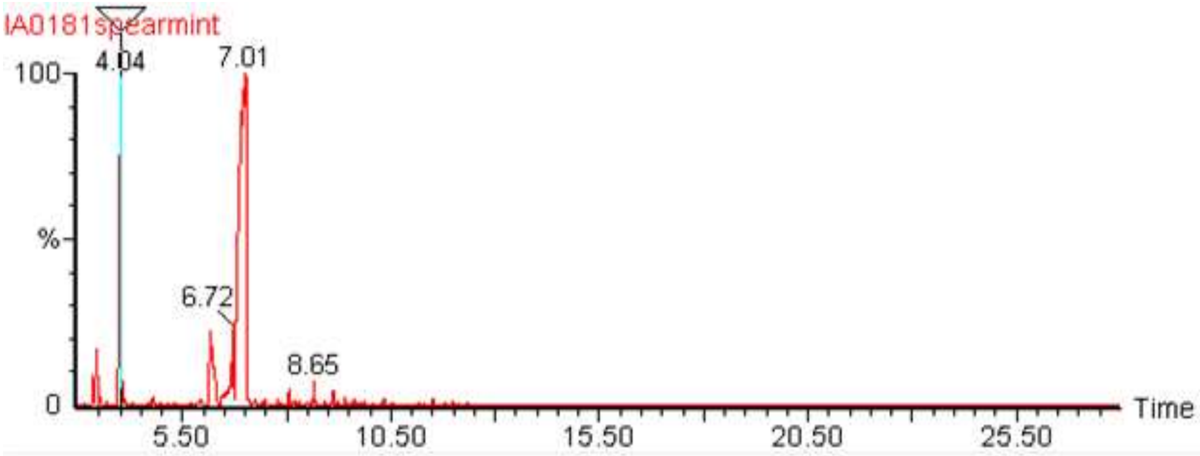


**Figure 6** GC-MS Chromatogram of Extracted Spearmint Essential Oil by Hydrodistillation

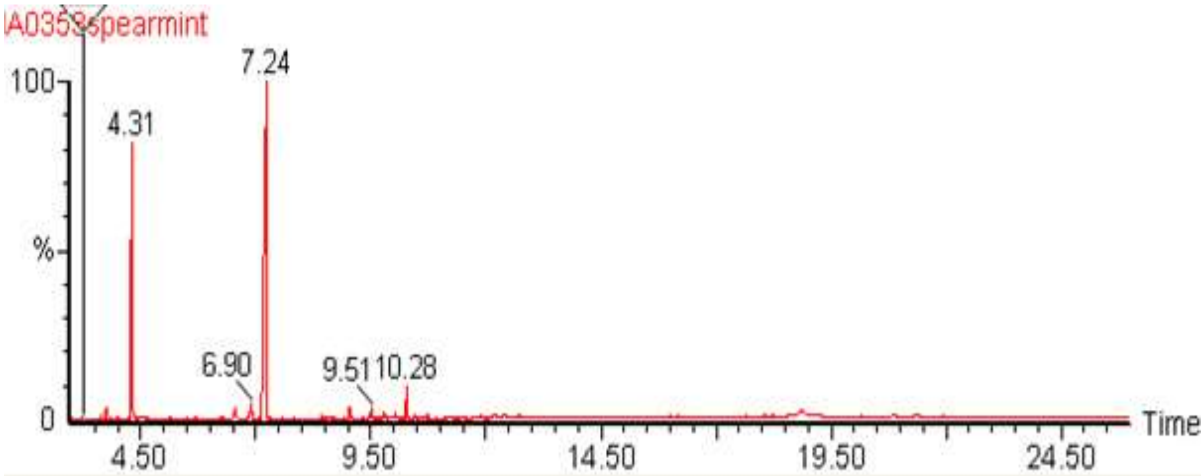


**Figure 7** GC-MS Chromatogram of Extracted Spearmint Essential Oil by Microwave-assisted Hydrodistillation

## Conclusion

In this research work, essential oils were successfully extracted by steam distillation, hydrodistillation and microwave-assisted hydrodistillation methods. Microwave-assisted hydrodistillation offered great advantages over conventional methods such as steam distillation and hydrodistllation. Microwave-assisted hydrodistillation technique was found to require shorter extraction time. In addition, GC-MS results proved that there were no significant difference between the components of essential oil extracted by microwave-assisted hydrodistillation and those obtained by steam distillation. However, the number of extracted components obtained by hydrodistillation method was found to be less than the other two methods. According to the substantial odor and yield of essential oil, saving of time, cost and energy with no significant changes in its constituents, microwave-assisted hydrodistillation technique was found to be a good alternative way in the extraction processes of essential oils from spearmint plants.

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