Comparison of chemical composition of essential oils obtained by hydro-distillation and microwave-assisted extraction of Japanese mint (*Mentha arvensis* L.) grown in Vietnam

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**Abstract.** The Japanese mint (*Mentha arvensis* L.) is known as a culinary and medicinal herb widely cultivated in several countries. The mint essential oil is considered as one main source of menthol. In this study, the Japanese mint collected from the Central Highlands of Lam Dong Province (Vietnam) was extracted the essential oil by hydro-distillation (HD) and microwave-assisted extraction (MAE). Different extraction parameters were investigated, involving mint leaf drying periods (30, 60, and 90 mins) at 50 °C, material to water ratios (1:4, 1:6, 1:8, and 1:10 for HD and 1:1, 1:1.5, 1:2, and 1:2.5 for MAE), extraction durations (60-180 for HD and 10-25 mins for MAE), and microwave energy (230, 380, 540, and 700 W) in order to discover the conditions with the highest yields for both HD and MAE. The GC-MS was used to determine the oil chemical constituents. For dried mint leaves (nearly 40 % moisture), the highest oil yield of 0.0832 mL g⁻¹ (dried weight) was recorded, and the content of menthol was up to 70.60 % extracted by MAE whilst 0.0510 mL g⁻¹ of yield (dried weight) and lower menthol (62.80 %) were obtained from HD. Besides, menthol, trans- and cis-menthone were found at relatively high concentrations for both HD and MAE (20.19-16.30 and 4.62-5.01 %, respectively). Compared to HD, MAE exhibited higher oil yields and menthol contents, but lower distillation time, resulting in more effective extraction performance of MAE.

1. **Introduction**

Essential oils and extracts obtained from various plants have recently been achieving increasing attention in society and scientific interests. They have been utilized as functional ingredients in food, drug, and perfumery [1, 2]. Botanically, Japanese mint (*Mentha arvensis* L.) belongs to the *Lamiaceae* family in the genus of *Mentha*. Its major producers can be listed as India, China, Vietnam, and Brazil due to its high economic values [3, 4]. As an herb, the aerial parts of mint, in general, have been used in cooking, cocktails, and confectionery, as well as in the preparation of medicinal liquors [5]. The essential oils, which are known as secondary metabolites of this plant, are obtained mostly by hydro-distillation of its aerial parts and are widely employed due to their antioxidant, antimicrobial, anti-inflammatory, and antitumor properties. Mint oil is composed primarily of menthol and menthone as well as many other minor constituents, including camphene, 1,8-cineole, and limonene [5, 6].

There are many distillation methods available to extract the essential oils from plants and herbs in general and from the mint in specific. These techniques can be classified into (i) classical and conventional methods and (ii) innovative techniques or non-traditional ones [7, 8]. Hydro-distillation
(HD), steam distillation (SD), solvent extraction, Soxhlet extraction, and cold pressing method are the roughly traditional and generally used methods [7, 8]. Modern and innovative extraction methods include supercritical fluid extraction (SFE), microwave-assisted hydro-distillation or extraction (MHD or MAE), ultrasound-assisted extraction (UAE), and microwave hydro diffusion and gravity (MHG) [7]. Among these methods, HD, SD, and MHD/MAE seem to be the most commonly used to extract the essential oils due to the reasonable cost, simple system, reduced extraction durations, saved time and energy consumption, less or no solvent use, especially organic solvents, and decreased carbon dioxide emission [7, 9].

In the literature, several works have been reported on dehydration effects on not only the essential oil yields but also the chemical compositions for various medicinal herbs and plants. There was a study of Omidbaigi et al. (2004) [10] about the influences of different drying methods, involving sun-drying, shade-drying, and oven-drying at 40 °C, on the qualitative and quantitative traits of the essential oils derived from Roman chamomile (Chamaemelum nobile L. All. var. flora plana). In this group’s study, although the oil contents of the shade-dried materials were the highest (1.9 %) followed by oven-drying (0.9 % w/w) and sun-drying (0.4 % w/w), drying methods had no effects on the number of chemical components of the oils but had a significant effect on the proportion of the various components. The research of Pirbalouti et al. (2013) [11] investigating the contents and chemical composition of the essential oils of the purple and green basil landraces aerial parts under sunlight, shade, oven, microwave, and freeze-drying treatments showed that, for both landraces, the highest yields were achieved from shade-dried samples. Moreover, there was a significant loss of most of monoterpene hydrocarbons in the essential oils for dried materials compared to fresh ones. Blanco et al. (2000) [12] dried peppermint leaves at temperatures of 40 °C, 60 °C, and 80 °C, and indicated that the higher drying temperature sharply decreased the essential oil yields. Zheljazkov et al. (2010) [13] assessed the effects of harvest time and drying on Japanese corn mint (Mentha Canadensis L.) to optimize the technology. Kurilov et al. (2009) [14] carried out a study to compare the chemical composition of the essential oils extracted from Mentha piperita and Mentha arvensis species grown in the Tsitsin Main Botanical Garden, Russian Academy of Sciences. Menthol contents of the essential oils extracted by these materials varied from 30 to 43 %. Verma et al. (2010) [3] also conducted a study to compare the essential oil chemical components of Mentha piperita and Mentha arvensis grown in the Kumaon region of western Himalaya. Their study performed various concentrations of menthol, ranging from 22.56 to 42.83 %. Zheljazkov et al. (2010) [15] studied Japanese corn mint in Mississippi and indicated that the concentrations of menthol were 50 % and 67-78 % in 2007 and 2008, respectively. Kohari et al. (2020) [16] used solvent-free microwave extraction for fresh Japanese mint (Mentha arvensis L.) and compared the essential oil yields and compositions obtained from those by traditional HD. The results of this research showed that the oil yields ranged from 0.88 to 0.95 % by treatment at 100-500 W within 20-90 minutes, which were similar to the figures derived from HD.

In Vietnam, there are few publications about Japanese mint essential oils. According to Do (1985) [17] and Le (2003) [18] in their books about essential oils, Mentha arvensis species can grow naturally mostly in mountainous regions in several provinces such as Son La, Lai Chau, Lao Cai, and so on. The main chemical components of Japanese essential oils could be listed as β-pinene (1.1 %), limonene (2.87 %), menthone (19.8 %), neomenthol (7.65 %), acetate menthyl (2.53 %), and menthol (51.8 %). These quantitative figures can be varied due to differences in plantation conditions such as weather, soils, farming methods. Cao et al. (2019) [19] performed a study on Japanese mint essential oils collected from the mountainous region in Lam Dong, Vietnam, and showed the content of menthol in the achieved essential oils up to 73.46 % in case of MAE.

HD and MHD/MAE are two distillation methods commonly applied in the extraction of essential oils, in which they represent for a conventional and modern extraction method, respectively. They all have their own advantages and disadvantages, then resulting in the variation of the chemical constituents present in the obtained essential oils. HD requires longer extraction durations but seems to be easily utilized in large scale industry due to its simple setup and proper extraction performance. Meanwhile, MHD/MAE appears to be more suitable for investigations and testing in laboratory scale.
because of reduction in the extraction times and used solvents, also with high yields. The main objectives of this study were to (i) assess the effects of several parameters related to the mint leaf essential oil extraction by HD and MAE (various drying periods at 50 °C, material to water ratios, distillation durations, and microwave power only for MAE) on the oil yields to discover the main and basic differences between two distillation methods and the optimized extraction conditions for both of them, then (ii) to compare and assess the chemical compositions of essential oils obtained from HD and MAE.

2. Materials and methods

2.1. Mint material collection, pre-treatment, and storage

Four-month Japanese mint (Mentha arensis L.) samples were collected at the highland mint garden in Duc Trong, Lam Dong Province (Vietnam) in February 2020, and transferred to Ho Chi Minh City within 6 hours for pre-treatment steps. Firstly, rubbish, crushed branches, brown leaves, and roots were manually removed from the raw materials. After that, the fresh leaves were separated from the plants, collected, washed by distilled water, and let drain at ambient temperature for thirty minutes. In this study, only mint leaves were used for essential oil extraction. The prepared leaf materials were stored in a refrigerator at 4 °C until the drying experiment was proceeded.

2.2. Drying experiments

The convective drying method was selected to dry the mint leaves materials. The leaves were taken out of the refrigerator and waited until they reached the ambient temperature. In our drying procedure, a laboratory-scale dryer was employed. 200 g of the mint leaves was used as a thin layer with an approximate thickness of around 10 mm. During the experiments, the mint leaves were dried at a constant airflow rate of 0.4 m s⁻¹ and a fixed temperature value of 50 °C for various lengths of time, including 30, 60, and 90 minutes in order to study the effect of drying on the essential oil yields and compositions (only for fresh and dried leaves with the highest yield). The moisture contents of dried materials for different periods of time were determined and recorded. Eventually, the dried leaves were ground to obtain 1-mm mint leaves before the extraction of the mint essential oils by both HD and MAE.

2.3. Extraction of mint essential oil

The essential oils were extracted from mint leaves by two different methods, involving (i) hydro-distillation (HD) and (ii) microwave-assisted extraction (MAE). Several basic extraction parameters related to the yields of mint essential oils were investigated for both HD and MAE, including ratios of material to water, extraction time, and microwave power (only for MAE), which was summarized in table 1.

| Table 1. Investigated parameters of the essential oil distillation procedure. |
|-----------------------------|----------------------|----------------------|------------------|
| Extraction parameters | Material:water ratios (g:mL, w/v) | Extraction time (min) | Microwave power (W) |
| Hydro-distillation (HD) | 1:4; 1:6; 1:8 and 1:10 | 60, 90, 120, 150, and 180 | |
| Microwave-assisted extraction (MAE) | 1:1, 1:1.5, 1:2 and 1:2.5 | 10, 15, 20, and 25 | 230, 380, 540, and 700 |

2.3.1. Extraction of essential oils by hydro-distillation (HD). HD using a Clevenger apparatus was applied for the extraction of the essential oils from mint leaf materials (figure 1).
100 (±0.1) g of ground mint leaves (prepared in item 2.2) was put into a 1000-mL round-bottom flask (figure 1), then 12 g of sodium chloride and a particular volume of distilled water (400, 600, 800 and 1000 mL) were added to investigate the effects of material to water ratios (1:4, 1:6, 1:8 and 1:10, w/v) on the yields. The mixture was stirred thoroughly for five minutes and soaked within two hours. The flask containing the mint leaves was heated by a heating mantle with a temperature of 130 °C for different extraction time periods of 60, 90, 120, 150, and 180 minutes (table 1). The extraction time used for the investigation of material to water ratios was kept at 60 minutes in order to save time and energy. The yields among these various experimental conditions were recorded and compared to achieve the optimized essential oil extraction condition for HD.

2.3.2. Extraction of essential oils by microwave-assisted extraction (MAE). MAE was utilized by a sort of Clevenger-type distillation device, and this adapted to global warming by microwave radiation through a Sanyo fan-assisted microwave oven 1200 W model (figure 2). A certain amount of 100 (±0.1) g of ground mint leaves (prepared in item 2.2) was also used to extract the essential oils as in the situation of HD. In order to optimize the parameters for MAE, microwave powers (230, 380, 540, and 700 W), extraction time periods (10, 15, 20, and 25 minutes), and ratios of material to water (1:1, 1:1.5, 1:2, and 1:2.5, w/v) (table 1) were changed and investigated to achieve the parameters resulting in the highest essential oil yield.
The essential oils obtained from HD and MAE were dried over anhydrous sodium sulfate and stored in sealed dark-glass ten mL-vials in a freezer (–4 °C) until chromatography analyses were performed.

2.4. Identification of the chemical composition
The identification and quantification of the mint essential oil chemical composition were performed on gas chromatography (GC), Trace 1310 Thermo Fisher Scientific, coupled with tandem mass spectrometer, TSQ 9000. A non-polar fused silica capillary column TG-5MS (30 m x 0.25 mm), 0.25 μm film thickness (Thermo Fisher Scientific, USA) was used. The MS scanning (1 scan s⁻¹) was performed in the mass range of 50-550 amu with electron impact ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1.2 mL min⁻¹ in a split ratio of 1:250 (0.2 μL). The GC oven temperature was kept at 60 °C for 30 sec and programmed to 180 °C at a rate of 6 °C min⁻¹ and increased to 300 °C at a rate of 20 °C min⁻¹, then hold at 300 °C for another 10 minutes. The temperature of the injector, ion source, and MS interface was set at 250 °C, 290 °C, and 300 °C, respectively.

Most of the compounds present in mint essential oils were identified by their mass spectra (NIST 2.2 library collection) and compared with Adams spectra library as a reference source [20]. Individual peak areas were recorded, and the relative peak areas (%) were calculated for the quantification of mint essential oil chemical components. Chromatographic analyses were run in triplicate to assure repeatability among runs.
2.5. Statistical analysis and calculating equations

The moisture content of the mint leaves was determined by the following procedure: 50 (± 0.0001) g of mint leaves was weighed and placed in an oven at 105 ºC until constant mass. The moisture content was calculated based on the equation below [21]:

\[ M = \frac{W_0 - W_d}{W_0} \times 100 \% \]  \hspace{1cm} (1)

whereas \( M \) is the moisture content (%), \( W_0 \) and \( W_d \) are masses (g) of the fresh and dried matter, respectively. The moisture content values of mint leaves were used to calculate the essential oil yields (dried mint leaf weight).

The mint essential oil yield (mL g\(^{-1}\) dried mint leaf weight) was calculated by the following equation:

\[ \text{Yield} = \frac{V_{ex}}{W} \times \frac{100}{100 - M} \] \hspace{1cm} (2)

whereas \( V_{ex} \) is the volume of essential oil extracted (mL), \( W \) is the weight of mint leaves used to extract the essential oil (g), and \( M \) is the moisture content (%). From now on, all of the performed yield values in this paper are understood as mL g\(^{-1}\) dried mint leaf weight (considered as no water content in the materials) if there are not any extra notes or explanations.

The chemical composition of mint essential oils was calculated by the relative percentage of chromatography peak areas as below:

\[ \text{Constituent relative content (\%) = } \frac{A_i}{A_{total}} \times 100 \% \] \hspace{1cm} (3)

whereas \( A_i \) is the chromatography peak area of each compound, and \( A_{total} \) is the total chromatography peak area of all present constituents.

The percentage of peak area for each component was calculated by Microsoft Office Excel 2016 software. Relative standard deviations (% RSDs) were used to check the repeatability of the quantification results.

3. Results and discussion

3.1. Extraction of mint essential oils

3.1.1. Effects of drying periods on the mint essential oil yields. Fresh mint materials were dried at 50 ºC for various periods of time of 30, 60, and 90 minutes to assess the influences of drying times on the oil yields. The material moisture reached 65.2 %, 41.1 %, and 33.6 % when dried at 50 ºC for 30, 60, and 90 minutes, respectively. The essential oils obtained from the fresh and dried mint leaves were found to be a yellow liquid for both HD and MAE. The essential oil yields obtained from both HD and MAE were performed in figure 3.
In all cases within our study, MAE gave higher essential oil yields than HD, typically ranging from 0.029 to 0.033 mL g\(^{-1}\) and from 0.017 to 0.024 mL g\(^{-1}\) for MAE and HD, respectively (figure 3). Among various drying periods, compared to the fresh leaves, convection drying at 50 °C for 30, 60, and 90 minutes all led to higher oil yields (0.017-0.024 mL g\(^{-1}\) for HD and 0.029-0.034 mL g\(^{-1}\) for MAE compared to 0.010 mL g\(^{-1}\) for HD and 0.019 mL g\(^{-1}\) for MAE in the case of fresh materials). Similar observation between fresh and dried materials was also discovered in the study of Beigi et al. (2018) [6], reporting the average peppermint oil yield of the fresh leaves was lower than shade drying and hot-air drying at 50 °C and 60 °C. Sellami et al. (2011) [22] found that the total essential oil content of L. Nobilis was also increased after natural drying at ambient temperature. There was a study of Pirbalouti et al. (2013) [11] indicating shade-dried plants resulted in the highest essential oil yield in comparison with fresh samples. The highest yields for both HD (0.024 mL g\(^{-1}\)) and MAE (0.034 mL g\(^{-1}\)) were recorded with 60-minute drying.

Drying temperature and time were considered as two important parameters for interpreting essential oil yield variation [22]. However, we did not utilize higher drying temperatures and only changed the drying times in our experiments due to the results performed in some available references. Beigi et al. (2018) [6] investigated the effects of drying methods on the peppermint essential oil yields, and showed a decrease in the yields when the temperature of the convective dryer was risen from 50 °C to 70 °C (22.4, 19.5, and 15.26 g/kg dried matter at 50 °C, 60 °C, and 70 °C, respectively. The similar observation also found in the study of Blanco et al. (2000) [12] for peppermint where they determined essential oil content of the leaves dried at 40 °C, 60 °C, and 80 °C to be 1.0, 0.14, and 0.12 % (v/w), respectively. Chi et al. (2016) [23] reported that increasing drying temperature in the range from 40 to 80 °C would result in decreased peppermint essential oil contents.

Within our results, longer drying periods mainly led to the decrease of the moisture contents in the materials, then had certain effects on the essential oil yields. It could be explained by the hypothesis that the most popular component of moisture content was water, so the water molecules present in the materials could affect the extraction performances, and led to the variation in essential oil yields. When the moisture content was higher, the water molecule would block the pores of the substrates and interfered the steam or the water from outsiders to uplift the oil components [24], especially for conventional extraction as HD, leading to lower yields (figure 3). In this study, a 60-min drying period (nearly 40 % moisture) was chosen for further investigations due to the highest yields and time-saving.

![Figure 3. Effects of drying on essential oil yields for HD and MAE](image)
3.1.2. Effects of material to water ratios on the mint essential oil yields. In the essential oil distillation, the ratios of the matrix to solvent or water play an important role in the extraction. The influences of material to water ratios on the oil yields were evaluated for both HD and MAE, and performed in figure 4. Between these two distillation methods, HD employed higher water volumes (400, 600, 800, and 1,000 mL) than those of MAE (100, 150, 200, and 250 mL) for the same quantity of mint leaf materials (100g). In conventional extraction methods as HD or SD, higher ratios of solvent volume to a solid matrix or lower matrix to solvent ratios were necessary to obtain better extraction yields; whereas, in case of MAE, lower ratios of materials to solvents may give a lower yield due to non-uniform distribution and exposure to microwave energy [9]. In conventional heating, heat is transferred from the heating sources (outsides the sample flask) to the interior of the sample mixture, while in microwave heating, heat is dissipated volumetrically inside the irradiated space. Therefore, in MAE, the solvent or water volumes should be sufficient enough to immerse the materials completely in the solvent during the whole microwave irradiation period [9, 16].

In HD process, the ratios of materials to water were varied in the range of 1:4, 1:6, 1:8, and 1:10 (w/v) (figure 4a) while other extraction parameters were kept unchanged, including the heating temperature of 130 °C and the extraction duration of 120 minutes. The 1:4 ratio showed the smallest oil yield (0.0102 mL g⁻¹) among the investigated ratios. It might be explained due to the insufficient water immersion into the materials, leading to a lower extraction performance [9]. Moreover, the water content might not be sufficient enough to transfer the heat effectively from the outer to the inner side of the samples, resulting in the poor ability to attack and break the plant cell membrane to uplift the oil components from the plant matrix. The rest ratios of 1:6, 1:8, and 1:10 did not exhibit any remarkable differences in the oil yields, varied in a very narrow range from 0.0234 to 0.0245 mL g⁻¹ (figure 4a). However, more water as the extraction solvent would require more heat, extraction time, and the quantity of water used, particularly when the pilot scale was applied. Therefore, the material to water ratio of 1:6 (w/v) was used for further experiments.

In MAE procedure, the investigated ratios of materials to water were 1:1, 1:1.5, 1:2, and 1:2.5 (w/v), in which other extraction parameters were maintained involving the microwave energy of 380 W and the extraction period of 15 minutes. The extraction mixture with the least water content (1:1 ratio) also gave the smallest oil yield (0.0238 mL g⁻¹) among all the investigated ratios for MAE (figure 4b). It could also be explained as in the situation of HD because the ratio of 1:1 did not give
enough water to immerse the mint leaves, so the microwave was not effectively irradiated during the extraction process [9, 16]. Higher water volumes performed better yields (0.030, 0.0408, and 0.0409 mL g⁻¹ for the ratios of 1:1.5, 1:2, and 1:2.5, respectively). The ratios of 1:2 and 1:2.5 did not show any distinguishable differences in essential oil yields. Therefore, in order to minimize the water used, but still obtain the best extraction performance with the highest yield, the ratio of 1:2 was utilized for further investigations in this study.

3.1.3. Effects of extraction time on the mint essential oil yields. In the essential oil distillation, the extraction duration was one factor not only having the influences on the oil yields but also helping to save time and energy for the whole distillation procedure. For HD, the extraction duration was changed from 60 minutes to 180 minutes, and for MAE, this length of time was varied from 10 to 25 minutes. The oil yield obtained from each condition was recorded and performed in figure 5. Other extraction parameters were maintained in all-time investigation experiments involving the material to water ratios (1:6 and 1:2 (w/v) for HD and MAE, respectively), the heating temperature (130 °C for HD), and microwave power (380 W for MAE).

![Figure 5](image_url)

Figure 5. Effects of extraction times on the essential oil yields obtained from (a) hydro-distillation (HD) and (b) microwave-assisted extraction (MAE)

In the situation of HD, the oil yield increased from 60 minutes (0.0170 mL g⁻¹), reached the peak at 120 minutes (0.0510 mL g⁻¹), then decreased when the extraction duration lasted to 150 minutes (0.0408 mL g⁻¹) and 180 minutes (0.0340 mL g⁻¹). The yield increase from 60 to 120 minutes could be explained because longer distillation periods made the solvent have more time to contact to the plant cells, then assisted to completely extract the essential oils from the leaf matrix. However, if the duration was too long (150 and 180 minutes), the essential oils extracted could be evaporated into the outer environment, leading to lower essential oil yields (figure 5a). For MAE, the microwave energy helped to reduce extraction periods (10-25 minutes) in comparison with conventional distillations like HD (60-180 minutes); however, longer distillation time could reduce the oil yields and waste more microwave energy. The results showed that the same yield tendency was also recorded as in the case of HD (figure 5b). The oil yield rose from 10 minutes (0.0340 mL g⁻¹), reach its highest value at 20 minutes (0.0747 mL g⁻¹), then fell to 0.0679 mL g⁻¹ at 25 minutes. This could be explained as in the situation of HD that longer duration helped to completely extract the essential oils from the plant matrix; however, when the extraction period was prolonged, not only there could be a potential of essential oil evaporation into the outer environment, but also the material could be dried due to the lack of water, they would be burnt, causing lower oil yields. Therefore, to have the highest yield and also save time and energy, 120 and 20 minutes were chosen as the extraction periods for HD and
MAE, respectively, for further experiments.

3.1.4. Effects of microwave power on the mint essential oil yields. In MAE, microwave power has an important influence on the extraction performance [16]. In this study, four different power values (230, 380, 540, and 700 W) were used for essential oil extraction, and the yield in each condition was recorded and performed in figure 6.

![Figure 6. Effects of microwave power on the essential oil yields obtained from microwave-assisted extraction (MAE)](image)

In the experiment of investigating the microwave power, the other extraction parameters were used at their optimized parameters, including the material to water ratio of 1:2 (w/v) and the distillation duration of 20 minutes. As can be seen from figure 6, the oil yield increased from 230 W (0.0340 mL g⁻¹) to 540 W, and reached the highest value at the power of 540 W (0.0832 mL g⁻¹), then decreased when 700 W (0.0510 mL g⁻¹) was utilized as the microwave power. The rise in essential oil yields from 230 W to 540 W could be explained due to the incomplete extraction of mint oils at smaller microwave power values (230 and 380 W) when the same extraction duration was employed (20 minutes). In general, the required distillation time would be increased with the decreasing microwave power, which was also present in the study of Kohari et al. (2020) [16]. However, when the microwave power was raised to 700 W, the oil yield was lower than that of 540 W, but still higher than the yield achieved from 230 W (figure 6). The reason for this decrease may be due to the incomplete agglomeration. Because MAE at 700 W started heating with very high energy, the vaporized essential oils might not be completely coagulated [16]. Additionally, with a certain amount of water used as the extraction solvent (200 mL for 100 g mint leaf materials), the water reached its boiling point very quickly at high microwave power and became to evaporate mostly before the distillation duration finished. Then, at the end of the distillation process, the mint material was dried, and a certain material part was burnt, leading to the decrease in essential oil yields at 700 W. Therefore, to ensure the highest yield within 20-minute distillation, 540 W was chosen as the microwave power for extraction the mint essential oils.

3.2. The yields and chemical composition of mint oils obtained from HD and MAE

The essential oils were extracted from the mint leaves by both HD and MAE, then the oil yields and chemical constituents between these two distillation methods were compared and evaluated. In general, MAE showed higher yields than those of HD (the highest yields of 0.075 and 0.051 mL g⁻¹ dried weight for MAE and HD, respectively), but shorter distillation time (20 min for MAE compared to 120 min for HD) and water used (200 mL in case of MAE and 600 mL in case of HD for the same
sample quantity of 100 g). Therefore, it could be said that MAE performed as an efficient distillation method for obtaining essential oils rich in flavor components from Japanese mint. It could be explained by the mechanism of microwave that the absorption efficiency of the microwave power is dominantly related to the moisture contents of the materials because the water molecules inside the materials would absorb the irradiation from the microwave, then convert the microwave energy into heat, and result in a sudden temperature rise inside the materials. According to the study of Paré [25], when the plant cells were subjected to severe thermal stress and localized in high pressures, the pressure in the plant cells would exceed their capacity for expansion, and cause their dislocation more rapidly than in conventional extraction methods like HD in which heat is transferred from the outer sides to the inner of the sample mixtures. The water known as a polar solvent absorbs the irradiated microwave energy, then heats up until reaching its boiling point, diffuses into the sample matrix, and solubilizes the analytes in a more effective way. Moreover, the oil cells may have been destroyed immediately after the generation of water vapor by microwave irradiation. Then, the essential oils were discharged from the plant system to the solvent in a very short time. This hypothesis was supported by the study of Kohari et al. (2020) [16].

For chromatographic analysis, the GC oven temperature program was established based on the reference method from Dr. Admas [20] with certain modifications so that it could be possible with our available instrument and reduce the running time, but still with proper separation. The total GC-MS acquisition time lasted within approximately 30 minutes. Although the separation was completed in the first 20 minutes, the temperature program was prolonged to around 30 minutes (extra 10 minutes holding at 300 °C) to assure that there was no carry-over by the injected samples. The variations of retention time for all identified peaks obtained from triplicates injected to GC-MS were small, 0-0.02 min. In this study, there was no internal standard used; therefore, the repeatability was one of the most important criteria to be considered, and % RSDs among relative peak areas were calculated to check the repeatability of GC-MS. The main components (contents higher than 1 %) performed lower % RSDs (0.81-4.5 %), indicating good repeatability. A typical chromatogram of Japanese mint essential oil obtained from MAE was present in figure 7, and their chemical components (calculated by relative percentage, %) were performed in table 2. Only the essential oils from fresh leaves (the moisture content of nearly 80 %) and dried leaves at the optimized condition (item 3.1.1, the moisture content of around 40 %) were used for chromatographic analysis to assess their chemical composition.

![Figure 7. Chromatogram of Japanese mint essential oil obtained by MAE with some main compositions listed as trans-menthone (16.3 %, tR = 8.55 min), cis-menthone (5.01 %, tR = 8.79 min), menthol (70.6 %, tR = 9.08 min).](image-url)
Table 2. Chemical composition (%) of mint essential oils obtained from HD and MAE.

| No. | Rt (min) | Constituent          | This study (Cong-Hau et al.) | Verma et al. [3] | Kohari et al. [16] |
|-----|----------|----------------------|------------------------------|------------------|-------------------|
|     |          |                      | HD Fresh | Dried* | HD Fresh | Dried* | HD Fresh | Dried** | Fresh | MAE Fresh | Dried* | HD Fresh | Dried** | Fresh | MAE Fresh | Dried* |
| 1   | 4.18     | α-pinene             | 0.53     | 0.13  | 0.08      | 0.06   | 0.69-1.40 | 0.20     | 0.18  | 0.18     |
| 2   | 4.43     | camphene             | 0.01     | ND    | ND        | ND     | -         | -        | -     | -        |
| 3   | 4.84     | sabinene             | 0.26     | 0.11  | 0.06      | 0.08   | <0.10-0.12 | 0.11     | 0.14  | 0.11     |
| 4   | 4.92     | β-pinene             | 0.34     | 0.21  | 0.14      | 0.14   | 0.40-0.94 | 0.24     | 0.30  | 0.23     |
| 5   | 5.11     | β-myrcene            | 0.18     | 0.12  | 0.09      | 0.09   | 0.27-0.56 | 0.16     | 0.16  | 0.14     |
| 6   | 5.16     | 3-octanol            | 0.30     | 0.37  | 0.67      | 0.57   | -         | 0.23     | 0.59  | 0.58     |
| 7   | 5.79     | o-cymene             | 1.12     | ND    | 0.01      | 0.02   | -         | -        | -     | -        |
| 8   | 5.88     | d-limonene           | 1.44     | 1.40  | 0.27      | 0.27   | 0.66-2.21 | 0.25     | 0.61  | 0.44     |
| 9   | 5.94     | 1,8-cineole          | 0.12     | ND    | ND        | 0.11   | <0.1      | -        | -     | -        |
| 10  | 6.01     | trans-β-ocimene      | 0.26     | 0.2   | 0.03      | 0.02   | <0.1      | 0.07     | 0.20  | 0.07     |
| 11  | 7.11     | p-mentha-2,4(8)-diene| 0.05     | 0.03  | 0.02      | 0.05   | -         | -        | -     | -        |
| 12  | 7.31     | linalool             | 0.41     | 0.38  | 0.09      | 0.09   | <0.1-0.20 | <0.01    | <0.01 |<0.01     |
| 13  | 8.36     | camphor              | 1.50     | 0.50  | 0.46      | 0.46   | -         | -        | -     | -        |
| 14  | 8.57     | trans-menthone       | 18.83    | 20.19 | 13.75     | 16.30  | 1.61-1.82 | 8.27     | 9.51  | 10.54    |
| 15  | 8.79     | cis-methone          | 4.22     | 4.62  | 4.81      | 5.01   | 2.88-3.32 | 3.05     | 3.19  | 2.97     |
| 16  | 9.09     | (-)-menthol          | 56.89    | 62.80 | 73.46     | 70.60  | 74.24-82.18 | 60.13    | 66.82 |59.32     |
| 17  | 9.38     | α-terpineol          | 0.17     | 0.16  | 0.28      | 0.25   | <0.1-0.34 | 0.18     | 0.27  | 0.18     |
| 18  | 10.33    | cis-3-hexenyl isovalerate | 0.18 | 0.14  | 0.14      | 0.14   | -         | -        | -     | -        |
| 19  | 10.46    | pulegone             | 0.56     | 1.50  | 0.90      | 0.89   | <0.10-0.77 | -        | -     | -        |
| 20  | 10.80    | piperitone           | 4.52     | 3.17  | 1.28      | 1.23   | 0.46-1.56 | 1.43     | 1.72  | 2.11     |
| 21  | 11.65    | methyl acetate       | 0.65     | 0.64  | 0.03      | 0.05   | 1.62-3.53 | 2.56     | 5.70  | 2.80     |
| 22  | 13.51    | α-copaene            | 0.01     | ND    | 0.02      | 0.03   | -         | -        | -     | -        |
| 23  | 13.72    | (-)-β-bourbonene     | 0.23     | 0.28  | 0.15      | 0.17   | <0.01     | <0.01    | -     | -        |
| No. | Rt (min) | Constituent       | This study (Cong-Hau et al.) | Verma et al. [3] | Kohari et al. [16] |
|-----|----------|-------------------|-----------------------------|------------------|-------------------|
|     |          |                   | HD  | MAE  | HD  | HD  | MAE  |
|     |          |                   | Fresh | Dried* | Fresh | Dried* | Fresh | Fresh | Dried** | Fresh |
| 24  | 14.46    | caryophyllene    | 0.72  | 0.65  | 0.55 | 0.54 | <0.10 | 0.64  | 0.35  | 0.48  |
| 25  | 15.74    | germacrene d     | 1.39  | 1.69  | 1.62 | 1.65 | <0.10-0.25 | -    | -    | -    |
| 26  | 16.56    | cadina-1(10)-4-diene | 0.15  | 0.04  | 0.04 | 0.04 | -    | -    | -    | -    |
| 27  | 17.61    | germacrene d-4-ol | 0.24  | 0.20  | 0.25 | 0.35 | -    | -    | -    | -    |
| 28  | 18.38    | epicubenol       | 0.07  | ND    | ND   | ND   | -    | -    | -    | -    |
| 29  | 19.12    | α-cadinol        | 0.16  | ND    | ND   | ND   | -    | -    | -    | -    |
|     |          | **Total identified** | 95.51% | 99.53% | 99.20% | 99.21% |

(*) dried at 50 °C for 60 minutes to reach the moisture content of approximately 40% before oil extraction
(**) dried at 50 °C for 9 hours until the constant mass before oil extraction
ND: not detected in this study
“-”: not reported or published in reference documents
As shown in table 2, for the mint essential oils in our study, there were maximum of 29 compounds identified, accounting for 95.51-99.53 % of the total volatiles. In fresh and dried materials extracted by both HD and MAE, the principal components were (-)-menthol (56.89-73.46 %), trans-menthone (13.75-20.19 %), cis-menthone (4.22-5.01 %), piperitone (1.23-4.52 %), and germacrene D (1.39-1.69 %). The chemical constituents of our essential oils were compared to those from the studies of Verma et al. (2010) [3] and Kohari et al. (2020) [16], which showed the similar main constituents, except for germacrene D (<0.10-0.25 and not identified in Verma et al. (2010) [3] and Kohari et al. (2020) [16], respectively) and methyl acetate (1.62-5.70 % in references). The mint essential oils reported by Verma et al. (2010) [3] exhibited higher (-)-menthol ranges (74.24-82.18 %), but lower trans-menthone (1.61-1.82 %) and cis-menthone (2.88-3.32 %) compared to our study. Kohari et al. (2020) [16] reported lower (-)-menthol (59.32-66.82 %), trans-menthone (8.27-10.54 %), and cis-menthone (2.97-3.19 %). The variation among results for chemical components of Japanese mint essential oils could be due to natural chemical differences called chemotype. This phenomenon occurs in the secondary metabolism of plants and could be contributed by several environmental factors listed as plantation soil types, growing altitude, sun exposure, rain, gather or seasonal variation, and in accordance with the study of McGimpsey et al. (1994) [26], could also be affected by genetic factors.

The most concerned components of Japanese mint essential oils were (-)-menthol and its derivatives, in which trans- and cis-menthone were considered as two flavor impact compounds. In this study, the essential oil obtained from MAE exhibited higher contents of (-)-menthol than those from HD (70.60-73.46 % and 56.89-62.80 % for MAE and HD, respectively). It might be due to the longer distillation time of HD, leading to the degradation of (-)-menthol present in oils. For HD, it was found that the essential oils obtained from fresh leaves contained more (-)-menthol than those of dried leaves (56.89 and 62.80 % for fresh and dried leaves, respectively), which was similar to the results of Kohari et al. (2020) [16]; however, for MAE, there was a reversed trend that dried leaves gave higher contents of (-)-menthol in the essential oils than fresh leaves (73.46 and 70.60 % for fresh and dried leaves, respectively). In contrast to (-)-menthol, both trans- and cis-menthone performed their lower contents in the case of MAE compared to the situation of HD (table 2).

Generally, the chemical compounds could be categorized into four main groups: (i) oxygenated monoterpenes, (ii) monoterpane hydrocarbons, (iii) sesquiterpene hydrocarbons, and (iv) oxygenated sesquiterpenes [6]. The relative percentage of the main chemical groups for Japanese mint essential oils was performed in figure 8.

![Figure 8](image_url)  
**Figure 8.** Comparison of the main chemical groups (%) of mint essential oils obtained from fresh and dried leaves extracted by HD and MAE.
As can be seen from figure 8, the chemical composition of the fresh and dried mint leaf essential oils was characterized basically by the presence of oxygenated monoterpenes (87.22-95.03 %). The levels of monoterpane hydrocarbons (0.70-4.19 %), sesquiterpene hydrocarbons (2.38-2.66 %), and oxygenated sesquiterpenes (0.20-0.47 %) were present at lower ranges. The results showed that although the drying process dehydrated the mint leaves, it did not lead to the decrease in the contents of oxygenated monoterpenes as in the research of Mohsen et al. (2018) [6], Khangholi and Rezaeinodohi (2008) [27], and Reza et al. (2012) [28], even higher in HD (87.22 and 93.32 % for fresh and dried leaves, respectively) and nearly the same contents in MAE (95.03 and 94.94 % for fresh and dried leaves, respectively). However, our results were similar to the study of Kohari et al. (2020) [16], in which oxygenated hydrocarbons were 71.63 and 79.79 % for fresh and dried leaves, respectively. Among our results, the minimum value of oxygenated monoterpenes (87.22 %) was obtained in HD-fresh leaves while this group concentration was higher in the case of MAE (94.94-95.03 %). The differences in the contents of oxygenated monoterpenes were mainly due to the differences in the main constituents of this group, especially (-)-menthol, trans- and cis-menthone (table 2). The concentrations of the monoterpane hydrocarbons from HD (2.20-4.19 %) were higher than those from MAE (0.70-0.73 %), in which HD-fresh leaves performed the highest content of this group (table 2). For sesquiterpane hydrocarbons, HD and MAE both performed nearly the same ranges (2.38-2.66 %), and the essential oils from dried leaves exhibited a little higher contents of this group (2.50 and 2.66 % for fresh and dried leaves in HD case, 2.38 and 2.43 % for fresh and dried leaves in MAE case). The growth in sesquiterpane hydrocarbons after drying has also been reported in the studies of Diaz-Maroto et al. (2003) [29], Sellami et al. (2012) [30], and Ghasemi Pirbalouti et al. (2013) [31]. For the last group of oxygenated sesquiterpenes, there was a reversed trend between two extraction methods (figure 8), in which HD-fresh performed the highest (0.47 %).

4. Conclusions

In this present work, HD and MAE were used as the distillation methods to extract the essential oils from (-)-menthol-rich Japanese mint leaves. The influence of the drying period at 50 °C was evaluated and showed that 60-minute drying to reach the moisture of around 40 % exhibited the highest yields for HD and MAE. Several parameters related to the distillation process were also investigated for both methods to assess their effects on the oil yields. The optimized extraction conditions were: (i) material to water ratio of 1:6 (w/v), distillation duration of 120 minutes at 130 °C for HD and (ii) material to water ratio of 1:2 (w/v), extraction time of 20 minutes, and microwave power of 540 W in the case of MAE. The main advantage of MAE over conventional extraction technique as HD is that MAE performs reduced solvent consumption (three times less than HD), shorter operation time (only 20 minutes at the microwave power of 540 W compared to 120 minutes at 130 °C in the case of HD), and better extraction performance (the highest yields of 0.0832 and 0.0510 mL g⁻¹ for MAE and HD, respectively), so MAE is possibly an effective option for further investigations into mint essential oils. In addition, MAE improved the contents of oxygenated monoterpenes, especially (-)-menthol (the highest values were 70.6 and 62.80 % for MAE and HD, respectively).

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