



Comparative Study of Microwave-assisted Extraction and Hydro-distillation Methods for Extracting Essential Oil from *Melaleuca cajuputi* in Southern Vietnam: Yield, Composition, and Efficiency Analysis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to the extraction of *Melaleuca cajuputi* essential oils by microwave-assisted extraction method. The comparison for productivity of microwave-assisted extraction method and traditional hydro-distillation method was also established to figure out the optimum condition for the isolation process.

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Methodology: Microwave-assisted extraction method was employed for the isolation of *Melaleuca cajuputi* essential oils. The components contained in *Melaleuca cajuputi* essential oils were analyzed by gas chromatography-mass spectrometry. Standard methods were applied to investigate the characteristics of extracted essential oils, including density, acid index, saponification index and ester index.

Results: The major components in the product included caryophyllene (21.45%), γ -terpinene (6.14%), α -terpinolen (7.12%), 1R- α -pinene (3%), α -caryophyllene (10.75%), α -terpineol (0.64%), 4-terpinenol (1.34%), eucalyptol (0.86%) and α -terpinen (0.77%). The highest yield of 0.42% (w/w) was obtained at optimum condition, which was water to raw material ratio of 3.5 mL/g, and extraction time of 210 minutes.

Conclusion: The comparison of extraction process performed by microwave-assisted extraction and traditional hydro-distillation method proved that microwave-assisted extraction was effective for essential oil extraction in both quality and quantity.

Keywords: Essential oils; *Melaleuca cajuputi*; microwave-assisted extraction; GC-MS.

1. INTRODUCTION

Essential oils (EOs) are natural volatile products extracted from different parts of a wide variety of plants. It is estimated 4000 EOs are known of which approximately 500 are of commercial importance [1,2]. EOs are highly concentrated substances used for their flavor and therapeutic or odoriferous properties, in a wide selection of products such as food, medicines and cosmetics. Nevertheless, these EOs have currently found utility in the medicinal field principally as insect repellents or insecticides [3,4]. With the increasing demand of EOs in the world presently, numerous species of trees previously exploited as source of timber have now been studied to find out if they have any ethereal EOs [5]. These efforts have often yielded good results with some parts of many trees having been found to produce significant, amounts of EOs which has diverse applications (depending on the species of trees) in many fields.

Melaleuca cajuputi (*M. cajuputi*) is a member of the Myrtaceae family. *M. cajuputi* is found in Northern Australia, Papua-New Guinea, Indonesia, Thailand and Vietnam and the main source of *M. cajuputi* EOs, which is widely used in the folk medicine of Southeastern Asia [6]. The species is highly adaptable and can grow in a wide range of milieu. They can survive in marshy soils, drain lines and in seasonally flooded soils, but they can also occur in dry, rocky and infertile soils. In Vietnam, *Melaleuca* trees can grow on acid land that stretches in a large portion of lands in the Mekong Delta region.

The leaves are highly aromatic and bitter to taste. *M. cajuputi* EOs from the leaves are usually greenish tint due to the trace of copper.

The compositions determined by gas chromatography-mass spectrometry (GC-MS) method reveal the *M. cajuputi* EOs to contain terpenoids 1,8 cineole (40-65%) as major component, with α -pinene, α -terpineol, nerolidol, limonene, benzaldehyde, valeraldehyde, dipentene and various sesquiterpene. Basically, 1,8-cineole is the major constituent of the EOs extracted [7]. In fact, the quality of the *M. cajuputi* EOs depends on the percentage of this particular constituent. It is found that poor growth conditions contributed to better EO quality with higher 1,8 cineole content which suggested that a defense mechanism where high amounts of secondary substance were generated for survival purpose. The other shows low concentration (31%) of it, and another chemotype does not show the presence of this terpene [8]. In Brazil, a chemotype with concentration of 1,8-cineol was identified (44%) [9]. The economic value of these EOs is directly related to the levels of this terpene. In Indonesia, EOs with concentration of 1,8-cineole above 55% are considered of first quality, and if below this value, they are considered standard EOs [8]. *M. cajuputi* EOs are used for the treatment of headache, toothache, rheumatism, convulsions and as insect repellent [10,11,12].

This increasing demand for EOs mainly also has opened up wide opportunities for its global marketing, leading to the requirement of competitive product in market. These products are often expected to come with all the advantages in terms of cost, quality and its production time. Since EO is a volatile component, it is vital to identify the best extraction technique, so that the higher yield of EOs with good quality can be extracted. At present, extraction method of EOs commonly

applied is steam distillation and solvent extraction. With the low yield, low purity, toxic solvent residue and other shortcomings, it is difficult to meet the current EO industry to the requirements of high-quality EOs, so the potential to explore new alternatives has become a must [13]. Microwave-assisted extraction (MAE) technology is one of the most effective method of extracting high quality EOs and a considerable potential process for development of new extraction techniques. In comparison to traditional methods, MAE has more advantages in terms of fast process, environmental safety and high quality, so it may become a dominant technology in industrial production of EOs. MAE has recently been used for the isolation of target ingredients [14,15].

This study thus focused on the extraction of *M. cajuputi* EOs by MAE method. The comparison for productivity of MAE and traditional hydro-distillation method was also established to figure out the optimum condition for the isolation process. In addition, several properties of the EOs were examined to investigate the effectiveness of the extraction method as well as economic value of extraction products.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of the Plant Material

A bulk sample of fresh *M. cajuputi* leaves was collected from a particular location in Binh Duong province, Southern Vietnam. The plant was authenticated by The Institute of Tropical Biology, Vietnam and a voucher specimen of (No. AB Bio – 06 – 08 – 02) had been deposited in the herbarium of Applied Biochemistry Laboratory, Department of Applied Biochemistry, School of Biotechnology, International University Vietnam National University – Ho Chi Minh City, Vietnam. The leaves were picked off the stalks and mixed thoroughly to form a homogeneous and representative stockpile samples. All were scaled exactly 200 g/sample and kept in air tight plastic bag.

2.2 Microwave-assisted Extraction

Microwave-assisted extraction (MAE) was performed in Milestone's NEOS – Essential Oils

System. This apparatus was a multimode microwave reactor 2.45 GHz with a maximum delivered power of 900 W variable in 10 W increments. The 1-L extraction vessel was made of Pyrex. A Clevenger system was used to collect the condensed vapors, EOs and return the distilled water in the extraction vessel [16].

MAE procedure was performed at atmospheric pressure. A fixed volume of 700 mL of distilled water as solvent was used with a selected amount of *M. cajuputi* leaves, determined by solid/liquid ratio and time extracted optimization. The plant material and solvent were added in the extraction vessel. The time and power of microwaves were selected. Once the extraction was done, the volatile organic compounds (VOCs) were collected from the Clevenger, dried on sodium sulfate, filtered and weighted on an analytical balance within a 1% error margin. VOCs collected were stored in amber glass vial at 4°C until used. An aliquot of the extract was made for analysis, filtered on 0.2 µm PTFE filter and stored in amber glass vial at -20°C until used.

2.3 Hydro-distillation method (HD)

Fresh *M. cajuputi* leaves were placed in a 1-L flask containing distilled water. The material was extracted by HD for 300 minutes using a Clevenger-type apparatus [17].

Quantitative characterization of the Eos: Different operating parameters were studied to investigate their effects on EO yield, including the ratio of solvent volume and material weight, and the duration of the extraction.

The volume of water was varied from 500 mL to 900 mL using an interval of 100 mL. The duration of the extraction was varied from 120 to 270 minutes using 30-minute interval.

The yields of the extraction were calculated from the relation between the mass of the EO extracted and the mass of raw material used in the extraction as the below formula:

$$\text{Yield of essential oil (\%)} = \frac{\text{amount of EO (g) obtained}}{\text{amount of raw materials (g) used}} \times 100\%$$

The EOs were then dried over anhydrous sodium sulfate, weighed and stored in at 4°C for later use.

2.4 Investigation of Physicochemical Properties of *M. cajuputi* EOs

Evaluation of physicochemical properties of *M. cajuputi* EOs includes the values of proportion, acid index, saponification index, and ester index [18].

2.4.1 Density

A certain amount of *M. cajuputi* EOs was taken by micropipette and weighted at 25°C. The density d (in gram/mL) was calculated by the formula:

$$d = \frac{m}{V}$$

In which, m (in gram) and V (in milliliter) was the mass and volume of EO, respectively.

The density could be used to investigate the chemical composition as the following criteria:

- If $d < 0.9$, EO content was hydrocarbon compounds, mainly linear forms.
- If $d > 1$, EO content was the compounds that contain oxygen, mainly phenol.
- If $d = 0.9$ to 1 , EO content was the compounds of alcohol, aldehyde, and ketone.

2.4.2 Acid index (IA)

The IA value of EOs was determined by titration method with 0.1 N potassium hydroxyl solution as the titrant. One gram of anhydrous *M. cajuputi* EOs was placed into an Erlenmeyer flask with 10 mL of absolute ethanol, then the mixture was shaken until completely dissolved. Ten drops of phenolphthalein were added as an indicator. The value of IA was measured by the amount of KOH (mg) reacting to the acid in one gram of sample.

2.4.3 Saponification index (IS)

The IS value was the amount of KOH (mg) needed to saponify 1 g of EOs which was determined by titration method. An appropriate amount of 0.5N KOH solution was added to 1 g of EOs (sample A). Another sample contained the same amount of KOH as in sample A and 1 mL of distilled water (sample B). Both samples were boiled in 50 minutes and let cool at room temperature. Three drops of 1% phenolphthalein were then added to each of two samples as an indicator and both of them were then titrated with HCl until the pink color disappearing. The IS value was calculated by the following formula:

$$IS = \frac{(V_B - V_A) \times C_{M(HCl)} \times M_{KOH}}{g}$$

Where V_B , V_A was the volume of HCl solution used to titrate sample B and A, respectively (mL); $C_{M(HCl)}$ was the concentration of HCl solution (mL) (N); M_{KOH} was the molecular mass of KOH (g/mol); g was the mass of tested EO (g).

2.4.4 Ester index (IE)

The ester index (mg/g) was the differential between the IA and IS, as the formula below:

$$IE = IS - IA$$

2.4.5 Chemical composition analysis

The components contained in *M. cajuputi* EOs were analyzed by Gas Chromatography Mass Spectrometry (GC-MS) which an Agilent 6890 gas chromatography instrument was coupled to an Agilent 5973 mass spectrometer and an Agilent Chem in order to identify their chemical constituents [19]. The operating parameters used for analysis consisted of a capillary GC column HP-5MS 5% phenylmethyl siloxane (30 x 0.25 mm i.d. x 0.25 mm film thickness), a carrier gas Helium (flow rate 1.2 mL min⁻¹) and a split-less injection mode. Injector temperature was 250°C; oven temperature was set initially 50°C for 2 min, then was increased 20°C/min to 80°C, continued to increase by 50°C/min to 200°C, and finally increased by 20°C/min to 300°C for 5 min till the end of the analysis. The eluted analytes detected using mass selective detector and Electron Impact ionization (EID) was carried out at 70 eV.

2.5 Statistical Analysis

All experiments were conducted in triplicate, and the results were expressed in terms of Mean \pm Standard Error of Mean (SEM). Statistical analysis was performed by SPSS and analysis of variance (ANOVA) with the level of significance $p = .05$.

3. RESULTS

3.1 Optimization of *M. cajuputi* EO Extraction Process

The EO extraction performed by MAE was more significantly effective than HD, in which, from the same amount of original material, the process of MAE took shorter time and gave more product than the traditional HD method (Table 1).

Table 1. Productivities of extraction by MAE and HD method

Method	Extraction time (min)	Yields (mL/100 g)
Microwave-assisted	210	0.35
Hydro-distillation	300	0.15

At a constant microwave power of 900W and the ratio of 3.5 mL solvent per one gram of material, the product yields increased by the prolonging of extraction period. The highest amount of EO was collected after 270 minutes (4.5 hours). However, the extend of extraction time led to the drop of product.

3.2 Determination of *M. cajuputi* EO Properties

Sensory properties: The obtained EO of *M. cajuputi* is transparently light yellow oily liquid at room temperature. It has a camphoraceous odor, and with a spicy taste (Table 2).

Physicochemical properties of *M. cajuputi* EOs are shown in Table 3.

3.3 Chemical Composition Analysis

Using GC-MS analysis, both EOs obtained from two methods, MAE and HD, contained 36 and 31 chemicals, respectively. The comparison of major compounds found in products including caryophyllene, γ -terpinene, α -terpinolen, 1R- α -pinene, α -caryophyllene, α -terpineol, 4-terpinenol, eucalyptol, α -terpinen and some minor components are shown in Table 4. α -terpineol, or eucalyptol – which were used to evaluate the quality of *M. cajuputi* EO - had almost similar concentration for both methods. Moreover, the EO compositions revealed that

higher amounts of oxygenated monoterpenes presented more in the EOs isolated by MAE than in HD. However, the opposite was found in the case of α -terpinen content.

4. DISCUSSION

The main factor causing the effectiveness of MAE was the application of microwave power, which broke down the plant cell membranes and allowed the EOs to drain outward. In addition, the products could be collected continuously in the condensing tube, which was more convenient than the traditional HD system. Therefore, more EOs could be extracted in shorter time. The only drawback of this modern method was that it can degrade the polar constituents. The proportion of EOs shown in previous parts also indicated the effect of microwave radiation of MAE and prolonging of heating time of HD method. It would be reasonable to believe that the more polar compounds, the more readily the microwave irradiation was absorbed, and the better the interaction between the electromagnetic wave and matters was established. This interaction would lead to more polar aromatic components obtained. The comparison of chemical composition could prove that microwave greatly accelerated the extraction process without causing considerable alternation in the volatile EO components, and even produced the desired EOs with more preferable quality.

Table 2. The sensory properties of *Melaleuca cajuputi* Eos

Sensibility	Color	Odor	Taste
Transparent Oily liquid	Light yellow	Camphor-like note	Spicy

Table 3. Physicochemical properties of *Melaleuca cajuputi* Eos

No	Physicochemical parameter	Value
1	Density (mg/mL)	0.91 \pm 0.01
2	IA (mg/g)	5.30 \pm 0.83
3	IS (mg/g)	16.75 \pm 2.79
4	IE (mg/g)	11.45 \pm 2.91

The data are presented as mean \pm standard deviation and $p = .05$

Table 4. The chemical compositions of *Melaleuca cajuputi* Eos

Entry	Retention Time (min)	Compound	Relative content (%)	
			MAE	HD
1	7.29	α -Thujene	0.42	1.55
2	7.53	1R- α -Pinene	3.00	12.80
3	9.40	β -Pinene	-	0.55
4	10.84	α -Phellandrene	0.34	0.52
5	11.51	α -Terpinen	0.77	1.37
6	11.96	m-Cymol	1.96	3.80
7	12.17	D-Limonene	0.65	1.26
8	12.29	Eucalyptol	0.86	2.68
9	13.97	γ -Terpinene	6.14	9.41
10	15.74	α -Terpinolen	7.12	9.07
11	20.68	4-Terpinenol	1.34	1.67
12	21.35	α -Terpineol	0.64	1.14
13	29.49	Caryophyllene	21.45	15.54
14	29.98	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	0.34	-
15	30.45	α -Caryophyllene	10.75	8.17
16	31.05	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene- and β -Maaliene	1.01	0.61
17	31.17	α -Amorphene	0.30	-
18	31.35	β -Eudesmene	2.36	1.35
19	31.60	α -Selinene	2.67	1.42
20	31.93	β -Cadinene	0.39	-
21	32.35	delta-Cadinene	0.36	-
22	33.25	Elixene	0.30	-
23	33.54	Palustrol	0.40	0.36
24	33.97	Caryophyllene oxide	5.02	4.86
25	34.18	Viridiflorol	1.02	0.94
26	34.32	Guaiol	3.06	1.94
27	34.48	Ledol	4.64	4.14
28	34.64	1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	2.04	2.02
29	35.02	Juniper camphor	0.52	0.39
30	35.17	Agarspirol and γ -Eudesmol	3.19	2.12
31	35.36	Hisnesol	0.64	0.21
32	35.64	β -Eudesmol	2.90	1.71
33	35.72	α -Eudesmol	4.62	3.03
34	36.04	Bulnesol	0.88	0.43
35	38.62	Unidentified	4.35	2.54
36	38.80	Unidentified	3.58	2.39

The initial increase in EO yields by the prolonging extraction time could be explained by the high dielectric properties of polar solvents such as water, when they were subjected to significant temperature increase. The decrease in yield after 210 minutes could, however, be associated with the possible degradation of the plant material as the extraction time becomes prolonged. This might therefore lead to undesired evaporation of the volatile component in the *M. cajuputi* EOs which could inevitably lead to a decrease in the extraction yield.

Another parameter that is believed to play an important role in the productivities of isolation process was the ratio of water to material. Generally, the main function of water in distillation was to prevent the raw material from being thermal degradation and also to act as the carrier of EOs during the evaporation before the condensation process took place. Larger amount of solvent may consume more energy, and more time would be needed to condense the extraction solution. Furthermore, it might cause excessive thermal stress due to rapid heating of the solvent

[20]. On the other hand, smaller volume could lead to incomplete extraction of the target substances. This could be in the form of failure on the part of the solution to withstand high microwave intensities, especially when the extraction time was prolonged. This could lead to the burning off part of the plant material in the flask.

The physicochemical properties of *M. cajuputi* EOs could be used to investigate the effectiveness of extraction methods. The density of *M. cajuputi* EOs at 25°C was less than 1, which indicated that the EO was lighter than water and it contained many less polar and volatile compounds. Therefore, *M. cajuputi* EO was less thermostable and it should be stored at low temperatures to limit the adverse changes affecting its quality. The IA of this EO was low which indicated that it was quite durable, and difficult to be asphalt or metamorphic. On the other hand, a high IE value proved the content of ester in the EOs, which was one of the major components contributing to the aroma of product. Thus, the extraction by MAE resulted in less change in the nature as well as the composition of the EOs.

5. CONCLUSION

Time of extraction was proved to be a crucial parameter in distillation, in which 210 minutes was the optimal time frame. The yield of the product was observed to have a positive correlation with increase in time. The ratio of water to plant material was found to have a considerable effect on the quantity of the EOs extracted and no effect on the overall quality of the EOs. A ratio of 3.5 mL water/1 g material was found to be optimal, as there was no further significant increase in the yield of the EOs with the increase in the amount of excessive water. The *M. cajuputi* EOs were found to possess compounds with known biochemical properties applicable in the medicinal world. These compounds had individual antifungal and/or antibacterial properties capabilities, but their effectiveness could be enhanced synergistically. Moreover, MAE method had the advantage of shortening the distillation time and saving energy.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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