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Determination of Chemical Composition and Antimicrobial Activity of *Melaleuca cajuputi* Essential Oil from Quang Tri Province, Vietnam

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The essential oil of *Melaleuca cajuputi* was obtained by hydrodistillation method. This work aims to adopt water as a solvent in a batch process to extract essential oil from *Melaleuca cajuputi* fresh leaves. The leaves are collected from Quang Tri Province, Vietnam. Analysis of constituents was performed by GC/MS. The maximum yield ranged from 0.6 to 0.7%. Several compounds have been identified in high quantities and meaningful qualitative and quantitative differences have been observed under different conditions. The main components of the *M. cajuputi* essential oil included eucalyptol (27.512%), γ-terpinene (8.59%), terpinolene (9.047%), β-eudesmene (3.359%), α-selinene (3.889%), α-terpineol (4.108%), 1R-α-pinene (2.158%), caryophyllene (6.48%) and α-caryophyllene (3.522%). This study has confirmed that the essential oil of *M. cajuputi* essential oil is a promising bactericidal agent on several Gram-positive and Gram-negative bacteria.

Keywords: Essential oil, *Melaleuca cajuputi* leaves, Physico-chemical, GC-MS, Chemical composition, Antibacterial activities.

INTRODUCTION

The essential oils are refined liquid that is commonly obtained by distillation by steam or water from leaves, stems, flowers, bark, roots, or other components of plant materials. Essential oils are mixtures of volatile substances that often contain a characteristic aromatic component. The rapid development of the essential oil industry has made essential oils an indispensable part of human life and in industries such as aromatherapy, food, cosmetics and pharmaceuticals [1-6].

Melaleuca tree (*Melaleuca cajuputi* Powell) is a plant species which belongs to the Myrtaceae family and has a wide ecological distribution range. Melaleuca forest is usually distributed on river mouths, coastal marshes in hot, humid tropics and grows well in the temperature range of 17-33 ºC. Areas with a concentrated distribution of Melaleuca usually have an average rainfall of 1.3-1.7 mm and typical monsoon. The leaves of the plant are in the form of a large leaf or bamboo leaves, 7-8 cm long, 2 cm wide. The *M. cajuputi* essential oil has been long known for its spicy properties, warmth, sweating and pain-reducing qualities. The essential oil also has a strong antiseptic effect on many pathogenic bacteria. In folk medicine, the oil plays an important role in treatment of flu, wounds and itchy rashes and is especially effective when being coordinated with other plants to produce rheumatoid arthritis medicine.

To obtain essential oils from plant materials, a variety of extraction methods such as cold pressing, water distillation and solvent extraction could be employed. Among those, water distillation is conventional and the most commonly used method for extraction of essential oils from melaleuca plant materials. Main constituents that are commonly found in melaleuca essential oils consist of alkenes, acids, alcohols, aldehydes, esters, ketones, phenols and nitrogen compounds [7]. However, different varieties of the plant and extraction method used could contribute to the discrepancy in chemical composition and extraction of the obtained oils [8]. The extraction of essential oils from *M. cajuputi* leaves may result in the yield ranging from 0.3-0.6% depending on the quality of the leaves used. Major components in the essential oil of the plant may include 1,8-cineole (46.9-57.9%) with monoterpenic alcohol (-)α-terpineol, (-)-linalool and (-)-terpinene-4-ol. There is also a high content of hydrocarbon monoterpenes (27.8%), a small amount of sesquiterpene hydrocarbons and alcohol [9-11]. Studies have revealed the antimicrobial activity of *M. caguputi* essential oil against Gram-positive and Gram-negative bacterial strains in disc diffusion and minimum inhibitory concentration test. To be specific, bacterial species that were shown to be effectively inhibited by the *M. caguputi* essential oil were *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *Staphylococcus aureus* and *Streptococcus pyogenes* [12-14].

Most of the studies on *M. cajuputi* have mainly focused on the extraction process of essential oils from the leaves, leaving physico-chemical activity and chemical composition of the obtained oil unexplored. Therefore, this study attempt the analysis of volatile components from essential oil extracted from fresh *M. cajuputi* leaves *via* GC-MS method. Moreover, the antioxidant and antibacterial activities of the obtained essential oils were assayed. The study results are expected to contribute to subsequent comparative studies and future development on industrial quality assessment of *M. cajuputi*.

EXPERIMENTAL

In this study, *M. cajuputi* leaves were selected and collected in Quang Tri Province, Vietnam in April 2019. The area is a tropical monsoon area with the hot, humid and rainy habitat. Raw materials were collected under dry conditions to ensure uniformity in the survey samples. Leaves are kept fresh during the experiment by preservation in a cool cabinet condition.

Isolation of *M. cajuputi* essential oils: The leaves after being collected are preliminarily processed (rinse, remove deep leaves, waterlogged leaves) and chopped 1-2 mm or pureed to increase the exposure area to water. Then, materials were transferred into a flask with the raw materials/water ratio of 1:4, followed by distillation in 75 min at 105 °C. The essential oil started to evaporate along with the steam through the condenser tube and was cooled by cold water whereas steam and essential oil were condensed into a small flask. The obtained product was the water-containing essential oil with two layers in which the upper layer was the essential oil and the lower layer was the water. The essential oil was removed from water using the funnel and essential oils were refined with Na_2SO_4. The final product was stored in an amber vial until sample analysis.

Physico-chemical analyses of essential oils: Some basic physical and chemical parameters of the essential oil analyzed by TCVN including density of essential oils, acid index (TCVN 8450:2010, ester index (TCVN 8451:2010), rotator power and refractive index. The experiments were repeated three times.

Gas chromatography-mass spectrometry (GC-MS) analyses: Chemical composition of the essential oil was determined by GC-MS analysis using GC Agilent 6890 N instrument coupled with HP5-MS column and MS 5973 inert. The pressure of the head column was 9.3 psi. 25 µL of essential oil was added with 1.0 mL *n*-hexane and dehydrated with Na_2SO_4. The flow rate was constant at 1 mL/min. Injector temperature is 250 °C and the rate of division is 30. Thermal program for samples: 50 °C kept for 2 min, followed by increase of 2 °C/min to 80 °C, continued to increase by 5 °C/min to 150 °C, continued to increase by 10 °C/min to 200 °C, increase 20 °C/min to 300 °C hold for 5 min.

Antibacterial activity testing: In this study, the antibacterial activity of the obtained *M. cajuputi* essential oil was assayed against five strains of bacteria including Gram-negative and Gram-positive. Gram-positive strains were *Bacillus cereus* NRRL-B-354 and *Staphylococcus aureus* NRRL-B-313. Gram-negative strains were *Escherichia coli* NRRL B-409, *Samonella typhimurium* NRRL-B-2354 and *Pseudomonas aeruginosa* NRRL B-14781 at concentrations of 1×10^8 CFU/mL [bacterial density is determined by optical density measurement (OD) method at a wavelength of 600 nm]. The nutrient agar LB was poured into petri dishes with a diameter of 90 mm until the thickness of the agar is 4 mm to prevent diffusion of the tested products. For each bacterial solution, 0.1 mL of solution was infused into the plate distributed evenly by the sterile gauze method. The panels are allowed to stand for 15 min. Simultaneously, a sterile 6mm diameter filter paper plate containing *M. cajuputi* essential oil was placed on agar plates and a 6 mm diameter disk with distilled water medium was used as the negative control. Another sterile paper disk containing amoxicillin antibiotic (400 µg/mL) is a positive control. The plates are symmetrically placed on the medium with sterile tweezers. The plates were incubated for 24 ± 2 h at 37 °C under anaerobic conditions. Antibacterial activity was indicated by the area without bacterial growth and was calculated using the following formula:

$$\text{Inhibition value} = \frac{\text{Inhibition diameter (mm)} - \text{Disk diameter (6 mm)}}{2}$$

Experiments were performed in triplicate.

RESULTS AND DISCUSSION

The extraction of the essential oil by hydrodistillation method from fresh leaves of *M. cajuputi* achieved the yield of around 0.6-0.7%. In other reports, hydrodistillation of *Melaleuca cajuputi* collected from six locations in Narathiwat (Korea) gave different essential oil yields. The maximum oil yield was 0.97%, which is much higher than the yield in the present study [15]. In contrast, the present results are higher than those of the same species collected from the Trengganu (Malaysia) areas. To be specific, essential oil extraction from leaves and twigs of *M. cajuputi* resulted in yield of 0.46% (leaves) and 0.02% (tree branches) respectively. Jajaei *et al.* [16] conducted the extraction of essential oil from *Melaleuca cajuputi* (Trengganu, Malaysia) by the supercritical extraction method (carbon dioxide) and solvent extraction method (hexane). The maximum yield for the two method was 4.2 and 6.0% respectively. At room temperature, the essential oil is liquid, almost insoluble in water but soluble in alcohol and organic solvents, partially soluble in alkaline solutions. Most melaleuca essential oil was either colourless or light yellow. The physico-chemical properties and sensory evaluation of melaleuca essential oils are shown in Table-1 below. Melaleuca oil is lighter than water with the density of essential oils (0.9497) < 1. The acid index of 0.524 indicated the low free acid content and the freshness of essential oils. The ester index of 6.851 is an important component of the aroma of essential oils. This index is also indicative of the small molecular weight and the low fatty acid content.

TABLE-1
PHYSICO-CHEMICAL PROPERTIES
OF *M. cajuputi* ESSENTIAL OIL

Organoleptic characteristics	*M. cajuputi* essential oil
Aspect	Liquid
Colour	Clear light yellow liquid
Odour	Specific
Density 20 °C	0.9497
Acid index	0.524
Ester index	6.851

The essential oil of *Melaleuca cajuputi* leaves were qualitatively analyzed using GC-MS with NIST 14 from the universal library. Table-2 shows the chemical composition of the essential oil and its component content. A total of 50 compounds were discovered in melaleuca oil, of which, 11 compounds were indicated as unidentified. Fifty retention periods are given in Table-2, corresponding to the 50 peaks in Fig. 1. Components having relatively large peaks of 11.956, 13.556 and 15.292 were constituents of high content in melaleuca oil. Results of chemical composition showed that eucalyptol (27.512%), γ-terpinen (8.59%), terpinolene (9.047%) were the main components of *M. cajuputi* leaf oil. Other components are β-eudesmene (3.359%), α-selinene (3.889%), α-terpineol (4.108%), 1R-α-pinene (2.158%), caryophyllene (6.48%), α-caryophyllene (3.522%). Pino *et al.* [17] showed that 38

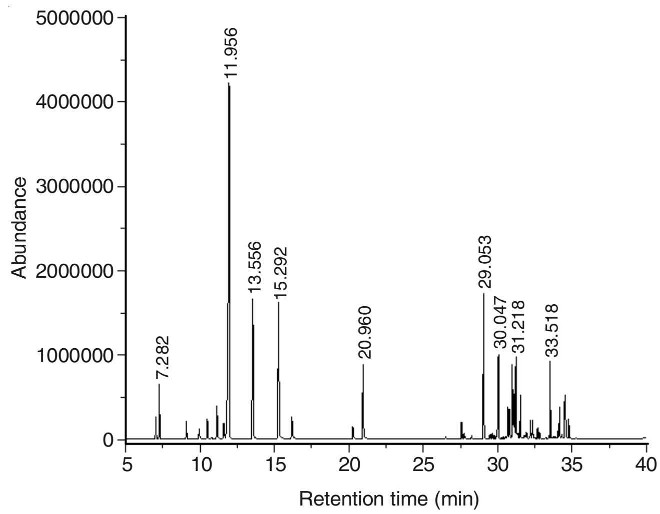

Fig. 1. Chromatogram of the *Melaleuca cajuputi* essential oil from leaves

TABLE-2
CHEMICAL COMPOSITION OF *Melaleuca cajuputi* LEAVES BY GC-MS

Peak	Retention time (min)	Compound	Pct Total	Peak	Retention time (min)	Compound	Pct Total
1	7.042	α-Thujene	0.880	26	30.695	Unknown	1.820
2	7.282	1R-α-Pinene	2.158	27	30.810	α-Amorphene	1.293
3	9.091	(-)-β-Pinene	0.796	28	30.977	β-Eudesmene	3.359
4	9.959	β-Myrcene	0.605	29	31.051	Unknown	0.755
5	10.492	α-Phellandrene	1.050	30	31.113	Cadinene	1.858
6	11.151	α-Terpilene	1.885	31	31.228	α-Selinene	3.889
7	11.601	β-Cymene	1.090	32	31.532	Unknown	1.959
8	11.956	Eucalyptol (1,8-cineole)	27.512	33	31.793	Eudesma-3,7(11)-diene	0.132
9	13.556	γ-Terpinen	8.559	34	31.856	Unknown	0.132
10	15.292	Terpinolene	9.047	35	31.919	δ-Cadinene	0.284
11	16.181	Linalol	1.569	36	31.971	Unknown	0.229
12	20.270	Terpinen-4-ol	0.776	37	32.190	Unknown	0.706
13	20.960	α-Terpineol	4.108	38	32.253	Unknown	0.242
14	27.579	Ylangene	0.735	39	32.337	Eudesma-3,7(11)-diene	0.837
15	27.725	Copaene	0.250	40	32.682	Germacrene B	0.421
16	27.809	Isoledene	0.144	41	32.818	Unknown	0.277
17	28.248	β-Elemen	0.155	42	33.424	Epicubebol	0.111
18	29.053	Caryophyllene	6.480	43	33.529	Guaiol	2.686
19	29.482	γ-Elemene	0.209	44	34.052	Unknown	0.263
20	29.618	α-Guaiene	0.257	45	34.135	Unknown	0.627
21	29.754	Guaia-6,9-diene	0.175	46	34.156	γ-Eudesmol	1.088
22	29.921	Aromandendrene	0.124	47	34.292	Hinesol	0.213
23	30.005	α-Caryophyllene	0.868	48	34.491	β-Eudesmol	1.278
24	30.047	α-Caryophyllene	3.522	49	34.543	α-Eudesmol	1.734
25	30.402	Unknown	0.107	50	34.773	Bulnesol	0.749

compounds, including viridiflorol (28.2%) and 1.8-cineole (21.3%) were the major components in Cuban *M. cajuputi* essential oil. *M. cajuputi* essential oil from South India contained significant amounts of (E)-nerolidol (76.58-90.85%), β-caryophyllene (1.52-4.49%), viridiflorol (0.19-2.79%), (E)-β-farnesene (≤ 0.10-2.67%) and α-humulene (0.22-1.03%), as shown by Padalia *et al.* [18]. These results suggest that the composition of the essential oil of *M. cajuputi* could vary depending on geographical regions and the origin of the plant. In melaleuca essential oils in Thailand, main components were 1,8-cineole (42-60%), α-terpineole (4-18%), caryophyllene (0.6-11%), α-pinene (3-12%)) and limonene (0-5%) [15].

However, *M. cajuput* essential oil from Yogyakarta (Java island) contains a significant amount of 1,8-cineole (55.16%), as well as caryophyllene homologue (9.09%) and α-caryophyllene (4.64%) [19]. The 1.8-cineole content in present study was lower than most of regional reports. The discrepancy in terms of component content and composition was possibly due to changes in metabolite production of the plant that is affected by geographical and environmental conditions.

Table-3 indicated that significant average antibacterial activity of the essential oil was found against all strains compared to the negative control and the positive control. Results in Table-3 showed that the most susceptible organism was *Salmonella typhimurium*, which shows the maximum zone of inhibition 7 mm in diameter, followed by 6 mm in diameter for *Staphylococcus aureus* strain. In addition, the zone of inhibition of three strains of *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* was 5 mm in size. The inhibition zone of the essential oil against all five strains of bacteria was much lower than those of antibiotics (amoxicillin). The antibacterial and antifungal activity of *Melaleuca* spp is mainly assigned to monoterpenes 1,8-cineol and terpinen-4-ol, the main component of essential oils, as well as secondary components of methyl eugenol [20]. Carson *et al.* [21] reported antifungal activities against *C. albicans* of melaleuca oil. However, the current study showed no inhibition against *C. albicans* and *R. nigricans*, which could possibly due to the compositional difference of essential oils. The composition of essential oils has been found to be affected by climate, seasonal and geographical conditions, regulating growth and harvesting stage [7,22]. In addition, the zone of inhibition may not be clearly visible for essential oil wells, which could be attributed to the evaporation of the active ingredient, oxidation of the image or the inadequate number of active ingredients. Based on the findings of this study, it can be concluded that essential oils from *M. cajuput* possess antibacterial activities against various clinical isolates and can be used in the treatment of various bacterial infections.

TABLE-3
ANTIBACTERIAL ACTIVITY OF CAJUPUTI ESSENTIAL OIL COMPARED WITH SELECTED ANTIBIOTIC TESTED ON FIVE STRAINS OF BACTERIAL PATHOGENS

Tested bacteria	Zone of inhibition (mm)		
	Essential oil clove	Control	
		Negative*	Positive**
Bacillus subilits	5	6	13
Staphylococcus aureus	6	6	11
Escherichia coli	5	6	9
Salmonella typhimurium	7	6	24
Pseudomonas aeruginosa	5	6	17

*H_2O as a negative control; **Amoxicillin (400 μg/disk)

Conclusion

The extraction of essential oil from *M. cajuputi* leaves gave 0.6-0.7% oil by hydrodistillation. The obtained essential oil was light yellow and almost transparent. The oil was lighter than water, had acid index of 0.524 and ester index of 6.851. The chemical composition of *Melaleuca cajuputi* essential oil was determined by gas chromatography-mass spectrometry and by comparison of retention time to the mass spectral library collection. Results of mass spectrometry (GC-MS) analysis showed that the essential oil extracted from fresh leaves of *M. cajuputi* oil contains 50 components in which eucalyptol (27.512%), γ-terpinen (8.59%), terpinolene (9.047%) were the components with the highest content. Antimicrobial activity of the obtained oil is demonstrated through the strongest zone of inhibition on *Salmonella typhimurium* and *Staphylococcus aureus* bacterial strains. The activity observed towards *M. cajuputi* indicates that this essential oil may represent a promising antimicrobial drug, especially in the management of antimicrobial resistance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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