Bull. Chem. Soc. Ethiop. **2021**, 35(3), 677-683.
DOI: https://dx.doi.org/10.4314/bcse.v35i3.18

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

SHORT COMMUNICATION

PHYSICOCHEMICAL PROPERTIES, CHEMICAL COMPONENTS, AND ANTIBACTERIAL ACTIVITY OF *MELALEUCA CAJUPUTI* POWELL ESSENTIAL OIL LEAVES FROM QUANG TRI PROVINCE, VIETNAM

Le Pham Tan Quoc*

Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City, Vietnam

(Received April 3, 2021; Revised January 5, 2022; Accepted January 6, 2022)

ABSTRACT. Essential oil (EO) was extracted from *Melaleuca cajuputi* Powell leaves using the steam distillation method. The main aim of this study is to determine several physicochemical properties of EO, including acid value, saponification value, ester value, relative density, absolute density, and freezing point. In addition, the chemical components were also analyzed using the gas chromatography–mass spectrometry (GC–MS) method. The obtained 1,8-cineol content is quite high (71.83%). In addition, these components in EO had an antioxidant capacity and antibacterial activity, including gram-positive and gram-negative bacteria (using the paper disc diffusion method for antibiotic susceptibility testing). Particularly, this EO inhibited the growth of *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076), and *Staphylococcus aureus* (ATCC 25923).

KEY WORDS: Antimicrobial activity, Antioxidant capacity, GC-MS, Essential oil, *Melaleuca cajuputi* Powell.

INTRODUCTION

Melaleuca cajuputi Powell (common name: cajuput tree or white tea tree) is a vigorous plant adapted to nutrient-poor soils, especially acid soils, cracking black clay, black peaty sand, and clay loam [1, 2]. This plant has a high economic value in Vietnam; it can be used as building materials, made into paper powder, or the EO extracted from its leaves can be applied in pharmaceutical technology [1]. This is a medium to tall tree (2-46 m), with papery bark and grey, brownish, pink-tan, or whitish tree. Leaves are long- or short-petiolate; blade glabrescent, narrowly elliptic, 40–140 mm long, and 7.5–60 mm wide. Flowering is recorded from March to November. This plant is found in Western and Northern Australia, Indonesia, East Timor, Vietnam, etc. [2].

Currently, the cajuput tree is distributed everywhere in Vietnam, especially Hue, Quang Binh, and Quang Tri province, etc. because the demands for EO in the drug, cosmetic, and flavoring field have increased significantly. The main component of this EO is 1,8-cineol (eucalyptol), and it has many uses such as treating bronchitis, sinusitis, chronic rhinitis, and asthma [3]. In addition, Atta *and* Alkofahi [4] also reported that *M. cajuputi* EO has antioxidant, anti-inflammatory, and antimicrobial activities.

Nowadays, many scientists have studied EO from *M. cajuputi* leaves. The results indicate that the chemical composition and yield of extraction of EO depend on different extraction methods and sources of the plant [5, 6]. The chemical composition significantly affects the physicochemical properties, antioxidant capacity, and antibacterial activity of EO [7, 8]. However, until now, there have been no studies on the chemical components, antibacterial activity, and physical properties of EO of *M. cajuputi* leaves from Quang Tri province (Vietnam). Therefore, the major purpose of this study is to clarify these issues mentioned above.

*Corresponding author. E-mail: lephamtanquoc@iuh.edu.vn

EXPERIMENTAL

Material and EO extraction. Fresh leaves of *M. cajuputi* were collected and harvested from Quang Tri province (Vietnam). Materials were distilled using the steam distillation method at 100 ºC. Then, the obtained EO was finally collected after decantation and stored at 4 ºC in dark until further analyses.

Bacteria strains. Four bacteria strains were tested in this study, including one gram-positive bacteria (*Staphylococcus aureus*, ATCC 25923) and three gram-negative bacteria (*Salmonella enteritidis*, ATCC 13076; *Escherichia coli*, ATCC 25922; *Pseudomonas aeruginosa*, ATCC 27853).

Determination of the relative density (RD) and absolute density (AD) of EO. According to ISO 279:1998 [9], the RD was determined by the proportion of the mass of a given volume of the EO to the mass of an equal volume of distilled water at 20 ºC, while the AD was determined by the proportion of the mass of a given volume of the EO to the same volume.

Determination of the freezing temperature of EO. 5 mL of the obtained EO was added to the test tube. Next, it was put into a freezing container. The temperature in the freezing container was decreased slowly until the EO appeared to crystallize. The freezing temperature was recorded at this moment [10].

Determination of acid value (AV) of EO. The AV was performed according to the procedure of Quoc [8]. The obtained EO (1 g) was dissolved in 5 mL of 96% ethanol, and a few drops of 1% phenolphthalein was added to the mixture. The KOH solution (0.1 M) was used to titrate this mixture until it turned pink.

$$AV = \frac{V_{KOH} \times 0.1 \times 56.1}{Mass\ of\ essential\ oil}$$

Determination of saponification value (SV) of EO. For the determination of SV, 2 g of EO and 25 mL of the ethanol solution of KOH (0.5 M) were mixed in a glass flask (250 mL). This mixture was heated for 60 min in the condenser system. Next, 25 mL of distilled water and a few drops of 1% phenolphthalein were added to the mixture. The HCl solution (0.5 M) was used to titrate this mixture until it turned colorless [8].

$$SV = \frac{(V_{blank} - V_{sample}) \times 56.1 \times 0.5}{Mass\ of\ essential\ oil}$$

Determination of antioxidant capacity (AC) of EO. The procedure to determine the AC of the EO to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was described according to Kirby and Schmidt [11] with some minor changes. The EO from *M. cajuputi* leaves was dissolved in ethanol (96%, v/v) to obtain different concentrations (512, 256, 128, 64, 32, and 16 mg/mL). 0.3 mL of the obtained solution and 2.7 mL DPPH in ethanol solution (concentration of 40 µg/mL) were mixed together. The mixture was kept in the dark for 30 min at room temperature. The AC was recorded by observing the decrease in absorbance at 517 nm against a control sample (containing only DPPH in ethanol solution without the tested sample). The AC of EO was compared to that of ascorbic acid as a standard. Percent inhibition was plotted against EO concentrations to estimate the concentration providing 50% inhibition (IC_{50}). The AC was calculated using the following expression:

$$\%inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Determination of antibacterial activity (AA) of EO. In this study, the paper disc diffusion method for antibiotic susceptibility testing was used to test the AA of EO according to the method of Bauer *et al.* [12] with some small corrections. Firstly, 100 µL of bacteria suspension (0.5 McFarland standard, approximately 1.5×10^8 CFU/mL) were spread on MHA media (Mueller-Hinton agar) by a sterile hockey stick. Next, the sterile paper discs (6 mm diameter) were impregnated by the obtained EO (5 µL), while gentamicin (10 µg/disc) and dimethylsulfoxide (DMSO) solution (5%, v/v) were used as positive and negative controls, respectively. Then, all dishes were incubated for 24 h at 37 ºC, and the inhibition zones were expressed in mm including the disc diameter of 6 mm.

Gas chromatography-mass spectrometry (GC–MS) analysis. The chemical composition of EO was analyzed using the GC–MS method. 1 µL of EO was injected into a gas chromatograph (Agilent HP 6890N, USA) with a capillary column (HP-5ms, 30 m×0.25 mm×0.45 µm, Agilent Technologies, USA) equipped with a quadrupole mass analyzer (Agilent HP 5972, USA). Helium was used as a carrier gas at a constant flow rate of 0.5 mL/min, and a split ratio of 10:1. The injection temperature was 250 ºC and the temperature program was set as follows: initial temperature of 50 ºC, held for 2 min, increased until 300 ºC at a rate of 10 ºC/min, and held for 5 min. Mass spectra were recorded at the ionization energy of 70 eV in EI mode.

Statistical data analysis. All experiments were conducted in triplicate and the results were expressed as a mean ± standard deviation (SD). One-way analysis of variance (ANOVA) method with Fisher's least significant difference (LSD) procedure was carried out to test significant differences between the means ($p < 0.05$). The data were analyzed using Statgraphics Centurion XV software (version 15.1.02, Statgraphics Technologies, Inc., USA).

RESULTS AND DISCUSSION

Determination of the physicochemical properties of M. cajuputi leaves EO. *M. cajuputi* leaves EO is a pale-yellow liquid with a characteristic odor. Some physicochemical properties of *M. cajuputi* leaves EO are presented in Table 1. The pH value of EO reached approximately 4.46, which was significantly different than values reported from previous studies; for instance, the pH values of EOs of *Ceratonia siliqua* pulp and seeds were 4.3±0.5 and 5.2±0.3, respectively [7]. This can be explained by the fact that the pH value depends significantly on the chemical composition of EOs. It is observed that the obtained EO is a little denser than that of other materials, showing RD of 0.9102±0.0002, whereas that of *Skimmia laureola* leaves EO and *Zanthoxylum armatum* leaves EO were 0.792±0.001 and 0.816±0.001, respectively [13]. In addition, the AD for EO of *M. cajuputi* leaves (0.9086±0.0002 g/mL) was not significantly higher than the EO of *C. siliqua* seeds (0.910±0.04 g/mL) [7] and *Myrtus communis* leaves (0.894±0.001 g/mL) [14]. As for the freezing point of EO, it can be seen in Table 1 that this value is quite low (approximate -45 ºC). Considering that previous scientific reports on the freezing point of this EO are nonexistent, it is difficult to make some further comments regarding our results. However, the chemical composition of EO is the most important factor that extremely affects these EO properties.

Table 1. Some physicochemical properties of *M. cajuputi* leaves EO.

No	Physicochemical properties	Value
1	pH	4.46±0.01
2	Relative density	0.9102±0.0002
3	Absolute density (g/mL)	0.9086±0.0002
4	Freezing point (ºC)	-45
5	Acid value (mg KOH/g EO)	0.59±0.15
6	Saponification value (mg KOH/g EO)	28.05±2.55

The AV recorded in this study was 0.59±0.15, which was lower than that *S. laureola* leaves EO (1.78±0.01) and *Z. armatum* leaves EO (1.98±0.01) [13], and *M. communis* leaves EO (4.451±0.710) [14]. Besides, the SV was also quite low (28.05±2.55), and it was found to be in accordance with the conclusion of Barkatullah *et al.* [13], who indicated that the SV value of plants is usually lower than the range of 188 to 196. In general, both the AV and SV values are two basic elements to evaluate the EO quality.

Determination of the chemical compositions of M. cajuputi leaves EO. GC–MS analysis was used to determine the chemical compositions of *M. cajuputi* leaves EO. The obtained results indicate that there are 23 different components in EO that comprised approximately 98% of the total chemical constituents of the EO (Table 2). All compounds were detected under retention time (RT) ranging from 6.3 min to 21.94 min. The compounds occupying the highest content in EO included 1,8-cineol (71.83%), *p*-menth-1-en-8-ol (6.01%), *p*-cymene (2.87%), γ-terpinene (2.73%), (+)-4-carene (2.7%), linalool (2.65%), (1R)-(+)-α-pinene (2.23%), and tyranton (2.03%). These components play an important role in EO quality. These chemical compositions in *M. cajuputi* leaves EO were very different from those in EO from leaves collected in various regions. Some compounds in the obtained EO were similar to those from many previous reports. The authors also studied the same material, for instance, 1,8-cineol, γ-terpinene, α-pinene, β-pinene, β-myrcene, benzaldehyde, caryophyllene [6], linalool, and p-cymene [15]. In general, there were minor changes in the proportion of components but there were no differences in major components, especially 1,8-cineol.This is the most important compound to evaluate the quality of EO from *M. cajuputi* leaves. Many previous studies reported that 1,8-cineol usually presents in *M. cajuputi* leaves EO with a high yield such as 7.3–23.2% [6], 46.9–57.9% [15], and 41.6–59.9% [16]. In some cases, there is no 1,8-cineol in *M. cajuputi* leaves EO [17], or the EO contained a low quantity (<3%) of this compound with the material from other places [5]. The difference in the chemical composition of EO can be explained by differences in the climatic conditions, time of harvesting, extraction method, and age of the plant, etc. [8].

Table 2. Chemical composition of *M. cajuputi* leaves EO.

No	Compound	RT (min.)	(%)
1	Tyranton	6.3	2.03
2	4-Methyl-1-(1-methylethyl)bicyclo[3.1.0]hexane didehydro deriv.	8.38	0.47
3	(1R)-(+)-α-Pinene	8.58	2.23
4	Benzaldehyde	9.2	0.17
5	β-Pinene	9.68	1.5
6	β-Myrcene	9.93	1.5
7	α-Phellandrene	10.34	0.36
8	δ-Terpinene	10.66	0.53
9	*p*-Cymene	10.88	2.87
10	1,8-Cineol	11.12	71.83
11	γ-Terpinene	11.73	2.73
12	(+)-4-Carene	12.5	2.7
13	Linalool	12.71	2.65
14	Methylenecyclooctane	14.5	0.11
15	4-Terpinenol	14.77	0.66
16	*p*-Cymen-8-ol	14.9	0.11
17	*p*-Menth-1-en-8-ol	15.1	6.01
18	β-Sesquiphellandrene	19.39	0.09
19	Caryophyllene	20.51	0.5
20	d,l-trans-4-methyl-5-methoxy-1-(1-methoxy-1-isopropyl)-cyclohex-3-ene	21.07	0.11
21	α-Caryophyllene	21.24	0.3
22	2,5,9,9-Tetramethyl-3,4,4a,5,8,9a-hexahydrobenzo [7] annulene	21.72	0.2
23	1H-Cyclopropa[a]naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-, [1aS-(1aα,3aα,7aβ,7bα)]-	21.94	0.37

Determination of the antioxidant capacity (AC) of M. cajuputi leaves EO. By the DPPH technique, with an increase in EO concentration from 16 to 512 mg/mL, a significant increase in the AC of *M. cajuputi* leaves EO was detected (Figure 1). The AC peaks at 38% for an EO concentration of 512 mg/mL, while the AC of the original EO was approximately 40.88±0.85%. This revealed that the IC_{50} was not found in the EO concentration ranges tested, while the AC of ascorbic acid (IC_{50} of 16 µg/mL) was more efficient than that of the EO concentrations obtained (Figure 2). In general, the AC depends significantly on the chemical components, especially 1,8-cineol, γ-terpinen, and 4-carene. They are classified as terpene groups and can be found in large amounts in *M. cajuputi* leaves EO. Many previous studies reported that these compounds have a strong AC [18-20]. Essentially, there are no published studies on the AC of *M. cajuputi* leaves EO in the literature, it was not possible to compare our results.

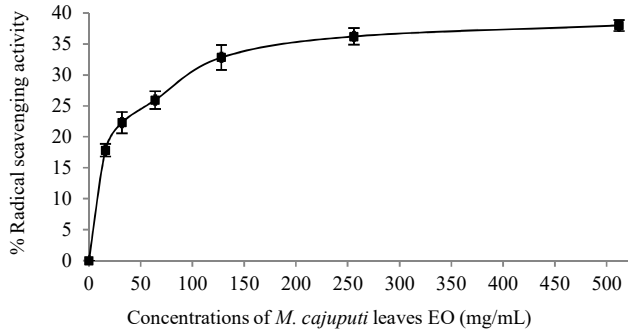

Figure 1. Radical scavenging activity of *M. cajuputi* leaves EO

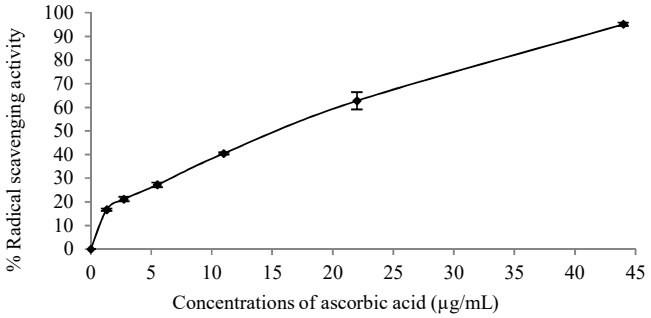

Figure 2. Radical scavenging activity of ascorbic acid

Determination of the antibacterial activity (AA) of M. cajuputi leaves EO. *M. cajuputi* leaves EO is considered a non-toxic agent. Beyond this, it has been noticed that cajuput EO is a disinfectant against bacteria [21]. In this study, the AA of cajuput EO is arranged in descending order: *S. aureus* > *S. enteritidis* > *E. coli* > *P. aeruginosa* (Table 3). However, cajuput EO did not inhibit the growth of *P. aeruginosa*, whereas it had a strong AA for the other three bacteria. In particular, the AA of EO for *S. aureus* and *E. coli*is significantly stronger than that of the positive controls. The same tendency of the AA of cajuput EO for *S. aureus*, *E. coli*, and *P. aeruginosa* is also observed in the study of Bharat *and* Praveen [22].

Although there is wide variation in the chemical composition of cajuput EO, the most important bioactive compound still is 1,8-cineol. The obtained EO in this study contains substantial amounts of 1,8-cineol (71.83%), and it was the main active component responsible for

its AA [23]. In addition, there are many minor compounds that exist in EO that also inhibit the growth of bacteria, for instance, linalool, γ-terpinene [21], myrcene [24], etc. This also proved that the AA can be a result of the synergistic action of major and minor components of the EO.

Table 3. The AA of *M. cajuputi* leaves EO.

No	Microorganisms	Diameter of the inhibitory zones of EO (mm)	Diameter of the inhibitory zones of positive control (gentamicin, mm)
1	*S. enteritidis*	23.67 ± 0.58^{Ab}	23 ± 0^{Ac}
2	*E. coli*	21.33 ± 1.15^{Ba}	15 ± 0^{Aa}
3	*P. aeruginosa*	-	17.67 ± 0.58^{b}
4	*S. aureus*	27.33 ± 0.58^{Bc}	25.67 ± 0.58^{Ad}

Different lowercase letters in the same column indicate significant differences between microorganisms at the $p < 0.05$ level. Different capital letters in the same row indicate significant differences between samples at the $p < 0.05$ level.

CONCLUSION

In general, the *M. cajuputi* leaves EO collected in Quang Tri province revealed that its physicochemical properties are very different compared to those of other EOs. It had high AC and AA. By the GC-MS method, 23 major components were identified in cajuput EO. This is a natural, rich source of 1,8-cineol that could be widely used in the cosmetic, food, and pharmaceutical industry.

ACKNOWLEDGMENTS

This research was performed at the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City (Vietnam). The author would like to thank Le Thi Anh Duong and Nguyen Thi Phuong Dung for their helpful advice on various technical issues examined in this paper.

REFERENCES

1. Hang, P.T.; Toan, N.B. Micropropagation of *Melaleuca cajuputi* Powell. *Can Tho Univ. J. Sci*. **2011**, 20B, 89–96. (In Vietnamese)
2. Brophy, J.J.; Craven, L.A.; Doran, J.C. *Melaleucas: Their botany, essential oils and uses*, Australian Centre for International Agricultural Research (ACIAR): Canberra, Australia; **2013**; p 415.
3. Juergens, U.R.; Stöber, M.; Schmidt-Schilling, L.; Kleuver, T.; Vetter, H. Antiinflammatory effects of eucalyptol (1,8-cineole) in bronchial asthma: inhibition of arachidonic acid metabolism in human blood monocytes ex vivo. *Eur. J. Med. Res*. **1998**, 3, 407–412.
4. Atta, A.H.; Alkofahi, A. Anti-nociceptive and anti-inflammatory effects of some Jordanian medicinal plant extracts. *J. Ethnopharmacol*. **1998**, 60, 117–124.
5. Kim, J.H.; Liu, K.H.; Yoon, Y.; Sornnuwat, Y.; Kitirattrakarn, T.; Anantachoke, C. Essential leaf oils from *Melaleuca cajuputi*. *Acta Hortic*. **2005**, 680, 65–72.
6. Van, H.T.; Phan, U.T.X.; Tran, G.B. Chemical diversity of the *Melaleuca cajuputi* leaf oils from six locations in southern Vietnam. *Agric. Conspec. Sci*. **2019**, 84, 391–397.
7. Ouis, N.; Hariri, A. Antioxidant and antibacterial activities of the essential oils of *Ceratonia siliqua*. *Banat's J. Biotechnol*. **2018**, 9, 13–23.
8. Quoc, L.P.T. Physicochemical properties and antibacterial activity of essential oil of *Ageratum conyzoides* L. leaves. *Agric. Conspec. Sci*. **2020**, 84, 391–397.
9. ISO 279:1998. *Essential oils - Determination of relative density at 20 °C*. International Organization for Standardization, Geneva, Switzerland.

10. ISO 1041:1973. *Essential oils - Determination of freezing point*. International Organization for Standardization, Geneva, Switzerland.
11. Kirby, A.J.; Schmidt, R.J. The antioxidant activity of Chinese herbs for eczema and of placebo herbs. *J. Ethnopharmacol*. **1997**, 56, 103–108.
12. Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C.; Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*. **1966**, 45, 493–496.
13. Barkatullah; Ibrar, M.; Rauf, A.; Rahman, I.U. Physicochemical characterization of essential and fixed oils of *Skimmia laureola* and *Zanthoxylum armatum*. *Middle-East J. Med. Plants Res*. **2012**, 1, 51–58.
14. Hagos, Z.; Mulugeta, A.; Gopalakrishnan, V.K.; Krishna, C.K.; Nagaraju, B. Chemical composition and physicochemical properties of essential oil from *Myrtus communis*. *Int. J. Pharm. Clin. Res*. **2017**, 9, 439–443.
15. Motl, O.; Hodatova, J.; Ubik, K. Composition of Vietnamese cajuput essential oil. *Flavour Fragrance J*. **1990**, 5, 39–42.
16. Sutrisno; Retnosari, R.; Asmaningrum, H.P. Profile of the Indonesian essential oil from *Melaleuca cajuputi*. *Adv. Eng. Res*. **2018**, 171, 14–19.
17. Sharif, A.M.; Kamal, A.F.; Jalil, N.J. Chemical composition of *Melaleuca cajuputi* Powell. *Int. J. Eng. Adv. Technol*. **2019**, 9, 3479–3483.
18. Smeriglio, A.; Denaro, M.; Barreca, D.; Calderaro, A.; Bisignano, C.; Ginestra, G.; Bellocco, E.; Trombetta, D. In vitro evaluation of the antioxidant, cytoprotective, and antimicrobial properties of essential oil from *Pistacia vera* L. variety Bronte hull. *Int. J. Mol. Sci*. **2017**, 18, 1212.
19. Miri, S. Phytochemistry, antioxidant, and lipid peroxidation inhibition of the essential oils of *Lavandula officinalis* L. in Iran. *Int. J. Food Prop*. **2018**, 21, 2550–2556.
20. Guo, Y.; Baschieri, A.; Amorati, R.; Valgimigli, L. Synergic antioxidant activity of γ-terpinene with phenols and polyphenols enabled by hydroperoxyl radicals. *Food Chem*. **2021**, 345, 128468.
21. Cuong, N.D.; Xuyen, T.T.; Motl, O.; Stráhnský, K.; Presslová, J.; Jedlicková, Z.; Serý, V. Antibacterial properties of Vietnamese cajuput oil. *J. Essent. Oil Res*. **1994**, 6, 63–67.
22. Bharat, C.S.; Praveen, D. Evaluation of *in vitro* antimicrobial potential and phytochemical analysis of spruce, cajeput and jamrosa essential oil against clinical isolates. *Int. J. Green Pharm*. **2016**, 10, 27–32.
23. Vuuren, S.F.V.; Viljoen, A.M. Antimicrobial activity of limonene enantiomers and 1,8-cineole alone and in combination. *Flavour Fragrance J*. **2007**, 22, 540–544.
24. Inoue, Y.; Shiraishi, A.; Hada, T.; Hamashima, H.; Shimada, J. The antibacterial effects of myrcene on *Staphylococcus aureus* and its role in the essential oil of the tea tree (*Melaleuca alternifolia*). *Nat. Med*. **2004**, 58, 10–14.