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Chemical Composition and Antimicrobial Activity of *Melaleuca cajuputi* Essential Oil from Vietnam

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Abtract: *Melaleuca cajuputi Powell is an aromatic plant used in Vietnam for its antiseptic and therapeutic purposes. To date, there is almost no scientific evidence for the Vietnamese plant material M. cajuputi essential oil. This study aims at the in vitro determination of the antimicrobial activity of Vietnamese M. cajuputi essential oil. Antimicrobial activity of essential oils obtained from hydrodistillated fresh leaves of M. cajuputi was tested in vitro for representative gram-positive and gram-negative bacteria and some fungus using standard procedures. Essential oil obtained from M. cajuputi leaves tested in this study showed varying degrees of antimicrobial activity and was found to be active on all the tested microorganisms. Specifically, there was stronger antimicrobial activity against gram-positive bacteria, where inhibition zones were larger, but there was moderate activity against gram-negative and fungal strains. This study showed that the essential oil of M. cajuputi is used for its antimicrobial properties, supporting the findings of previous studies. Essential oils of M. cajuputi in Vietnam is a potential candidate for the development of antimicrobial products made from essential oil and for research on its antimicrobial properties.*

Keywords: Melaleuca cajuputi; essential oil; antimicrobial activity; terpinen-4-ol; Candida albicans; cosmetic application.

1. Introduction

Antimicrobial resistance and the decrease in usefulness of usual antibiotics have resulted in the advancement of alternative antimicrobial products. This has created a global health challenge of increasing magnitude. With the scientific interest of antimicrobial products shifting toward the widely used and biogradable essential oils of plants, the use of traditional phytotherapy is being reconsidered. The compounds of essential oils are complex and volatile, and they impact multiple targets which, in turn, mitigates the formation of resistant strains. Considerable work has been done with essential oils of the Myrtaceae family and the antimicrobial studies of eucalyptus for the primary Myrtaceae members have a documented history and proven ethnomedical use as antiseptic. The Melaleuca genus is of particular interest as a high number of studies have proven the essential oils of the species to inhibit a variety of pathogenic fungi and Gram-positive and Gram-negative bacteria. This elevated antimicrobial activity has been the basis for the use of these species in traditional medicine, as well as topical and cosmetic formulations.

Research about the antibacterial and antifungal properties of the essential oil from Melaleuca alternifolia (Tea tree) has shown effectiveness against Staphylococcus aureus, Escherichia coli, and Candida albicans. As a result of the evidence of the oil's ability to restrict the growth of microbes within a limited concentration over the course of several in vitro studies, it has been deemed a natural antimicrobial substance. As a result of these findings, other Melaleuca species with similar ethnomedicinal properties have reported further studies. *Melaleuca cajuputi* Powell, or Cajuput tree, has primary roots and distribution in Southeast Asia, particularly in Vietnam, Indonesia, and Malaysia. Cajuput oil has been used in Traditional Medicine for the treatment of respiratory infections, skin diseases, and minor wound injuries, in addition to being a topical antiseptic. It has been documented in several regions of the world that the essential oil from *M. cajuputi* has antimicrobial activity against a few selected bacterial and fungal species, but the degree of activity differs from one study to another. This has been reasoned to differences in the geographical origin of the plant, the environmental conditions it was subjected to, and the methods used in plant extraction. Prior research demonstrates that essential oils from *M. cajuputi* have the ability to deter some pathogenic microbes, including certain Gram-positive bacteria and some opportunistic fungi. *M. cajuputi* information, however, is still scant compared to M. Alternifolia. A significant portion of the research that does exist has centred on Indonesian and Malaysian materials. Investigations of *M. cajuputi* essential oil derived from Vietnamese materials are also scant, but it is widely and traditionally used in Vietnam. Essential oils are typically assessed in vitro for their antimicrobial activity/signature using screening methods such as the agar diffusion and broth micro-dilution assay. There is a public demand for antimicrobial essential oils that the screening method could help address. These methods offer a first look at microbial resistance and authenticity of claims in traditional medicine. Several studies have documented the antimicrobial properties of essential oils from various Melaleuca species and demonstrated their ability to inhibit and control microbial growth in a laboratory setting.

Due to the widespread traditional application of *M. cajuputi* in Vietnam and the scarcity of scientific data assessing its antimicrobial properties, additional research is necessary. Consequently, the current study seeks to evaluate the in vitro antimicrobial efficacy of *Melaleuca cajuputi* essential oil derived from Vietnamese plant material against specific bacterial and fungal strains. This study aims to enhance the body of evidence regarding the antimicrobial properties of *M. cajuputi* essential oil by contextualizing the findings within the framework of prior research, thereby establishing a scientific foundation for its further investigation as a natural antimicrobial agent.

2. Materials and methods

2.1. Plant Material

Fresh leaves of *Melaleuca cajuputi* Powell were collected from Thua Thien Hue of Vietnam. From these fractions, initial essential oil has been prepared by simple mixing the fractions of 0% and 98% terpinen-4-ol to receive the mixtures of about 25%, 50%, 75% and 90% terpinen-4-ol.

2.2. Essential Oil Extraction

The essential oil was extracted from *Melaleuca cajuputi* leaves using hydrodistillation with a Clevenger-type apparatus. Approximately 5000 g of fresh leaves were distilled with distilled water for [3–4] h. The obtained essential oil was separated from the aqueous phase, dried over anhydrous sodium sulfate, and stored in airtight amber vials at 4°C until antimicrobial testing. The oil yield was calculated as a percentage (v/w) based on the fresh weight of plant material.

2.3. Microorganisms

The antimicrobial activity of the essential oil was evaluated against selected microbial strains, including Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*), and a fungal strain (*Candida albicans*). Escherichia coli and Staphylococcus aureus microorganisms are supplied by the Department of Biotechnology of Hochiminh City University of Technology. Pseudomonas aeruginosa and Candida albicans are supplied by the Pasteur Institute at Hochiminh City.

2.4. Antimicrobial Activity Assay

Antimicrobial activity was determined using the agar diffusion method, following established procedures with minor modifications. Briefly, microbial suspensions were adjusted to a standardized turbidity equivalent to approximately 10^6 CFU/mL and uniformly spread onto Mueller–Hinton agar plates (for bacteria) or Sabouraud dextrose agar plates (for fungi).

Sterile paper discs (6 mm diameter) were impregnated with defined volumes of *Melaleuca cajuputi* essential oil and placed onto the inoculated agar surfaces. Plates were incubated at 37 °C for 24 h for bacterial strains and at 30 °C for 48 h for fungal strains. Antimicrobial activity was evaluated by measuring the diameter of inhibition zones (mm), including the disc diameter.

3. Results and discussion

3.1. Qualitative evaluation of antibacterial activity using the well diffusion method

The well diffusion method was used to examine the antibacterial activity of tea tree oil fractions against the selected microorganisms. The applied concentration of each fraction was expressed as the volume (µL) added to each well. The inhibition zones observed for *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Pseudomonas aeruginosa* are presented in Figures 1a, 1b, 1c, and 1d, respectively.

To evaluate whether evaporation of the essential oil affected the antibacterial results, additional experiments were carried out in which the agar surface was exposed only to essential oil vapor. The results, summarized in Table 1, show that no clear inhibition was observed under these conditions. This indicates that the antibacterial activity mainly resulted from the diffusion of the essential oil into the agar medium rather than from vapor-phase effects.

At lower concentrations of essential oil, the diameters of the inhibition zones increased rapidly as the applied volume increased. At higher concentrations, the inhibition zones continued to enlarge, but at a slower rate. This behavior can be explained by the non-polar nature of essential oils, which limits their diffusion in the polar agar medium, particularly at higher concentrations.

In general, an increase in terpinen-4-ol content led to larger inhibition zones. However, for *Staphylococcus aureus* and *Pseudomonas aeruginosa*, this trend was not observed at very high terpinen-4-ol levels (98%). This may be related to the reduced presence of other active components in highly purified fractions, as well as differences in the cell structures of the tested microorganisms.

3.2. Quantitative evaluation of antibacterial activity using the microorganism counting method

To obtain a quantitative assessment of antibacterial activity, the microorganism counting method was applied. Three concentrations of tea tree oil (0.5%, 0.25%, and 0.1% v/v) were tested against each microorganism. The corresponding results for *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Pseudomonas aeruginosa* are shown in Tables 2, 3, 4, and 5, respectively.

At concentrations of 0.5% and 0.25% (v/v), all tested oil fractions showed high antibacterial effectiveness, with inhibition levels close to 90% for most microorganisms. This included the fraction containing 98% terpinen-4-ol, although slightly lower effectiveness was observed against *Pseudomonas aeruginosa*. These results suggest that the microorganism counting method provides a more reliable quantitative evaluation than the well diffusion method, particularly for non-polar substances such as essential oils.

When the concentration was reduced to 0.1% (v/v), the antibacterial activity remained high against *Escherichia coli* (above 80%), *Staphylococcus aureus* (above 85%), and *Candida albicans* (above 90%). Notably, fractions with lower terpinen-4-ol content still showed strong antibacterial effects, indicating that components other than terpinen-4-ol also contribute to the antimicrobial activity of tea tree oil.

3.3. Evaluation of tea tree oil application in cosmetic products

Because tea tree oil is not soluble in water, Tween-80 was added as an emulsifying agent at a concentration of 1%. Two

concentrations of tea tree oil (0.25% and 0.1% v/v) were incorporated into body soap and shampoo formulations. The evaluation results are presented in Tables 6, 7, 8, and 9.

The results show that formulations containing 0.1% tea tree oil maintained a pleasant odor and did not exhibit any visible color changes. This indicates that tea tree oil can be incorporated into cosmetic products at low concentrations when an appropriate emulsifier is used, supporting its practical use as an antibacterial ingredient in cosmetic formulations.

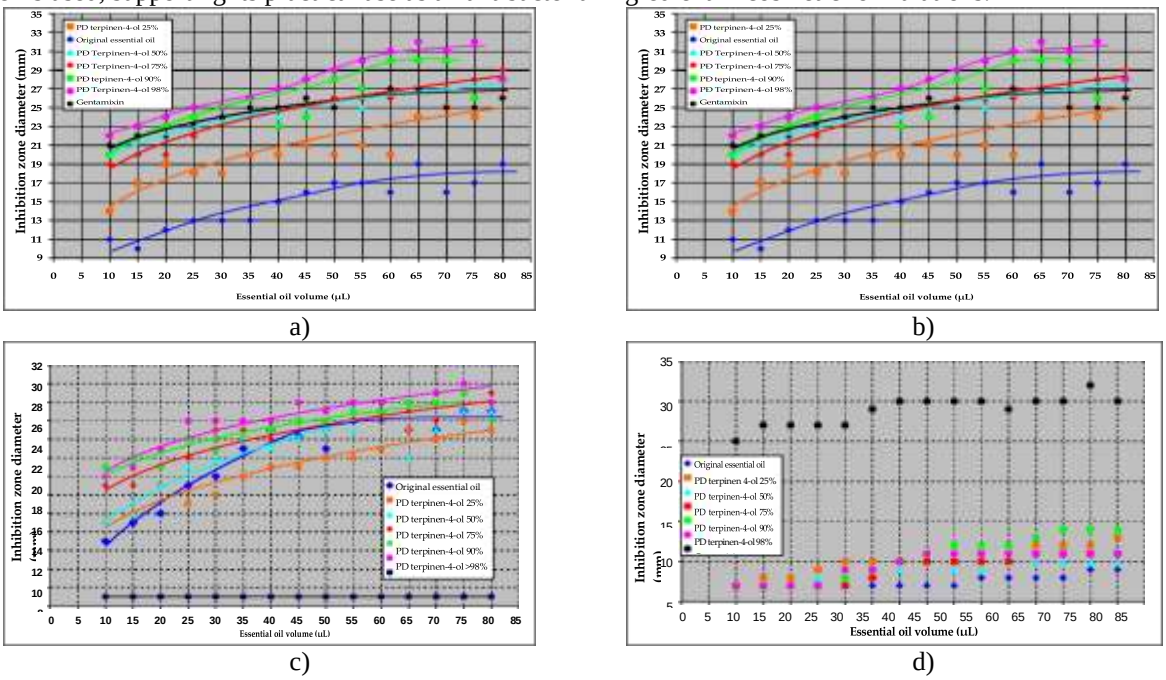

Figure 1. *Dependence of the antibacterial cycle diameter on the used amount of essential oil*
a) Escherichia coli, b) Staphylococcus aureus, c) Candida albicans, d) Pseudomonas aeruginosa

Table 1. *Antibacterial effect of essential oil diffusion and evaporation*

Used amount of essential oil (µl)	Diffusion only	Evaporation only	Both diffusion and evaporation
		Diameter of the antibacterial cycle (mm)	
25	19	7	19
25	19	7	19
25	19	7	19
		Average	
25	19	7	19

Table 2. *Investigation result for antibacterial activity to Escherichia coli*

Sample	Concentration (v/v)	Bacterium before contact (cfu/ml)	Alive bacterium (cfu/ml)	Percentage of killed bacteria (%)
Original essential oil	0.1	5.10^6	882.000	83.3
	0.25	5.10^6	640.000	87.2
	0.5	5.10^6	375.000	92.5
raction of 25% Terpinen-4-ol	0.1	5.10^6	744.000	80.4
	0.25	5.10^6	800.000	78.9
	0.5	5.10^6	546.000	85.6
raction of 50% Terpinen-4-ol	0.1	5.10^6	594.000	84.4
	0.25	5.10^6	480.000	87.4
	0.5	5.10^6	276.000	92.7
raction of 75% Terpinen-4-	0.1	$3.8.10^6$	516.000	86.4

ol	0.25	$3.8.10^6$	468.000	87.7
	0.5	$3.8.10^6$	246.000	93.5
raction of 90% Terpinen-4-	0.1	$3.8.10^6$	450.000	88.2
ol	0.25	$3.8.10^6$	392.000	89.7
	0.5	$5.6.10^6$	351.000	93.7
raction of 98% Terpinen-4-	0.1	$5.6.10^6$	648.000	88.4
ol	0.25	$5.6.10^6$	396.000	92.9
	0.5	$5.6.10^6$	333.000	94.1

Table 3. *Investigation result for antibacterial activity to Staphylococcus aureus*

Sample	Concentration (v/v)	Bacterium before contact (cfu/ml)	Alive bacterium (cfu/ml)	Percentage of killed bacteria (%)
Original essential oil	0.1	$3.02.10^7$	2.160.000	92.8
	0.25	$3.02.10^7$	1.383.000	95.4
	0.5	$3.02.10^7$	826.000	97.3
Fraction of 25% Terpinen-4-ol	0.1	$1.62.10^7$	2.032.000	87.5
	0.25	$1.62.10^7$	549.000	96.6
	0.5	$1.62.10^7$	350.000	97.8
Fraction of 50% Terpinen-4-ol	0.1	$1.62.10^7$	231.600	85.7
	0.25	$1.62.10^7$	504.000	96.9
	0.5	$1.62.10^7$	312.000	98.1
Fraction of 75% Terpinen-4-ol	0.1	$8.8.10^6$	795.000	91.0
	0.25	$8.8.10^6$	729.000	91.7
	0.5	$8.8.10^6$	454.000	94.8
Fraction of 90% Terpinen-4-ol	0.1	$8.8.10^6$	1.450.000	83.5
	0.25	$8.8.10^6$	795.000	91.0
	0.5	$8.8.10^6$	382.000	95.7
Fraction of 98% Terpinen-4-ol	0.1	$3.02.10^7$	2.630.000	91.3
	0.25	$3.02.10^7$	2.240.000	92.6
	0.5	$3.02.10^7$	1.130.000	96.3

Table 4. Investigation result for antibacterial activity to *Pseudomonas aeruginosa*

Sample	Concentration (v/v)	Bacterium before contact (cfu/ml)	Alive bacterium (cfu/ml)	Percentage of killed bacteria (%)
Original essential oil	0.1	$2.32.10^7$	7.080.000	69.5
	0.25	$2.32.10^7$	3.300.000	85.8
	0.5	$2.32.10^7$	3.024.000	87.0
Fraction of 25% Terpinen-4-ol	0.1	$2.32.10^7$	4.920.000	78.8
	0.25	$2.32.10^7$	2.120.000	90.9
	0.5	$2.32.10^7$	1.085.000	95.3
Fraction of 50% Terpinen-4-ol	0.1	$2.32.10^7$	5.784.000	75.1
	0.25	$2.32.10^7$	3.140.000	86.5

	0.5	$2.32.10^7$	1.764.000	92.4
Fraction of 75% Terpinen-4-ol	0.1	$2.32.10^7$	5.760.000	75.7
	0.25	$2.32.10^7$	3.220.000	86.1
	0.5	$2.32.10^7$	1.764.000	92.4
Fraction of 90% Terpinen-4-ol	0.1	$1.12.10^7$	3.696.000	67.0
	0.25	$1.12.10^7$	3.159.000	71.79
	0.5	$1.12.10^7$	1.750.000	84.4
Fraction of 98% Terpinen-4-ol	0.1	$1.12.10^7$	4.074.000	63.6
	0.25	$1.12.10^7$	3.636.000	67.5
	0.5	$1.12.10^7$	2.580.000	77.0

Table 5. *Investigation result for antibacterial activity to Candida albicans*

Sample	Concentration (v/v)	Bacterium before contact (cfu/ml)	Alive bacterium (cfu/ml)	Percentage of killed bacteria (%)
Original essential oil	0.1	$7.2.10^6$	588.000	91.9
	0.25	$7.2.10^6$	500.000	93.1
	0.5	$7.2.10^6$	320.000	95.6
Fraction of 25% Terpinen-4-ol	0.1	$7.2.10^6$	720.000	90.0
	0.25	$7.2.10^6$	580.000	91.9
	0.5	$7.2.10^6$	224.000	96.9
Fraction of 50% Terpinen-4-ol	0.1	$7.2.10^6$	552.000	92.3
	0.25	$7.2.10^6$	280.000	96.2
	0.5	$7.2.10^6$	200.000	97.2
Fraction of 75% Terpinen-4-ol	0.1	$5.8.10^6$	444.000	92.4
	0.25	$5.8.10^6$	330.000	94.3
	0.5	$5.8.10^6$	280.000	95.2
Fraction of 90% Terpinen-4-ol	0.1	$5.8.10^6$	396.000	93.2
	0.25	$5.8.10^6$	300.000	94.9
	0.5	$5.8.10^6$	240.000	95.9
Fraction of 98% Terpinen-4-ol	0.1	$1.4.10^7$	456.000	96.7
	0.25	$1.4.10^7$	450.000	96.8
	0.5	$1.4.10^7$	288.000	98.9

Table 6. *Investigation result for antibacterial activity of the essential oil in cosmetics to E.coli*

Sample	Concentration (v/v)	Bacterium before contact (cfu/ml)	Alive bacterium (cfu/ml)	Percentage of killed bacteria (%)
Body soap	0	$3.12.10^7$	5.000.000	84.0
	0.1	$3.12.10^7$	1.048.000	96.6
	0.25	$3.12.10^7$	4.560.00	98.5
Shampoo	0	$3.12.10^7$	5.920.000	81.0
	0.1	$3.12.10^7$	1.144.000	96.3
	0.25	$3.12.10^7$	520.000	98.3

Table 7. *Investigation result for antibacterial activity of the essential oil in cosmetics to Staphylococcus aureus*

Sample	Concentration (v/v)	Bacterium before contact (cfu/ml)	Alive bacterium (cfu/ml)	Percentage of killed bacteria (%)
Body soap	0	$2.24.10^7$	5.000.000	77.78
	0.1	$2.24.10^7$	93.000	95.8
	0.25	$2.24.10^7$	496.000	97.8
Shampoo	0	$2.24.10^7$	5.920.000	73.6
	0.1	$2.24.10^7$	456.000	98.0
	0.25	$2.24.10^7$	336.000	98.5

Table 8. *Investigation result for antibacterial activity of the essential oil in cosmetics to Candida albicans*

Sample	Concentratio n (v/v)	Bacterium before contact (cfu/ml)	Alive bacterium (cfu/ml)	Percentage of killed bacteria (%)
Body soap	0	$6.6.10^6$	1.480.000	77.6
	0.1	$6.6.10^6$	420.000	93.6
	0.25	$6.6.10^6$	330.000	95.0
Shampoo	0	$6.6.10^6$	1.560.000	76.4
	0.1	$6.6.10^6$	480.000	92.7
	0.25	$6.6.10^6$	344.000	94.8

Table 9. *Investigation result for antibacterial activity of the essential oil in cosmetics to Pseudomonas aeruginosa*

Sample	Concentration (v/v)	Bacterium before contact (cfu/ml)	Alive bacterium (cfu/ml)	Percentage of killed bacteria (%)
Body soap	0	$2.8.10^7$	6.960.000	75.7
	0.1	$2.8.10^7$	2.808.000	90.2
	0.25	$2.8.10^7$	1.152.000	96.0
Shampoo	0	$2.8.10^7$	7.080.000	75.2
	0.1	$2.8.10^7$	2.832.000	90.1
	0.25	$2.8.10^7$	1.248.000	95.6

The antimicrobial properties of tea tree oil noted in this study are consistent with previous research on the antimicrobial effectiveness of essential oils derived from Melaleuca species. The notable inhibitory effects on Escherichia coli and Staphylococcus aureus, in addition to the antifungal activity against Candida albicans, support earlier in vitro studies demonstrating the extensive antimicrobial properties of tea tree oil. The reduced effectiveness against Pseudomonas aeruginosa may be attributed to the microorganism's intrinsic resistance, commonly associated with diminished membrane permeability and efficient efflux mechanisms, as indicated in previous research.

The relationship between terpinen-4-ol concentration and antimicrobial efficacy observed in this study corroborates prior research that recognized terpinen-4-ol as a crucial component in the antibacterial characteristics of tea tree oil. Nonetheless, the present findings further support the notion that the antimicrobial properties of tea tree oil cannot be ascribed to a single component. The overall inhibitory activity is likely the result of the synergistic effects of multiple components. This implies that the constituents of the essential oil may synergistically enhance their efficacy. There has been a lot of talk in the literature on essential oils about this

kind of synergy, which is thought to be an important part of how they work in the body.

It is important to find a safe use concentration of 0.25% for tea tree oil from a practical point of view. Previous research and regulatory frameworks have shown that this concentration is safe and effective for topical and cosmetic use. It strikes a good balance between antimicrobial effectiveness and safety. The successful incorporation of tea tree oil at this concentration, in conjunction with 1% Tween-80, further demonstrates its suitability for cosmetic formulations, where solubility and stability are critical considerations.

These results are good, but there are some problems with this study. We used tests in a lab to see how well the antimicrobial worked. These tests give us some initial proof, but they don't show us how it really works. Quantitative metrics, including minimum inhibitory concentration values and thorough chemical characterization, were also omitted from this study. Future research should focus on comprehensive antimicrobial assessments and formulation studies to elucidate the potential applications of tea tree oil.

The results of this study confirm earlier evidence regarding the antimicrobial properties of tea tree oil and provide additional data supporting its traditional application and practical use in antimicrobial cosmetic formulations.

4. Conclusion

Based on the experimental findings, several key observations can be highlighted.

Essential oil demonstrated significant antibacterial efficacy against Escherichia coli and Staphylococcus aureus, alongside antifungal properties against Candida albicans; however, it exhibited a relatively diminished inhibitory effect against Pseudomonas aeruginosa. The findings also show that the essential oil works better against bacteria when it has more terpinen-4-ol in it. However, terpinen-4-ol is not the only reason why tea tree oil has antimicrobial properties. Other parts of the oil also help it fight bacteria.

For practical purposes, a concentration of 0.25% was found to be safe for all tested fractions of tea tree oil. Also, adding 1% (w/w) of the emulsifying agent Tween-80 made it possible to use 0.25% tea tree oil in cosmetic formulations, which supports its possible use as an antibacterial agent in cosmetics.

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