STUDY ON THE ANTIBACTERIAN CHARACTERISTICS OF TEA TREE OIL AND ITS APPLICATION IN COSMETICS

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ABSTRACT: *The article describes the investigation results on the antibacterial activity of the Australian-originated tea tree (Alternifolia melaleuca) oil and its application in cosmetics production. The research points out that not only terpinen-4-ol but also other components of the oil have rather high antibacterial activity. With a dose of about 0.25%, all fractions of the tea tree oil could destroy 90% the typical bacteria as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albacans. A test of adding 0.25% of the oil into cosmetics shows that the product retains its antibacterial activity and pleasant odor as well. The investigation results contribute to its application prospect in producing detergents, cosmetics and pharmaceuticals of suitable antibacterial activity.*

Keyword: *Tea tree oil; terpinen 4-ol; antibacterial;*

1.INTRODUCTION

Tea trees have been widely planted in the Mekong River Delta area of Vietnam. The investigation has pointed out that its essential oil composition is of about 2% by weight. The essential oil could be easily recovered and purified by steam distillation followed by fractional one [9].

It has been reported that tea tree oil has rather high antibacterial activity [9,12,14,17,18] for its high composition of terpinen-4-ol. Nevertheless, it has been found that not only terpinen-4-ol but also other components have the activity. It is necessary to find out the available minimum dose of the essential oil in practical use that the antibacterial activity could be retained. Besides, it is important to point out the available ability to use different fractions of the essential oil for antibacterial purpose.

2.MATERIALS AND METHODS

2.1.Materials

The original tea tree essential oil is recovered and fractionally distillated from the tea trees planted in the Mekong River Delta area 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.Microorganisms

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.

2.3.Methodology

Antibacterial activity of the essential oil, its distilled fractions or of essential oil based cosmetics are determined by the method of diffusion well (hole boring) and the method of microorganism counting. In the case of cosmetic products, the method of microorganism

counting is applied, provided that the water-undissolved essential oil is emulsified by Tween 80 as the emulsifying reagent.

3.RESULTS AND DISCUSSION

3.1.Qualitative investigation of antibacterial activity by diffusion well method

The hole boring method is applied to all types of chosen microorganisms. The concentrations of the used essential oil fractions are represented by the number of microlitters applied to the hole. The investigated results are shown on Figures 1a, 1b, 1c, 1d for Escherichia coli, Staphylococcus aureus, Candida albacans and Pseudomonas aeruginosa, respectively.

Checking the effect of essential oil evaporation by contacting the agar medium with the essential oil vapor is shown in Table 1. It has pointed out that only the diffusion of the oil determines the antibacterial characteristics.

It is pointed out from the experimental results that at lower concentration of essential oil, diameters of the antibacterial cycles rapidly increase with its used amounts. At higher concentration of essential oil, the diameters increase also but more slowly. It has the reason of non-polar nature of the essential oil, so that its diffusion in polar water at higher concentration is more difficult.

By the way, diameters of the antibacterial cycles increase with the concentration of terpinen-4-ol in the essential oil, with the exception of Staphylococcus aureus and Pseudomonas aeruginosa at very high (98%) concentration of terpinen-4-ol. It may be caused by the less composition of other components in the essential oil and the different cell structures of different types of bacteria.

3.2.Quantitative investigation of antibacterial activity by microorganism counting method

To quantitatively investigate the antibacterial activity of different concentrations of essential oil, microorganism counting method has been applied. Three concentrations (0.5%, 0.25% and 0.1% v/v) have been tested to every chosen type of bacterium. The results are shown in Tables 2,3,4,5 for Escherichia coli, Staphylococcus aureus, Candida albicans and Pseudomonas aeruginosa, respectively.

It is clear from experimental results that with 0.5% and 0.25% v/v, all fractions of the essential oil have the antibacterial efficiency of about 90%, including of the fraction of 98% terpinen-4-ol to all chosen types of bacteria, except the little lower efficiency for the case of Pseudomonas aeruginosa. This means that the method in more quantitative than the hole boring method, where the non-polar essential oil is less diffusive in polar water media.

With the concentration of 0.1% v/v, the antibacterial effect is still rather high to Escherichia coli (more than 80%), Staphylococcus aureus (more than 85%) and Candida albican (more than 90%). The high antibacterial activity of the fractions of lower terpinen-4-ol composition points out that beside terpinen-4-ol, other components in the tea tree oil have also the added effect of antibacterium.

3.3.Investigation of tea tree oil application in cosmetics

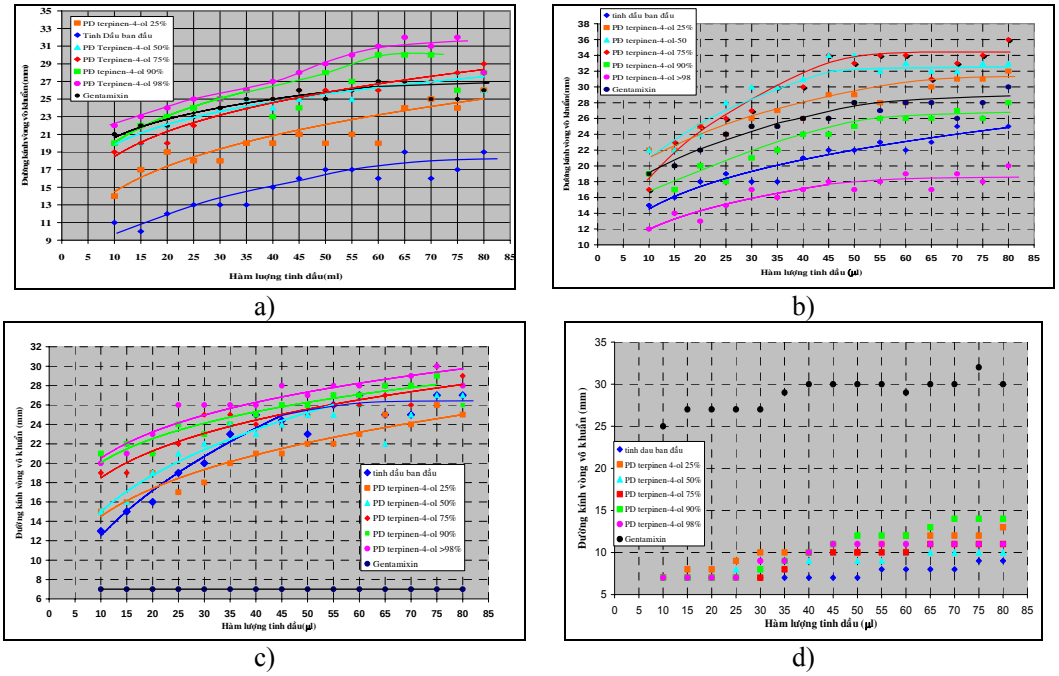
Since tea tree oil is not dissolved in water, the emulsifying reagent Tween-80 is applied with the concentration of 1%. Two concentrations of essential oil have been used: 0.25% and 0.1%. The tests have been carried out to body soap and shampoo. Investigation results are shown in Tables 6,7,8,9.

It has been shown that with only 0.1% of the essential oil, the product is kept with pleasant odor and no colour-change.

4.CONCLUSION

Through the experimental results, it could be pointed out:

- Tea tree oil has strong antibacterial activity to Escherichia coli, Staphylococcus aureus and Candida albacans, and a little weaker to Pseudomonas aeruginosa.
- The more concentration of terpinen-4-ol in the essential oil is, the more effective its antibacterial activity is. Nevertheless, not only terpinen-4-ol but also other components in the tea tree oil have the antibacterial effect.
- The accepted dose of all fractions of the tea tree oil in practical use is 0.25%.
- By adding 1% of the emulsifying reagent Tween-80, 0.25% of the tea tree oil could be applied in the cosmetic production for antibacterial purpose.



a) b) c) d)

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
Fraction 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
Fraction 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
Fraction of 75% Terpinen-4-ol	0.1	$3.8.10^6$	516.000	86.4
	0.25	$3.8.10^6$	468.000	87.7
	0.5	$3.8.10^6$	246.000	93.5
Fraction of 90% Terpinen-4-ol	0.1	$3.8.10^6$	450.000	88.2
	0.25	$3.8.10^6$	392.000	89.7
	0.5	$5.6.10^6$	351.000	93.7
Fraction of 98% Terpinen-4-ol	0.1	$5.6.10^6$	648.000	88.4
	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	0.1	$8.8.10^6$	795.000	91.0

75% Terpinen-4-ol	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%	0.1	$7.2.10^6$	720.000	90.0
	0.25	$7.2.10^6$	580.000	91.9

Terpinen-4-ol	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	Concentration (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

NGHIÊN CỨU ĐẶC TÍNH KHÁNG KHUẨN CỦA TINH DẦU TRÀM TRÀ VÀ KHẢ NĂNG ỨNG DỤNG TRONG MỸ PHẨM

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TÓM TẮT:*Bài báo trình bày các kết quả nghiên cứu về đặc tính kháng khuẩn của tinh dầu tràm trà giống Úc trồng tại Đồng Tháp Mười của Đồng Bằng Sông Cửu Long và khả năng sử dụng nó trong sản xuất các mỹ phẩm. Kết quả nghiên cứu chỉ ra rằng không chỉ có terpinen 4-ol mà các cấu tử khác có mặt trong tinh dầu tràm trà đều có khả năng kháng khuẩn. Với hàm lượng sử dụng 0,25%, các phân đoạn khác nhau của tinh dầu tràm đều có khả năng tiêu diệt các chủng Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa và Candida albacans là các chủng điển hình đến 90%. Việc ứng dụng thử nghiệm các phân*

đoạn khác nhau của tinh dầu tràm trà trong sản xuất mỹ phẩm cho thấy với hàm lượng 0,25%, hoạt tính kháng khuẩn của tinh dầu luôn được đảm bảo mà vẫn có mùi dễ chịu. Kết quả này góp phần củng cố triển vọng ứng dụng của tinh dầu tràm trà trong việc sản xuất các chất tẩy rửa, mỹ phẩm và dược phẩm.

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