Extraction and refining of essential oil from Australian tea tree, Melaleuca alternifolia, and the antimicrobial activity in cosmetic products

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Abstract. Tea tree oil (TTO) comes from the leaves of Melaleuca alternifolia that belongs to the myrtle family (Myrtaceae). It is one of the most powerful immune system stimulants and sorts out most viral, bacterial and fungal infections in a snap, while it is great to heal wounds and acnes. In Vietnam, Melaleuca trees can grow on acid land that stretches in a large portion of lands in the Mekong Delta region. So, there are some Melaleuca plantations developed under the Vietnamese government plans of increasing plantation forests now. However, TTO contains various amounts of 1,8-cineole that causes skin irritant. So TTO purification is very necessary. In this study, the purification of TTO that meet International Standard ISO 4730 was carried out via two steps. The first step is steam distillation to obtain crude TTO (terpinen-4-ol 35% v/v) and the average productivity is among 2.37% (v/wet-wt) or 1.23% (v/dry-wt). In the second step, the cleaned TTO is collected by vacuum distillation column and extraction yield of the whole process is about 0.3% (w/w). Besides, high concentration essential oil was applied in the cosmetic products to increase its commercial value.

1. Introduction

Melaleuca alternifolia, commonly known as Australian tea tree and Melaleuca tree, grow fast and can be harvested as early as six months. They have been used medicinally for centuries by Australian aboriginal people. Tea tree oil (TTO) that comes from their leaves, contains over 100 components (mostly monoterpenes, sesquiterpenes and terpene alcohols). [4], [5] The studies demonstrated that terpinen-4-ol, a monoterpene is the most abundant (minimum 30%) and responsible for most of the antimicrobial activity, besides TTO also contains various amounts of 1,8-cineole that causes skin irritant. In an agriculture-based country like Vietnam, the acid land is actually not used for cultivation except for Melaleuca trees. So develop techniques to extract and refine essential oil from Melaleuca alternifolia leaves and research its cosmetic applications will improve the living standard of families in the area, especially for the farmers living on acid land.

Most essential oils available today are extracted by steam distillation among, although there are various extraction methods such as distillation, CO₂ supercritical extraction, and solvent extraction. It's
the oldest form of essential oil extraction, quite simple and the best method for distilling leafy materials. Moreover, this process not only causes minimum changes to the essential oil composition during extraction, but also the steam is readily available, cheap, not hazardous and can be recycled. So steam distillation is used to collect crude TTO (35 – 45% of volume of Terpinen-4-ol) [1].

After steam distillation, the commercial value of TTO is not high, so it should be refined to increase its commercial value and fit with tea tree oil standard. Some refinement methods are vacuum distillation, crystallization, chromatography column... In this experiment, we focus on vacuum distillation to decrease TTO boiling point, thus limiting degradation of heat-sensitive volatiles.

Tea Tree Oil has always been appreciated for its biological activity. So TTO is used as antiseptic, antifungal to treat cuts, burns, abrasions and acne in a range of cosmetic products such as antiseptics, deodorants, shampoo and soaps. In this study, we used TTO as preservatives, germicidal ingredient in two cosmetic products: hand cleaner and body lotion. [3]

2. Materials and methods
The technical process for extracting and refining TTO was illustrated in Figure 1

![Figure 1: Technical process for TTO extraction and refinement](image)

2.1. Essential oil extraction – steam distillation process [2]
Materials in this experiment is Australian melaleuca trees that were planted in Tan Phuoc District - Tien Giang Province - Vietnam.

TTO were produced by steam distillation from the leaves and terminal branches of Melaleuca tree. Dry materials were obtained after 24 hours of drying, then determined the moisture of these materials (representative sampling). We distilled both dry and wet materials to exam impact of moisture in raw materials to essential oil amount extracted. Steam for distillation is supplied from outside boiler of which capacity is 350 kg water per hour. The main device for this process is distillation vessel of which dimensions are 1110 mm diameter, 1689 mm height. The steam flowrate into this one is adjusted automatically by controller connected to the temperature sensor and the pressure sensor. The operation parameters of distillation vessel are 120°C and 2 bar in 3 hours.

![Figure 2: (A) Melaleuca Tree in Vietnam, (B) two isomers (S) and (R) of terpinen-4-ol](image)
Mixture from condenser includes water and essential oil. Because of the difference of their specific gravity, they will become two liquid phases in the separator. The amount of essential oil that dissolved in the distillate water is small (negligible) and therefore recovery of essential oil dissolved in the water is unnecessary.

2.2. Essential oil refinement – vacuum distillation process [2]

Materials for vacuum distillation process are crude TTO (35 – 45% of volume of Terpinen-4-ol) from steam distillation.

The purpose of TTO purification is to create product that has low concentration of 1,8-cineole and high concentration of terpinen-4-ol. In this experiment, we carried out in conditions: 6000 ml of feed volume, 5 mmHg and a reflux ratio of around 1:1 (1 part returned condensate to 1 part condensate take off).

First, feed the crude essential oils into the reboiler of the vacuum distillation column. Then, the feeds are boiled by indirect heating resistor via thermal oil heater. A vacuum generator is connected to the top of distillation tower to create low-pressure in system. In front of the vacuum generator, we put the liquid separator, which is cooled by liquid nitrogen, in order to condense completely essential oil entrained by the vacuum line and to protect this engine.

The reflux ratio was set by adjusting On/Off time of solenoid valve to take TTO out. When the valve closes (timer Off), TTO vapour was condensed at the top of the tower and made a reflux stream back to the tower. Conversely, when the valve opens (timer On), TTO vapour was brought to condenser. Because On/Off cycle is very short (in a few seconds), it can be considered as continuous circulation process. Two thermometers are put on the bottom and top of distillation column to determine each interval exactly.

2.3. Cosmetic applications of TTO

Materials are some cosmetic ingredients from Mai Phuong Cosmetic Company – Vietnam, TTO with high concentration of terpinen-4-ol that is produced at RPTC.

First, we created the formulations for two cosmetic products: body lotion and hand cleaner. [6]

**Table 1: Formula of cream base**

| Materials                  | Concentration % w/w |
|----------------------------|---------------------|
| Emulpharma 165            | 1                   |
| Beeswax                   | 4                   |
| MC30 (Hydrogenated polyisobutene) | 2             |
| 1,3 Butylene Glycol       | 4                   |
| Floraeste K-20W           | 0.5                 |
| Aquagel 45                | 3.5                 |
| Salacos 99                | 2                   |
| TC-Carbomer 380           | 0.4                 |
| Water                     | 82.6                |

**Figure 3:** Procedure for body lotion
Table 2: Formula of hand cleaner

| Materials                             | Concentration % w/w |
|---------------------------------------|---------------------|
| Sodium lauryl sulfate (SLS)           | 20                  |
| Cocamido propyl betain (CAPB) 30%    | 1                   |
| Coconut Diethanolamine (CDE)         | 0.06                |
| Beeswax                              | 1                   |
| Ethanol                              | 0.5                 |
| Glycerin                             | 3                   |
| TC-Carbomer 380                      | 1                   |
| Water                                | 73.24               |

Next, these products were examined anti-microbial (for E. coli, Staphylococcus aureus) at the different ratios of the formulation and cleaned TTO. General principle was counting quantity of bacteria before and after using cosmetic products. From there, the suitable content was chosen. This survey was carried out at Bio-Technology Laboratory – Ho Chi Minh City University of Technology.

Besides, customer’s evaluations were also collected (based on the satisfaction of 28 female customers and the product characteristics). Finally, in order to ensure product quality and safety for customer, these cosmetic products were inspected by 3113/1999/QĐ-BYT method (according to Vietnamese Standard) at Ho Chi Minh City Pasteur Institute – Vietnam.

3. Results and Discussions:
3.1. TTO extraction and refinement

For essential oil extraction, we carried out 19 batches to determine average productivity of TTO. The results were shown in Figure 5.

Table 3: Component of steam-extracted TTO before purification

| Component   | % Concentration (v/v) |
|-------------|-----------------------|
| α-pinen     | 1.96 - 2.72           |
| Terpinen-4-ol| 31.8 - 47.02          |
| α-terpinen  | 8.65 - 9.04           |
| Limonen     | 3.49 - 4.68           |
| p-cymen     | 1.56 - 7.215          |
| 1,8-cineol  | 4.44 - 7.141          |
| γ-terpinen  | 16.747 - 19.20        |
| Terpinolen  | 5.58 - 13.71          |
| α-terpineol | 2.79 - 3.32           |
The yield of essential oil is typically 1 to 2% of wet material weight [1]. In this study, the average productivity is among 2.37% (v/wet-wt) or 1.23% (v/dry-wt), when Melaleuca trees were planted in Tien Giang province – Vietnam. The results are also consistent with some studies in other provinces of Vietnam (Long An, Can Tho, An Giang…). The essential oil percentage decreased significantly after several days, so either the wet material or the dry one can be used for essential oil extraction. The GC used in this analysis was an Agilent 6890N with a flame ionization detector. Separation took place in DB-5 column (0.32mm × 30m × 0.25μm), using nitrogen as carrier gas. The GC oven temperature was initially controlled at 50°C, then it was increased at a rate of 5°C per min to 80°C which was held for 1 min, finally it was increased at a rate of 20°C per min to a final temperature 220°C which was held for 10 min. According to Table 3, two significant components of TTO after steam distillation meet International Standard ISO 4730 (terpinen-4-ol >30% and 1,8-cineol <15). However, we still continued refining crude TTO and compared the benefits between crude TTO production and cleaned TTO production in order to determine the TTO type that we will apply in industrial scale.

For essential oil refinement, the results were described in Figure 6. We can divide this process into 5 intervals. The main component of Interval 1 which was from the beginning of distillation to 30 min and 0-15% oil volume, was α-terpinen (8-12% v/v). The main components of Interval 2 which was from end of interval 1 to 210 min and 15-30-35% oil volume, were α-terpinen (20-30% v/v) and γ-terpinen (25-30% v/v). The main component of Interval 3 which was from 210-330 min and about 30-60-65% oil volume, was γ-terpinen (46-52% v/v). The main component of Interval 4 which was 330-480 min and 60-65%-75-80% oil volume, was terpinen-4-ol (78-95% v/v). Finally, Interval 5 contains amount of TTO that was still in the reboiler of the vacuum distillation column, has 75-80%-95-100% oil volume. This interval has about 50-70% (v/v) of terpinen-4-ol and 10-15% (v/v) α-terpineol.

![Figure 6](image1)

**Figure 6:** The essential oil components during refinement

![Figure 7](image2)

**Figure 7:** The essential oil components during refinement when mixing interval 5 of many batches

The percentage of terpinen-4-ol in different intervals from refinement process are different. The highest concentration of terpinen-4-ol is in interval 4 and lower in previous ones. The concentration of terpinen-4-ol decreases after interval 4, so we shutdown the operation here. Interval 5 that has significant amounts of terpinen-4-ol, will be mixed with interval 5 in many batches and purified again. According to Figure 7, we divided this process into 2 intervals. The main component of first interval that was from the beginning of distillation to 210 min, 0-45% oil volume, was 90-95% (w/w) of terpinen-4-ol. The main component of final interval was α-terpineol that has 35-40% (w/w). Extraction yield of whole process is about 0.3% (w/w) means we can obtain about 3kg of cleaned TTO (minimum 95% v/v) from 1 ton of Melaleuca leaves.
Table 4 showed that producing TTO can bring high economic efficiency. Besides, ROR of cleaned TTO product is about 1.5 times higher than ROR of crude TTO product, but cleaned TTO purification need more complex technology and more money than crude TTO. Depend on economic abilities and technical conditions, we will choose kind of TTO product.

Table 4: Benefits from TTO

| (1) Products | (2) Price (VND/liter) | (3) Yield (liter/day) | (4) Cost /liter | (5) Total costs (VND/day) | (6) Revenues (VND/day)b | (7) Income (VND/day)c | (8) Return on revenue (ROR) (%)d |
|-------------|----------------------|----------------------|----------------|--------------------------|-------------------------|----------------------|--------------------------|
| Crude TTO   | 1.000                | 4.47                 | 620            | 2.770                    | 4.470                   | 1.700                | 38                       |
| Cleaned TTO | 8.000                | 1.34                 | 3.276          | 4.390                    | 10.720                  | 6.330                | 59                       |

*a* The lowest price of TTO in Vietnamese market (2010)

*b* Revenues (VND/day) = (2) * (3) means amount of money received by selling TTO during a certain time period (a day)

*c* Income (VND/day) = (6) - (5) means revenues minus total cost, over a given period of time (a day)

*d* Return on revenues (ROR) % = (7)/(6)*100 . This is a means of measuring the profitability of a business by dividing the income by the amount of revenue

1 USD = 21014VND

3.2. Two cosmetic products

For body lotion, it has quite high anti-microbial ability even in small concentrations, Figure 8. Based on economic and anti-microbial ability, we choose it containing 0.5% (w/w) of cleaned TTO. However, anti-microbial requirements in body lotion product are lower than the cleaning products, especially the smell product is very important. TTO has bit unpleasant odor, so the TTO percentage in body lotion should be only about 0.3% (w/w).

![Figure 8: Anti-microbial ability of TTO in Body Lotion](image)

Figure 8: Anti-microbial ability of TTO in Body Lotion

Anti-microbial ability of hand cleaner product is as good as body lotion product, Figure 9. The ability to kill *Staphylococcus aureus* is lower than *E. coli*. But, it is quite high generally. Therefore, we chose 0.5% (w/w) of cleaned TTO in hand cleaner.

Besides, these cosmetic products are appreciated by 28 female customers. According to microbial test result of Ho Chi Minh City Pasteur Institute, they were not infected bacteria and satisfied microbial standards for cosmetic field.

![Figure 9: Anti-microbial ability of TTO in Hand Cleaner](image)

Figure 9: Anti-microbial ability of TTO in Hand Cleaner

![Figure 10: Some cosmetic products from TTO produced in RPTC](image)

Figure 10: Some cosmetic products from TTO produced in RPTC
Four cosmetic products from TTO: crude TTO, cleaned TTO, body lotion and hand cleaner were studied in RPTC, Figure 10. All of them were made to improve commercial value of Melaleuca trees, so living quality of farmers in Mekong Delta region would increase.

4. Conclusion:
Both TTO types satisfy the requirements given in International Standard ISO 4730. The concentrations of crude and cleaned TTO are about 35-48%, 95-100% of volume of terpinen-4-ol, respectively. Among them, cleaned TTO has higher commercial value and its ROR is about 1.5 times higher than ROR of crude TTO. The technologies for these processes are simple with cheap materials and quality of TTO is high. Besides, the antimicrobial activity of TTO in cosmetic products was still kept so the economic value of TTO increased. Therefore, TTO and its cosmetic products actually bring benefits for Vietnamese farmers who live on agriculture in acid land area.

*Acknowledgement:* We would like to thank Dr. Seiji Iwasa for not only giving us the opportunity to work in his laboratories but also being a great mentor and acknowledge Jica’s Office, Tien Giang Department of Science Technology for their financial supports.

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