Enriching terpinen-4-ol from tea tree (Melaleuca alternifolia) oil using vacuum fractional distillation: Effect of column and packings on the separation

M T Le¹,², N M Nguyen¹,², X T Le¹,²*

¹Department of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), Ho Chi Minh City, Vietnam
²Vietnam National University Ho Chi Minh City, Ho Chi Minh City, Vietnam
*Corresponding Author, tien.le@hcmut.edu.vn

Abstract. Two types of columns, three types of packings, and four levels of column height were considered to investigate how column and packings affected the separation in the vacuum fractional distillation process of raw tea tree (Melaleuca alternifolia) oil (TTO). This study discussed those effects on purity, yield, and overall discovery to select the most excellent operating conditions for enriching terpinen-4-ol. After the experiments, the essential oil was successfully separated into two fractions, in which the second one composed mostly the main TTO constituent, terpinen-4-ol. The best result was achieved by conducting the distillation on a 300-mm Hempel column filled with small Fenske helices (10 mm × 2 mm i.d.) at the system pressure of 60 mmHg. GC/MS analysis showed an almost 2.5-fold increase in the content of terpinen-4-ol, from 39.23% to 95.77% after fractionation. Meanwhile, there was 75% of terpinen-4-ol successfully recovered from its parental oil. Hence, the vacuum fraction distillation could be an effective method to enrich the terpinen-4-ol content in TTO.

1. Introduction
Melaleuca alternifolia, or tea tree, is an Australian endemic species belonging to the Myrtaceae family [1]. The extraction and purification of its essential oil attracted a plethora of publications owing to a significant number of various valuable commercial applications and bioactivities [2]. Traditionally, tea tree oil (TTO) was observed as a remedy in Australia, The United States, and several countries in Asia folk medicine records for treating bruises, insect bites, skin infections, common colds, and flu [3]. TTO could also be used to treat dermatological disorders, resulting from its antimicrobial activities against various micro-organisms with little development in drug resistance [1]. Recently, many reports provided reliable evidence for TTO antifungal [4-6], anti-inflammatory [7, 8], and antiviral [6, 9, 10] properties. This oil even inhibited the growth of drug-resistant cancer cells and prevented them from spreading out in the human body [11]. TTO could be exploited in the agriculture, food, cosmetics, and perfumery industry in the commercial field due to its pleasant aromatic properties [12].
Those TTO bioactivities originated from its main constituent, terpinen-4-ol. The TTO composition analysis found that terpinen-4-ol presented with the content ranged between 30% to 45%, together with a considerable amount of p-cymene (3–4%), 1,8-cineole (3–6%), α-pinene (7–10%), and γ-terpinene (17–25%) [13-15]. However, such coexisted compounds raised the safety concern of tea tree oil on the human body. Several of them were potent dermatitis allergic substances such as α-pinene, α-terpinene and 1,8-cineole [12]. Those presences were also found to decrease the activities of terpinen-4-ol. Loughlin et al. (2007) discovered that terpinen-4-ol prevented the growth and eliminated bacterial skin better than its parental oil. Therefore, terpinen-4-ol should be applied as a single agent in topical treatment products [16]. Furthermore, the content of terpinen-4-ol and the others vary in the genetic, habitation, weather, and soil condition. Thus the oil quality and properties could not be easily maintained and controlled, limiting its usage in the fine chemical industry as the pharmaceutical producing process despite its significant bioactivities [17]. To solve this problem, a purification (or refining) of TTO is necessary to ensure terpinen-4-ol purity and quality.

Freezing crystallization [18-20], fractional distillation [21, 22], and solvent extraction [23] techniques were commonly considered to separate a target component from the essential oil. Freezing crystallization was typically observed as the primary essential oils refining technique on the industrial scale due to its plain, low-waste, and non-corrosive operation [18]. Though there is a lack of studies on TTO, the experiments based on other essential oils revealed optimistic results. In recent research, refining eucalyptus oil’s final products could reach up to 99% content of 1,8-cineole from nearly 80% [18-20]. Nevertheless, the purity of the feed oils for crystallization is also a challenge of using this technique. Generally, it is required to be larger than 80% of the main constituents before feeding to the system [18-20]. As the raw oils may contain a considerable amount of low melting point impurities, the freezing temperature would decrease steeply [24], leading to significant energy consumption and complicated equipment.

For the solvent extraction methods, solvent extraction is the most crucial technique in classic organic chemistry to isolate a specific group or individual [25]. However, the large-scale extraction process consumes a massive volume of organic solvents and raises the cost and pollution concern [26]. Among those, fractional distillation becomes a cost-effective, simple technique. With a considerable difference in the volatility between terpinen-4-ol and other constituents (Table 1), this method could permit an almost complete separation of the main components if the distillation is performed on a single column on batch mode [27]. The fractional distillation efficiency on TTO had also been discussed in a study with a promising result. Huynh et al. (2012) fractionated [21, 22] raw TTO on a batch of 6000 mL under the system pressure of 5 mmHg and the reflux ratio of nearly 1:1. The fractionation column was 1.5-m in height and fully packed with wire mesh structure packings. Through a series of replicate experiments, the main fraction was identified by collecting the distillate after 330 minutes of distillation. The final products contained nearly 78 to 95% of terpinen-4-ol. On other types of essential oils, the distillation technique also provided an attractive result. For instance, Warsito et al. witnessed an improvement in citronellal purity, from 31.63% in the feed to 88.43% in the main fraction. The experiments were carried on a 2-m plain column filled with Raschig rings at 10 mmHg (approximately 1.33 kPa) [28]. The fractionation procedure is often operated under deep diminished pressure to prevent the decomposition of constituents from the thermal degradable and considerably shorten the distillation time [29].

Though being a potential solution for refining TTO, regardless of a growing amount of fractional distillation studies, there is a lack of investigations on the effect of variables on this process and the distillation optimization on TTO. Hence, in this study, the fractional distillation of TTO at different column heights, packings, and system pressure would be carried to understand their effect on the process and determine the distillation conditions achieving the highest terpinen-4-ol purity and recovery.
2. Materials and methods

2.1. Source of Essential Oil
The leaves and branches of *Melaleuca alternifolia* plants were collected from Ninh Binh province, Vietnam, and extracted by steam distillation method. The TTO was then stoked at ambient temperature in sealed containers that avoided direct sunlight.

2.2. Fractional Distillation Apparatus
The distillation system consists of two central units: fractionation and utility. In the fractionation unit, TTO would be fractionated on a packed column (4), which is joined with a 250-mL flask (2) at the bottom containing the feed. At the top of the column, a Liebig condenser (7) is coupled to the system via a three-way adapter. Eventually, a multi-limb receiver (8) is connected to the condenser outlet. This receiver could be rotated manually to divert the products into different receiving flasks.

![Figure 1. Schematic diagram of the laboratory-scale fractional distillation system.](image)

1 Electric mantle and oil bath, 2 Feed flask, 3 Bottom thermometer, 4 Distillation column, 5 Three-way adapter, 6 Top thermometer, 7 Liebig condenser, 8 Multi-limb product receiver and receiving flasks, 9 Cold trap, 10 Vacuum pump, 11 Pressure controller, 12 Chiller

To supply heat, vacuum, and cooling water during the process, the utility unit consists of three device sets: a heating mantle (1), a vacuum system (9-11), and a chiller (12). The mantle (1) provided them to the feed flask through an oil bath. Meanwhile, a chiller (12) is used to constantly maintain the low temperature of the cooling water circulating the Liebig condenser. Finally, a vacuum pump (9) is installed between a cold trap (10) and a controller (11) to produce and maintain the interval vacuum. The process variables such as temperatures were signalized by two thermometers at the column's top (3) and bottom (6).

2.3. Fractional Distillation Operating Procedure
To initiate the experiment, 100.0 grams of TTO decanted by sodium sulfate (1.0 grams) was charged to the feed flask (2). The compounds removed from the column would be traced by the temperature at the three-way adapter (5). This thermometer would be recorded on a 60-second interval after the first distillate droplets appeared. The fractions were then split into different receiving flasks based on the step-change in the temperature profile at the top of the column. The fractionation would last until the temperature at the distilling head started to drop more than 20 °C. Following then, the products would then be weighed and sent for GC/MS analysis.
2.4. Study of the TTO Fractionation Pattern
Before investigating the effect of the variables, a set of preliminary experiments were performed to identify the number of fractions, their composition profile, and temperature range for the later operation. Those experiments would be performed on a 300-mm Hempel column using small helices as packing beds (Figure 2) at the system pressure of 60 mmHg. The temperature profile at the top of the column is also recorded during the procedure. After the refining process, all fractions (including the bottom one) were analyzed quantitatively and qualitatively via the GC/MS system.

![Image of fractionation columns and packings]

**Figure 2.** Structure and specifications of fractionating columns and packings in the study. [30, 31]

2.5. Investigation of the effect of variables on distillation characteristics
The effect of different columns, packing types, and column height would be considered, respectively. When a change in any one factor was compared, all other factors remained constant. The 300-mm Hempel column filled with three different packings (large helices, small helices, and mesh rings as illustrated in Figure 2) together with the Vigreux column would be used in the first set of experiments. The distillations were then performed on the column with different heights of 100, 200, and 300 mm to study their effect. All experiments were performed on triplicate.

All fractions mass and composition were analyzed to calculate the yield of each fraction and terpinen-4-ol recovery. Terpinen-4-ol purity and recovery were then calculated as the below formulas to select the operating conditions allowing the greatest performance.

The yield of a fraction is the percentage of the materials transferred to that fraction from the initial feed. As illustrated in Equation 1, it is determined by dividing the overall weight of the favored fraction (Fraction Weight) by that of the raw essential oils (Feed Weight).

Meanwhile, the recovery of a constituent in a fraction is the percentage of that constituents distilled into that fraction from the feed. Equation 2 explains how the terpinen-4-ol recovery is determined, in which Fraction Terpinen-4-ol Content and Feed Terpinen-4-ol Content are the content of terpinen-4-ol in the selected fraction and in the feed, respectively.

\[
\text{Yield} = \frac{\text{Fraction Weight (g)}}{\text{Feed Weight (g)}} \times 100\%
\]

**Equation 1**

\[
\text{Recovery} = \frac{\text{Fraction Terpinen-4-ol Content}}{\text{Feed Terpinen-4-ol Content}} \times 100\%
\]

**Equation 2**
2.6. GC/MS Chromatographic Analysis

The samples were analyzed on a Gas Chromatography system (Agilent G1530A, Agilent Technologies, United States), coupled with an HP-5MS column (30m × 0.25 mm i. d. ×0.25 μm film thickness). The Mass Spectrum (Agilent 5973N, Agilent Technologies, United States) were used as a detector. The column used helium as the carrier gas with a flow rate of 1 mL/min. The scanning, which rate was 1 scan/s, was performed on the mass range between 40 and 400 amu, the ionization energy of 70 eV, and the ionization source temperature of 220 °C. The temperature program was set as follows: maintaining at 60 °C for the first 3 minutes, increasing at 10 °C/min to 280 °C, and kept constant at this temperature for 10 minutes. The sample was prepared in acetone with a concentration of 1000 ppm. The injection volume was 1.0 μL with a split ratio of 1:50. The peaks in the chromatogram were compared with the data from the NIST2.3 library to identify the constituent structure.

3. Results and discussion

3.1. Composition

The raw TTO was extracted from *Melaleuca alternifolia* leaves and branches by steam distillation and sent to GC-MS analysis to clarify its chemical composition (Table 1). The result reported the presence of eight major constituents accounted for 99.15% of the total content. The main active component of TTO, terpinen-4-ol, favored for the most significant content (39.23%), followed by three major impurities: γ-terpinene (28.69%), α-terpinene (14.64%) and β-cymene (6.04%). Terpinolene (4.36%), α-pinene (2.71%), α-terpineol (2.00%), and 1,8-cineole (1.48%) were also reported. Compared to ISO standard (Table 1) [32], the composition almost concurred with the provided range, especially with the content of terpinen-4-ol, the key constituent. However, the amount of α-terpinein and γ-terpinene exceeded the maximum allowed value.

| RT (min) | Constituent         | %   | ISO °C | BP °C |
|----------|---------------------|-----|--------|-------|
| 3.75     | α-pinene            | 2.71| 1.0–6.0| 155   |
| 4.81     | α-terpinene         | 14.64| 5.0–13.0| 174   |
| 4.91     | β-cymene            | 6.04| 0.5–8.0| 177   |
| 5.03     | 1,8-cineole         | 1.48| tr–15.0| 176   |
| 5.39     | γ-terpinene         | 28.69| 10.0–28.0| 183   |
| 5.82     | terpinolene         | 4.36| 1.5–5.0| 186   |
| 7.11     | terpinen-4-ol       | 39.23| 30.0–48.0| 209   |
| 7.29     | α-terpineol         | 2.00| 1.5–8.0| 219   |

° Retention time (min)

b GC/MS area percentage

ISO Standard – Essential oil of Melaleuca, terpinen-4-ol type (Tea Tree oil) (ISO 4730:2017) [32]

Boiling point (°C) at ambient pressure (760 mmHg) [33]

Since most undesirable constituents were more volatile than terpinen-4-ol, the fractionation may need to split the distillate into two fractions. The first fraction would compose of all compounds with a boiling point lower than that of terpinen-4-ol. Terpinen-4-ol was then directed to the second fraction until the end of the process. Besides, the boiling of terpinolene is 23 °C different from terpinen-4-ol and that of α-terpineol is 10 °C. Thenceforth, the significant deviation between the boiling point of
terpinen-4-ol and two adjacent components proposed considerable relative volatility [24] and promising separation efficiency.

3.2. TTO Rectification Pattern
Figure 3 illustrated the temperature profile at the top and bottom of the column against time after the first distillate drop was formed. At the early stage, the temperature at the distilling head faced a significant increase from 44 °C to around 88 – 90 °C (interval A) before reaching the plateau (interval B). Following this, the top temperature rose sharply to 115 °C (interval C) and slightly fluctuated afterward around 115 – 117 °C (interval D). Thenceforth, the products would be split into four fractions based on the temperature trend: during the transition stages (interval A and C) and stability (interval B and D). It is noteworthy to notice that the distillate collected in intervals B and C would have the exceptional purity of either a single or a group of constituents. The vapor temperature from the boiling phase keeps constant only if its composition is maintained [24]. Thenceforth, the fractions collected during the stable stage of temperature profile would have the slightest change in the component during the distillation and consist of mainly several specific compounds with high purity.

![Figure 3. The temperature profile of the TTO fractional distillation at 60 mmHg](image)

The GC/MS analytical result validated that projection. During interval D (F2 fraction), the fraction composed a great percentage of terpinen-4-ol (95.77%). Consequently, 115 – 117 °C was the optimal temperature range at the distilling head to isolate terpinen-4-ol. Since terpinen-4-ol is the only favored constituent for the other intervals, splitting fractions between intervals A, B, and C is unnecessary. As a result, the products during the first three intervals would be gathered as the F1 fraction.

Table 2. Characteristics of packing types in this study

| Description     | Specifications | Packing density (unit/m³) | Specific surface area (m²/m³) |
|-----------------|---------------|--------------------------|-------------------------------|
| Mesh rings      | 10 mm × 2 mm i. d. | 2,252,000                | NA                            |
| Large helices   | 10 mm × 4 mm i. d. | 1,121,000                | 1250                          |
| Small helices   | 10 mm × 2 mm i. d. | 2,252,000                | 1250                          |

3.3. Influence of column and packing types on the fractionation
In this section, the effect of different fractionating columns and packings was considered on the TTO fractionation. The experiments were performed on the Hempel column filled with three types of packing and the Vigreux column at the absolute pressure of 60 mmHg. Mesh rings, large helices, and small helices (Figure 2) were applied to the packed bed. The fraction composition, yield, and terpine-
4-ol recovery were monitored and illustrated in Figure 4, Figure 5, and Figure 6, respectively, for the evaluation.

In Figure 4, the separation performed on the Vigreux column was the worst, leading to the lowest terpinen-4-ol content in the F2 fraction (86%). Meanwhile, this value of the Hempel column filled with large Fenske helices, small Fenske helices, and wire mesh was 92%, 95%, and 94%, respectively. The low efficiency of the Vigreux column could be explained due to the low contact area between the two phases. The Vigreux column surface area originated only from the spurs on the sidewalls, leaving a massive void at the central column. On the other hand, the packed columns provided the additional surface area of packings in this void [25]; therefore, providing more excellent separation [34].

Regarding the different packing types, the small helices showed the most amazing terpinen-4-ol content (95%), almost the same as mesh rings (94%). It is noteworthy that those packings had the same packing density, i.e., the same packing units per column volume, due to their exact sizing (Table 2). On the contrary, the terpinen-4-ol content in the main segment was the lowest for the large helices. It may be attributed to its low packing units per volume (Table 2), declining contact area, and separation efficiency [34].

The fraction yield in Figure 5 indicated that the F2 fraction collected from the experiment with the Vigreux column was also the lowest among the experiments, achieving only 28%. The result also revealed that the least amount of terpinen-4-ol-rich fraction was obtained when using mesh rings. This resulted from the structure of this packing. The grid structure on the surface of packing holds a sufficient volume of liquid to those pores, increasing the liquid hold-up and therefore decreased the fraction yield. For the helices packing, the lower packing density of the large ones lessens the number of pores on the packing surface and the liquid hold-up. Thenceforth, there was a slight improvement in the main fraction yield of the large helices compared to the small one.

In conclusion, Figure 6 indicated that the Vigreux column performance in the fractionation was worse than the Hempel one, both in terpinen-4-ol fraction purity and yield; therefore, it gave the lowest terpinen-4-ol recovery (54%). For the effect of the packings, despite the considerable separation efficiency, mesh rings still expressed a low terpinen-4-ol recovery (almost equivalent to Vigreux column) since the grid structure on the surface triggered a large liquid hold-up and decreased the yield steeply. For the helices packings, there was a reverse trend in the performance of large and small Fenske helices. The small one presented a little higher efficiency while a slight decrease in fraction yield and vice versa. Hence, their recovery was similar and most significant in this set of experiments (75%). As a result, they are both appropriate to be used in the fractionating of TTO.
3.4. Influence of the column height on the fractionation

The change in the terpinen-4-ol content and the yield of the main fraction against the different heights of the Hempel column, namely 100 mm, 200 mm, and 300 mm, were depicted in Figure 7, Figure 8, and Figure 9 respectively.

![Figure 6](image6.png)  
**Figure 6.** Effect of different column and packing types on the terpinen-4-ol purity and recovery in F2 fraction

![Figure 7](image7.png)  
**Figure 7.** Effect of different column height on the terpinen-4-ol purity and recovery in F2 fraction

![Figure 8](image8.png)  
**Figure 8.** Effect of different column height on the composition of F2 fraction

![Figure 9](image9.png)  
**Figure 9.** Effect of different column height on the yield of each fraction

The experiments were performed at the operating pressure of 60 mmHg with the helices packing of both sizes on the Hempel column. The result indicated that the increase in height for the same packing did not provide a sufficient effect on the separation efficiency. In Figure 8, for the large helices, the main fraction was composed of 94%, 93%, and 92% of terpinen-4-ol as the column height increased from 100 mm to 300 mm. On the other hand, this value increased minimally from 93% at 100 mm to 96% at 200 mm and 95% at 300 mm when using small helices as packings.

This influence trend is inconsistent with several previous reports, such as Warsito et al. (2019), and Do et al. (2021). Both studies concluded that the increase in column height would provide better separation. The former witnessed an almost three-fold rise in the citronellal content, from 31.63% to 88.43%, after carrying the fractionation of *Cymbopogon winterianus* on a 2-m Raschig-packaging...
column instead of a 1-m one [28]. This effect could also be seen in the study by Do et al. [29]. The author reported that the citral content increased slightly from 89% to 93% as the column varied from 200 mm to 400 mm in the distillation of Cymbopogon citratus on a glass plate column [29].

The reason for those inconsistent may originate from the column construction. The rise of column height would permit a longer residence time for the contact of the liquid and vapor phase, thus usually enhancing the mass transfer between two-phase [29]. However, the uneven height and diameter of the Fenske helices may attribute to the poor packing distribution. As a result, in the rundown through packing beds, the liquid could travel in a completely different channel to the vapor and avoid the component transfer [35]. Furthermore, the liquid in the column tends to flow toward and along the column walls to deal with the least resistance while the vapor passes up through the void between packing beds, limiting their contact [35]. The build of a liquid distributor, though necessary, in such a small-diameter glass column is intricate. Therefore, in a plain Hempel column, the higher the column is, the higher chance the liquid flows around the column sidewalls, downgrading the separation efficiency against the long columns and balancing the positive effect of the increase in column height.

The fraction yield result (Figure 9) illustrated different trends between the experiments with two types of packing. Regarding the large helices, the F2 fraction yield increased from around 23% at 100 mm column height to 31% at 300 mm. In reverse, the B fraction decreased its amount against the increase in column height. This value reached 18%, 16%, and 12%, with the column height of 100, 200, and 300 mm, respectively. In this case, the more extended column typically leads to a better separation between the main and bottom fractions [29]. Hence, more terpinen-4-ol partially purified from the bottom to the F2 fraction.

However, the tendency of large helices was different from that of the small one. While the F2 fraction yield remained stable at around 30% against various column heights, the bottom fraction yield of 10%, 12%, and 15% was recorded at 100-, 200-, and 300-mm column height, respectively. The reason for this trend may result from the high pressure drop to pass through beds. Compared to large helices (Table 2), the higher packing density gave much greater resistance to the vapor flow [35]. Consequently, the vapor pressure at the distilling pot was required to be higher to pass through the dense packing beds, which means a higher energy load to the system [24]. As a result, the amount of vapor escaping the bottom feed declined within the same power input.

The terpinen-4-ol recovery pattern (Figure 7) shared the same tendency with the F2 fraction yield. The terpinen-4-ol content increased from 59% (100 mm) to around 75% (300 mm) for the large helices. On the other hand, the F2 fraction was composed of around 75% of terpinen-4-ol for all levels of column height in the small helices experiments. However, since the purity achieved at the highest column (300 mm) was the most significant (96% compared to 94% at 100 mm), the optimum conditions were selected at 300 mm column height and small helices packings. This is also the optimum operating variable for the fractionation of TTO.

The composition analysis of each fraction was proposed in Table 3. The composition profile of F2 fraction indicated that terpinen-4-ol was refined successfully. There was an absence of foreign impurities, which suggested that the thermal degradation of constituents was either unlikely to happen or occur with a trace level. However, though the lighter constituents (α-terpinene, β-cymene, γ-terpinene, and terpinolene) was removed to less than 1% of the content, α-terpineo presented with a relatively high amount (2.44%). This level of impurity content prevented terpinen-4-ol from being considered as a pure chemical. Since boiling point of α-terpineo was only 10 °C larger than that of terpinen-4-ol (Table 1), the removal of this compound may require a different purification technique such as crystallization.
Table 3. Chemical composition of each fraction in the optimal experiment

| Component         | Composition (%) | Raw | F1  | F2  | B   |
|-------------------|-----------------|-----|-----|-----|-----|
| α-terpinene       | 14.64           | 17.99 | 0.09 | -   |     |
| β-cymene          | 6.04            | 20.37 | 0.23 | 0.53 |     |
| 1,8-cineole       | 1.39            | 2.93  | -   | -   |     |
| γ-terpinene       | 28.69           | 40.99 | 0.92 | 0.20 |     |
| terpinolene       | 4.36            | 5.34  | 0.41 | -   |     |
| terpinen-4-ol     | 39.23           | 0.76  | 95.77 | 31.53 |     |
| α-terpineol       | 2.00            | -     | 2.44 | 7.44 |     |
| NA*               | -               | -     | -   | 60.30 |     |

*Not available

4. Conclusion
This paper provided a vacuum fractional distillation procedure to separate terpinen-4-ol from its parental TTO. The effect of changing the types of columns, packings, and column height on the separation and terpinen-4-ol recovery was discussed. The optimal fractionation conditions were determined: 300-mm Hempel column filled with small Fenske helices (2 mm × 10 mm) at the system pressure of 60 mmHg. The following parameters allowed terpinen-4-ol to witness a 2.5-fold increase in its purity, from 39.23% in the raw oil to 95.77% in the main fraction. Meanwhile, 75% of terpinen-4-ol was recovered from the natural oil. Removing skin-irritant agents such as α-pinene, α-terpinene, and 1,8-cineole was also efficacious, decreasing their content in the second fraction to less than 1%.

5. Acknowledgements
This work was funded by the Vingroup Innovation Foundation (VINIF) under the project code DA120-15062019/year 2019. We acknowledge the support of time and facilities from Ho Chi Minh City University of Technology (HCMUT), VNU-HCM and Notessen Co., LTD for this study.

References
[1] Yadav E, Kumar S, Mahant S, Khatkar S, Rao R J Essent Oil Res 2016 29 1-13.
[2] Carson C F, Hammer K A, Riley T V Clin Microbiol Rev 2006 19 50-62.
[3] Sharifi-Rad J, Salehi B, Varoni E M, Sharopov F, Yousaf Z, Ayatollahi S A, et al. Phytoterror res 31 1475-94.
[4] Nenoff P, Haustein U F, Brandt W Skin Pharmacol Physiol 1996 9 388-94.
[5] Terzi V, Morcia C, Faccioli P, Valè G, Tacconi G, Malnati M Lett Appl Microbiol 2007 44 613-8.
[6] Chao S, Young D, Oberg C J Essent Oil Res 2000 12 639-49.
[7] Hart P H, Brand C, Carson C F, Riley T V, Prager R H, Finlay-Jones J J Inflamm Res 2000 49 619-26.
[8] Salvatori C, Barchi L, Guzzo F, Gargari M Oral Implantol 2017 10 59-70.
[9] Schnitzler P, Schön K, Reichling J Pharmazie 2001 56 343-7.
[10] Bishop C D J Essent Oil Res 1995 7 641-4.
[11] Bozzuto G, Colone M, Tocciacieli L, Stringaro A, Molinari A Planta medica 2011 77 54-6.
[12] Larson D, Jacob S Dermatitis 2012 23 48-9.
[13] Swords G, Hunter G L J Agric Food Chem 1978 26 734-7.
[14] Brophy J J, Davies N W, Southwell I A, Stiff I A, Williams L R J Agric Food Chem 1989 37 1330-5.
[15] Johns M R, Johns J E, Rudolph V J Sci Food Agric 1992 58 49-53.
[16] Loughlin R, Gilmore B F, McCarron P A, Tunney M M Lett Appl Microbiol 2008 46 428-33.
[17] Zhang K, He C, Chen Z, Deng Q, Wu M, Li H, Standardized recombinant tea tree oil and preparing method thereof China patent CN101829074A 2010.
[18] Wang Z, He G, Wu J, Method and device for preparing high-purity cineole China patent CN1436783A 2003.
[19] Zheng X, Yang Z, Li J, The extracting method of highly pure eucalyptus oil China patent CN1064700A 1992.
[20] Gu L, Liu W, Wan H, Method for crystallizing and purifying low-concentration eucalyptus oil patent CN104761566A 2015.
[21] Huynh Q, Phan T D, Thieu V Q Q, Tran S T, Do S H J Phys Conf Ser 2012 352 012053.
[22] Huynh Q, Phan T, Thieu V Q Q. Research on Distillation Technology to Extract Essential Oil from Melaleuca Alterfornia (TTO). Int. Proc. Chem. Biol. Environ. Eng. 2012 (Singapore: IACSIT Press) 43 125-30.
[23] Ririh Y W Universitas Negeri Yogyakarta 2009.
[24] Mortimer R G 2008 Physical Chemistry 3rd ed (USA: Academic Press).
[25] Mohrig J, Alberg D, Hofmeister G, Schatz P, Hammond C 2014 Laboratory Techniques in Organic Chemistry 4th ed (New York: W.H. Freeman).
[26] Rowe D 2009 Chemistry and Technology of Flavours and Fragrances (USA: CRC Press).
[27] Mujtaba I M 2004 Batch Distillation: Design And Operation (London: Imperial College Press).
[28] Warsito W, Cahyani C, Sukardi S, Cahayo M IOP Conf Ser: Mater Sci Eng 2019 546 22-33.
[29] Do D N, Nguyen D P, Phung V-D, Le X-T, Le T M, Do V M, et al. Processes 2021 9 593-604.
[30] Ahluwalia V K, Bhagat P, Aggarwal R 2013 Laboratory Techniques in Organic Chemistry (India: I.K. International Publishing House).
[31] Normag-Laboratory Glassware Distillation accesory vol 7 2005.
[32] Essential oil of Melaleuca, terpinen-4-ol type (Tea Tree oil), ISO 4730:2017, 2017.
[33] Haynes W M 2016 CRC Handbook of Chemistry and Physics 97th ed (New York: CRC Press).
[34] Prada R J, Martínez E L, Wolf Maciel M R Comput Aided Chem Eng 2012 30 1113-7.
[35] Carney T P 1949 Laboratory Fractional Distillation (New York: Macmillan).