Isolation of guaiene from crude and distillate patchouli oil extracted by molecular distillation

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Abstract. Guaiene is one of the components of sesquiterpenes that affects the patchouli oil fragrance. It is commonly used in the pharmaceutical industry and is also used as a flavoring and fragrance agent in the food industry. The objective of this study was to obtain the highest content of guaiene and to investigate the effect of the isolation stage by the molecular distillation process. Crude and distillate fraction patchouli oil samples were introduced into the molecular distillation system. The process conditions applied were first-stage and second-stage molecular distillation. Parameters observed were in terms of chemical composition and color. Results obtained the highest content of α-guaiene in the patchouli oil fraction distillate provided by second-stage molecular distillation (23.53%). However, the highest content of Δ-guaiene was revealed in second-stage molecular distillation residues (33.15%) of patchouli oil fraction distillates samples. The chromaticity value of the second stage distillate was yellow while the residue was yellow-red, respectively.

1. Introduction
Patchouli oil is a fragrant plant native to Southeast Asia that is now widely cultivated in many tropical and subtropical countries, including China, Indonesia, India, Brazil, Vietnam, Malaysia, Mauritius, the Philippines, and Thailand[1]. Patchouli is one of the leading commodities of Indonesian essential oil because it supplies nearly 90% of the world’s patchouli needs. The development of patchouli oil production tends to increase every year. Along with the continued development of the world’s essential oil needs, the need for downstream essential oil derivative products is also growing rapidly. Patchouli oil has several chemical compounds, namely patchouli alcohol, Δ-guaiene, α-guaiene, seychellene and α-patchoulene [2]. The main compound contained in patchouli oil is patchouli alcohol which is a determinant of quality, while Δ-guaiene, α-guaiene, and seychellene are sesquiterpenes which affect the aroma of patchouli oil.

Guaiene is a sesquiterpenoid compound which has the molecular formula C15H24 with a molecular weight of 204,357 g/mole and PubChem CID 5317844 [3]. The guaiene compound is a bicyclic
compound consisting of a decahydroazulene groups, substituted with two methyl groups and 1 1-methylethyl group at positions 1, 4, and 7 [4]. Guaiene compounds that are found in patchouli oil are \( \alpha \)-guaiene and \( \Delta \)-guaiene[5]. The chemical structure of \( \alpha \)-guaiene and \( \Delta \)-guaiene is presented in Figure 1.

![Chemical structures of \( \alpha \)-guaiene and \( \Delta \)-guaiene](image)

**Figure 1.** (a) \( \alpha \)-guaiene and (b) \( \Delta \)-guaiene [5].

Physical properties of \( \alpha \)-guaiene include boiling point 281-282°C at 760 mmHg, specific gravity 0.897-0.9093 at 25°C, refractive index 1.4930-1.4990 at 20°C, soluble in water and alcohol, and recommended as an ingredient flavor and fragrance in food [6]. The role of \( \alpha \)-guaiene in industrial applications is as a surfactant and emulsifier[4]. In contrast to \( \alpha \)-guaiene, technical grade \( \Delta \)-guaiene compounds (purity > 85%) have properties including optical activity -1 ± 0.5°, refractive index 1.505, boiling point of 274°C at 760 mmHg with storage temperatures of 2-8°C[3]. \( \Delta \)-guaiene compounds have anti-inflammatory activity against PAF (Platelet Activating Factor) a mediator phospholipid produced by various cells when exposed to allergies, inflammation, asthma, and others[7].

Guaiene has a potential application both in food and pharmaceutical industries. With the amount of guaiene content that occupies the second position after patchouli alcohol, so it has the opportunity to be separated into a highly concentrated or single compound of guaiene. Pure compounds have certain vapor pressure values related to the vaporization temperature. Therefore, efforts are needed to separate guaiene by engineering process namely molecular distillation.

Molecular distillation is also one of the physical separation techniques distillation which is usually used for the removal of low molecular substances, high molecular substances, or impurities in a mixture. The molecular distillation technique has been performed based on the nature of the evaporation of molecules, where the ease of evaporation depends on the vapor pressure for each molecule. The vapor pressure of each molecule varies depending on the weight of each molecule. The advantages of using this molecular distillation technology are the use of low evaporation temperatures, high vacuum pressure, and short periods of separation intended to avoid damage to a molecule[8]. It is also characterized by a small distance between evaporator and condenser[9]. It is considered as an alternative to maintaining product quality with a higher separation rate than traditional distillation. The most important parameters in molecular distillation are vaporization temperature, flow rate, wiper speed and vacuum operating pressure[10].

Previous studies focused only on patchouli alcohol and patchoulool outcome. Therefore, in this study guaiene was isolated. Several works on the molecular distillation of patchouli oil have been done previously by[11], that the study effectively raises patchouli alcohol and pogostone contents 27-47%. This technique had been applied to isolate patchouli alcohol up to 73.37% according to[12]. Recently, molecular distillation process optimization by response surface method resulting 62.344% patchoulool contents[13]. Regardless, the application of the engineering distillation method for guaiene isolation is limited. Previous studies on guaiene isolation have been done using vacuum fractional distillation [14], while patchouli alcohol purification and analysis have been performed by vacuum fractionation distillation [15]. There is no known research reported on the performance of molecular distillation in treating guaiene isolation. Therefore, the objective of this study was to investigate the effect of the
isolation stage by molecular distillation on the yield and quality of patchouli oil while obtaining the highest guaiene compounds.

2. Material and methods
The crude essential oil used was a local patchouli (*Pogostemon cablin* Benth) oil, obtained from Garut distiller, West Java, Indonesia. The steam distillation process was used to extract the patchouli essential oil from the patchouli plants. Meanwhile, the distillate fraction patchouli oil sample was a product of previous research conducted by[16]. They investigated the fractional distillation process to increase patchouli alcohol content of crude patchouli oil to produce PA chrystal. They used only fraction 3 to produce *patchouli alcohol* chrystal. Therefore, the by-product (fraction 1) which is distillate fraction patchouli oil processed using vacuum fractional distillation under fractionation temperature 230-283°C is used during this study. The rationale to use this distillate fraction of patchouli from the previous study is because it is still rich in guaiene compound, so we need an effort to isolate it to be single compound guaiene. In addition, the supporting chemical material needed is a mixture solution of liquid nitrogen and ethanol 96% with (1:1) w/w (analytical grade) to maintain the temperature inside the cold trap.

The distillation equipment used a molecular distillation film apparatus KDL-5 Short Path Distillation, manufactured by UIC-GambH, Alzenau, Germany. The core component of KDL-5 is made of borosilwth. Evaporation occurs when the material flowed forms a thin layer due to mechanical agitation of the wiper. The thin layer that forms, is pushed to form turbulent flow by the wipers and then drops along the walls of the evaporator column because of the gravitational force and gaps in the wipers. As long as the material flows to the heater, the molecules evaporate depending on the characteristics of the material and the temperature of the evaporator used. Therefore, in molecular distillation operations, there are two streams, namely distillate, and residue. Unevaporated molecules will flow down as residues, while evaporated material is condensed and separated into distillates. The molecular distillation process was carried out with two treatments namely first-stage molecular distillation dan second-stage molecular distillation. The schematic diagram for the overall process with the flow stream for each component (feed, distillate, residue) is shown in Figure 2.

![Figure 2. The schematic diagram of molecular distillation process and scheme of sequential distillation performed by KDL-5 short path distillation.](image)

2.1. Molecular distillation
The following procedure was done to begin the operation. In the beginning, the breaker level was lifted to switch on the operation. The vacuum and pressure indicator was turned on, all valves on the heater wiper condense, and feed oil line. The feed oil line is connected to the heater wiper condense, and the distillate fraction patchouli oil from the previous study is used as the feed oil.

- **MD-1**: First Stage Molecular Distillation
  - R1 (78°C): Residue
  - D1 (78°C): Distillate

- **MD-2**: Second Stage Molecular Distillation
  - R2 (85°C): Residue
  - D2 (85°C): Distillate

R(1-2) : Residue  D(1-2) : Distillate

MD-1 : First Stage Molecular Distillation
MD-2 : Second Stage Molecular
device must be completely closed. A mixture of liquid nitrogen and ethanol 96% were filled into the cold trap. All the heaters were turned on and process conditions included desired temperature (feed, evaporator, and discharge temperature) were set. Moreover, the flow of the condenser cooler and the pressure were adjusted to the desired setting. A 200 ml patchouli sample was inserted into the boiling flask from the top, then the flow rate was adjusted by turning the tap. After the process condition were set up, the stirrer was turned on and the distillation process was started. All the samples will slowly distill all, then the results of distillate and residue were collected in a separate flask. When the vacuum has finished and the pressure indicator was turned off. Afterward, lower the breaker to switch off the position.

Distillation time was also investigated. Distillation time is the time needed to distill all of the samples into two parts, distillate fraction and residue fraction. The variables set up used in the molecular distillation process in this study were presented in Table 1.

Table 1. Variables of molecular distillation set up.

| Process                    | Parameter set up                  | Value          |
|----------------------------|-----------------------------------|----------------|
| First-Stage Molecular Distillation | Running Pressure (mbar)           | 3.1-3.3        |
|                            | Temperature Feed (°C)             | 40             |
|                            | Temperature Evaporator (°C)       | 78             |
|                            | Temperature Discharge (°C)        | 60             |
|                            | Rotation (rpm)                    | 356            |
|                            | Feed Rate (drops/second)          | 4              |
|                            | Initial Volume (mL)               | 200            |
| Second-Stage Molecular Distillation | Running Pressure (mbar)           | 1.2-1.8        |
|                            | Temperature Feed (°C)             | 40             |
|                            | Temperature Evaporator (°C)       | 85             |
|                            | Temperature Discharge (°C)        | 60             |
|                            | Rotation (rpm)                    | 356            |
|                            | Feed Rate (drops/second)          | 4              |

2.2. Gas chromatography-mass spectroscopy (GC-MS) analysis

The results of essential oils from the molecular distillation process were performed by gas chromatography-mass spectroscopy. Patchouli oil sample of 1 μL was injected on gas chromatography-mass spectrophotometer (Shimadzu GC-MS-QP2010 SE, Japan) with the following operating conditions: column used was Rtx-5MS (length 30 m, diameter 0.25 mm and film thickness 0.25 μm). The initial temperature of oven temperature in the column was set at 60 °C for 14 minutes to the final temperature of 200 °C. The injector temperature was the same as the detector temperature, which was 250 °C. The carrier gas used was Helium with a flow rate of 101.3 ml/min, a pressure of 36.2 kPa. While the type of ionizing was electron impact (EI). Next, the components are identified by the mass spectral library which is integrated with the MS system.

2.3. Determination of color

Color ordinates which expressed the reflectance spectrum were quantified using a Spectrophotometer UltrascanPro (D65, Hunter Lab, USA). This equipment was directly connected to a computer and Universal software version V.4.10 was used to demonstrate the value of color perceptions. Hunter color standard Illuminant D65 10° Observer (L*=93.33, a*= -0.91, b*=1.46) was served as the standard light source. This system was standardized with white and black glass prior to color testing. 5 ml samples were put in the optical glass and CIE L* (lightness), a* (redness), b* (yellowness) values were straightforwardly obtained from computer display. The color was evaluated in terms of lightness (L*), redness/greenness (a*) and yellowness/blueness (b*), chroma and hue angle. The experiments
were made in triplicate and mean values were remarked. The Hue degree is adjusted according to the color chromaticity range in Table 2.

**Table 2. Hue value and chromaticity color range area.**

| Hue value | Chromaticity color range area          | Hue value | Chromaticity color range area          |
|-----------|---------------------------------------|-----------|---------------------------------------|
| 342 – 18  | Red purple (RP)                       | 162 – 198 | Green (G)                             |
| 18 – 54   | Red (R)                               | 198 – 234 | Blue green (BG)                       |
| 54 – 90   | Yellow red (YR)                       | 234 – 279 | Blue (B)                              |
| 90 – 126  | Yellow (Y)                            | 279 – 306 | Blue purple (BP)                      |
| 126 – 162 | Yellow green (YG)                     | 306 – 342 | Purple (P)                            |

3. Results and discussion

3.1. Chemical composition of raw materials

The raw material used in first-stage molecular distillation was crude patchouli oil. While the second-stage molecular distillation uses distillate product resulting from the first-stage molecular distillation. Both crude patchouli and distillate fraction patchouli oil were identified by GC-MS analysis. According to GC results, each material has a different material compositions. The results of both GC-MS are presented in Table 3 and Table 4.

**Table 3. Composition of crude patchouli oil.**

| Retention time (min) | Component              | Percentage (%) |
|----------------------|------------------------|----------------|
| 26.459               | 4,7-Methanoazulene     | 2.57           |
| 32.317               | Alpha.-guaiene         | 16.84          |
| 32.613               | Caryophyllene          | 3.68           |
| 34.657               | 1H-3a,7-Methanoazulene | 4.33           |
| 34.854               | Seychellene            | 13.71          |
| 38.237               | Aciphyllene            | 2.38           |
| 38.950               | Delta.-guaiene         | 17.37          |
| 51.354               | 4H-Inden-4-one         | 1.24           |
| 52.630               | Patchouli alcohol      | 34.77          |
| 53.030               | Aromandendrene         | 1.74           |

It can be seen that crude patchouli oil consists of 10 components with 3 dominant components namely *Patchouli alcohol* 34.77%, *Δ*-guaiene 17.37%, followed by *α*-guaiene 16.84% (Table 3). There were 16 constituent components of crude patchouli oil, with 3 dominant components as follow *Patchouli alcohol* (42.75%), *Δ*-guaiene (28.30 ), and *α*-guaiene (20.48%) [17]. This patchouli oil content was varied might be due to the essential oil processing unit, it commonly applied a simple/traditional technology. Meanwhile, from Table 4 distillate patchouli oil consists of 30 components with 3 dominant components namely *patchouli alcohol* 25.53%, *Δ*-guaiene 16.71%, *α*-guaiene 18.80%, respectively.
Table 4. Composition of distillate fraction of patchouli oil.

| Peak | Retention time (min) | Component | Percentage (%) |
|------|----------------------|-----------|----------------|
| 1    | 7.848                | Patchouli alcohol | 25.53 |
|      |                      | 6-isopropenyl-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1h-naphthalen-2-one | 0.35 |
| 2    | 9.885                | Delta-elemene | 0.19 |
| 3    | 10.125               | Beta-patchoulen | 0.56 |
| 4    | 10.513               | 1(10),4-aramenedradiene | 0.43 |
| 5    | 10.727               | Beta-patchoulen | 2.77 |
| 6    | 10.874               | (-)-Beta-elemene | 1.54 |
| 7    | 10.915               | Seychellene | 1.12 |
| 8    | 11.313               | Trans,(beta.)-caryophyllene | 3.30 |
| 9    | 11.390               | Alpha-guaiene | 18.80 |
| 10   | 13.250               | Seychellene | 10.27 |
| 11   | 11.802               | Alpha-humulene | 0.75 |
| 12   | 11.873               | Alpha-patchoulen | 9.83 |
| 13   | 11.976               | Patchoulen | 1.93 |
| 14   | 12.058               | Pentadecane | 0.30 |
| 15   | 12.178               | Eremophilen | 0.66 |
| 16   | 12.297               | Delta-guaiene | 16.71 |
| 17   | 12.537               | (E)-1,2,4,4-tetramethyl-3-(3’-methyl-1’,3’-butadienyl)-2-cyclohexen-1-ol | 0.17 |
| 18   | 12.726               | Alpha-cubebe | 0.34 |
| 19   | 12.793               | (-)-Caryophyllene oxide | 0.16 |
| 20   | 12.846               | 2-methyl-4-(2,6,6-trimethyl-cyclohex-1-enyl)-but-2-enal | 1.12 |
| 21   | 13.170               | (-)-Spathulenol | 0.82 |
| 22   | 13.342               | (-)-Caryophyllene oxide | 1.43 |
| 23   | 13.403               | 4,4-Dimethyl-3-(3-methyl-3-buten-1-yliden)-2-methylidenbicyclo[4.1.0]heptane | 0.20 |
| 24   | 13.585               | 4,4-Dimethyl-3-(3-methyl-3-buten-1-yliden)-2-methylidenbicyclo[4.1.0]heptane | 0.25 |
| 25   | 13.679               | Valerenol | 0.19 |
| 26   | 13.795               | Valerenol | 0.25 |
| 27   | 13.952               | Valerenol | 0.19 |

3.2. Composition of each distillate and residue
It was observed that first and second-stage molecular distillation process could significantly change the α-guaiene and Δ-guaiene content for both crude and distillate fraction patchouli oils. Table 5 described the highest α-guaiene content (22.61%) achieved for residue crude patchouli oil which were subjected to first-stage molecular distillation. However, the highest Δ-guaiene content (19.50%) was the residue of second-stage molecular distillation. Compared to initial α-guaiene and Δ-guaiene in crude patchouli, both first-stage and second-stage molecular distillation have not shown good
performance in separating guaiene components from crude patchouli oils. Meanwhile, the first-stage molecular distillation could isolate the highest patchouli alcohol in the first distillate stream (52.88%).

**Table 5.** Percentage $\alpha$-guaiene, $\Delta$-guaiene and patchouli alcohol content in crude patchouli oil (PO), distillates, and residues during first stage molecular distillation and second molecular distillation.

| Raw materials               | Molecular distillation | $\alpha$-guaiene | $\Delta$-guaiene | Patchouli alcohol |
|-----------------------------|------------------------|-------------------|------------------|------------------|
| Crude patchouli oil         | Distillate 1st stage   | 16.84             | 17.37            | 34.77            |
|                             | Residue 1st stage      | 9.28              | 12.67            | 52.8             |
|                             | Distillate 2nd stage   | 22.61             | 18.28            | 5.92             |
|                             | Residue 2nd stage      | 16.63             | 17.52            | 22.77            |
|                             |                        | 12.21             | 19.50            | 38.21            |

In contrast, the residue first-stage molecular distillation from distillate fraction patchouli oil could reveal the highest patchouli alcohol (24.84%), the value was lower than the initial patchouli alcohol in distillate fraction of patchouli alcohol (25.53%). This first-stage molecular distillation process then decreases the PA content by 2%. The performance of second-stage molecular distillation using distillate fraction patchouli oil could isolate better guaienes components. The $\Delta$-guaiene content of second-stage molecular distillation in the residue was the highest (33.15%). Table 6 would mean that there was a 16.44% increase in $\Delta$-guaiene content of the second residue compared with the initial $\Delta$-guaiene distillate fraction PO. In addition, we found that distillate fraction patchouli oil which was introduced second-stage molecular distillation exhibit the highest $\alpha$-guaiene content (23.53%), so there were an increase of almost 4.86% from the initial $\alpha$-guaiene content. Therefore, second-stage molecular distillation could produce higher purity of both $\alpha$-guaiene and $\Delta$-guaiene content. This was maybe due to the experimental setup in terms of evaporator temperature and running vacuum pressure which could lead to successful guaiene separation. Hence, pressure-temperature (P-T) diagram should be taken into consideration since it is reflected in the sample phase of any pure substance. Vacuum conditions during the fractionation distillation are used to minimize damage to components separated by high temperatures so that the vacuum conditions are needed so that the distillation process temperature was not too high [18].

**Table 6.** Percentage $\alpha$-guaiene, $\Delta$-guaiene and patchouli alcohol content in the distillate fraction crude patchouli oil (PO), distillates, and residues during first stage molecular distillation and second molecular distillation.

| Raw materials               | Molecular distillation | $\alpha$-guaiene | $\Delta$-guaiene | Patchouli alcohol |
|-----------------------------|------------------------|-------------------|------------------|------------------|
| Distillate fraction patchouli Oil | Distillate 1st stage   | 18.67             | 16.71            | 25.53            |
|                             | Residue 1st stage      | 21.03             | 23.63            | 15.89            |
|                             | Distillate 2nd stage   | 18.71             | 15.30            | 24.84            |
|                             | Residue 2nd stage      | 23.53             | 23.70            | 10.69            |
|                             |                        | 19.84             | 33.15            | 3.91             |
3.3. Yield
Distillate is a product that is rich in components that evaporate with lower boiling points, while residue is a product that contains components that are difficult to evaporate and have high boiling points. The percent yield of distillates and residues is presented in Table 7. The first-stage molecular distillation process always show higher total yield than second-stage processes. The residue of crude PO which were processed by second-stage molecular distillation showed the highest yield (68.16%). In contrast, the residue of distillate fraction PO by first-stage molecular distillation process (63.40%) revealed the highest yield. The difference in the amount of residue and distillate is thought to be due to differences in the composition of volatile and non-volatile materials. It can be seen that there were some losses during the distillation process because there were some amount of mass that stick inside the molecular distillation apparatus.

Table 7. Yield of first-stage and second-stage molecular distillation.

| Raw materials      | Molecular distillation | Distillate yield (%) | Residue yield (%) | Total yield (%) |
|--------------------|------------------------|----------------------|------------------|-----------------|
| Crude patchouli oil| First-stage            | 56.95                | 36.79            | 93.74           |
|                    | Second-stage           | 18.59                | 68.16            | 86.75           |
| Distillate fraction| First-stage            | 34.71                | 63.40            | 98.11           |
| Patchouli oil      | Second-stage           | 58.91                | 29.61            | 88.52           |

3.4. Distillation time
Generally, the time required to isolate guaiene by second-stage molecular distillation was longer than by the first-stage molecular distillation. This happened because the distillation time is directly related to the evaporator temperature and the pressure applied. So the lower the evaporator temperature during the process the longer time required to distill the guaiene component. As temperature increases and pressure decreases, these escaping tendencies usually increase and the substance evaporates. The force generated by these escaping molecules is referred to as the vapor pressure of that material at a particular temperature and pressure. It was because of the relative difference in vapor pressure of substance that dictates how easily a complex compound can be separated into its constituent compounds [19]. The fastest process was obtained by first-stage molecular distillation using distillate fraction PO with 1.18 h processing time. The detailed data in terms of molecular distillation time were tabulated in Table 8.

Table 8. Molecular distillation time.

| Raw materials                  | Molecular distillation | Time (h) |
|--------------------------------|------------------------|----------|
| Crude patchouli oil            | First-stage            | 1.45     |
| Distillate fraction patchouli oil| Second-Stage         | 1.53     |
|                                 | First-stage            | 1.18     |
|                                 | Second-Stage           | 1.43     |

3.5. Color characteristics
The color of distillate and residue fraction was analyzed in CIE Lab color scale. Hue notation is the value obtained from the values a * and b * and represents the wavelength of the dominant color. It can be concluded that the results of the chromaticity value of patchouli oil which was processed by molecular distillation was varied from red to yellow. The appearance of raw patchouli oil, distillates, and their residues are presented in Figure 3 and Figure 4. This emphasized that when the higher
evaporator temperature and more molecular distillation time were applied the trends of color changed. In this overall study, it was concluded that the trend in color was CIE b* toward higher b* and exhibited positive correlation, where as a* tend to negatively correlated when were subjected to distillation treatment. Meanwhile, the detailed of color characteristic are reported in Table 7 and Table 8.

**Figure 3.** Color of crude patchouli oil, distillates, and residues.

**Figure 4.** Color of the distillate fraction patchouli oil, distillates, and residues.

**Table 9.** Color characteristic of crude patchouli oil, distillates and residues.

| Properties | Crude patchouli oil | Distillate 1st. Stage md | Residue 1st. Stage md | Distillate 2nd. Stage md | Residue 2nd. Stage md |
|------------|---------------------|--------------------------|-----------------------|--------------------------|-----------------------|
| L*         | 14.84               | 14.86                    | 17.88                 | 16.07                    | 15.32                 |
| a*         | 0.77                | 0.45                     | -0.79                 | -0.53                    | 0.34                  |
| b*         | 0.46                | 0.39                     | 1.33                  | 0.85                     | 0.21                  |
| C          | 1.04                | 0.60                     | 1.55                  | 1.01                     | 0.40                  |
| H          | 41.90               | 41.33                    | 120.61                | 122.38                   | 31.90                 |
| Chromaticity| red                 | red                      | yellow                | yellow                   | red                   |
Table 10. Color characteristic of distillate fraction of patchouli oil, distillates and residues.

| Properties | Distillate fraction patchouli oil |
|------------|----------------------------------|
|            | Raw mat | Distillate 1st stage md | Residue 1st stage md | Distillate 2nd stage md | Residue 2nd stage md |
| L*         | 16.64   | 15.41               | 17.04             | 16.72              | 16.29              |
| a*         | -0.72   | -0.43               | -0.66             | -0.89              | 0.53               |
| b*         | 2.64    | 1.43                | 1.18              | 2.39               | 2.52               |
| C          | 2.74    | 1.50                | 1.35              | 2.55               | 2.57               |
| H          | 105.38  | 106.84              | 119.14            | 110.49             | 78.09              |
| Chromaticity | yellow | yellow | yellow | yellow | Yellow red |

4. Conclusions
The stage of molecular distillation could affect the yield, composition of α-guaiene and Δ-guaiene production, molecular distillation time, and also color characteristics of both distillate and residues. We concluded to select distillate fraction PO rather than crude PO to isolate better guaiene compounds. Isolation of guaiene from distillate fraction patchouli oil produces a higher purity than from crude patchouli oil. These results revealed that second-stage molecular distillation treatment was suitable in isolating guaiene components due to its higher purity of guaiene. Based on the results, distillate fraction patchouli oil which experienced second-stage molecular distillation contained the highest α-guaiene of (23.53%), while its residue contained the most Δ-guaiene (33.15%). There was slightly increases in the α-guaiene and Δ-guaiene content of distillates fraction patchouli oil after some distillation process will also significantly affect the color quality of the final product.

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