Enriching 1,8-Cineole Content in *Eucalyptus camaldulensis* D. Raw Essential Oil: An Investigation on Optimizing Vacuum Fractional Distillation Process

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Abstract: *This study addressed the gap in optimizing the 1,8-cineole content in* Eucalyptus camaldulensis *essential oil, an area with a limited focus in existing literature. While previous research has explored distillation methods for essential oils, this study is the first to systematically investigate the effects of key operational parameters on cineole enhancement using batch vacuum fractional distillation. The optimization process was carried out using a single–factor method focusing on operating pressures (60, 80, 100, 120, 140, and 160 mmHg), column types (Vigreux and Hempel), packing materials (wire mesh, large strings, small strings), and column heights (300, 400, 500, and 600 mm). After each distillation experiment, the essential oil samples were analyzed using GC-MS to quantify the concentration of 1,8-cineole and other components. The best result, achieved at 60 mmHg with a 500 mm Hempel column packed with small metal helixes (2 × 10 mm), increased 1,8-cineole content from 47.9% to 74.6%, with 97% recovery and over 60% yield. These findings highlight the impact of distillation parameters on cineole concentration, marking a 1.5-fold improvement. This optimized distillation process offers an efficient alternative for producing high-bioactivity cineole oil, with potential pharmaceutical and personal care applications, reducing reliance on complex synthesis.*

Keywords: *1,8-cineole; fractional distillation;* Eucalyptus camaldulensis*; essential oil; enriching*

■ INTRODUCTION

Eucalyptus camaldulensis Dehnh, which belongs to the Myrtaceae family, is one of the most widely cultivated eucalypti in a range of arid, temperate, and tropical regions, including Australia and the Mediterranean [1-2]. Eucalyptus essential oil, derived from leaf extraction, fulfills a wide range of medical demands, including the treatment of sore throats and providing antibacterial remedies for respiratory and urinary areas. Eucalyptus oil-based ointments have been employed in Aboriginal medicine for both wound healing and the management of fungal infections [3]. Additionally, *E. camaldulensis* resin has been traditionally used as an antiseptic for mouth and teeth and in antidote drugs [4].

The bioactivity of eucalyptus oil is primarily attributed to 1,8-cineole (also known as eucalyptol), a versatile compound derived from its natural sources. This versatility is applied across pharmaceutical, food, and personal care fields. The 1,8-cineole has demonstrated excellent inhibition of leukemia cell growth without exerting considerable toxic effects on the human body [5]. Additionally, due to its distinctive spicy taste and aroma, 1,8-cineole is favored as a flavoring agent in food preparation and a fragrance in perfumes and cosmetics [6, 7]. The approval by the US Food and Drug Administration (FDA) for the safety of 1,8-cineole in food and pharmaceuticals [2] permits even higher consumption in these industries.

However, the use of 1,8-cineole in its natural form, essential oils, presents significant disadvantages due to the presence of other coexisting constituents. The content of 1,8-cineole in raw *E. camaldulensis* essential oil has been reported to range from 20% to 60% [8]. In the previous study, other constituents such as limonene, terpinene-4-ol, and α-terpineol are also commonly present in desirable percentages (1–8%) [9]. The presence of these unsaturated compounds is related to the adverse effects reported when eucalyptus oil is used [10]. The ingestion of raw eucalyptus oil can lead to the reduction of unsaturated compounds triggered by enzymes, inducing oxygen radicals from these components, damaging tissues, and provoking inflammation [1-2]. Additionally, allergic and neurological symptoms stimulated by another impurity, *p*-cymene, have been described [11]. Therefore, to eliminate the detrimental effects of other impurities in the original oils and maximize 1,8-cineole's potential in pharmaceutical applications, there is a need to enhance the concentration of 1,8-cineole from its parental sources.

The optimal conditions for vacuum fractional distillation are influenced by pressure, temperature, column design, and packing material separation efficiency. Vacuum pressure reduces boiling points, allowing for easier separation without thermal degradation. For instance, Le et al. [12] demonstrated that a vacuum pressure of 60 mmHg increased the purity of terpinene-4-ol from *Melaleuca alternifolia* tea tree oil to 95.77%. Column design and packing material, such as Hempel columns with Fenske helixes, enhance separation by increasing surface area contact. Additionally, a high reflux ratio improves the purity of α-guaiene from patchouli oil, as shown by Widyasanti et al. [13]. These results underscore the necessity for meticulous optimization in vacuum fractional distillation to achieve maximum efficiency.

Despite the complex composition of raw *E. camaldulensis* oil, the effective separation of 1,8-cineole from other components is feasible due to its significantly lower boiling point compared to common impurities like terpinene-4-ol and α-terpineol [14]. This allows 1,8-cineole to evaporate and be recovered first, achieving optimal separation during fractional distillation, especially under vacuum pressure. Recent studies have explored fractional distillation of cineole oils, primarily from rosemary and myrtle. Silvestre et al. [15] successfully increased the 1,8-cineole content from 26% to 62% in *Rosmarinus officinalis* L. essential oil from South Brazil, using a Hempel column packed with Raschig rings at 10 kPa (75 mmHg), and collected the product when the bottom temperature reached 125 °C. Meanwhile, Farah et al. [16] achieved a 96% purity of 1,8-cineole from raw *Myrtus communis* L. essential oil, which initially contained about 43% 1,8-cineole, using a Hempel column with glass rings under atmospheric pressure. The highest 1,8-cineole content was obtained after 30–50 min.

While *E. camaldulensis* oil contains cineole at levels similar to those in rosemary and myrtle oils, there is a lack of research on the fractionation of this oil. Most existing studies have focused on simulating the fractionation of *Eucalyptus globulus*, which has a higher cineole content and may not directly apply to *E. camaldulensis*. For example, Almeida et al. [17] simulated a process that increased cineole from 85% to 99% in *E. globulus*. Still, these findings may not be suitable for *E. camaldulensis* due to the lower cineole concentration in the latter. To address this gap, it is essential to develop an optimized fractional distillation process specifically for *E. camaldulensis* and examine how different operational parameters affect cineole separation. This study proposes a method to achieve high purity of 1,8-cineole from *E. camaldulensis* oil and explores the influence of operational conditions on the effectiveness of the separation process.

■ EXPERIMENTAL SECTION

Materials

The study utilized essential oils derived from the leaves of *E. camaldulensis* using the steam distillation method provided by Notessen Co., Ltd Vietnam. The preparation and storage procedures followed the previous method [18].

Instrumentation

Gas chromatography and mass spectrometry (GC-MS) analysis was performed using an Agilent 6890 GC

system (G1530A, Serial: US00002778) coupled with an Agilent 5973N MSD. The GC was equipped with an HP-5MS column (30 m × 0.25 mm × 0.25 μm), with helium as the carrier gas at 1 mL/min. The oven temperature was initially set at 60 °C and increased by 10 °C/min. The Agilent MS (Model 5973N MSD) operated in EI mode at 70 eV, with an ion source temperature of 220 °C. The mass scan range was 40 to 400 amu. A 1:100 (v/v) diluted sample in acetone was injected (1 μL) at a concentration of 1000 ppm with a split ratio of 1:50 and a scan rate of 1 s/scan. Compound identification was based on standard terpinene-4-ol and the NIST library, with quantification by peak area integration.

Procedure

Vacuum fractional distillation procedure

Vacuum was employed during the distillation process to lower the boiling points of the components, thereby preventing the thermal degradation of sensitive compounds such as 1,8-cineole. This approach facilitates effective separation at lower temperatures, minimizing the risk of decomposition and ensuring higher yield and purity of the target product. The essential oil distillation system used was based on the study by Le et al. [19]. The raw essential oil was initially dried with 1.0 g of sodium sulfate and then transferred to a flask, using 100 g per batch. The cooling water was set to 5 °C, and the operating pressure was maintained through a pressure regulator. To begin the process, the heater was switched on with a constant heating rate. Once the first drop of condensed sample appeared, temperatures at the top and bottom of the column were recorded at 1-min intervals. Fractionation was based on the top temperature, and only the vapor from the highest point of the distillation column was collected. Fractionation was determined based on the top temperature, with the first fraction collected when the top temperature was below 82 °C and the second (main) fraction collected when it reached or exceeded this temperature (Table 1). The remaining oil in the feed flask was considered the bottom product. The system was shut down when the top temperature dramatically decreased to around 60 °C, indicating that no more essential oil was condensing. Finally, both the collected product and the raw essential oil were analyzed using GC-MS.

Optimization of fractional distillation conditions for E. camaldulensis

Four critical variables affecting separation efficiency were considered, i.e., vacuum pressure, column type, packing material, and column height (Table 2). The effect of each variable was investigated individually by conducting experiments where all other operating parameters were kept constant except for the variable under study. The value of each variable that yielded the highest product purity and 1,8-cineole recovery was selected for subsequent experiments. The products were weighed to calculate the yield and were subjected to chromatographic analysis to assess their chemical composition and 1,8-cineole content. The yield of 1,8-cineole was determined by calculating the ratio of the mass of the fraction to the mass of the feed. Recovery was determined through the multiplication of the yield with the ratio of the component concentration in the fraction to that in the feed [11].

Table 1. Top temperature of the main fraction with respect to pressure

Pressure (mmHg)	60	80	100	120	140	160
Temperature (°C)	82–91	90–95	97–100	101–103	105–108	109–119

Table 2. Summary of varied parameters in the experimental design

Column type	Vigreux, Hempel
Vacuum pressure (mmHg)	60, 80, 100, 120, 140, 160
Packing type	Wire mesh, big metal helixes, small metal helixes
Column height (mm)	300, 400, 500, 600

■ RESULTS AND DISCUSSION

Fractional Distillation of 1,8-Cineole from *E. camaldulensis* Essential Oil

E. camaldulensis essential oil was fractionated under various vacuum conditions to optimize eucalyptol separation. Initially, the effect of vacuum pressure on the Vigreux column's fractionation process was explored. Following this, the impact of vacuum pressure was assessed on the Hempel column, which features random packing with stainless steel springs (4 × 10 mm). The study then investigated the effect of three different packing types, i.e., wire mesh, stainless steel springs (4 × 10 mm), and springs (2 × 10 mm). Lastly, the effect of column height on the fractionation process was analyzed. These experiments identified the optimal conditions for distilling eucalyptol from the essential oil.

Composition of the Raw Essential Oil

E. camaldulensis oil, extracted from leaves and branches by steam distillation, containing around 0.45% essential oil, was analyzed using GC-MS. Significant peaks were detected by comparing them with the NIST mass spectral database. The composition and identification of these peaks are listed in Table 3. The proportion of eucalyptol in raw essential oil ranges around only 45% (GC-MS percentage of peak area), while the minimum eucalyptol concentration must be at least 70% based on Australian standard AS 2247.1 – 1999. The primary chemotypes of this essential oil are 1,8-cineole, D-limonene, and α-pinene, accounting for about 80% of the chromatogram area. Due to its lowest boiling point (155 °C) and a 10 °C difference from 1,8-cineole, α-pinene is expected to appear mainly in the first fraction, not significantly affecting the separation of 1,8-cineole. However, D-limonene, present at 14.4%, may co-elute with 1,8-cineole due to their close boiling points [14]. The concentrations of 1,8-cineole and D-limonene are 38.5 and 10.9% [8], respectively, which also showed that both are the major components of the essential oil. The significant presence of D-limonene and its similar boiling points to the desired compound, 1,8-cineole, may compromise the separation process and reduce the flavor and purity of the final product.

Table 3. Chemical composition of raw *E. camaldulensis* essential oil

Compound	%[a]	BP[b]	Ref
1*R*-α-Pinene	18.4	155	[20]
β-Pinene	1.9	163–166	[20]
O-Cymene	1.8	177	[21]
D-Limonene	14.4	176	[21]
1,8-Cineole	47.9	176	[21]
γ-Terpinene	1.2	183	[21]
Terpinolene	2.1	186	[11]
L-Pinocarveol	0.8	215–217	[21]
α-Terpineol	1.8	219	[20]
α-Terpyl acetate	3.4	220	[21]
Aromadendrene	2.6	258–259	[21]
epi-β-Caryophyllene	1.1	264–266	[21]
Bicyclogermacrene	1.9	267–268	[21]
Globulol	0.8	283	[21]

[a]Percentage area in GC-MS chromatogram; [b]Boiling point (BP) at ambient pressure, 760 mmHg

Impact of Vacuum Pressure and Column Types on Distillation

Samples were tested using either a Vigreux or Hempel column adapted from Mohrig et al. [22] (Fig. 1) under six different vacuum conditions. Both columns had a diameter (D) of 22 mm and a height (H) of 400 mm. The experimental conditions for each test are detailed in Table 4. The main fraction ranges in Table 4 were determined by observing step changes in the top temperature and the distillation time profile. Each step change in top temperature corresponds to a change in the primary component of the distillate, indicating a distinct fraction.

Fig 1. The (a) Vigreux and (b) Hempel columns

Table 4. Experimental conditions to study the impact of vacuum pressure and column type

Experiment	Column type	Packing fill	Pressure[a] (mmHg)	Main fraction range[b] (°C)
1	Vigreux[c]	No	60	82–89
2	Vigreux	No	80	90–95
3	Vigreux	No	100	97–100
4	Vigreux	No	120	101–103
5	Vigreux	No	140	105–108
6	Vigreux	No	160	109–111
7	Hempel[c]	Big metal helixes	60	82–89
8	Hempel	Big metal helixes	80	90–95
9	Hempel	Big metal helixes	100	97–100
10	Hempel	Big metal helixes	120	101–103
11	Hempel	Big metal helixes	140	105–108
12	Hempel	Big metal helixes	160	109–111

[a]Absolute pressure, [b]Based on temperature measured at the top of the column, [c](D × H = 22 mm × 400 mm)

Fig. 2 illustrates the composition of 1,8-cineole in the main fraction across different reduced pressures with two types of columns. The highest purity, nearly 63%, was recorded in Experiment 11 using the Hempel column at 140 mmHg. Reducing the operating pressure from 140 mmHg decreased 1,8-cineole purity, reaching a low of approximately 57% at 100 mmHg. For the Vigreux column, the effect of operating pressure was less distinct, with 1,8-cineole purity fluctuating between 56% and 60% without a clear trend. At lower pressures, the temperature difference between the Vigreux and Hempel columns was minimal, resulting in similarly low separation efficiencies for 1,8-cineole, with no significant distinction between the two columns. However, as the pressure increased (from 120 to 160 mmHg), the temperature difference became more pronounced [23]. The design of the Hempel column, which allows for the addition of packing materials (Fig. 1(b) and Table 5), increased the surface area for liquid-vapor interaction. This facilitated more efficient condensation and evaporation processes, leading to superior separation of 1,8-cineole compared to the Vigreux column, particularly at higher pressures. The impact of pressure on the vacuum fractional distillation process was also investigated by Widyasanti et al. [13] in their study on the separation of α-guaiene from patchouli oil. Specifically,

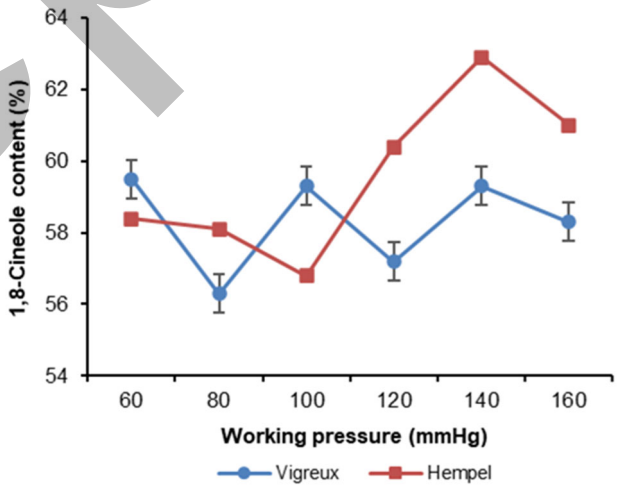

Fig 2. 1,8-Cineole content in the fractionated products of experiments 1 to 12

Table 5. Experimental conditions to study the impact of packings

Experiment	Packing fill	Dimension[a]	Density[b]	Surface area[c]	Main fraction range[c] (°C)
13	Wire mesh ring	4 × 10	0.44	$1250\ \frac{m^2}{m^3};\ N_o = \frac{1{,}121{,}000}{m^3}$	82–89
14	Big metal helixes	4 × 10	1.12	$No = 444{,}215\ m^3$	82–89
15	Small metal helixes	2 × 10	2.25	$1250\ \frac{m^2}{m^3};\ N_o = \frac{2{,}252{,}000}{m^3}$	82–89

the higher the applied run pressure, the greater the yield of α-guaiene. The optimal distillation conditions were achieved at a pressure of 14.8 mmHg, yielding a relative peak area for α-guaiene of 44.47%.

The Hempel column showed a slight improvement over the Vigreux column at higher pressures (120–160 mmHg), with a higher 1,8-cineole content at the same pressure. This enhancement may be attributed to the increased mass transfer area of the Hempel column, which improves the separation of 1,8-cineole from other components. However, the degree of vacuum did not lead to a significant improvement in 1,8-cineole concentration, as the difference between the highest and lowest 1,8-cineole content using the Hempel column was only 8% (56 and 63%, respectively). Fig. 3 presents the composition of 1,8-cineole (eucalyptol) and its main impurities. D-Limonene content varied between 20 and 25% in all experiments, which is higher than the 15% found in the feed stream. In contrast, α-pinene content significantly decreased, remaining around 10% with the Vigreux column and, in some cases, with the Hempel column. The Hempel column performed better at removing α-pinene at 120, 140, and 160 mmHg compared to the Vigreux column, with α-pinene levels reduced to about 7% at 120 and 160 mmHg and as low as 5% at 140 mmHg. This indicates that the substantial difference in boiling points between α-pinene and 1,8-cineole enables the effective removal of α-pinene, whereas D-limonene is less effectively removed by fractional distillation.

The recovery of 1,8-cineole during distillation using two types of columns at various pressures is illustrated in Fig. 4. The Vigreux column reached a peak recovery of approximately 58% at 100 mmHg, which then declined at other pressures. On the other hand, the Hempel column showed a decreasing recovery with increasing pressure, with a maximum recovery of about 80% at the lowest pressure of 60 mmHg and a minimum of 45% at the highest pressure of 160 mmHg. The Hempel column demonstrated a significant improvement in recovery compared to the Vigreux column at the same pressures, achieving up to 85%, which is 1.5 times higher than the best result obtained with the Vigreux column. In summary, the Hempel column outperformed the Vigreux column in terms of 1,8-cineole purity and recovery. Different pressures had varying effects on 1,8-cineole purity and recovery. A high absolute pressure of 140 mmHg yielded the highest 1,8-cineole content, whereas a low working pressure of 60 mmHg achieved the highest recovery. Specifically, using 60 mmHg instead of 140 mmHg reduced 1,8-cineole purity by only 4% (from 63 to 59%) while significantly improving recovery, reaching 80% at 60 mmHg compared to about 55% at 140 mmHg. Therefore, the optimal condition is the Hempel column at 60 mmHg.

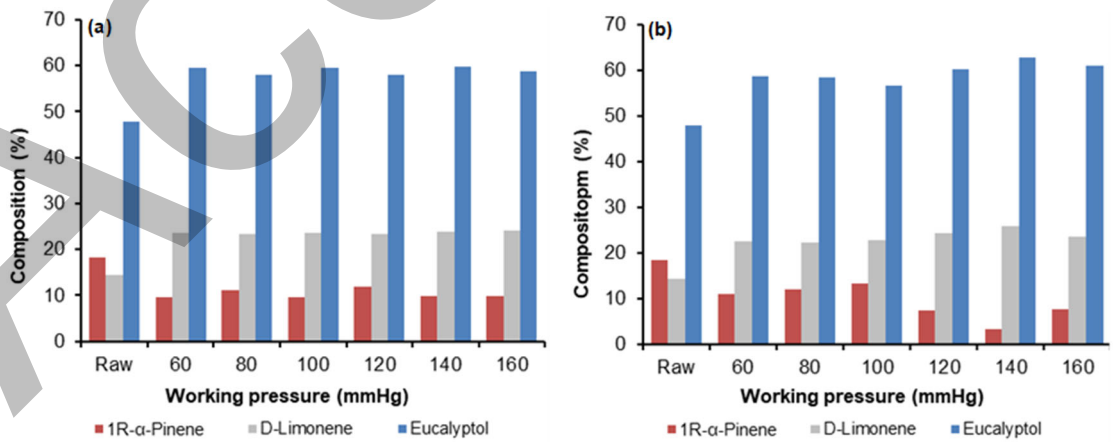

Fig 3. Composition of feed and fractionated products for experiments 1 to 12. (a) Experiments 1 to 6 using the Vigreux column, and (b) experiments 7 to 12 using the Hempel column

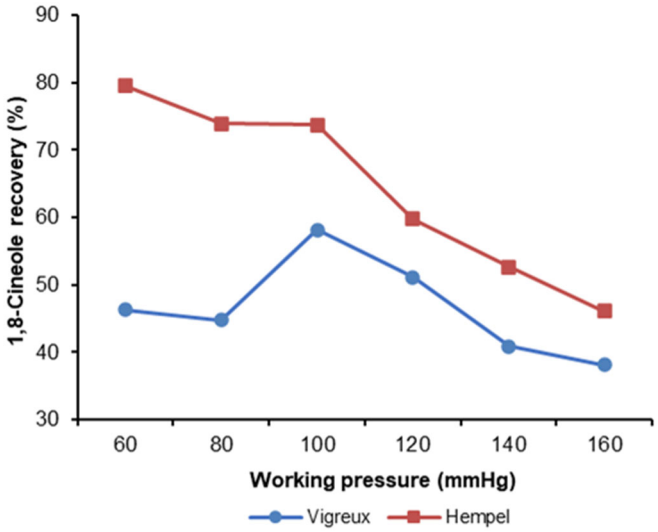

Fig 4. 1,8-Cineole recovery of the main fractions from experiments 1 to 12

Impact of Packing Type on Distillation

The impact of packing type was investigated through the fractionation of untreated eucalyptus oil using a Hempel column (D × H = 22 mm × 400 mm) at 60 mmHg. The column was packed with three different types of materials, i.e., wire mesh rings (D × H = 4 mm × 10 mm), large metal helixes (D × H = 4 mm × 10 mm), and small metal helixes (D × H = 2 mm × 10 mm). The cross-sectional views of each packing type are shown in Fig. 5. Additional details are provided in Table 4. Fig. 6 illustrates the composition of the feed and the main fractions obtained from experiments 13 to 15. The wire mesh packing demonstrated the highest performance, achieving a 1,8-cineole purity of 70%, which is superior to the helixes, which gained approximately 65 and 60% of 1,8-cineole with large and small dimensions, respectively. This improved performance can be attributed to the enhanced mass-transfer area provided by the wire mesh compared to the large and small helixes. Increased mass transfer facilitates a higher number of theoretical plates, which in turn improves the separation efficiency.

To further assess the separability of α-pinene and D-limonene from 1,8-cineole, the composition of the second fraction was analyzed, as shown in Fig. 7. The results indicate that the small metal helixes were the most effective at removing α-pinene from 1,8-cineole, as evidenced by the second fraction containing the highest α-pinene content, approximately 90%. In contrast, other packing types exhibited a notable decrease in separability, with significant amounts of 1,8-cineole remaining in the second fraction. These findings confirm the superior performance of the small metal helixes in selectively removing α-pinene while leaving a higher concentration of 1,8-cineole.

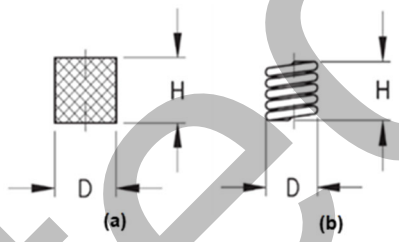

Fig 5. Different packing types of (a) wire mesh ring and (b) metal helix

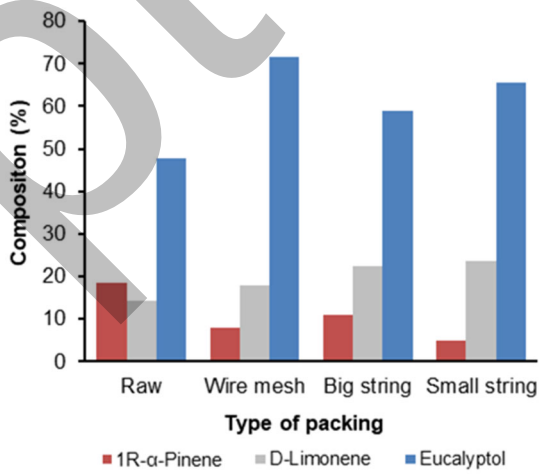

Fig 6. Composition of feed and main fractions from experiments 13 to 15

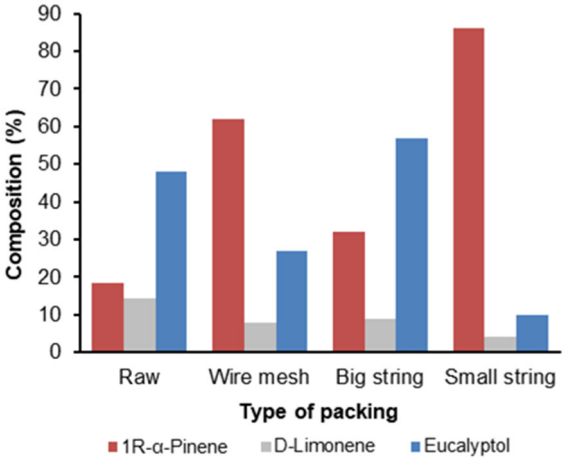

Fig 7. Composition of feed and second fractions from experiments 13 to 15

The recovery results for 1,8-cineole using three different packing types are shown in Fig. 8. The data indicate that the small metal helixes achieved the highest recovery rate, reaching up to 90%. In contrast, wire mesh rings yielded a lower recovery rate of approximately 65%. This discrepancy can be attributed to the large volume of liquid retained within the wire mesh rings, particularly at the string nodes, which reduces the mass yield. Therefore, small metal helixes are identified as the most effective packing material for 1,8-cineole fractionation due to their superior mass-transfer efficiency and minimal liquid retention.

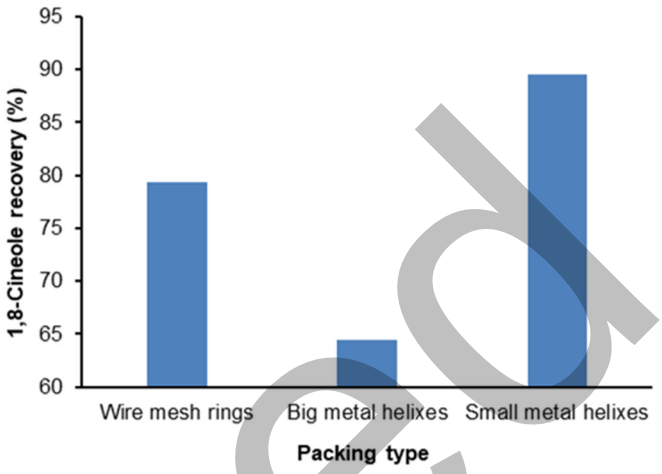

Fig 8. 1,8-Cineole recovery of the main fractions from experiments 13 to 15

The Impact of Column Height on Distillation

The effect of column height on the separation of 1,8-cineole was evaluated using a Hempel column packed with small metal helixes (D × H = 2 mm × 10 mm) with heights of 200, 300, 400, 500, and 600 mm under a pressure of 60 mmHg. The composition of the feed and main distillate products is illustrated in Fig. 9. The results indicate only minor differences between the various column heights. Notably, columns with 200, 300, and 400 mm heights demonstrated lower efficiency, with 1,8-cineole concentrations around 65%. In contrast, columns with heights of 500 and 600 mm achieved significantly higher 1,8-cineole concentrations, approaching 80%. The lower 1,8-cineole content observed in samples from shorter columns is attributed to insufficient column height, which results in inadequate separation and mixing of constituents between fractions. These findings underscore the importance of column height in optimizing the separation process for 1,8-cineole. Finally, the overall recovery of 1,8-cineole was directly proportional to the height of the column, as shown in Fig. 10. Specifically, columns with heights of 500 and 600 mm achieved the highest recovery rates, with nearly complete recovery of 1,8-cineole (over 97%). In contrast, the 300 mm column showed the lowest recovery, at just over 82%. The 400 mm column exhibited intermediate performance, with a recovery rate of approximately 90%. Given the time and energy savings and the minimal difference in recovery between the 500 and 600 mm columns, the 500 mm column was selected for the fractionation of eucalyptus oil due to its optimal balance between recovery efficiency and cost-effectiveness.

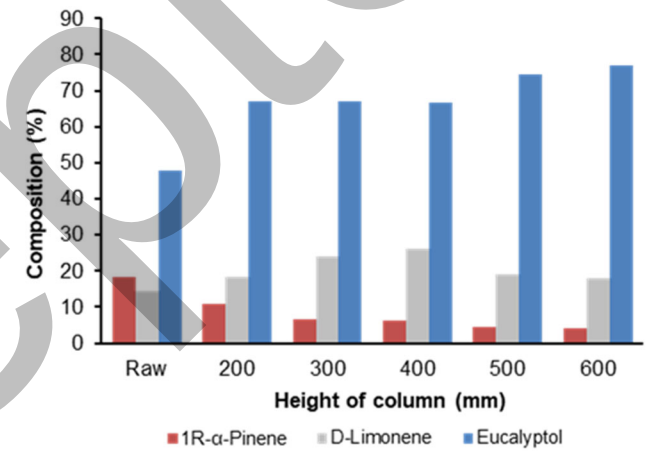

Fig 9. Composition of feed and main fractions according to column height

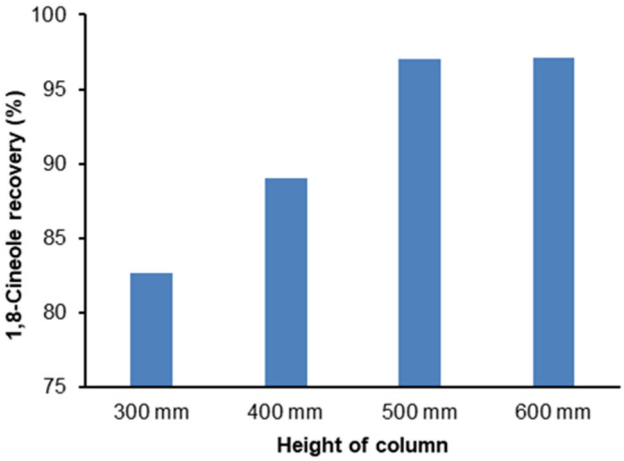

Fig 10. 1,8-Cineole recovery in main fractions as a function of column height

After conducting experiments on various column types, working pressures, packing materials, and column heights, the optimal conditions for the process were determined. The most suitable configuration was the Hempel column operated at a working pressure of 60 mmHg. The ideal packing material was identified as small spring packing with dimensions of 2 mm × 10 mm (D × L), and the most effective column height was established at 500 mm. Under these conditions, the purity of 1,8-cineole reached 74.6%, with a recovery rate of 97% and an efficiency exceeding 60%. In comparison, the study by Nikkhah et al. optimized the fractional distillation process for enriching essential oils from cumin seeds (*Cuminum cyminum*) and eucalyptus leaves (*E. globulus*), simulating the process using Aspen Plus based on data from a pilot-scale unit. Their optimization identified operating conditions (reflux ratio, pressure, time, jacket heating duty, and condenser temperature) that minimized costs, achieving efficiencies of 69.56 and 59.77%, respectively. These findings indicate that our experimental results align well with their theoretical predictions [24]. Silvestre et al. [15] investigated a vacuum distillation method utilizing a packed column to separate rosemary essential oil into different chemical components, focusing on vapor pressure, temperature, and separation time. Their staged separation technique effectively distilled fewer volatile compounds with higher boiling points, yielding substantial amounts in the early stages.

The GC-MS analysis results for the feed, base, and final product of the optimal distillation are presented in Table 6. Specifically, the purity of 1,8-cineole was increased to 86.1% from an initial concentration of 47.9% in the raw material. The GC-MS analysis demonstrated the difference between the raw essential oil of *E. camaldulensis* and the oil following the fourth fractional distillation. In Fig. 11(a), the chromatogram showed the presence of undesirable compounds, such as terpinene-4-ol and α-terpineol, as evidenced by small peaks between retention times of 10 to 14 min. Conversely, Fig. 11(b)

Table 6. Composition of feed, base, and fractions product after fractionating *E. camaldulensis* oil at optimal condition

Component	Raw[a]	F2[b]	F4[c]	B[d]
1R-α-Pinene	18.4	39.3	2.0	-
β-Pinene	1.9	6.3	1.4	-
β-Myrcene	1.8	1.9	1.1	-
d-Limonene	14.4	0.2	0.67	-
1,8-cineole	47.9	40.4	86.1	0.4
Linalool	1.2	0.1	1.0	-
Terpinene-4-ol	2.1	-	-	2.9
α-Terpineol	0.8	-	-	22.6
Caryophyllene	1.8	-	-	0.3
γ-Eudesmol	3.4	-	-	21.3
β-Eudesmol	2.6	-	-	20.5
α-Eudesmol	1.1	-	-	3.2

[b]Second fraction, [c]Fourth fraction, [d]Base remaining after distillation, [a, b, c, d]Percentage area in GC-MS analysis

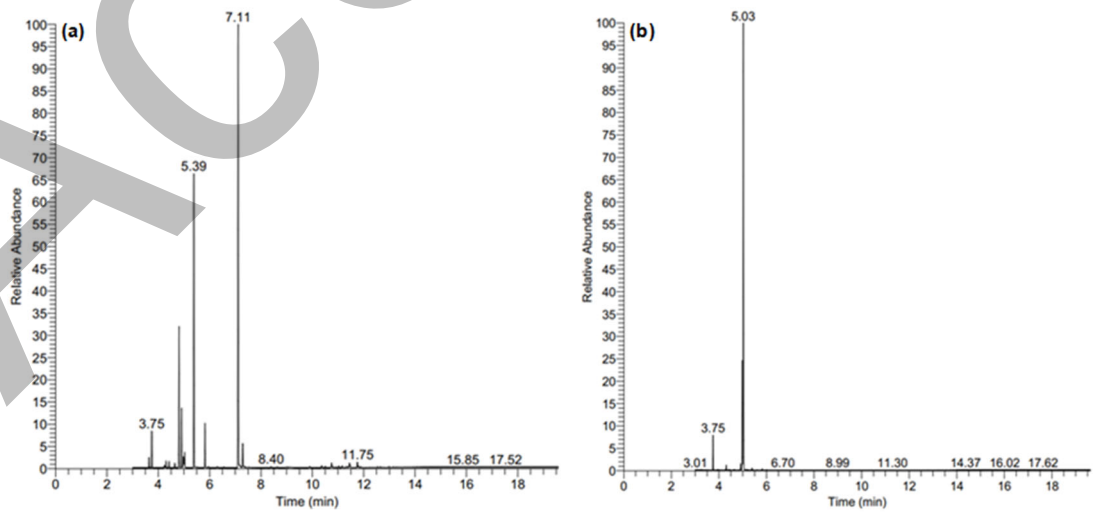

Fig 11. *E. camaldulensis* GC-MS chromatogram for (a) raw essential oil and (b) 4th fraction

revealed the absence of these compounds, highlighting the effectiveness of the fractional distillation process in purifying 1,8-cineole. The elimination of impurities in Fig. 11(b) not only increased the purity of the product but also confirmed the efficiency of the fractional distillation technique utilized. Chauhan et al. [25] demonstrated that the fractional vacuum distillation process effectively separated 1,8-cineole from *E. hybrida* essential oil, achieving a purity of 90% in the B fraction compared to the A fraction, where other compounds like α-pinene and β-pinene were predominantly present. This finding supports our research, which similarly shows that vacuum fractional distillation significantly improves the purity of target compounds in essential oils.

■ CONCLUSION

The 500 mm Hempel column, packed with 2 mm × 10 mm metal helixes and operated at 60 mmHg, achieved the highest efficiency for separating 1,8-cineole from *E. camaldulensis* essential oil. The 1,8-cineole content increased from 47.9 to 74.6%, with a recovery rate of 97% and a yield exceeding 60%. This configuration achieved the most excellent purity and recovery of 1,8-cineole, with the main fraction collected at a top temperature of 82.5 °C. The study demonstrates the significant influence of vacuum pressure, column configuration, packing material, and column height on the yield and purity of 1,8-cineole. The optimized distillation conditions identified here provide a scalable approach for industrial applications, particularly in the pharmaceutical, food, and cosmetic industries, where the efficient production of high-purity cineole is essential.

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■ CONFLICT OF INTEREST

We declare that there are no conflicts of interest regarding the publication of this paper.

■ AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to this research and have approved the final version of the manuscript. Tien Xuan Le provided funding, managed the project, and finalized the manuscript. Minh Nhat Nguyen conducted the experiments. Trung Minh Le drafted the initial manuscript and Minh Chau Vu Pham revised and completed the paper.

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