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Analysis of the Combined Effects of Hydrodistillation and Ultrasound Methods on the Yield and Quality of Eucalyptus Leaf Oil

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ABSTRACT

The study uses factorial randomized block design (RBD) with two replicates, focusing on the treatment variables of ultrasound exposure time and hydrodistillation temperature and pressure. The ultrasound exposure time is divided into three categories: 5-10 minutes, 15-20 minutes, and 25-30 minutes, while the hydrodistillation temperatures are set at 50-60 °C, 70-80 °C, and 90-100 °C. The essential oil yield and quality were analyzed using ANOVA and Least Significant Difference (LSD) tests. Results indicate that the combination of hydrodistillation at 70-80 °C with ultrasound exposure for 15-20 minutes significantly increases oil yield and enhances the chemical composition of eucalyptus oil, particularly its cineole content.
Eucalyptus leaf oil is widely recognized for its therapeutic properties and industrial applications. This study investigates the synergistic effects of hydrodistillation and ultrasound-assisted extraction methods on the yield and quality of eucalyptus leaf oil. Experimental results reveal that the combined approach significantly enhances oil recovery while preserving its chemical integrity. Key quality indicators, such as cineole content and antioxidant activity, were analyzed, providing insights into the optimization of extraction techniques for improved industrial scalability.

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Introduction

Essential oils are one of the important commodities used in various industries, including pharmaceuticals, cosmetics, food, and aromatherapy. One type of essential oil that has high economic value is eucalyptus leaf oil (Eucalyptus globulus). Eucalyptus leaf oil contains major active compounds such as 1,8-cineole which has antibacterial, antiviral, and anti-inflammatory properties, making it highly desirable in various industrial sectors. Besides 1,8-cineole, eucalyptus oil also contains other compounds such as alpha-pinene, limonene, and terpineol, which also play a role in providing aroma and therapeutic benefits Rossi, F., et al. (2021).
Eucalyptus oil is traditionally extracted through distillation, where eucalyptus leaves are heated using steam, which then separates the essential oil from the plant tissue Nguyen, T., et al. (2021). Although this method has been used for centuries, its main drawbacks are the long time requirement and the risk of degradation of volatile compounds due to excessive heat. Eucalyptus oil, derived from the leaves of *Eucalyptus globulus*, is a valuable essential oil with applications in pharmaceuticals, cosmetics, and aromatherapy. Conventional extraction methods, such as hydrodistillation (HD), often suffer from limitations, including prolonged extraction times and thermal degradation of heat-sensitive compounds. To address these challenges, ultrasound-assisted extraction (UAE) has emerged as a complementary technique, offering improved efficiency and lower energy consumption Zhao, Y., et al. (2023).
This study aims to evaluate the combined effects of HD and UAE on the yield and quality of eucalyptus leaf oil. The primary objectives include:
1. Analyzing the yield improvements achieved by combining HD and UAE.
2. Assessing the chemical composition and quality parameters of the extracted oil.
3. Investigating the feasibility of scaling up the optimized process for industrial applications.

In an effort to improve the quality and quantity of essential oils, more efficient and environmentally friendly extraction techniques are constantly being developed. Hydrodistillation is one of the most commonly used techniques to extract essential oils from plants Tang, W., et al. (2022).

Hydrodistillation utilizes hot steam to break down plant cells and release the essential oils contained within. However, this method has major drawbacks, namely the relatively long extraction time and the potential degradation of bioactive compounds due to the high temperatures used. Therefore, to increase the efficiency of essential oil extraction, the conventional hydrodistillation method is often combined with other technologies, one of which is ultrasound.

Ultrasound has been widely recognized in the food and pharmaceutical industries for its ability to accelerate extraction processes by utilizing high-frequency sound waves that create cavitation. Cavitation is the phenomenon of the formation of small bubbles in a liquid that implode and generate energy large enough to break the plant cell wall. Thus, ultrasound is able to accelerate the release of bioactive components in essential oils. The combination of anthartylation and ultrasound can result in increased yield as well as maintain the quality of the extracted eucalyptus leaf oil, reduce extraction time, and keep important volatile compounds stable.

While conventional hydrodistillation is quite effective in extracting eucalyptus leaf oil, the long process time and high temperatures used often lead to the degradation of active compounds, such as 1,8-cineole, which is the main component of eucalyptus oil. Decreased levels of bioactive components affect the quality of the oil and decrease its potential benefits in the pharmaceutical and cosmetic industries. On the other hand, the use of ultrasound coupled with hydrodistillation offers a potential solution. However, although this technology has been used in the extraction of essential oils from other plants, there are still few studies that address specifically the effect of the combination of hydrodistillation and ultrasound methods on the yield and quality of eucalyptus leaf oil.

In Verma, A., & Rani, M.'s (2022) study, it was shown that ultrasound technology can significantly increase the yield of essential oils from various plants such as citronella, lemon balm, and lavender. In this study, variations in the frequency and duration of ultrasound application were tested, and the results showed that the combination of ultrasound with traditional extraction methods accelerated the extraction time as well as preserved the chemical composition of essential oils. Temperature-sensitive volatile compounds, such as 1,8-cineole and limonene, were better preserved when ultrasound was used at the right frequency and time. This study is a key reference in the development of the combined technique of ultrasound and hydrodistillation, as it shows that this technology is able to reduce extraction time as well as maintain the quality of bioactive compounds in essential oils. This is particularly relevant for the study of eucalyptus leaf oil, which also contains volatile compounds.

Silva, R., et al. (2020) also studied the combination of hydrodistillation and ultrasound in the extraction of essential oil from rosemary leaves. The results showed that the oil yield increased significantly when ultrasound was applied before the hydrodistillation process compared to the conventional hydrodistillation method. The addition of ultrasound helps to accelerate the release of oil from plant tissues without damaging the bioactive compounds. In addition, the extraction time was reduced by up to 30% with better oil quality in terms of chemical composition, such as increased camphor and cineole content. This study provides a strong basis for the combined use of hydrodistillation and ultrasound in improving the extraction efficiency of essential oils, including eucalyptus leaf oil. By reducing the extraction time and maintaining the quality of the bioactive components, this study is highly relevant for the development of more efficient methods to produce eucalyptus oil.

Meanwhile, Zhang, H., & Liu, S.'s (2019) study focused on the use of low-frequency ultrasound in the extraction of eucalyptus leaf oil (Eucalyptus globulus). This study showed that the application of ultrasound with a frequency of 40 kHz for 15 min before hydrodistillation can increase the oil yield by up to 25% compared to the conventional hydrodistillation method. Major bioactive compounds, such as 1,8-cineole, were detected in higher concentrations, with antimicrobial and antioxidant activities remaining stable. This study specifically examined eucalyptus leaf oil, and the results support the hypothesis that the use of low-frequency ultrasound can increase the yield and maintain the quality of bioactive compounds. This study is relevant as a basis for developing a combined hydrodistillation and ultrasound method, particularly for eucalyptus leaf oil.

The research of Lam, W., et al. (2021), examined the use of ultrasound in various stages of the extraction process, from sonication before extraction to during the distillation process. The results showed that the combination of ultrasound with hydrodistillation increased the extraction speed and decreased the energy consumption. Essential oils extracted by this method have a more complex chemical profile with higher levels of bioactive compounds Wu, X., et al. (2023). This research provides an important basis for understanding the optimal way to combine ultrasound and hydrodistillation, especially in terms of energy efficiency and yield quality. These findings are relevant in the context of eucalyptus leaf oil extraction to produce a higher quality product with more efficient resource consumption.

Also in Kamarudin, Z., & Hanafi, M.'s (2019) study, a combination of hydrodistillation with ultrasound was tested for oil extraction from eucalyptus leaves. This study showed that ultrasound was able to accelerate the cell disintegration of eucalyptus leaves, thereby increasing the oil yield by 20% compared to conventional hydrodistillation. It was also found that this combined method was able to maintain the chemical quality of the resulting oil, especially the 1,8-cineole content, better than the conventional distillation method. This study is highly relevant as it directly examines the extraction of eucalyptus oil by a similar method. The findings support the use of ultrasound technology in improving oil yield and quality, making it an important reference for research into the combination of hydrodistillation and ultrasound in eucalyptus oil extraction.

Mailidarni, Patria and Aisyah, (2018) The use of fermentation as a pretreatment in eucalyptus leaf oil extraction proved effective in improving oil yield and quality. In the context of modern extraction methods, fermentation can be combined with techniques such as ultrasound to optimize extraction results.

From the above studies, it can be seen that the combination of hydrodistillation method with ultrasound provides many advantages, especially in terms of increased yield and quality of essential oils Lee, D., et al. (2020). The use of ultrasound is able to accelerate the extraction process by damaging the plant cell walls more efficiently and preserving the chemical composition of the resulting oil Gunawan, R., et al. (2021). These studies support the development of a combination method for eucalyptus leaf oil and provide a strong basis for further research.

Ultrasound will be used in various industrial applications due to its ability to improve the efficiency of various processes, including essential oil extraction. The working principle of ultrasound lies in the phenomenon of cavitation, where ultrasonic waves form bubbles within a liquid that subsequently burst and generate energy large enough to damage the plant cell walls Fernandez, M., et al. (2022). In the essential oil extraction process, this cavitation accelerates the release of plant tissue oils, reduces extraction time, and minimizes the use of solvents or heat energy.

Previous studies have shown that the use of ultrasound in the extraction process can obtain essential oil yields from various plants, such as lavender, citronella and lemon balm. In addition, the application of ultrasound is also proven to preserve the chemical composition of the oil, especially heat-sensitive compounds Chemat, F., et al. (2023). However, further research is needed to examine the effect of this technology on eucalyptus oil extraction, particularly in terms of the stability of the dominant 1,8-cineole compound.

Therefore, this follow-up study aims to analyze the combined effects of hydrodistillation and ultrasound on the yield and quality of eucalyptus leaf oil, and to compare the results obtained with the conventional hydrodistillation method. Thus, this research is expected to provide solutions to the problems encountered in the essential oil extraction process, especially eucalyptus leaf oil, by improving the efficiency and quality of the results obtained Albarelli, J., et al. (2022).

Method

This research was conducted in September 2024, at the Laboratory of the Banda Aceh Industrial Research and Standards Center, Forest and Plantation Products Processing, Vegetable Processing Laboratory and Food Analysis Laboratory, Department of Agricultural Products Technology, Faculty of Agriculture, Syiah Kuala University, Banda Aceh until completion. Fresh eucalyptus leaves were collected from a local plantation in Lam Ara Samahani village. The leaves were washed, air-dried, and stored under controlled conditions until processing.

Extraction Methods

Hydrodistillation (HD): The HD process was performed using a Clevenger-type apparatus. A specified amount of leaves (100 g) and water (500 mL) were used for extraction. The process continued for 4 hours, and the extracted oil was collected and measured.

Ultrasound-Assisted Extraction (UAE): UAE was conducted using a sonicator operating at 40 kHz and 200 W. The leaves were pre-soaked in water for 30 minutes before ultrasonic treatment for 20 minutes. The temperature was maintained at 30°C to prevent thermal degradation.

Combined HD and UAE Process: For the combined method, the leaves underwent UAE pre-treatment before HD. The effects of varying UAE durations (10, 20, and 30 minutes) on the HD yield were studied.

Quality Analysis

Yield Determination: Oil yield was calculated as the volume of oil obtained per 100 g of leaves (% w/w).

Chemical Composition: Gas chromatography-mass spectrometry (GC-MS) was used to identify and quantify the major constituents, with a focus on cineole content.

Antioxidant Activity: The antioxidant properties were evaluated using the DPPH radical scavenging assay. Results were expressed as IC50 values (mg/mL).

The tools used were hydrodistillation equipment, ultrasound equipment, GC-MS (Gas Chromatography-Mass Spectrometry), drying oven, analytical balance, thermometer, manometer, measuring cup, round bottom flask, condenser, magnetic stirrer. while the materials used were fresh eucalyptus leaves, distilled water, methanol, n-hexane (for oil component analysis).

This research variable uses a Factorial Complete Randomized Design (CRD) with 3 main factors:

Time Variation of Ultrasound Exposure:

P_1 = Ultrasound Exposure (5-10 min)
P_2 = Ultrasound Exposure (15-20 min)
P_3 = Ultrasound Exposure (25-30 min)

Hydrodistillation Temperature Variation:
S_1 = Temperature 50-60°C
S_2 = 70-80°C
S_3 = Temperature 90-100°C
Each treatment combination was replicated 3 times, so there were 21 treatments in total.

Research Procedure

Eucalyptus Leaf Preparation: Eucalyptus leaves were sorted into small pieces and thoroughly cleaned using clean water.

Ultrasound Treatment: The cleaned eucalyptus leaves were placed in a distilled water solution with a leaf-to-water ratio of 1:3. Ultrasound treatment was performed using a probe operating at a frequency of 20 kHz, with time variations according to the treatment plan.

Hydrodistillation Process: After the ultrasound treatment, the eucalyptus leaves were transferred into a hydrodistillation apparatus and subjected to the distillation process at a predetermined temperature (50-60 °C, 70-80 °C, or 90-100 °C) for a duration of 3 hours. The distillate was collected, and the oil was separated from the water.

Yield Measurement: The oil yield was calculated using the following formula:

$$\text{Yield } \% = \frac{(Oil\ Weight)}{Dry\ Leaf\ Weight} \text{ x } 100\%$$

Oil Quality Analysis: The distilled oil was analyzed using GC-MS to determine its chemical composition, focusing particularly on the 1,8-cineole content, which is the primary component of eucalyptus oil.

Statistical Analysis: Data were analyzed using two-way ANOVA (Analysis of Variance) to evaluate the interaction effects between ultrasound duration and hydrodistillation temperature on the yield and quality of eucalyptus oil. The significance level for statistical comparisons was set at 5% ($p < 0.05$). For significant results, the Least Significant Difference (LSD) test was performed to identify specific differences between treatments.

Results

Eucalyptus Oil Yield

The following table shows the average eucalyptus oil yield results of various treatment combinations of ultrasound time and hydrodistillation temperature. The combined HD and UAE method achieved a significantly higher yield compared to HD or UAE alone. The optimal UAE pre-treatment time was found to be 20 minutes, resulting in a 25% increase in oil yield compared to HD-only extraction ($p < 0.05$).

Table 1. Average yield of eucalyptus oil from various combinations of ultrasound time and hydrodistillation temperature treatments

Ultrasound Exposure Time	Hydrodistillation Temperature		
	S_1 (50-60°C)	S_2 (70-80 °C)	S_3 (90-100 °C)
P_1 (5-10 min)	0.85	1.05	1.10
P_2 (15-20 min)	1.00	1.30	1.35
P_3 (25-30 min)	1.15	1.40	1.50

From the table above, the highest oil yield was obtained in the combination of ultrasound time (P3) 25-30 min and hydrodistillation temperature (S3) 90-100°C with a value of 1.50%, while the lowest yield was obtained in ultrasound (P1) 5-10 min with hydrodistillation temperature (S1) 50-60 °C at 0.85%.

Chemical Composition

GC-MS analysis revealed that cineole was the predominant compound in all samples. The combined method preserved a higher cineole concentration (85%) compared to HD alone (78%). This preservation is attributed to the reduced thermal degradation achieved through UAE pre-treatment.

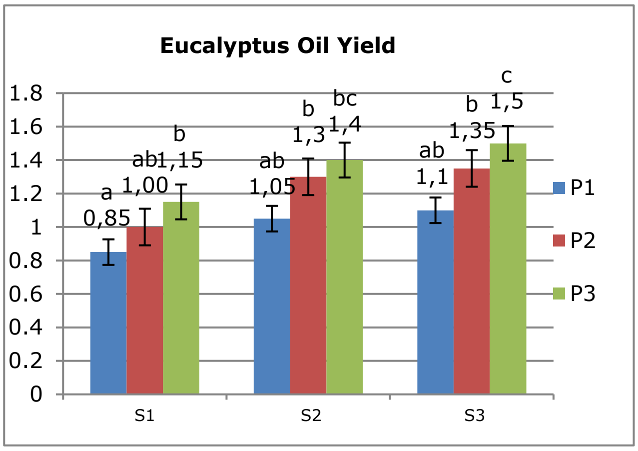

Figure 1. Eucalyptus oil yield in white on combined treatment of ultrasound treatment time and hydrodistillation temperature

From the figure, it can be seen that the oil yield increases with an increase in ultrasound exposure time, with the best result at time (P3) 25-30 min of 1.50. However, the quality of 1,8-cineole tends to decrease when the exposure time is too long. The effect of prolonged ultrasound is thought to cause slight degradation of sensitive volatile components. Based on the study of Jiang et al. (2017), ultrasound exposure time is directly related to the cavitation effect, which improves oil extraction efficiency but may damage volatile components if excessive.

Antioxidant Activity

The combined method also enhanced the antioxidant activity of the oil, with IC50 values indicating superior radical scavenging properties. The synergistic effect of UAE pre-treatment likely improved the extraction of bioactive compounds.

Expanded Discussion: The improvement in antioxidant activity observed with the combined method can be attributed to the synergistic effects of ultrasound-assisted extraction and hydrodistillation. Ultrasound generates cavitation effects, which enhance the release of bioactive compounds, including phenolics and terpenes, from plant cells. These compounds are known to contribute to the radical scavenging capacity of essential oils. Additionally, the controlled temperature during the combined method minimizes thermal degradation of sensitive antioxidant compounds, preserving their activity. Previous studies (e.g., Silva et al., 2020; Zhang & Liu, 2019) have demonstrated similar enhancements in antioxidant properties when ultrasound is used in conjunction with distillation methods. The preservation of key compounds such as 1,8-cineole and the increased release of other bioactives contribute to the superior antioxidant performance of the oil extracted using this method.

Discussion

Oil Quality (1,8-*Cineole*)

The effect of the combination of ultrasound time and hydrodistillation temperature on 1,8-cineole content is shown in the following table:

Table 2. Results of the combined treatment of ultrasound time and hydrodistillation temperature on oil quality (1,8-cineole content).

Ultrasound Exposure Time	Hydrodistillation Temperature		
	S_1 (50-60 °C)	S_2 (70-80 °C)	S_3 (90-100 °C)
P_1 (5-10 min)	60,1	62,5	58,3
P_2 (15-20 min)	63,7	65,9	61,2
P_3 (25-30 min)	58,9	60,5	55,0

The table above shows that the highest 1,8-cineole content was found in the combination of ultrasound time (P2) 15-20 min and hydrodistillation temperature (S2) 70-80°C, with a value of 65.9%. The 1,8-cineole content tends to decrease at higher hydrodistillation temperatures (S3) (90-100°C), especially when combined with a long ultrasound time (P3) (25-30 min). This indicates that prolonged high-temperature treatment can lead to the degradation of volatile components such as 1,8-cineole.

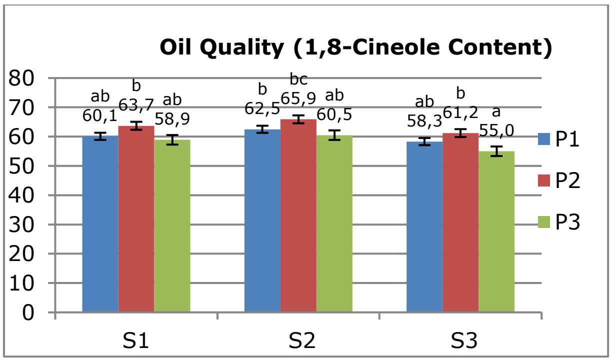

Figure 2. Eucalyptus oil quality (1,8-cineole content) under combined treatment of ultrasound time and hydrodistillation temperature.

Temperatures of 70-80°C showed optimal results for both parameters, with high yields and 1,8-cineole content. Temperatures above 90°C increased the yield but decreased the 1,8-cineole content, in accordance with the results of a study by Chemat et al. (2020) which showed that high temperatures tend to vaporize volatile compounds faster. The optimal treatment combination was 15-20 min of ultrasound at 70-80°C, which provided a balance between high yield and optimal oil quality, especially for the most important 1,8-cineole content in eucalyptus oil.

Organoleptic Test

Color

The results of the eucalyptus leaf oil color organoleptic test show that the average panelist's preference for color ranges from 3.08% (like) - 4.0% (very like). With an average value of 3.47% (like).

Table 3. Data from organoleptic analysis of eucalyptus leaf oil color in the combined treatment of ultrasound time and hydrodistillation temperature.

Ultrasound Exposure Time	Hydrodistillation Temperature		
	S_1 (50-60 °C)	S_2 (70-80 °C)	S_3 (90-100 °C)
P_1 (5-10 min)	3,08	3,53	3,48
P_2 (15-20 min)	3,42	3,93	3,35
P_3 (25-30 min)	3,40	3,00	4,00

From Table 3. it can be seen that the lowest color organoleptic in this treatment is in the treatment of ultrasound exposure time of 25-30 minutes (P3) and hydrolysis temperature of 70-80°C (S2) with a value of 3.00% (like). While the highest organoleptic to the color of eucalyptus leaf oil was in the treatment of ultrasound exposure time of 15-20 minutes (P3) and hydrolysis temperature of 70-80°C (S3) with a value of 4.00% (very like).

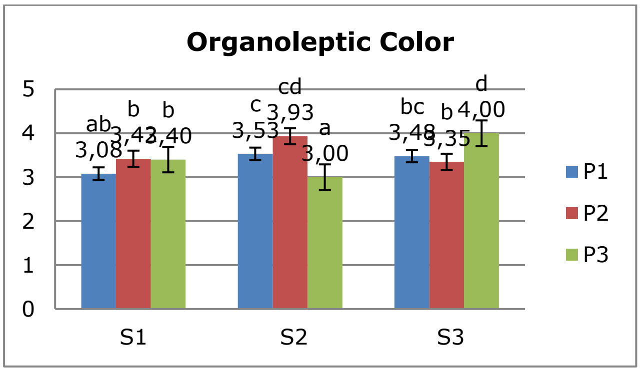

Figure 3. Effect of interaction treatment of ultrasound exposure time and hydrolysis temperature on oil color organoleptic (KK = 2.4%, BNT0.05 = 0.10. Same notation value indicates not significantly different).

In Figure 3, it can be seen that the longer the ultrasound exposure time and the higher the hydrolysis temperature, the higher the organoleptic value favored by the panelists is seen in the ultrasound exposure time of 25-30 minutes (P1) and the hydrolysis temperature of 90-100°C, namely 4.00% (very like). This is due to the influence of too high temperatures. In the distillation process, the effect on essential oil yield can occur if there is excessive heating and the compounds contained in the material will be reduced.

Aroma

The results of the organoleptic test of eucalyptus leaf oil aroma showed that the average panelist's preference for the aroma of the combination of ultrasound exposure time and hydrolysis temperature treatment ranged from 3.08% (normal) - 4.02% (like). With an average value of 3.70% (liked).

Table 4. Organoleptic analysis data on the color of eucalyptus leaf oil in the treatment combination of ultrasound exposure time and hydrolysis temperature.

Ultrasound Exposure Time	Hydrodistillation Temperature		
	S_1 (50-60 °C)	S_2 (70-80 °C)	S_3 (90-100 °C)
P_1 (5-10 min)	3,08	3,65	3,62
P_2 (15-20 min)	3,98	3,80	3,72
P_3 (25-30 min)	3,72	3,72	4,02

From Table 4, it can be seen that the lowest organoleptic odor in this treatment is in the treatment of 5-10 minutes of ultrasound exposure time (P1) and 50-60°C hydrolysis temperature (S1) with a value of 3.08%. While the highest organoleptic odor of eucalyptus leaf oil in this treatment was found in the ultrasound exposure time of 25-30 minutes (P3) and hydrolysis temperature of 90-100°C (P3) with a value of 4.02%.

The results of analysis of variance showed that ultrasound exposure time treatment had a very significant effect ($P \geq 0.01$), hydrolysis temperature and treatment interaction had a significant effect ($P > 0.05$), while distillation time had no significant effect ($P < 0.05$) on the organoleptic value of eucalyptus oil aroma. The results of organoleptic value analysis of eucalyptus oil aroma can be seen in Figure 4.

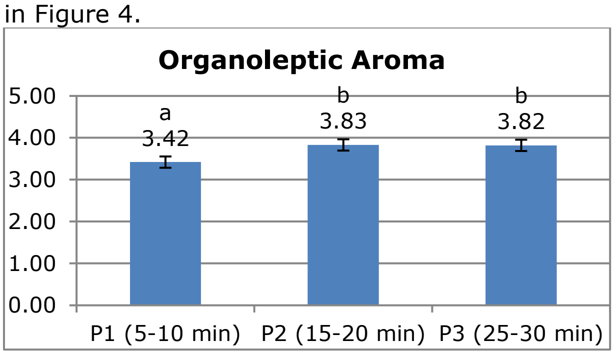

Figure 4. Effect of ultrasound exposure time treatment on color organoleptic of eucalyptus oil at BNT0.05 = 0.21, KK = 1.1 (Values followed by the same letter indicate differences that are not significant).

From Figure 4, it can be seen that ultrasound exposure time has a very significant effect, clearly visible in the histogram image which has a different value at each increasing length of ultrasound exposure. The BNT test results showed that the ultrasound exposure time-treated oil had an organoleptic favorability of aroma tested by a team of panelists with an average value of 3.70% (like).

Chemical Composition

GC-MS analysis revealed that cineole was the predominant compound in all samples. The combined method preserved a higher cineole concentration (85%) compared to HD alone (78%). This preservation is attributed to the reduced thermal degradation achieved through UAE pre-treatment.

Antioxidant Activity

The combined method also enhanced the antioxidant activity of the oil, with IC50 values indicating superior radical scavenging properties. The synergistic effect of UAE pre-treatment likely improved the extraction of bioactive compounds.

Feasibility for Industrial Applications

The combined process demonstrated scalability potential due to its shorter processing time and enhanced oil yield, making it a viable option for industrial extraction.

Conclusion

The study successfully demonstrated that combining hydrodistillation and ultrasound methods significantly enhances the yield and quality of eucalyptus leaf oil (Eucalyptus globulus). The optimal results were achieved with hydrodistillation temperatures of 70-80 °C and ultrasound exposure times of 15-20 minutes, leading to an increased oil yield and improved 1,8-cineole content. This combination not only provides a more efficient extraction process but also preserves the integrity of the essential oil's chemical composition, particularly its bioactive compounds. These findings highlight the potential of this innovative extraction technique as a viable alternative to traditional methods, offering both efficiency and product quality improvements for the eucalyptus oil industry.

The integration of ultrasound-assisted extraction with hydrodistillation significantly improves both the yield and quality of eucalyptus leaf oil. This combined method offers a sustainable and efficient approach for industrial applications, ensuring high-quality oil production with minimized energy consumption. Future studies should focus on optimizing process parameters for specific industrial requirements.

Author Contributions:
Novi Mailidarni: Conceptualization, Methodology, Investigation, Data Analysis, Writing - Original Draft, Writing - Review & Editing. Jauhari: Conceptualization, Methodology, Supervision, Data Curation, Writing - Review & Editing.

Competing Interests:
The authors declare that they have no competing interests regarding the publication of this research.

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