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Research Article

Extraction of Basil Leaves Essential Oil using Microwave Assisted Hydrodistillation Method: Physical Characterization and Antibacterial Activity

Ekstraksi Minyak Atsiri Daun Kemangi menggunakan Metode Microwave Assisted Hydrodistillation: Uji Karakteristik Fisik dan Aktivitas Antibakteri

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Abstract

Basil oil can be obtained from basil leaves by non-extraction methods, namely Microwave Assisted Hydrodistillation (MAHD). Therefore, this research aims to determine the yield percentage, essential oil composition by GC-MS, physical characteristics, and antibacterial activity of basil essential oil. The highest yield of 0.3076% was obtained at the optimum condition, which included a microwave power of 300 W, a mass-to-volume solvent ratio (F/S) of 0.75 g/mL, a raw material size of ± 1.75 cm, and an extraction time of 90 min. The results of the analysis of variance showed that all process parameters used had a significant effect on the yield obtained. Basil oil exhibited a larger inhibition zone against *Escherichia coli* bacteria (16.38 mm) which tended to be stronger than *Staphylococcus aureus* (5.95 mm) and was classified as moderate. The main components contained in the basil oil were E-Citral (46.79%) and Z-Citral (38.17%). The physical characteristic test showed that the basil oil was soluble in 96% ethanol after a ratio of 1:9, with 1 ml of basil oil compared to 9 ml of ethanol. The density of basil oil at 0.961 g/mL also complied with the standard value according to the Essential Oil Association (EOA) of *Ocimum basilicum* Essential Oil. These results showed revealed that the parameter analyzed using oil yields at operating conditions produced the most optimum yield value.

Keywords: antibacterial; Basil oil; MAHD; physical characteristics test

Abstrak

Minyak kemangi dapat diperoleh dari daun kemangi dengan metode ekstraksi non-konvensional yaitu Microwave Assisted Hydrodistilation (MAHD). Tujuan penelitian ini adalah untuk mengetahui persentase rendemen, komposisi minyak kemangi dengan GC-MS, uji karakteristik fisik, dan aktivitas antibakteri pada minyak kemangi. Rendemen tertinggi 0,3076% diperoleh pada daya 300 W, waktu 90 menit, ukuran daun ± 1,75 cm, serta rasio F/S 0,75 g/mL. Hasil analisis varian menyatakan bahwa semua parameter proses yang digunakan memiliki pengaruh yang signifikan terhadap rendemen yang dihasilkan. Minyak kemangi memiliki zona hambat lebih besar terhadap bakteri Escherichia coli (16,38 mm) yang cenderung lebih kuat dibandingkan Staphylococcus aureus (5,95 mm) yang tergolong sedang. Komponen utama yang terkandung dalam minyak kemangi yaitu E-Citral (46,79%) dan Z-Citral (38,17%). Uji karakteristik fisik menunjukkan bahwa minyak kemangi larut dengan etanol 96% saat perbandingan 1:9 yaitu 1 ml minyak kemangi dibandingkan 9 ml etanol. Densitas minyak kemangi 0,961 g/mL telah sesuai dengan standar nilai menurut Essential Oil Association (EOA) of Ocimum basilicum Essential Oil). Parameter analisis menggunakan hasil minyak pada kondisi operasi dengan nilai rendemen paling optimum.

***Kata kunci**: antibakteri; MAHD; minyak kemangi; uji karakteristik fisik*

1. Introduction

Basil is an indigenous vegetable that is quite dense, with a height of 100 cm [1] and is widely available across Indonesia, particularly in Jember, East Java. This vegetable belongs to the Ocimum genus, *Ocimum basilicum* L species, Magnoliopsida class, Lamiales orde, and Spermatophyta subdivision. Furthermore, basil is one of the indigenous products recognized by the Directorate General of Horticulture, according to the Decree of the Minister of Agriculture of Indonesia Number 511/Kpts/PD.310/9/2006.

Basil leaves contain phenolic compounds, saponins, flavonoids, and essential oils. In the health sector, basil can be used as an antipyretic, analgesic, antifungal, antiseptic, hepatoprotector, and immunomodulator [2]. The composition of basil oil consists of alcohol compounds, phenol (1–19% eugenol, iso-eugenol), oxides, phenolic ethers (3–31% methyl clavicol, 1-9% methyl eugenol), hydrocarbons, ketones, and esters [3]. Furthermore, the main component is the aldehyde group, citral, which acts as an antibacterial pathogen [4].

Due to its antibacterial properties, basil oil has the potential to be developed as a source of active ingredients for antibacterial products [5]. Pathogenic bacteria such as *Staphylococcus aureus*, which is a gram-positive bacteria, and *Escherichia coli*, a gram-negative, usually attack humans [6]. Therefore, an antibacterial activity test is conducted to determine the various kinds of antibacterial activities contained in basil oil.

The selection of the appropriate solvent for the extraction process is very important. In this research, water is used as the solvent because it has a high dielectric constant, which optimizes microwave absorption [7]. The use of water as a solvent has the benefit of being a "green solvent" because it is relatively cheap, environmentally friendly, non-flammable, non-toxic, and allows for clean processing and pollution prevention [8].

The application of the "green technique" in essential oil extraction becomes more effective to overcome the drawbacks of conventional extraction methods, such as the long extraction time and increased energy consumption. The Microwave Assisted Hydrodistillation (MAHD) method is more effective because the process is short, produces a high yield, and minimizes the use of solvents [9]. According to Definiujemy [10], the use of MAHD with fresh basil and a 1 cm cut, 500 W power, 1000 ml aquadest volume, and a 5-7 hours time span produced a yield of 0.2948%. This research aims to conduct antibacterial activity tests, process variable differences, GC-MS analysis, and optimization using the Response Surface Methodology (RSM). Microwaves are used to heat and evaporate water from cells, causing the cells to swell, stretch, burst, and release metabolic components for solvent extraction. [11].

The extraction of basil leaves using a MAHD method was optimized with Box-Behnken Design (BBD) - Response Surface Methodology (RSM). The RSM was used to minimize costs, shorten the number of runs, optimize response, reduce research time, and as an improvement from previous research [12]. This technique can also be used to evaluate the relative significance of several factors influencing complex interactions between independent variables [13].

2. Research Methods

2.1 Tools and Materials

This research used several pieces of equipment, namely the Electrolux EMM-2007X type microwave, 20 L, 220 V, maximum power 800 W, wave frequency 2450 MHz, 1000 mL round bottom flask, modified clavenger (Figure 1), and condenser. The PTFE-coated microwave cavity measures 46.1 x 28 x 37.3 cm. The materials used were water, 96% ethanol, and fresh basil (*O. basilicum* L.) leaves with a water content of 85.8% obtained from Tanjung Market, Jember, East Java, Indonesia.

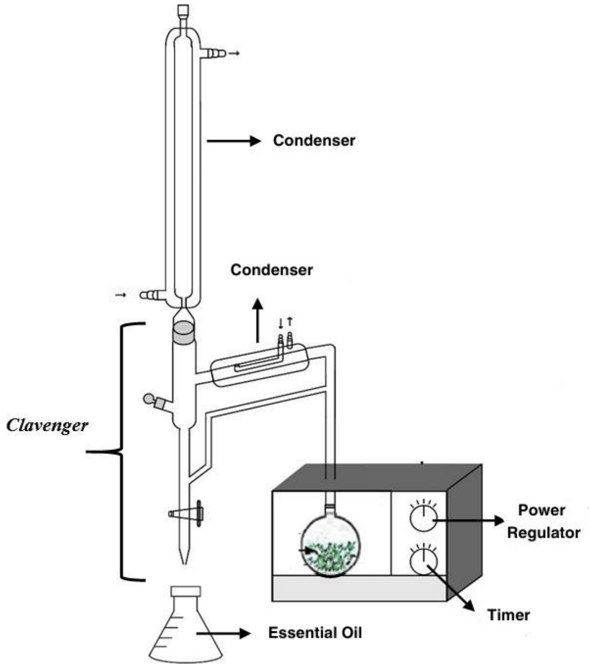


Figure 1. Modified *Clavenger* [14]

2.2 Essential Oil Extraction Process

Fresh basil leaves were weighed using an analytical balance and cut according to their sizes of 0.5 cm, 1.75 cm, and 3 cm. The feed-to-solvent (F/S) ratio used was 0.25, 0.5, and 0.75 g/mL with a solvent volume of 200 mL. The extraction process was operated at a power of 150, 300, and 450 W for 30, 60, and 90 minutes, respectively. The extract was separated using a separatory funnel on the clavenger and put into a 10 mL vial.

2.3 Measurement of Essential Oil Yield

Yield is the ratio of the mass of oil produced to the mass of the raw material used (equation 1). The higher the yield obtained, the more basil oil is produced [15].

$$Yield = \frac{mass\ of\ essential\ oil}{mass\ of\ raw\ material}\ x\ 100 \quad \text{.......(1)}$$

2.4 Physical Properties Analysis

The analysis of physical properties was carried out by analyzing the density of the sample using a pycnometer (equation 2).

$$\rho = \frac{m}{v} \quad \text{.................(2)}$$

The second analysis was the evaluation of solubility in alcohol, which is carried out by dripping ethanol on the sample.

2.5 Essential Oil Composition Analysis

The GC-MS (Gas Chromatography and Mass Spectroscopy) test was used to determine the chemical content of basil oil. The compounds contained in the basil oil mixture were separated in the chromatography column. The advantages of this method included fast identification time, high sensitivity, good separation, and long-term use of tools [16].

2.6 Antibacterial Activity Test

The method used in the antibacterial test was agar diffusion [17]. In this method, the basil extract was placed in a petri dish, each of which had been added with *E. coli* ATCC 25933 and *S. aureus* ATCC 25923. Subsequently, the petri dish was placed in an incubator for 24 hours at 36°C, and antibacterial activity was observed based on the zone of inhibition (halo diameter).

2.7 Statistic Analysis

The optimization analysis used the RSM method, aided by the Design Expert 13 application. The RSM model used was the Box-Behnken Design with four process parameters, namely F/S ratio, microwave power, extraction time, and leaf size. A total of 29 experiments were carried out and the effect of the extraction parameters used on the extract yield was observed using analysis of variance (ANOVA) [18].

3. Results and Discussion

3.1 Effect of Process Parameters on Basil Oil Yield

3.1.1 Effect of Microwave Power and Time on the Yield of Basil Oil

Microwave power in the extraction process functioned as a controller for the capacity of heat energy to be received by the material. The greater the power used, the higher the system temperature during extraction, thereby reducing the time needed to reach the boiling point of water [19]. In the research (Figure 2), using an F/S ratio of 0.75 g/mL, leaf size 1.75 cm, power 300 W, and time 90 minutes, produced a yield of 0.3076%. Meanwhile, with an F/S ratio of 0.75 g/mL, leaf size 1.75 cm, power 450 W, and time of 60 minutes, a yield of 0.247% was obtained. This indicated that longer extraction time and moderate microwave power produced higher yields [20]. This showed that the higher the power used, the greater the yield of oil obtained. However, the thermal degradation of the basil oil components and the materials used caused a decrease in the yield from 300 W to 450 W. An increase in extraction temperature also occurred at high power, which reduced solvent effectiveness in the absorption of the essential oil compounds in basil [21]. The time parameters used range from 30 to 90 min, showing an increase in yield gain. The best yield was obtained at an F/S ratio of 0.75 g/mL, leaf size 1.75 cm, 300 W power, and 90 minutes of 0.3076%. This occurred because the longer extraction time caused the cell walls of basil leaves to break down, taking a long time for the solvent to extract the compound content in the leaves [22].

3.1.2 Effect of F/S Ratio and Leaf Size on Basil Oil Yield

The F/S ratio was one of the important parameters in the extraction process. In this research (Figure 3), the basil oil extraction process that the greater mass of the material used produced a higher yield obtained. The use of an F/S ratio of 0.75 g/mL, leaf size 1.75 cm, power 300 W, and time of 90 minutes, obtained a yield of 0.3076%. Meanwhile, with an F/S ratio of 0.5 g/mL, leaf size 3 cm, power 300 W, and time 90 minutes, a yield of 0.237% was obtained. This showed that a greater F/S ratio and smaller leaf size produced a higher yield. In a previous research, a greater mass of the material, oil yield, and smaller leaf size produced a higher leaf surface area compared to the whole size [23].

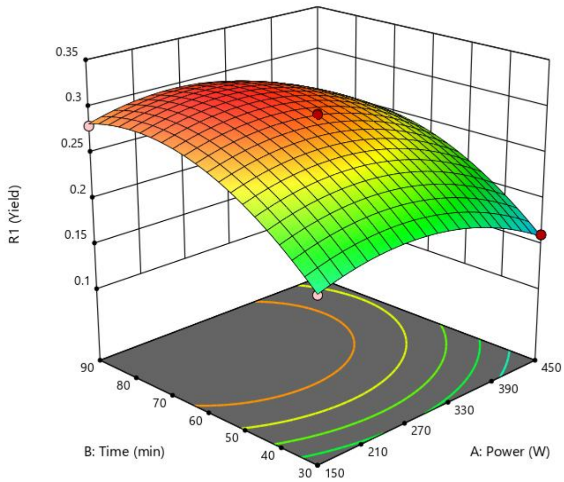


Figure 2. Effect of power and time on the yield of basil oil

3.1.3 Effect of Time and F/S Ratio on Basil Oil Yield

The parameters of time and F/S ratio showed a significant effect on the yield of basil oil produced. Increasing the extraction time also improved the extraction yield, while a higher F/S ratio produced more basil oil. The effect of time and ratio was proven by increasing the yield from 0.29% at an F/S ratio of 0.5 g/mL, with a time of 60 minutes to 0.3076% at an F/S ratio of 0.75 g/mL at 90 minutes, with all other parameters being kept constant. These values were the highest yield obtained from the experimental results (Figure 4). The ratio used was related to the density of the raw material when included in the distiller flask for optimal oil extraction and evaporation processes [14].

3.1.4 Effect of Time and Leaf Size on Basil Oil Yield

The content of essential oils in plants was found in the sac vessels, glandular hairs, and oil glands [5]. In this research, the variation in leaf size was carried out to open the oil glands and facilitate the evaporation of essential oils. Therefore, as the leaf size became smaller, the surface area increased, making it easier to come into contact with moisture [24]. The effect of time and leaf size (Figure 5) was proven by decreasing the yield at 90 minutes with a leaf size of ± 1.75 cm from 0.3076% to 0.2525% obtained at 60 minutes with a leaf size of ± 0.5 cm using the parameters and processes. This was caused by the shorter time and the leaf enumeration factor, which was excessively small. Factors that caused a decrease in this yield were the release of oil during the enumeration process, oil left in the equipment, and the evaporation process [25]. Therefore, the longer the extraction time and leaf size, the greater the yield of basil oil.

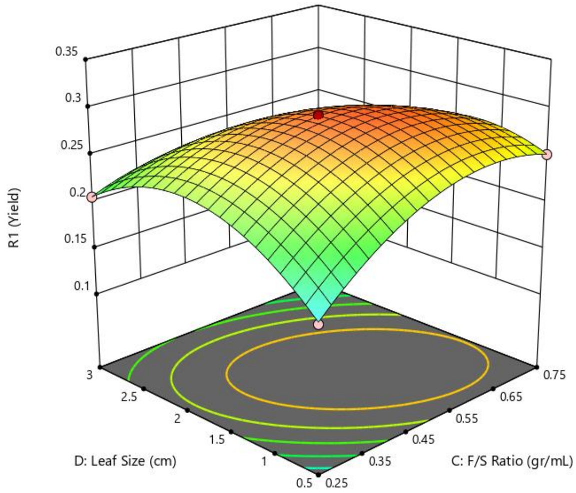


Figure 3. Effect of F/S ratio and leaf size on basil oil yield

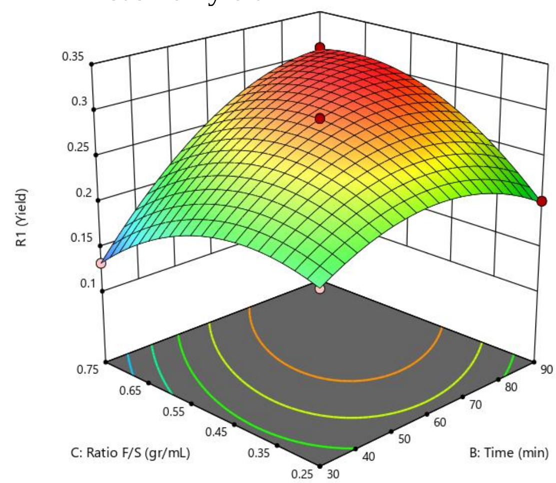


Figure 4. Effect of time and F/S ratio on basil oil yield

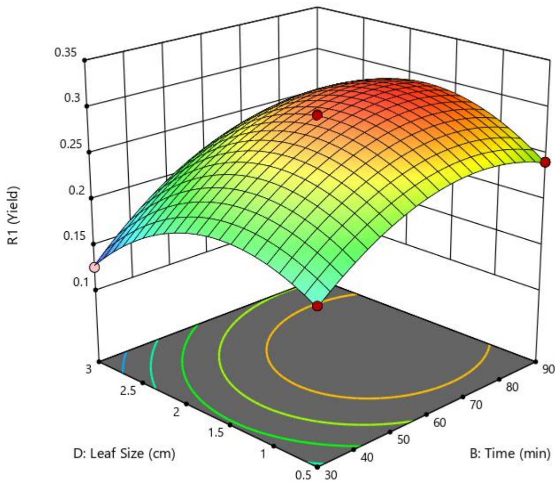


Figure 5. Effect of time and leaf size on basil oil yield

3.2 Basil Oil Analysis

3.2.1 Basil Oil Yield

The process of extracting basil oil using the MAHD method produced the smallest and largest yield of 0.1257% and 0.3076%, respectively, with clear yellow oil color. The diversity of yield values obtained was based on the existence of several different treatments between process variables. However, the variation in the yield of basil oil showed a significant difference. Table 1 showed a comparison of the largest and smallest basil oil yields with different parameters.

Table 1. The yield of the largest and smallest basil oil

Power (W)	Time (min)	F/S Ratio (g/mL)	Leaf Size (cm)	Yield (%)
300	30	0.5	± 3	0.1257
300	90	0.75	± 1.75	0.3076

3.2.2 GC-MS Analysis

The components contained in basil oil were identified by Gas Chromatography–Mass Spectrometry (GC–MS) analysis. The results of basil oil analysis at 300 W, 90 minutes, 1.75 cm leaf size, and 0.75 g/mL F/S ratio were shown in Table 3, indicating that the basil oil contained 20 chemical constituents.

Based on GC-MS analysis (Figure 6), the components contained in basil oil consisted of five groups of compounds, namely monoterpenes 0.34%, sesquiterpenes 6.9%, oxygenated monoterpenes 91.56%, oxygenated sesquiterpenes 0.69%, and other oxygenated compounds 0.52%.

Oxygenated compounds had more influence on the aroma of essential oils than other components. The highest levels of basil oil were obtained in E-citral at 46.79% and Z-citral at 38.17%, which were the constituent of the aldehyde group of citral. According to Putri and Rahmawati [24], the majority of the composition of the basil oil contained citral compounds, including E-Citral at 33.7 and Z-Citral at 27.9%.

3.2.3 Antibacterial Activity

The inhibition activity of bacteria was determined by measuring the diameter of the inhibition zone formed. Based on the inhibition activity criteria of Lingga et al [26], inhibition zones formed between ≥ 20 mm were considered to have very strong inhibition, 10-20 mm as strong, 5-10 mm as moderate, and ≤ 5 mm as weak.

Table 2. Bacterial inhibition zone

Isolate	Inhibition Zone (mm)
E. coli	16.38
S. aureus	5.95

The inhibition zone values (Table 2) showed that the basil oil had a larger inhibition zone against *E. coli* bacteria, namely 16.38 mm. This indicated a stronger effect compared to *S. aureus*, which was only 5.95 mm and was classified as moderate. In this research, citral content was the main component of basil oil, which acted as a pathogenic antibacterial, thereby inhibiting the test bacteria. Aldehyde compounds can activate proteins by forming covalent cross-links with groups of functional organic matter in proteins [27].

Basil oil also contained flavonoids and phenolic compounds, which have different mechanisms of action against bacteria. The flavonoid compounds can damage bacterial membranes by forming complex compounds that dissolve with extracellular proteins. Meanwhile, phenol compounds can break peptidoglycan bonds when passing through the cell wall [28]. The variation in their mechanism also occurred due to differences in the sensitivity of the bacteria used.

E. coli bacteria are gram-negative, while *S. aureus* bacteria are gram-positive. Gram-negative bacteria have a hydrophilic side, namely amino acids, hydroxyls, and carboxyls, which caused them to be sensitive to polar compounds [29]. Hydrophilic molecules such as flavonoids and alkaloids more easily pass through lipopolysaccharides compared to hydrophobic [5]. Therefore, the use of water as a polar solvent for extraction caused *E. coli* bacteria to have a larger inhibition zone diameter than *S. aureus*.

In gram-negative bacteria, the peptidoglycan layer on the cell membrane was thinner compared to gram-positive bacteria. The outer membrane of gram-negative bacteria was composed of phospholipids and lipopolysaccharides for antibacterial substances that interfered with the integrity of the cell membrane to easily affect gram-negative bacteria by dissolving the phospholipids. Phospholipids break down into glycerol, carboxylic acids, and phosphoric acids, causing the membrane to lose its shape and allowing uncontrollable entrance and exit of the cell, leading to disruption of metabolism by bacterial lysis [30].

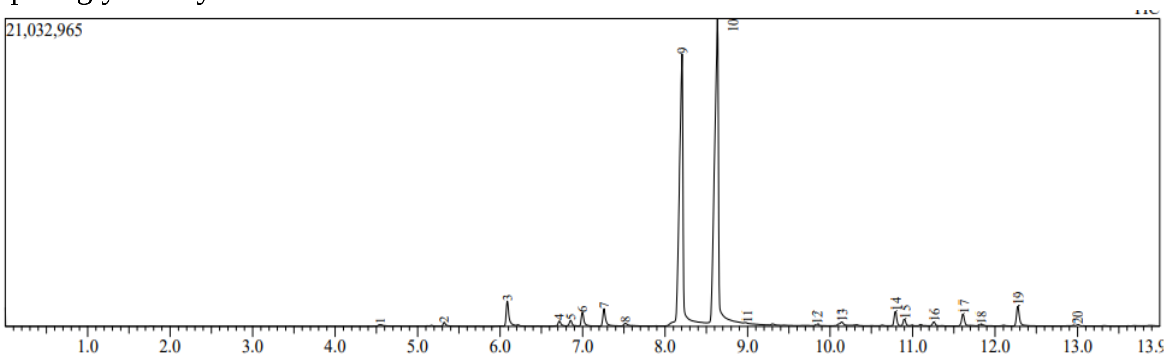


Figure 6. GC-MS analysis result

Table 3. GC-MS test result

Peak	Compound	Class	Retention Time	% Area
1	Methyl heptanone	OOM	4.548	0.18
2	β-Ocimene	M	5.322	0.34
3	LINALOOL-L	OM	6.087	2.67
4	Geranial	OM	6.718	0.50
5	Cyclohexene	S	6.854	0.54
6	Isogeraniol	OM	6.997	1.33
7	α-Sicositral	OM	7.259	1.77
8	α-Terpineol	OM	7.517	0.33
9	Z-Citral	OM	8.204	38.17
10	E-Citral	OM	8.635	46.79
11	trans-Myrtanyl acetate	OOC	8.985	0.15
12	Neryl acetate	OOC	9.839	0.19
13	β-Bisabolene	OS	10.140	0.55
14	trans-Caryophyllene	S	10.793	1.47
15	α-Bergamotene	S	10.902	0.67
16	α-Caryophyllen	S	11.258	0.42
17	Germacrene-D	S	11.610	1.26
18	Bicyclogermacrene	S	11.818	0.27
19	α-Humulene	S	12.275	2.27
20	(-)-Caryophyllene oxide	OS	12.994	0.14

Gram-positive bacteria have a thicker peptidoglycan layer than gram-negative bacteria, which results in lower cell wall permeability. The sensitivity of bacteria to antibiotics also depends on differences in the arrangement of the cell wall. Therefore, it is more difficult for the active substance of essential oils to penetrate the cell membrane of gram-positive bacteria, leading to a less optimal antibacterial effect [31].

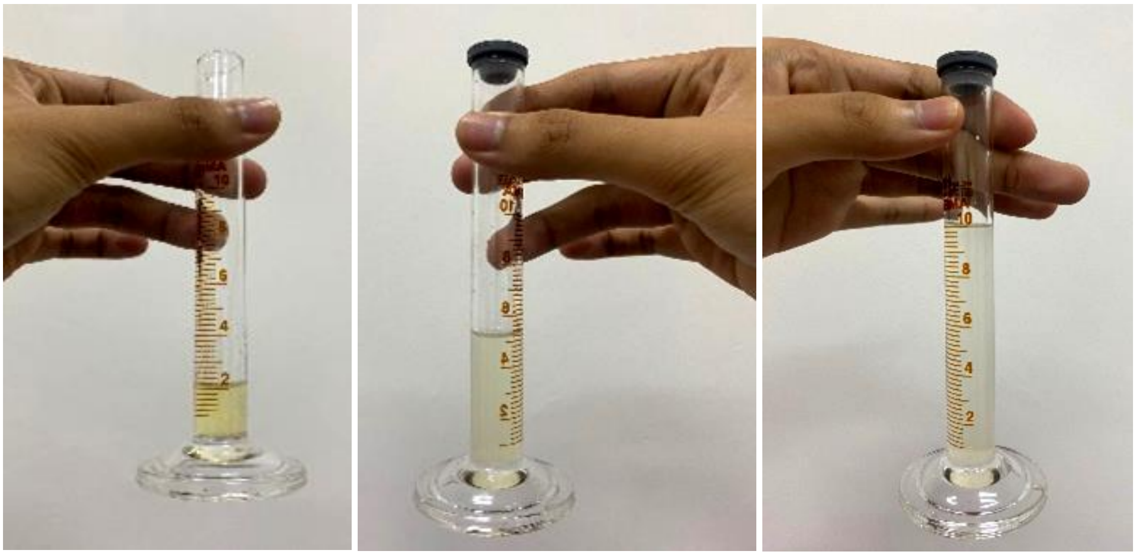
3.2.4 Physical Properties Analysis

Analysis of physical properties was carried out through visual observation or testing. According to the Essential Oil Association of *O. basilicum* Essential Oil, the average density of basil oil ranged from 0.952 to 0.973 g/mL. From the calculation results, the density of basil oil was 0.961 g/mL, which was in line with the standard value.

The thermogravimetric method was used for the alcohol solubility analysis of basil oil, with a ratio of 1:1, 1:5, and 1:9. To achieve a ratio of 1:1, 1 mL of basil oil and 1 mL of 96% ethanol was mixed and the solubility was observed when pure basil oil dissolved, as described by a change in color to yellowish white. When the ratio was increased to 1:5, the color of the basil oil was still cloudy due to the presence of undissolved oil (Figure 7). Subsequently, the ratio was increased to 1:9, and the resulting color was getting clearer, indicating that the oil was perfectly mixed with alcohol. This showed that the better the oil quality, the more difficult the solubility. Generally, basil oil containing oxygenated monoterpenes was more soluble in alcohol compared to non-oxygenated monoterpenes [28].

3.2.5 Analysis of Variance (ANOVA)

Statistical analysis was used to prove that the process parameters employed in the extraction of basil affected the resulting yield. In Table 4, significant parameters were obtained when the p-value of the ANOVA method had a value less than 0.05.



Ratio 1:1 Ratio 1:5 Ratio 1:9

Figure 7. Comparison of the solubility of essential oils in alcohol

The regression equation of the model given was used to predict the actual results of the research. This indicated that the effect of process parameters, namely extraction time, microwave power, F/S ratio, and leaf size affected the yield of basil oil produced. However, the interaction of power and time did not significantly affect the yield.

$$\text{Yield} = 0.2916 + 0.0184A + 0.0433B + 0.0092C - 0.0135D + 0.0397AC + 0.0222AD + 0.0434BC + 0.0127BD - 0.0317CD - 0.0315A^2 - 0.0412B^2 - 0.0408C^2 - 0.0532D^2 \quad \textbf{(3)}$$

The equation 3 indicated that time was directly proportional to the yield response. The ratio parameter also showed the same relationship because the constant was positive. Meanwhile, the yield response was inversely proportional to the power and leaf size because the constants were negative [5]. This indicated that the yield reduced as the size of the leaves decreased and more energy was used.

Table 5 showed that an R^2 value of 0.9992 was obtained. Since the value obtained was close to 1, the model was considered perfect and in line with the research results. The predicted R^2 value was 0.9958, which corresponded to the adjusted R^2 value of 0.9984, which showed a difference of less than 0.2. In the Figure 8, experimental data values spread around the line, indicating that there was a concordance between the models and the experimental data. Therefore, the regression model was considered suitable for use.

Table 5. Fit statistics ANOVA

Std. Dev.	0.0021	**R^2**	0.9992
Mean	0.2226	**Adjusted R^2**	0.9984
C.V. %	0.9523	**Predicted R^2**	0.9958
		Adeq Precision	116.6345

Table 4. ANOVA results

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0809	14	0.0058	1285.63	< 0.0001	significant
A	0.0040	1	0.0040	899.28	< 0.0001	
B	0.0225	1	0.0225	4999.55	< 0.0001	
C	0.0010	1	0.0010	227.69	< 0.0001	
D	0.0022	1	0.0022	487.94	< 0.0001	
AB	0.0000	1	0.0000	3.56	0.0801	not significant
AC	0.0063	1	0.0063	1401.32	< 0.0001	
AD	0.0020	1	0.0020	437.75	< 0.0001	
BC	0.0075	1	0.0075	1676.80	< 0.0001	
BD	0.0007	1	0.0007	144.72	< 0.0001	
CD	0.0040	1	0.0040	893.17	< 0.0001	
A^2	0.0064	1	0.0064	1431.29	< 0.0001	
B^2	0.0110	1	0.0110	2454.92	< 0.0001	
C^2	0.0108	1	0.0108	2407.52	< 0.0001	
D^2	0.0184	1	0.0184	4087.71	< 0.0001	
Residual	0.0001	14	4.493E-06			
Lack of Fit	0.0001	10	5.770E-06	4.44	0.0819	not significant
Pure Error	5.200E-06	4	1.300E-06			
Cor Total	0.0809	28				

A = Power; B = Time; C = F/S Ratio; D = Leaf Size

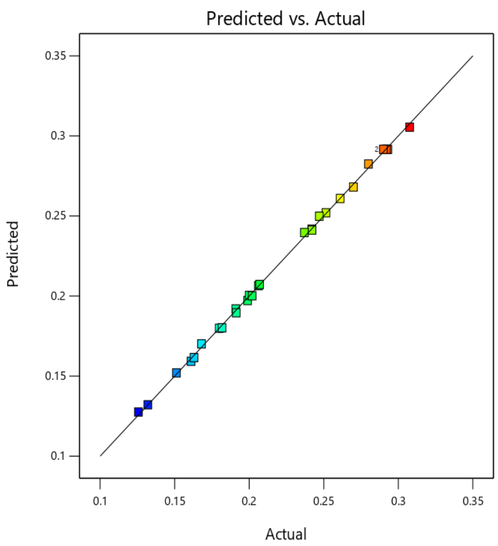


Figure 8. Predicted vs actual ANOVA

4. Conclusion

The optimum yield of basil oil was 0.3076% at 300 W power, 90 minutes, 1.75 cm leaf size, and an F/S ratio of 0.75 g/mL. The quality of basil oil was good, as indicated by its low solubility in 96% ethanol and a density value of 0.961 g/mL, which was in line with the standard value according to the Essential Oil Association (EOA). The main components of basil oil extracted using the MAHD method were E-citral at 46.79% and Z-citral at 38.17%. The basil oil obtained was more effective against *E. coli* bacteria because it had a larger inhibition zone than *S. aureus*.

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