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The Effect of Various Solvent in Soxhlet Extraction on The Characterictics of Basil Oil (*Ocimum Americanum L.*)

Luthfi Kurnia Dewi[1,2,✉], Siska Dwi Setyawati[1], Annisa Nur Fidyanti Pamuji[1], Syifa Indrayana[1], Chandrawati Cahyani[1,2]

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[1] Department of Chemical Engineering, Faculty of Engineering, Universitas Brawijaya, Jl. MT. Haryono No.167, Malang, 65145, Indonesia)
[2] Essential Oil Institute, Universitas Brawijaya, Jl. Veteran, Gd. Senat Lt. 1, Malang, 65145, Indonesia

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Abstract

Basil (*Ocimum americanum L.*) is a common plant in Indonesia used as the source of basil oil. Many methods to produce basil oil, such as solvent extraction using Soxhlet apparatus that called Soxhlet extraction. Determining solvent is a necessary factor in Soxhlet extraction because it affects the results of extraction in the form of quantity and quality of the essential oil produced. This shows that the selection of the type of solvent is an important factor to consider in the process of extracting basil essential oil, so this study was carried out to know the effect of solvent variations on the extraction results which were analyzed based on the yield and quality of essential oils including refractive index, specific gravity, and active compound. This research used three solvents with different polarities, ethanol, ethyl acetate and n-hexane. Basil leaves were separated from their flower and stem and dried in the oven at 102°C until their water content was 15-25%. Before extraction, dried basil leaves were cut to reduce size. The ratio of material to solvent that used was 1:50. Extraction was done at boiling point of each solvent for ten cycles. The rotary evaporator was used to separate basil oil and solvent. The highest yield is accomplished by basil oil that used ethanol (2,74%) followed by ethyl acetate (0,74%) and n-hexane (0,41%). The major active compounds obtained from each solvent were linalool 38.08% (ethanol), linalool 27.11% (ethyl acetate), and methyl chavicol 39.3% (n-Hexane). Basil oil from three solvents has a refractive index and specific gravity that suit commercial basil oil.

INTRODUCTION

Essential oils are natural extracts from certain types of plants, both from leaves, flowers, wood, seeds, and also flower pistils (Bhavaniramya et al., 2019). Essential oils provide a distinctive aroma and taste to plants (Burt, 2004). Around 60% of the world's use of essential oils as fragrance agents is added to household, beauty, and aromatherapy products (Tisserand & Young, 2014). One of the essential oil products used as a fragrance agent is basil oil (Güez et al., 2017). Basil oil has the potential to be developed in Indonesia due to the abundance of plants. Basil oil has a characteristic herbal aroma that is sweet, slightly spicy, and can warm (Rezzoug et al., 2019). In industry, basil oil is most often used as a fragrance agent in products such as cosmetics, detergents, mouthwash solutions, and dental creams (Sarkic & Stappen, 2018). The use of basil oil as a fragrance agent is due to the presence of the main components in the form of active compounds linalool and methyl chavicol which have a distinctive aroma of basil essential oil (Cai et al., 2022; Sundararajan et al., 2018). The composition of the active compound linalool in basil essential oil is 5-79%, and methyl chavicol is 5-29% (Preedy, 2014; Putu et al., 2019).

✉ Corresponding author:
E-mail: luthfikurnia@ub.ac.id

Production of basil oil can be done by Soxhlet extraction using a solvent. Soxhlet extraction of basil oil has the advantage that the resulting oil has a fragrance similar to natural fragrance (Guenther et al., 1987), providing high purity and yield, easy separation process at the end of the extraction process, as well as solvents used continuously in new conditions (Abed et al., 2018). In Soxhlet extraction, the type of solvent is an important factor that needs to be considered, because it can affect the extraction results in the form of quantity and quality of the essential oil produced. Based on the level of polarity, polar solvents can extract polar components with maximum extract yields, as well as non-polar solvents (Kumar, 2016).

The extraction of basil oil with polar and non-polar solvents resulted in the main component of Linalool in polar solvents of 3.15% and non-polar solvents of 0.57% (Dewi et al., 2018). This shows that the selection of the type of solvent is an important factor to consider in the process of extracting basil oil. This research was conducted to determine the effect of various solvents on the characteristics of basil oil including yield, refractive index, specific gravity, and active compounds.

MATERIALS AND METHODS

Materials

The local basil leaves and stems (*Ocimum americanum L.*) from the market in Malang - East Java, Aquadest, and three types of technical solvent (1) Ethanol, (2) Ethyl acetate and (3) n-Hexane.

Methods

Sample Preparation

The local basil leaves and stems (*Ocimum americanum L.*) was dried using an oven at a temperature of 102°C until the moisture content reaches 15±1%. Then the dried basil leaves and stems were chopped to a very small size.

Extraction Process

The process of producing basil oil using the Soxhlet extraction method. Samples was weighed for 20 grams and then wrapped in filter paper and extracted using various solvents (Ethanol, Ethyl acetate and n-Hexane) in Soxhlet extractor. The extraction with each solvent was carried out at different temperatures according to the boiling point of the solvent. The Soxhlet extraction with ethanol solvent was carried out at 83°C, ethyl acetate at 82°C and n-hexane at 74°C for 10 cycles and the average repetition was four times for each solvent variable. After the extraction process, the solvent was separated using a rotary evaporator. Separation was carried out at a temperature of 57°C with a pressure of 121 mBar for Ethanol, 169 mBar for Ethyl Acetate and 201 mBar for n-Hexane until all of the solvent had evaporated as indicated by the solvent no longer dripping on the round bottom flask and basil oil is obtained which is ready for further analysis.

Analysis of Basil Oil Characteristics

The characteristics of basil oil including yield, refractive index, specific gravity, and active compounds. Extracted basil oil uses three solvents that have been separated from the solvent and then the yield is measured. The yield of basil oil can be calculated using Eq. (1).

$$Yield = \frac{Mass\ of\ basil\ oil}{Mass\ of\ raw\ material} \times 100\% \quad (1)$$

The refractive index test was carried out using a refractometer at 20°C. Specific gravity was determined by comparing the density of basil oil and the density of water, so that basil oil and water were calculated at the same temperature using a pycnometer using Eq. (2).

$$Specific\ Gravity = \frac{Density\ of\ basil\ oil}{Density\ of\ water} \quad (2)$$

The composition of the active compound was identified using Shimadzu's Gas Chromatography-Mass Spectrophotometry (GC-MS). Comparison of basil oil from the research with commercial was determined based on the composition of the active compound's linalool and methyl chavicol.

RESULTS AND DISCUSSION

Effect of Various Solvent on The Yield of Basil Oil

Based on the results of calculations using Eq. (1), the yield from the extraction can be seen in Table 1. Table 1 shows the yield of basil oil extraction using ethanol as solvent of 2.74%, using ethyl acetate solvent of 0.74%, and using n-solvent.

Table 1. The Characteristics of Basil Oil with Various Solvent

Solvent	Yield (%)	Refractive Index	Specific Gravity
Ethanol	2.74	1.5047	0.9172
Ethyl acetate	0.74	1.5041	0.9353
n-Hexane	0.41	1.5041	0.9045

Hexane by 0.41%. The highest yield of basil oil extraction was obtained using ethanol as a solvent.

The extraction results can be influenced by the polarity of the solvent or the ability of the solvent to dissolve an extracted material (Kumar, 2016). The polarity of the solvent can be seen through the value of the dielectric constant. The greater the dielectric constant, the higher the polarity of a solvent (Sarker et al., 2006). Ethanol has a dielectric constant of 24.3, ethyl acetate 6.08, and n-hexane 1.89 (Abed et al., 2018). This shows that ethanol solvent has a higher polarity than ethyl acetate and also n-hexane so that the ability to dissolve polar compounds is higher. The solvent will more easily extract compounds that have almost the same polarity (Kumar, 2016). Basil oil contains the main active compounds, namely linalool as much as 59%, methyl chavicol as much as 29% (Preedy, 2014). Linalool as the active compound with the largest number is polar, and the presence of other polar active compounds including phenyl ethyl, menthol, and octadecane which cannot be extracted using Ethyl acetate and n-Hexane. Therefore, the extraction using Ethanol gets the highest yield.

Effect of Various Solvent on The Refractive Index of Basil Oil

The refractive index is the ratio between the speed of light in air and the speed of light in a substance at a certain temperature. The refractive index of essential oils is influenced by the components that compose them. The more components of long-chain carbon compounds such as sesquiterpenes or components that have double bonds, the density of the essential oil medium will increase so that the incoming light will be more difficult to refract. This causes the refractive index of oil to be larger (Benedicta et al., 2016). Based on Table 1, it can be seen that the refractive index of basil oil using ethanol solvent is 1.5047, ethyl acetate solvent is 1.5041, and n-Hexane solvent is 1.5041. The refractive index values of the three extractions did not have a significant difference. The value of the refractive index using ethyl acetate and n-hexane solvent has the same value, this can be due to the difference in the test temperature. The test temperature can affect the value of the refractive index. Where, the higher the test temperature, the more liquid it will be, and the refracted light will pass through the medium faster, so the value of the refractive index is getting smaller (Supriyana & Toifur, 2017). The refractive index values of the three extraction results are included the specification range of commercial basil essential oil refractive index, which is in the range of 1.490 up to 1.530 (Darjeeling, 2020).

Effect of Various Solvent on The Specific Gravity of Basil Oil

The value of the specific gravity of essential oils is defined as the ratio between the mass of oil and the mass of water in the same volume of water as the volume of oil. Specific gravity is related to the weight of the components contained in the essential oil. The greater the mass contained in the oil, the greater the value of its specific gravity (Sudarmadji & Haryono, 2007). Based on calculations using Eq. 2, the specific gravity value of basil oil can be seen in Table 1. The specific gravity value in Table1 shows that the extract of basil using ethyl acetate solvent has a value of 0.9353 then the extract of basil using ethanol is 0.9172 and the lowest is the extract of basil using n-hexane as a solvent, which is 0.9045. The difference in specific gravity values can be due to the weight fraction of components contained in basil essential oil, and the specific gravity of oxygenated terpene components is greater than that of unoxygenated terpenes (Sastrohamidjojo, 2020). Based on Table 1, the greatest number of compounds extracted using ethyl acetate solvent, and some of the compounds are oxidized terpene components including anethole, caryophyllene oxide, phenylacetaldehyde. Compared with the results of the extraction using ethanol and n-hexane as the solvent, this is what makes basil oil using ethyl acetate as solvent has the highest specific gravity value. From table 1 it can also be seen that the specific gravity value of basil oil is still within the standardized range according to commercial specifications in the range 0.9000 to 0.960

Table 2. The Active Compound of Basil Oil with Various Solvent

Active Compound	Molecular Formula	Composition (%)			
		Ethanol	Ethyl Acetate	n-Hexane	Commercial basil oil*
Linalool	$C_{10}H_{18}O$	38.08	27.11	16.83	17.65
Methyl Chavicol	$C_{10}H_{12}O$	34.39	24.38	39.3	74.87
Cyclohexanol	$C_{10}H_{20}O$	1.92	1.68	-	-
Citral	$C_{10}H_{16}O$	1.36	4.76	-	0.66
Trans Caryophyllene	$C_{15}H_{24}$	2.06	4.94	-	0.40
α-Bergamonten	$C_{15}H_{24}$	2.04	5.08	5.92	-
α -Humulene	$C_{15}H_{24}$	0.95	12.99	-	-
Phenylethyl	$C_8H_{10}O$	6.69	-	-	-
Terphenyl	$C_{18}H_{14}$	1.58	-	-	-
β- Farnesene	$C_{15}H_{24}$	1.06	2.77	-	-
Menthol	$C_{10}H_{20}O$	0.98	-	-	-
β- Bisabolene	$C_{15}H_{24}$	0.97	2.43	2.94	-
Octadecane	$C_{18}H_{38}$	0.6	-	-	-
Anethole	$C_{10}H_{12}O$	-	2.51	-	-
Benzenemethaneamine	$C_{14}H_{15}NO$	-	1.77	30.16	-
Caryophyllene oxide	$C_{15}H_{24}O$	-	1	-	-
Phenylacetaldehide	C_8H_8O	-	0.52	-	-
Hexadecane	$C_{16}H_{34}$	-	0.28	-	-
Methyl Eugenol	$C_{11}H_{14}O_2$	-	-	2.55	-
Benzene dicarboxylic	$C_8H_6O_4$	-	-	2.32	-
Total Active Compounds Identified		13	14	7	4

*Darjeeling Basil Essential Oil, 2020, CAS No: 8017-73-4

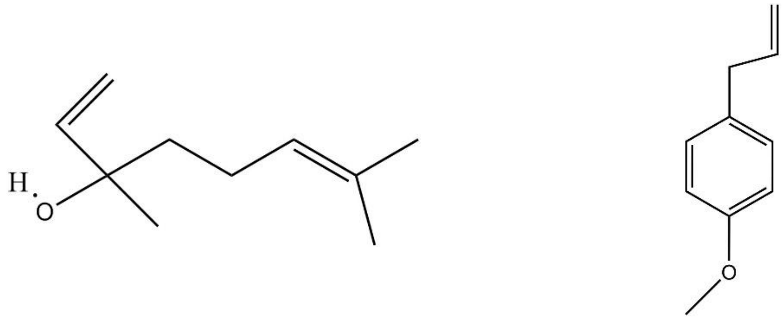
(Darjeeling, 2020), so that the basil oil produced from this extraction is included in the commercial qualification.

Effect of Various Solvent on The Chemical Compound of Basil Oil

The extraction result is closely related to the selectivity of the solvent and the solubility in the compound so that the three solvents used produce different compositions and types of active compounds extracted (Kumar, 2016). The composition of the active compounds contained in basil oil is shown in Table 2. Based on Table 2, it can be seen that the extraction using ethanol solvent contains 13 active compounds, the ethyl acetate solvent contains 14 active compounds, and the n-hexane solvent contains 7 active compounds. This shows that in the extraction process using a solvent, the selectivity factor of the solvent is very high affect the composition of the active compounds in it. However, if the results of this study are compared with commercial basil oil, they are very different because most commercial basil oils are included in the methyl chavicol base basil oil which contains 75 - 78% methyl chavicol (De Groot & Schmidt, 2016).

Linalool is also found in the extraction using n-hexane solvent which is non-polar at 16.83% this can occur due to the presence of an alkene group in linalool (Figure 1A) which makes linalool soluble in n-hexane solvent because of the soluble nature of alkenes. in non-polar solvents (Putu et al., 2019).

The composition of methyl chavicol in the basil oil using ethanol, ethyl acetate, and n-hexane solvents was 34.39%, 13.46%, and 39.3% respectively. The highest percentage of methyl chavicol composition in the basil oil using n-hexane is influenced by the formation of a benzene ring in the structure of the methyl chavicol compound and the presence of an alkene group that makes methyl chavicol non-polar (PubChem, 2023) and also the solvent of n-hexane which has the dielectric



(A) (B)

Figure 1. (A) Linalool (B) Methyl Chavicol (Zahra & Iskandar, 2017)

Table 3. Basil Oil Specification based on Methyl Chavicol and Linalool

Active Compound	Based on Methyl Chavicol (ISO 11043, 1998)		Based on Linalool (AFNOR 75-244, 1998)		Experimental data		
	Min	Max	Min	Max	Ethanol	Ethyl Acetate	n-Hexane
Methyl Chavicol	75.0%	87.0%	0.0%	30.0%	34.39%	24.38%	39.3%
Linalool	0.5%	3.0%	5.0%	62.0%	38.08%	27.11%	16.83%

constant is 1.89 so the ability to dissolve polar compounds is low and tends to be non-polar. Based on the principle of polarization, the compound will easily dissolve at the same polarity level in this case methyl chavicol and n-hexane are non-polar.

Methyl chavicol is also found in the composition of basil essential oil which is extracted using a polar ethanol solvent, this is because ethanol has a non-polar ethyl group so that it can extract methyl chavicol (Tekin et al., 2018). The amount of methyl chavicol in the extraction using ethanol is greater than in the extraction using ethyl acetate solvent. This shows that the ethyl group in ethanol is better at extracting methyl chavicol than the ethyl group in ethyl acetate solvent.

The results of the extraction using ethyl acetate solvent obtained the composition of the active compound linalool by 27% and methyl chavicol by 13.46%. The composition of the major active compounds in ethyl acetate is smaller than that of ethanol and n-hexane. This is because ethyl acetate solvent has a dielectric constant of 6.08, lower than ethanol and higher in n-hexane so that it is semi-polar. If observed from the total active compound composition, linalool is greater than methyl chavicol, indicating a tendency to be polar in ethyl acetate solvents.

Comparison between Basil Oil using Soxhlet Extraction with Commercial Basil Oil

Basil oil is used by industry as a fragrance agent because it contains the active compounds linalool and methyl chavicol so that which is typical of basil oil (NIRR, 2011). The composition of the active compound's linalool and methyl chavicol is the basis for the division of basil essential oil into linalool-based oil and methyl chavicol-based oil (De Groot & Schmidt, 2016). The specifications for the composition of the active compounds in each basil oil base can be seen in Table 3.

The composition of the active compound linalool extracted from basil oil using ethanol, ethyl acetate and n-hexane follows the specifications of linalool-based basil oil. Basil oil extraction using ethanol solvent has a composition of 38.08% linalool which complies with specifications, but the composition of methyl chavicol exceeds specifications. The extraction results using ethyl acetate solvent with a composition of 27.11% linalool and 13.46% methyl chavicol were both in accordance with the specifications, having the most extracted active compounds compared to the other two solvents, but the resulting yield was smaller than ethanol. The result of extraction with n-hexane solvent has a composition of 16.83% linalool which complies with specifications, but the composition of methyl chavicol exceeds specifications.

CONCLUSION

The variations of solvent in Soxhlet extraction affect the characteristics of basil oil. The highest yield was obtained from extraction using ethanol (2.74%), followed by ethyl acetate (0.74%) and n-hexane (0.47%). Basil oil with ethanol, ethyl acetate, and n-hexane solvents has a refractive index and specific gravity according to the specifications of commercial basil oil. Basil oil with ethanol solvent produced 38.08% linalool and 34.39% methyl chavicol, ethyl acetate solvent produced 27.11% linalool and 13.46% methyl chavicol, and n-hexane solvent produced 16.83% and methyl chavicol 39.3%.

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