Extraction of basil leaves (*Ocimum canum*) oleoresin with ethyl acetate solvent by using soxhletation method

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Abstract. The goal of this research is to produce oleoresin from basil leaves (*Ocimum canum*) by using soxhletation method and ethyl acetate as solvent. Basil is commonly used in culinary as fresh vegetables. Basil contains essential oils and oleoresin that are used as flavouring agent in food, in cosmetic and ingredient in traditional medicine. The extraction method commonly used to obtain oleoresin is maceration. The problem of this method is many solvents necessary and need time to extract the raw material. To resolve the problem and to produce more oleoresin, we use soxhletation method with a combination of extraction time and ratio from the material with a solvent. The analysis consists of yield, density, refractive index, and essential oil content. The best treatment of basil leaves oleoresin extraction is at ratio of material and solvent 1:6 (w/v) for 6 hours extraction time. In this condition, the yield of basil oleoresin is 20.152%, 0.9688 g/cm³ of density, 1.502 of refractive index, 15.77% of essential oil content, and the colour of oleoresin product is dark-green.

1. Introduction
Indonesia is a rich country in resources and natural products. Basil (*Ocimum canum*) is annual plant and herbs, with a height of 20 to 60 cm, have white-purple flowers. This plant comes from Asia, Africa, and South America and have been almost all over the world [1–4]. Basil is one of the resources that has not been used optimally. Basil is usually used as a flavor enhancer of food and as complementary foods [5–6]. One of the valuable contents in basil is oleoresin. Basil oleoresin contains the main component of volatile substances (essential oils) and non-volatile (resin and gum), each of that aims to determine the aroma and flavor [7–8]. Usually oleoresin is used as a flavouring agent in food, in cosmetic and ingredient in traditional medicine [9–10]. Oleoresin is derived from oleo, which means oil and resin, which means amber. Oleoresin is oil and resin that is a mixture of essential oils as an aroma carrier and a kind of resin as a carrier of flavor [7–8]. Basil oleoresin is dark-green oil and contains essential oil about 4 to 12 percents. In the trading, the quality of oleoresin is indispensable. The quality standard of basil oleoresin is dark-green color, typical aroma of basil, oil content of 4 % to 12 %, 1 g/cm³ of density, and 1,501-1,521 of refractive index [11]. To get the oleoresin contained in the basil leaves, one of methods is soxhletation, which is a standard technique where a fresh solvent is contact with sample periodically [12]. The efficiency of soxhletation method can be determine by several factors, such as the average size of the particles, the ratio of material and solvent, and the usage of polar or non-polar solvents [13–14], such as n-hexane, ethyl acetate, acetone, and ethanol [15]. The high of yield can be influence by extraction time and ratio of material and solvent [16]. Based on the above description, this research aims to produce oleoresin from basil leaves by using soxhletation...
method and ethyl acetate as solvent by varying extraction time and the ratio of material and solvent.

2. Methods
2.1 Raw Materials and Equipment
The sample in this research is basil leaves (*Ocimum canum*). Ethyl acetate is used as solvent. The equipment in this research is a hot plate, 40 mesh of sieve tray, funnel, condenser, round-bottom flask, blender, electric scales, thermometer, filter paper, rotary evaporator, measuring cups, and glass beaker.

2.2 Basil Leaves Powder Preparation Procedure
Basil leaves are washed using water, and then the samples are drying using the solar heat. After it is dried, the sample are crushed using a blender, and it is sieved using sieve (40 mesh) to obtain a basil leaves powder.

2.3 Basil Oleoresin Extraction Procedure
Extraction of basil leaves oleoresin has done using soxhletation method. In this method, the ethyl acetate is boiled at the boiling point, that is 77 °C as long as 3 hours, 4 hours, 5 hours, and 6 hours with the ratio of material and solvent (w/v) much as 1:5; 1:6; 1:7 and 1:8. Samples were finely put 50 g into a thimble located in the middle part of the soxhlet equipment. Ethyl acetate solvent used for the extraction process is put in a round bottom flask with a comparison between the material and solvent (w/v) 1:5; 1:6; 1:7 and 1:8. Oleoresin is extracted with soxhlet equipment for 3 hours, 4 hours, 5 hours, and 6 hours at temperature of 77 °C. Once extraction is complete, oleoresin has separated from the solvent using a rotary evaporator. The oleoresin is stored in the bottles for the analysis.
Analysis of the results in this research includes analysis of oleoresin yield, oleoresin density, oleoresin refractive index, and essential oil content.

3. Result and Discussion
3.1 Analysis of Oleoresin Yield

![Figure 1. The influence of ratio between material and solvent and extraction time to oleoresin yield](image)

Figure 1 is an overview of extraction, which shows the influence of the ration of material and solvent and extraction time to oleoresin yield. From Figure 1, we can see that in overall, the ratio of material and solvent and extraction time give an increasing effect to oleoresin yield,
which oleoresin concentration increases, with the increase ratio of material and solvent and extraction time. It can be explained that extraction time is required to obtain the higher oleoresin yield during prolong contact between basil leaves and solvent and giving basil leaves a chance to contact with solvent so that the oleoresin can be extracted optimally. At the time of extraction time unchanged, but the increase in the ratio of the material to the solvent, this has a major influence in producing oleoresin. To increase the oleoresin extract, much solvent should be available to maximize the diffusion of oleoresin yield. The increasing in the ratio of material and solvent with basil leaves affect the diffusion of oleoresin from basil leaves to the solvent, more solvent making diffusion of oleoresin will be greater, so solvent distribution to basil leaves will be greater. Equitably solvent distribution to basil leaves will increase the yield of oleoresin produced. The more solvents used will reduce the saturation level of the solvent, so that the extracted extracted component can be maximized [16]. The highest yield of oleoresin in this research is 20,434 % with the ratio of material and solvent 1:8 at 6 hours. At the same time, but with the ratio of material and solvent 1:6 obtained yield of 20,152 %, and lowest yield is 9,8676 % with the ratio of material and solvent 1:5 and extraction time at 3 hours. Effect of variations in the ratio of material and solvent and extraction time is different to each oleoresin yield product. The increasing in ratio of material and solvent and extraction time will increasing oleoresin yield.

3.2 Analysis of Oleoresin Density

![Figure 2](image_url)

Figure 2. The influence of ratio between material and solvent and extraction time to oleoresin density

Figure 2 is an overview of extraction which shown the influence of the ratio of material and solvent and extraction time to oleoresin density. We can see that in overall, the ratio of material and solvent and extraction time give effect to the density of oleoresin, which density of oleoresin increases with the increase in ratio of material and solvent and extraction time. For the ratio of material and solvent 1:5 at 3 hours has obtained oleoresin density of 0.9137 g/cm³. At 4 hours has obtained oleoresin density of 0.9373 g/cm³ and at 5 hours has obtained oleoresin density of 0.9470 g/cm³. At 6 hours, the oleoresin density has obtained about 0.9570 g/cm³. For the ratio material and solvent 1:6 at 3 hours has obtained oleoresin density of 0.9375 g/cm³. At 4 hours is obtained oleoresin density of 0.9477 g/cm³ and at 5 hours is obtained oleoresin density of 0.9554 g/cm³. At 6 hours has obtained oleoresin density of 0.9688 g/cm³. For the ratio of material and solvent 1:7 at 3 hours has obtained oleoresin density of 0.9442 g/cm³. At 4 hours has obtained oleoresin density of 0.9501 g/cm³ and at 5
hours has obtained oleoresin density of 0.9649 g/cm$^3$. At 6 hours, the oleoresin density has obtained about 0.9698 g/cm$^3$. For the ratio of material and solvent 1:8 at 3 hours has obtained oleoresin density of 0.9487 g/cm$^3$. At 4 hours has obtained oleoresin density of 0.9582 g/cm$^3$ and at 5 hours has obtained oleoresin density of 0.9649 g/cm$^3$. At 6 hours, the oleoresin density has obtained about 0.9731 g/cm$^3$. At this research, the density obtained are between 0.9137-0.9731 g/cm$^3$. Oleoresin density obtained in this research is lower when it was compared with the density according to Lluch Essence, that is 1,000 [11]. The density differences caused by oleoresin obtained in this study contains relatively small essential oils. The lower the essential oil content, the content of resins, fatty acids, and irregular compounds will be higher [7].

3.3 Analysis of Oleoresin Refractive Index

Figure 3 is an overview of extraction which shown the influence of the ration of material and solvent and extraction time to oleoresin refractive index. We can see at Figure 3 that with the ratio of material and solvent and extraction time given different of refractive index results. From Figure 3 we can see that more the amount of solvent and longer the time of extraction showed an increasing in the value of refractive index. For the ratio of material and solvent 1:5 at 3 hours is obtained oleoresin refractive index of 1,4938. At 4 hours is obtained oleoresin refractive index of 1,4950. At 5 hours is obtained oleoresin refractive index of 1,4963. At 6 hours is obtained oleoresin refractive index of 1,4977. For the ratio of material and solvent 1:6 at 3 hours is obtained oleoresin refractive index of 1,4992. At 4 hours is obtained oleoresin refractive index of 1,5001. At 5 hours is obtained oleoresin refractive index of 1,5009. At 6 hours is obtained oleoresin refractive index of 1,5016. At 7 hours is obtained oleoresin refractive index of 1,5021. For the ratio of material and solvent 1:8 at 3 hours is obtained oleoresin refractive index of 1,5010. At 4 hours is obtained oleoresin refractive index of 1,5012. At 5 hours is obtained oleoresin refractive index of 1,5018. At 6 hours is obtained oleoresin refractive index of 1,5023. At this research, refractive index are between 1,4938 – 1,5023. According to Lluch Essence, the value of refractive index allowed is 1,5010 – 1,5210 [11]. From the research has been done, it has been fulfil the standards of basil oleoresin refractive index.

![Figure 3. The influence of the ratio between material and solvent and extraction time to oleoresin refractive index](image-url)
3.4 Composition of Essential Oil in Basil Leaves Oleoresin

Identification of essential oil composition in basil leaves oleoresin when extraction time at 6 hours and 1: 6 of raw material and solvent ratio has shown at Figure 4. This result obtained by soxhletation method, and chromatogram obtained by using Gas Chromatography Mass Spectrometry (GC/MS). The components of basil leaves oleoresin are shown in Table 1, and the characteristics of basil leaves oleoresin are summarized in Table 2.

**Figure 4.** Basil leaves oleoresin chromatogram using GC/MS when extraction time at 6 hours and 1: 6 of raw material and solvent ratio

**Table 1.** Composition of Essential Oil in Basil Leaves Oleoresin at the Ratio of Material and Solvent 1:6 with Extraction Time 6 Hours

| Peak | R. Time | Area (%) | Component                  |
|------|---------|----------|----------------------------|
| 1    | 14,485  | 1,75     | Trans-alpha-bisabolene     |
| 2    | 15,111  | 0,77     | Undetected                 |
| 3    | 17,908  | 6,69     | Neophytadiene              |
| 4    | 17,981  | 1,17     | Phytol                     |
| 5    | 18,173  | 0,97     | Phytol                     |
| 6    | 18,374  | 1,35     | Phytol                     |
| 7    | 19,161  | 1,83     | Octadecenoic acid          |
| 8    | 20,725  | 4,83     | Phytol                     |
| 9    | 20,794  | 1,05     | Undetected                 |
| 10   | 20,958  | 2,23     | Methyl linolenate          |
| 11   | 21,710  | 0,82     | Phytol                     |
| 12   | 22,099  | 0,79     | Flavone                    |
| 13   | 23,133  | 2,15     | Beta-pinene                |
| 14   | 23,512  | 1,18     | Beta-pinene                |
| 15   | 25,750  | 1,80     | Methyl linolenate          |
| 16   | 25,926  | 0,78     | Undetected                 |
| 17   | 26,736  | 4,12     | Farnesol                   |
According to table 2, the characteristics of basil leaves oleoresin in this research meet the standard of basil oleoresin, but few of the value of the density and refractive index are still below the standard value [11], and basil leaves oleoresin contains essential oil about 15.77%.

Table 2. Basil Leaves Oleoresin Characteristics [11]

| Parameter          | Research Result  | Standard for Oleoresin |
|-------------------|------------------|-------------------------|
| Color             | Dark-Green       | Dark-Green              |
| Appearance        | Viscous Liquid   | Viscous Liquid          |
| Taste             | Characteristic of Basil | Characteristic of Basil |
| Essential Oil Content (%) | 15.77 | 4 - 12               |
| Density (g/cm³)   | 0.9137-0.9731   | 1                       |
| Refractive Index  | 1.4938-1.5024   | 1.5010-1.5210           |

4. Conclusion
The oleoresin of basil leaves can be obtained optimally at ratio of material and solvent 1:6 and extraction time at 6 hours which contains 20.152% of oleoresin yield, contain 15.77% of essential oil, 0.9688 g/cm³ of density, and 1.502 of refractive index and physical characteristics are dark-green color, viscous liquid appearance, and has aroma of basil characteristic.

5. References
[1] Eva K, Katerina H, Jana H, Tomas C, Jan P, and Martin K., 2008, *Aroma Profiles of Five Basil (Ocimum basilicum L.) Cultivars Grown Under Conventional and Organic Conditions*, Elsevier. Food Chemistry, Vol. 107, pp. 464-472.
[2] Jean C C and Mehmet M O., 2008, *Comparative Essential Oil of Flowers, Leaves, and Stems of Basil (Ocimum basilicum L.) Used as Herb*. Food Chemistry, Vol. 110, No.2, 501-503.
[3] Massimo L, Mariangela M, Bernardetta L, Fabrizio G, Mauro M, and Francesco S.. 2004, *Morphological Characterization, Essential Oil Composition and DNA Genotyping of Ocimum basilicum L. Cultivars*. Plant Science, Vol. 167, pp. 725-731.
[4] Mohammed C, Douniazaed E A, Njara R, Xavier F, and Farid C., 2016, *Comparative Study of Essential Oils Extracted from Egyptian Basil Leaves (Ocimum basilicum L.)
Using Hydro-Distillation and Solvent-Free Microwave Extraction. Molecules. MDPI. Vol.113, No.21.

[5] Philippe B, Patrick A N, Eric M B F, Gisèle A F D, and Joseph L T.. 2013, Chemical composition and residue activities of Ocimum canum Sims and Ocimum basilicum L essential oils on adult female Anopheles funestus ss. Journal of Animal & Plant Science. Vol. 19. No. 1.

[6] Mehmer Ö, Jean C C.. 2002, Essential Oil Composition of Ocimum basilicum L. and Ocimum minimum L. in Turkey. Czech J. Food Sci, Vol 20, No. 6.

[7] Seema R, Hindole G, and Muddasir B.. 2016, Phytochemical Characterization and Antioxidative Property of Ocimum canum: Effect of Ethanolic Extract of Leaves and Seeds on Basic Immunologic and Metabolic Status of Male Rats. Immuno Biol. Vol. 1. No. 2.

[8] Susheela R U.. 2000, Handbook of Spices, Seasonings and Flavoring. Lancaster-USA: Techonomic Publishing Co. Inc.

[9] Normalina A, Satriana, and Kiki R.. 2013, Extraction of Oleoresin from Waste of Nutmeg Oil Refining by Using Ultrasonic. Vol. 9, No. 4, pp.1412-5064.

[10] Yuva B, Fadila B, Abdelhanine, Zina D, Laid B, Abderrahim N, and Mokrane I O.. 2014, Antioxidant Activity of the Essential Oil and Oleoresin of Zingiber Officinale Roscoe as Affected by Chemical Environment. Vol. 16. No.6.

[11] Lluch E.. 2016, Essential Oils-Aromatic Chemicals-Flavors and Fragrances. Spain : El Prat de Llobregat-Barcelona.

[12] Sabel, W. & J.D.F. Wamen..1973, Theory and Practice of Oleoresin Extraction. In Proceedings at the Conference On Spices. London: Tropical Products Institute.

[13] Jibrin M D, Agus A, and Muhammad A A Z.. 2015, Characterization and Process Optimization of Castor Oil (Ricinus communis L.) Extracted by the Soxhlet Method Using Polar and Non-Polar Solvents. Journal of The Taiwan Institute of Chemical Engineers. Vol.47, pp.99-104.

[14] Sara B.. 2004, Essential Oils: Their Antibacterial Properties and Potential Applications in Foods-A Review. International Journal of Food Microbiology. Vol.94, 223-53.

[15] Kaibing Z, Hui W, Weni M, Xiaona L, Ying L, and Haofu D., 2011, Antioxidant Activity of Papaya Seed Extracts. Molecules Vol: 16, pp. 6179-6192.

[16] Muhammad D S, Anwar F, Pocut N A, and Normalina A.. 2011, Solvent Extraction of Ginger Oleoresin Using Ultrasound. Makara Seri Sains. Vol. 15. No. 2, pp. 163-167.