A COMPARATIVE ANALYSIS OF STEAM DISTILLATION AND ENFLEURAGE METHODS FOR THE EXTRACTION OF *Cananga odorata* FLOWER ESSENTIAL OIL

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Abstract

Essential oils are made from the natural extracts of many different kinds of plants. Indonesia has a lot of potential for making essential oils that could make it the leader in the essential oil market around the world. One of them is the essential oil from the *Cananga odorata* flower, which is used a lot in the pharmaceutical industry and others. The aim of this research was to compare methods of extracting essential oils from *C. odorata* leaves that can produce oils of good quality and that meet standards. In this study, steam distillation and enfleuration methods were used to extract the essential oil from the flower. Essential oil extracted with steam distillation had a yield value of 1.87%. Its characteristics were that it was light yellow in color, had a distinct fragrance, had a refractive index of 1.480, and had a specific gravity of 0.908 g/mL. The yield of essential oil from enfleurage was 2.87%, and it was dark yellow with a distinct fragrance. It had a refractive index of 1.510 and a specific gravity of 0.9012 g/mL. The GC-MS analysis of the essential oil from the steam distillation found 21 different compounds. The highest of these was β-cubebene (19.92%). The GC-MS analysis of the essential oil from enfleuration method found 15 different compounds, with caryophyllene (39.94%) being the highest. Based on the findings, it was concluded that the enfleuration method was more optimal for extracting essential oils from *C. odorata*.

***Keyword**: Cananga odorata, enfleurage, essential oil, steam distillation*

Background

Indonesia is a country that is home to many types of flora and fauna, making Indonesia a country with a very high level of biodiversity (Assidiq et al., 2021). Flora that grows in Indonesia has various species that can be used as phytopharmaceuticals and medicinal products (Isyraqi et al., 2020), but many Indonesian people do not know the benefits and uses of the plants around them (Rasna, 2010). One of the plants that has potential in medicine in Indonesia is the flower of *Cananga odorata*.

Cananga odorata Kait. F. & Thomson, commonly called *C. odorata*, is a plant commonly found in tropical Asia such as the Philippines, Malaysia, Indonesia, and several other islands in the Indian Ocean, especially the Comoro islands, Nossi Be, and Madagascar. This plant is famous for its fragrant flowers, and *C. odorata* essential oil has been widely used in the food industry as well as the perfume and aromatherapy industries (Tan et al., 2015; Junior et al., 2022).

Apart from that, this plant has pharmacological activities as antimicrobial, antioxidant, antibiofilm, insecticidal, anti-inflammatory, antimelanogenesis, insect repellent, relaxing, sedative, antihyperglycemic, antiviral, antidiabetic, and can improve mood and cognitive performance (Tan et al., 2015; Junior et al., 2022). This pharmacological benefit is obtained due to the secondary metabolite compounds contained therein (Thawabteh et al., 2019).

The major essential oil compounds from *C. odorata* are methyl benzoate, geraniol, eugenol, linalool, benzyl acetate, pinene, and caryophyllene (Tan et al., 2015). *C. odorata* essential oil generally comes from the flower parts of the plant (Oktavianawati, 2020). *C. odorata* oil is described as having a medium-to-strong initial aroma with a fresh, floral, slightly fruity, but soft scent. In addition, the flowers are also described as producing a very sweet aroma similar to jasmine (Goodrich, 2012; Junior et al., 2022). The extraction method to obtain essential oils will affect the quality and quantity of the oil produced (Oktavianawati, 2020). The extraction method commonly used to extract essential oils is distillation, either by hydrodistillation, steam-water distillation, or steam distillation (Oktavianawati, 2020). Apart from that, solvent extraction and enfleurization are also used to extract essential oils (Yuliani and Sahutu, 2012).

The aim of this research was to compare methods of extracting essential oils from *C. odorata* flowers that can produce oils of good quality and that meet standards. The characteristic test was done to determine the quality of each *C. odorata* essential oil, and then identified using the GC-MS instrument. The extraction method using steam distillation can be done simply and economically (Ariyani et al., 2017). This method is carried out by flowing water vapor into the mixture of ingredients contained in the components to be separated. The flow of water vapor will cause the oil to evaporate and be carried away with it by steam water, which is then condensed and separated by decantation (Sato, 2012). Whereas the enfleuration method is an ancient method from France that is utilized in making perfume. This method is suitable for producing essential oils that come from flowers. The process of extracting essential oils uses fat solids as an absorbent substance. The fat can be used as a sorbent, namely beef fat, pork fat, goat fat, fat chicken, butter yellow, butter white, and vaseline (Julianto, 2016).

Material and Methods

Material

Material used in this research include: *C. odorata* flowers, distilled water, 70% alcohol, filter paper, white butter (pulma), and n-hexane.

Steam Distillation

500 grams of fresh *C. odorata* flowers that have been prepared are placed on a filter in a distillation tank filled with water on the filter rack that has been boiled. Essential oils using the steam distillation method are more optimal when done for 6 hours (Setyawan et al., 2022). The distillation process is carried out once. The resulting oil is still mixed with water, so it must be filtered using a separating funnel and added to Na_2SO_4 so that the oil and water can be separated to obtain pure essential oil. The resulting *C. odorata* flower essential oil is stored in a dark, dry glass bottle and tightly closed. Next are yield calculations, characteristic tests, and identification using GC-MS (Sato, 2012).

Enfleuration

This research begins with preparing the container (chassis). The container is evenly smeared with butter with a thickness of 5 mm. On top of the butter layer, *C. odorata* flowers are arranged with the *C. odorata* kumtum facing down or sticking to the butter. Then cover with plastic wrap and leave for 7 days, and the flowers are replaced every day. After 7 days, the flowers are separated from the butter. The butter was then added to n-Hexane as a solvent and left for 24 hours. Finally, n-hexane and *C. odorata* oil were separated using a rotary vacuum evaporator (IKA RV10 Digital V). The resulting *C. odorata* flower essential oil is stored in a dark, dry glass bottle and tightly closed. Next, yield calculations, characteristic tests, and identification were carried out using GC-MS (Sato, 2012).

Yield Calculation

Yield is a comparison of the amount (quantity) produced from aromatic plant extracts. The higher the yield value produced, the more essential the oil extract produced (Aina et al., 2015). The yield calculation was carried out to find out how much extract was produced by dividing the weight of the oil extract by the weight of the simplicia in grams multiplied by 100% (Whika et al., 2017). The following is the yield formula:

$$\%\ \text{Yield} = \frac{\text{Sample Extract Weight (g)}}{\text{Sample weight (g)}} \times 100\ \%$$

Characteristics of C. odorata Essential Oil

a. Color Determination

Color observations are carried out visually using the sense of sight (eyes) directly to determine whether the color of the essential oil produced is in accordance with applicable standards according to SNI 06-3949-1005.

b. Smell

Odor observations are carried out visually using the sense of smell (nose) directly to determine the smell or aroma of the essential oil produced in accordance with applicable standards according to SNI 06-3949-1005.

c. Determination of refractive index

The determination of the refractive index is carried out using a refractometer. The prism on the tool is cleaned with alcohol, dried with a tissue, and then coated with the resulting essential oil. The scrub or slide is rotated, and you will get a line that clearly separates the dark and light areas. If this line meets the intersection point of the two intersecting line boundaries, then wait a few minutes before reading the refractive index.

d. Specific Gravity

The determination of the specific gravity of essential oils was carried out using a 10 ml pycnometer (IWAKI®). Pycnometers that are free of water and clean are weighed using an analytical balance (the weight of an empty pycnometer). Then fill it with distilled water slowly so that no bubbles form and weigh it (the weight of the pycnometer filled with water). The same thing is done with the essential oil results obtained to determine the weight of the pycnometer containing the sample. The following is the formula for calculating specific gravity:

$$BJ = \frac{m_2 - m}{m_1 - m}$$

BJ = Specific gravity
m = Mass, in grams, of empty pycnometer
m_1 = Mass, in grams, of a pycnometer filled with water
m_2 = Mass, in grams, of the oil- filled pycnometer

GC-MS Analysis

The chemical composition of *C. odorata* oil was analyzed using a Gas Chromatography-Mass Instrument Spectrometry (GC-MS) QP5050A (Shimadzu Co. Ltd., Kyoto, Japan) with a 15-meter-long TC-1701 capillary column. Helium gas was used as the mobile phase with a gas flow rate of 47.9 ml/minute with split injection, an injection volume of 1.0 µl, an injection temperature of 310 ºC, and a column temperature set at 70 ºC from 70 ºC to 310 ºC with a temperature increase rate of 15 ºC/minute. The results of the analysis of the chemical components contained in *C. odorata* oil are read through chromatogram analysis by comparing the retention time with the Kovats retention index and comparing them with the library literature: WILEY7 LIB.

The data analysis used in this research is descriptive analysis. Analysis data is presented in the form of tables or images according to the results obtained. Descriptive analysis was used to analyze data on the yield of essential oils, data on the characteristics of *C. odorata* essential oil according to SNI 06-3949-1005, and identify compounds using the Gas Chromatography-Mass Spectrometry (GC-MS) instrument.

Results and Discussion

The samples that will be used for research must first be determined with the aim of ensuring the correctness of the plant samples used in the research (Hasnaeni et al., 2019). Plant determination is the earliest step in the research process before proceeding to further steps (Primadiamanti et al., 2023).

Determination of *C. odorata* flower plants is carried out at the UPT. Materia Medika Batu, the results of the determination of *C. odorata* flowers are appropriate and can be proven by a letter from the UPT. Materia Medika Batu with letter number 067/376/102.20/2023.

Steam Distillation

Extraction using the steam distillation method to produce *C. odorata* flower essential oil was carried out for 6 hours with 1 cycle. This steam distillation method uses 500 grams of *C. odorata* flowers and produces 9 ml of essential oil.

Enfleuration

Extraction uses the enfleuration method to produce *C. odorata* flower essential oil for 7 days. The enfleurization process was carried out on 500 grams of *C. odorata* flowers using white butter as an adsorbent and 12 ml of essential oil was obtained.

Yield Calculation

Table 1. Yield Results for *C. odorata* Flower Essential Oil

Method	Simplisa weight (grams)	Extract Weight (grams)	Rendement
Steam Distillation	500 g	9.4806 g	1.89%
Enfleuration	500 g	14.3764 g	2.87%

Based on the table above, the yield of *C. odorata* oil using the enfleuration method was 2.87% and the yield of *C. odorata* oil using the steam distillation method was 1.89%. The higher the yield, the more essential oil extract will be produced. The quality of the essential oil produced is inversely proportional to the amount of yield produced. The higher the yield value produced, the lower the quality obtained (Aina et al., 2015).

Characteristics of C. odorata Essential Oil

Characterization of *C. odorata* essential oil aims to determine the quality of the essential oil produced. The characterization includes color, odor, refractive index measurement, and specific gravity. Based on data on the physical properties of *C. odorata* flower essential oil shown in Table 2, the color, odor and specific gravity meet the Indonesian National Standard (SNI) 06-3949-1005, while the refractive index of *C. odorata* essential oil does not meet the quality standards according to SNI 06-3949-1005.

Table 2. Results of Characteristics of *C. odorata* Flower Essential Oil

Test	Steam Distillation	Enphleuration	SNI 06-3949-1005
Color	Light yellow	Dark yellow	Light yellow-dark yellow
Smell	The smell of *C. odorata* flowers	The smell of *C. odorata* flowers	Typical smell of Kenanga
Refractive Index	1.510	1.480	1.493-1.503 (20 ^{o}C)
Specific gravity	0.912 g/ml	0.908 g/ml	0.904-0.920 g/ml (20 ^{o}C)

Based on **Table 2**, the results of the characteristics of *C. odorata* essential oil are said to not meet the Indonesian National Standard (SNI) 06-3949-1005 (Pujiarti et al., 2015), this is due to the refractive index results for the steam distillation method being 1.510 and the enfleuration method being 1.480. The factor that influences the refractive index value is the presence of water content in the oil. The more water content, the smaller the refractive index value. This is because water easily refracts incoming light. Apart from that, the small refractive index is due to the small oil content and the still presence of solvent in the results obtained (Yulistiani et al., 2020).

The refractive index of essential oils is closely related to the components contained in the essential oils produced. The more many long chain components such as sequiterpenes or oxygen group components also distilled, then the density of the oil medium essential oils will increase so that the light coming will be more difficult to refract. This causes the refractive index of oil to increase big. The more water content, the more the smaller the refractive index value. This is because The nature of water is that it is easy to refract incoming light (Ariyani et al., 2017).

Analysis of C. odorata Flower Essential Oil Components Using GC-MS

Results of gas chromatography analysis of *C. odorata* flower essential oil using steam distillation method obtained 22 peak and chromatography results method enfleuration is obtained 20 peak like Which seen on **Picture 1** and **Picture 2**.

Chromatogram Results of the Distillation Method for *C. odorata* Flower Essential Oil

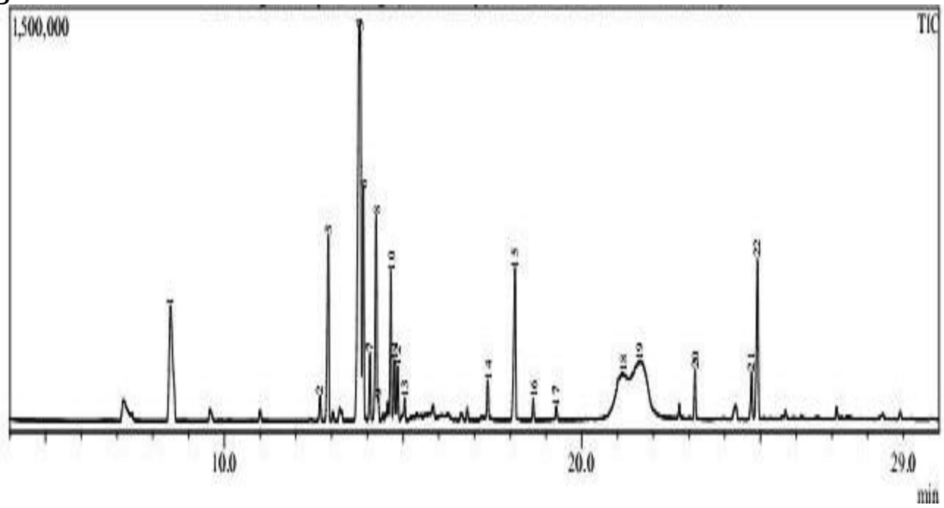

Figure 1. *C. odorata* Oil Profile Steam Distilled Methods

From the results of the analysis data according to **Figure 1**, 22 peaks were obtained with different retention times. In the steam distillation method, the compound detected first was the Linalool compound with an area % of 9.47% with a retention time of 8.497 minutes. The lower the boiling point of a compound, the faster it will be released/detected by the instrument compared to compounds that have a high boiling point (Al Rasyid and Nasir, 2020). From data results analysis obtained 22 peak with own time retention Which different. Complete data for each compound is listed in the table as following.

Table 3. Compounds detected from *C. odorata* Oil as a result of the distillation method

No.	Retention time	% area	SI	Molecular formula	Compound
1	8.497	9.47%	96	$C_{10}H_{18}O$	Linalool
2	12.682	0.92%	94	$C_{10}H_{12}O_2$	Phenol-2-methoxy-4-(2-propenyl)-(CAS) eugenol
3	12.909	7.29%	90	$C_{12}H_{20}O_2$	Geranyl acetets
4	13.776	19.92%	86	$C_{15}H_{24}$	β-cubebene
5	13.806	9.20%	93	$C_{15}H_{24}$	Trans(β)caryophyllene
6	12.889	7.17%	94	$C_{15}H_{24}$	β-cubene
7	14.075	2.20%	90	$C_{15}H_{24}$	Germacrene D
8	14.249	8.16%	92	$C_{15}H_{24}$	α-humulene
9	14.317	0.55%	88	$C_{15}H_{24}$	Germacrene
10	14.659	4.31%	94	$C_{15}H_{24}$	Farnesene
11	14.760	1.74%	89	$C_{15}H_{24}$	α-bergamotene
12	14.852	1.71%	90	$C_{15}H_{24}$	Germacrene
13	15.046	0.58%	89	$C_{15}H_{24}$	γ-cadinene
14	17.370	1.21%	91	$C_{15}H_{26}O$	Cis-fernesol
15	18.131	6.59%	97	$C_{14}H_{12}O_2$	Benzyl benzoate
16	18.644	0.60%	87	$C_{12}H_{20}O_2$	Geranyl acetate
17	19.290	0.38%	97	$C_{14}H_{12}O_3$	Bezoid acid
18	21.150	2.05%	89	$C_{18}H_{34}O_2$	9-octadecenoic acid
19	21.635	6.23%	91	$C_{18}H_{34}O_2$	9-octadecenoic acid
20	23.167	1.55%	88	$C_{37}H_{74}O_8$	Hexadecanoic acid 16

21	24.751	1.48%	86	$C_{39}H_{72}O_5$	DI-(9 octadecenoyl)
22	24.919	6.70%	78	$C_{14}H_{15}O$	-

Based on the analysis data above, 22 peaks with different retention times were obtained. The MS results obtained contained 21 known compounds and 1 compound The compound is not yet known. This happens because the SI (Similarity Index) value Which found <85%. The compounds in peak number 22 are not yet known, because the resulting SI value is 78. Compounds in numbers 4-13 are sesquiterpene compound groups ($C_{15}H_{24}$) and numbers 18-19 are fatty acid groups ($C_{18}H_{34}O_2$) including compounds isomerization, namely compounds that have the same molecular formula.

The highest peak appeared with a retention time of 1,776 with a % area of 19.92%, namely the β-Cubebene compound, this compound has great benefits in the health sector, one of which has neuroprotective effects (Kaplan and Çelikoğlu, 2020). The main components of *C. odorata* flower essential oil produced by the steam distillation method is the Linalool compound, β-Cubebene, Trans β Caryophylene, α-Humulene and Benzyl benzoate.

Chromatogram Results of the Enfleuration Method for *C. odorata* Flower Essential Oil

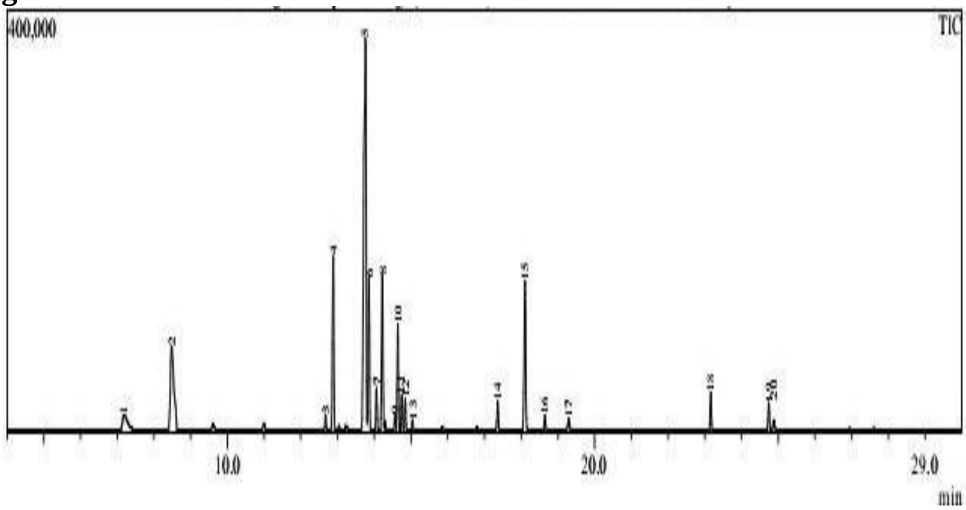

Figure 2. *C. odorata* Oil Profile Enfleuration Methods

From data results analysis obtained 20 peak with own time retention Which different. In the enfleuration method, the compound detected first was Benzene,1-methoxy-4-methyl with a % area of 2.20% with a retention time of 7,186 minutes. Complete data for each compound is listed in the table as following.

Table 4. Compounds detected from *C. odorata* Oil as a result of the enfleuration method

No.	Retention time	% area	SI	Molecular formula	Compound
1	7.186	2.20%	86	$C_8H_{10}O$	Benzene,1-methoxy-4-methyl
2	8.480	11.10%	91	$C_{10}H_{18}O$	Linalool
3	12.672	0.63%	83	$C_{10}H_{12}O_2$	-
4	12.880	9.04%	94	$C_{12}H_{20}O_2$	Geranil acetats
5	13.761	34.94%	96	$C_{15}H_{24}$	Caryphyllene
6	13.852	7.51%	91	C15H24	Germacrene-D
7	14.058	1.86%	91	$C_{15}H_{24}$	Germacrene-D

8	14.218	8.64%	89	$C_{15}H_{24}$	α-humulene
9	14.560	0.54%	74	$C_{15}H_{24}$	-
10	14.641	4.97%	90	$C_{15}H_{24}$	(Z,E)-α-farnesene
11	14.750	1.72%	90	$C_{15}H_{24}$	(-)-endo-2-6 dimethyl-6-(4-methyl-3 pentenyl)biciclo
12	14.841	1.38%	85	$C_{15}H_{24}$	Germacrene-D
13	15.041	0.38%	77	$C_{15}H_{24}$	-
14	17.358	1.41%	90	$C_{15}H_{26}$	2Z,6E-Fernesol
15	18.103	9.00%	97	$C_{14}H_{12}O_2$	Benzyl benzoate
16	18.645	0.60%	86	$C_{12}H_{20}O_2$	Neryl acetats
17	19.292	0.58%	99	$C_{19}H_{20}O_7$	5-O-Benzyl-2-o-tosyl-D
18	23.163	1.71%	79	$C_{13}H_{26}O_2$	-
19	24.741	1.34%	79	$C_{18}H_{34}O_2$	-
20	24.878	0.45%	98	$C_{12}H_{16}O_3$	4-(methoxy methoxy)

Based on the table above, 20 peaks with different retention times are obtained. The MS results obtained contained 15 known compounds and 5 compounds The compound is not yet known. This happens because of the SI value (Similarity Index) Which found <85%, namely at peak numbers 3, 9, 13, 18 and 19. Compounds with peak numbers 5-13 are isomeric compounds, namely compounds that have the same molecular formula, namely sesquiterpene compounds ($C_{15}H_{24}$).
The highest peak in the enfleuration method was the Caryophylene compound with a % area of 39.49%. Compound Caryophyllene has benefits Which big in the health sector, such as anticancer, antioxidant and antimicrobial (Kaplan and Çelikoğlu, 2020). The dominant compounds The products produced by the enfleuration method are Linalool, Geranil acetate, Caryophyllene, α-Humulene and benzyl benzoate.
From the results of Gas Chromatography-Mass Spectrometry data, only 5 compounds have similarities to *C. odorata* essential oil using the steam distillation method and the enfleuration method, these compounds are Linalool ($C_{10}H_8O$), Geranyl Acetate ($C_{12}H_{12}O_2$). α-Humulene ($C_{15}H_{24}$), Aromatic Benzyl Benzoate ($C_{14}H_{12}O_2$) and Germakren D ($C_{15}H_{24}$). The factors that cause the many differences in the compounds produced are due to differences in the extraction process carried out and use different solvents.

Conclusion

The enfleuration method is a more optimal extraction method for obtaining essential oil from *C. odorata* compared to the steam distillation method, because it has a high yield of 2.87%. all characteristics of essential oils resulting from extraction using steam distillation and enfluration methods are in accordance with SNI 06-3949-1005, except for the refractive index value. The β-Cubebene compound is the main compound in the essential oil extract of *C. odorata* flowers using the steam distillation method, while Caryophyllene is the dominant compound in the essential oil extract of *C. odorata* flowers using the enfleurization method.

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