Qualitative Analysis of Amomum cardamomum..

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Qualitative Analysis of *Amomum cardamomum* Essential Oil by Using Gas Chromatography-Mass Spectrometry (GC-MS) Through Its Fragmentation and Retention Indices Calculation

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Abstract. Cardamom plant (*Amomum cardamomum*) is an essential oil-producing plant in Indonesia that is rich in benefits. Due to its high antioxidant content, it has the potential to be a standardized herbal medicine. The purpose of this study was to compare two qualitative methods for determining the compound content of cardamom. Hydrodistillation and ultrasonication as a pretreatment (Ultrasonic Assisted Extraction Hydrodistillation/UAE-HD) were used to extract cardamom oil. Analyzed GC–MS (gas chromatography-mass spectrometry) was performed to obtain the essential oil content. Confirmation of chemical compounds is carried out using the Kovats equation. The retention index (RI) method introduced by Kovats expects the targeted compound to be identified correctly. The Retention Index (RI) calculation using different analytical methods and the test mixture of hydrocarbon compounds show that the difference with the RI library - RI NIST: 1.5 - 1.86% is not more than 20% to the provisions. Between the two methods used in this study, Method 2 allows faster analysis, as demonstrated by the shorter retention time (RT = 8.18) than Method 1 (RT =11.67).

INTRODUCTION

Cardamom plant (*Amomum cardamomum*) is a spice producer with many benefits for the community. Cardamom is widely used in the food to pharmaceutical industries. Cardamom is included in the nine major world spices commodities and is traded in essential oils and dried fruit [1]. In the health sector, cardamom has antihypertensive benefits with a diuretic effect, prevents chronic disease, overcomes digestive problems, antibacterial activity, and cytotoxic properties can also fight cancer cells [2].

The dynamic content of the compounds in essential oils has excellent potential as antioxidants and antimicrobials. Cardamom seed essential oil contains active compounds 1,8-cineol, p-simena, α-terpineol, α-pinene, and ß-pinene[1]. Several studies have shown that cardamom essential oil has the benefit of eliminating bad breath by adding it to toothpaste [3] and adding it to jelly candy [4] in cream preparations that have antibacterial benefits [2]. There is a dearth of research on essential oils' antioxidant and antibacterial properties in standardized herbal medicines. This is an excellent opportunity to research cardamom essential oil in conjunction with antioxidant and antibacterial ointment

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formulations to mitigate the side effects associated with the use of synthetic drugs. This research was conducted in line with Indonesian government programs in the health sector, particularly research, development, and utilization of local natural resources in supporting the acceleration program for Standardized Herbal Medicine and phytopharmaca.

Cardamom essential oil was extracted using Ultrasonic Assisted Extraction (UAE) technology to maximize extract yield. The UAE method is a new extraction method developed as an alternative method, with the advantages of accelerating the extraction time, reducing the use of solvents, and increasing the extract yield [5]. Furthermore, the mechanical effect caused by ultrasonic waves can improve the solvent's ability to penetrate the cell of the material, thereby increasing the number of cell components that diffuse into the solvent [6]. In addition, the unnecessary use of heat makes the UAE method suitable for extracting cardamom essential oil because it will not damage the chemical components in cardamom that are easily damaged by heat [7].

The research will be conducted using Ultrasonic Assisted Extraction (UAE) as a pretreatment technique [6]. Some studies about ultrasonic extraction of compounds on fennel hops, marigold, mint leaves, and lemon can increase 20-40% extraction yield compared to conventional extraction methods [8]. For example, corn extracted by ultrasound for 2 minutes obtained 55.2-67.8%, almost the exact yield obtained from heating water for 1 hour, 53.4%. Furthermore, the application of UAE technology is expected to improve the quality and yield of cardamom essential oil [9]. Using the extraction techniques investigated, the major aromatic compounds isolated from cardomom oil were α-terpinyl acetate and 1.8-cineol [10].

The purpose of this study is to compare two methods for qualitative analysis of cardamom's compound content. Identification of the composition of cardamom oil was performed using the GC-MS instrument. Confirmation of chemical compounds is carried out using the Kovats equation. The retention index (RI) method introduced by Kovats expects the targeted compound to be identified correctly. RI was the ratio between the anchoring time of the target compound and the holding time of the two alkane hydrocarbons eluted between the target compounds. The RI value of the compound will not change even though changes in the analysis process parameters are applied in the same column. Two kinds of conditions can be applied when calculating RI using a gas chromatograph. These conditions are programmed temperature conditions and isothermal. Formulas to determine the RI value in those two states are given by Equation 1 and Equation 2 for programmed temperature and isothermal conditions, respectively [11][12].

$$RI = \left[\frac{t_{r(unknown)} - t_{r(n)}}{t_{r(N)} - t_{r(n)}}\right] * (100\ x\ z) + (100\ x\ n) \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$RI = 100\ x\left[n + (N - n)\frac{\log(t_{r(unknown)}) - \log(t_{r(n)})}{\log(t_{r(N)} - log\ (t_{r(n)}))}\right] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

With :

RI = Retention index
n = Alkane eluted carbon chain before target compound
N = Alkane eluted carbon chain after target compound
t_r = Retention time
2 = Difference number between N and n carbon chain
$t_{r(n)}$ = Retention time of the preceding alkane
$t_{r(N)}$ = Retention time of the following alkane

MATERIAL AND METHODS

Material

The material used in this study was cardamom oil derived from cardamom seeds from Padang City, Indonesia, with the brand Tusanco. Cardamom seed oil was hydrodistilled for 6 hours to extract the essential oil completely from 100 grams of cardamom seeds with particle size 8 mesh parameters. Sonication pretreatment was performed at a 30% and 60% amplitude. The cardamom oil was extracted using Ultrasonic Assisted Extraction (UAE) technology with an amplitude of 30% (H3SF20) and 60% (H6SF20) for 15 minutes (SF20=Solvent Feed Ratio between Water and Cardamom seeds 20:1 (v/w)). To determine the RI, a test mixture containing the hydrocarbons $C_{11}H_{24}$ - $C_{22}H_{46}$ obtained from Sigma Aldrich was prepared at a concentration of ten parts per million. In addition, n-hexane and dichloromethane used for the solvent in the present study were acquired from Merck.

The instrument used 2 the analysis was Agilent 7890B/5977A Series Gas Chromatograph/Mass Selective Detector system. A DB-5MS-UI capillary column (30 m x 0.25 mm i.d. with a 0.25 (m) film thickness was employed in this study. The gas used was UHP grade helium gas with a flow rate of 1 ml/min. The injection method used was splitless mode, with the injector's temperature maintained at 250 °C. The ion source and interface temperature at the MS detector were set at 230 °C and 250 °C. Full scan mode was used from 40 to 600 m/z, with the ionization energy maintained at 70 eV. The database used is NIST 17.

Methods

Analysis of the target compound begins with injecting a blank sample into the GC-MS system. This is done to ensure the system in the instrument is clean and ready to be used for analysis (Fig. 1). Then, cardamom oil dissolved in n-hexane (10 µl/1 ml solution) was injected into the GCMS system [13]. The GC analysis parameters are described in Table 1.

The test mixture of hydrocarbons $C_{11}H_{24}$ - $C_{22}H_{46}$ was dissolved in n-hexane to obtain a concentration of 1000 ppm, followed by further dilution to get a standard solution of test mixture $C_{11}H_{24}$ - $C_{22}H_{46}$ 10 ppm, which will be used to determine the Retention Index (RI) value [11]. Hydrocarbon standards were also injected into the GC-MS system according to the same method parameters as cardamom oil. The retention time for each peak of both cardamom oil and hydrocarbon standards was recorded and calculated using the equation below:

TABLE 1. GCMS oven temperature program of cardamom oil analysis

Method	GCMS oven temperature program	References
1	Initial temperature 40 °C for 2 minutes, then increase at a rate of 5 °C/min to 210 °C, then held for 5 minutes	Nashwa F.S.Morsy[14]
2	Initial temperature 40 °C for 1 minute, then increase at a rate of 10 °C/min to 300 °C, then held for 4 minutes	Kurniawan and Pusfitasari[13]

RESULTS AND DISCUSSION

The results of the blank injection are shown in Fig. 1, which shows the clean system with no undesirable peaks in the readings of the GC-MS. The cardamom oil samples were then injected into the GCMS using two methods, as described in Table 1. Finally, the results of cardamom oil analysis are displayed in Fig. 2 and Fig.3.

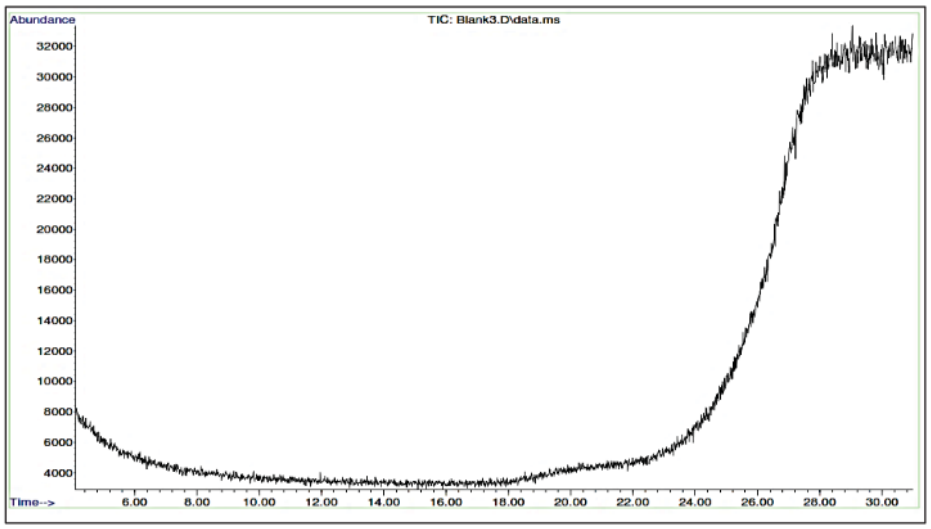

FIGURE 1. The results of the analysis of the empty sample injection

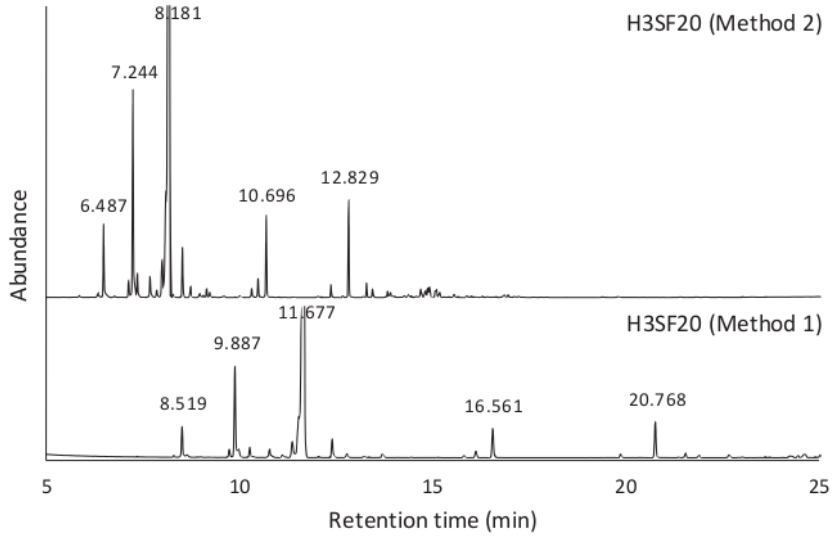

FIGURE 2. Chromatograms of Cardamom sample H3SF20

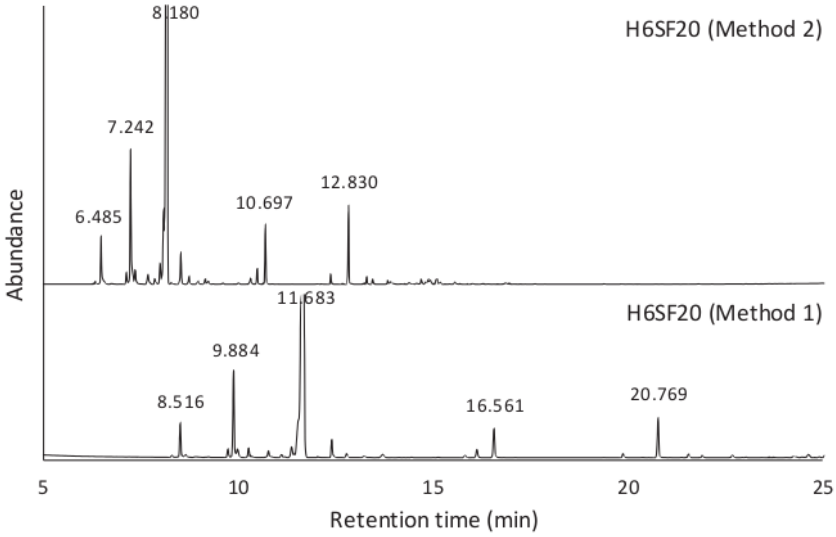

FIGURE 3. Chromatograms of Cardamom sample H6SF20

The identification of cardamom oil constituents using GC-MS (Table 2) shows that the dominant compound in cardamom is 1,8-cineol with a concentration of 66 - 78%. This can also be seen from the fragmentation results (Fig. 3), showing that the component is 1,8-cineol with a mass fragment of 154 m/z.

TABLE 2. Comparison of GCMS analysis results of cardamom oil samples with different methods

	Method 1						Method 2					
	H3SF20			H6SF20			H3SF20			H6SF20		
No	% Area	Retention Time	Compound	% Area	Retention Time	Compound	% Area	Retention Time	Compound	% Area	Retention Time	Compound
1	67.53	11.674	Eucalyptol	76.83	11.687	Eucalyptol	70.96	8.183	Eucalyptol	71.97	8.183	Eucalyptol
2	10.15	11.548	Limonene	6.14	9.885	beta.-Pinene	8.02	7.238	beta.-Pinene	7.18	7.238	beta.-Pinene
3	5.33	9.884	beta.-Pinene	3.11	20.774	alpha.-Terpinyl acetate	3.38	12.834	alpha.-Terpinyl acetate	4.84	8.095	Limonene
4	4.49	11.372	p-Cymene	2.53	16.564	alpha.-Terpineol	2.86	10.691	alpha.-Terpineol	3.59	12.834	alpha.-Terpinyl acetate
5	2.28	20.773	alpha.-Terpinyl acetate	2.3	8.511	alpha.-Pinene	2.63	6.482	alpha.-Pinene	2.74	10.704	alpha.-Terpineol
6	1.99	16.564	alpha.-Terpineol	1.45	12.405	gamma.-Terpinene	2.03	8.007	o-Cymene	2.62	6.482	alpha.-Pinene
7	1.54	8.523	alpha.-Pinene	1.16	11.372	o-Cymene	1.72	8.523	gamma.-Terpinene	1.51	8.536	gamma.-Terpinene
8	1.25	12.405	gamma.-Terpinene	0.94	9.985	beta.-Pinene	1	7.364	beta.-Pinene	1.27	7.994	o-Cymene
9	0.66	10.779	alpha.-Phellandrene	0.71	16.123	Terpinen-4-ol	0.99	7.692	alpha.-Phellandrene	0.87	7.364	beta.-Pinene
10	4.78		Other	4.83		Other	6.41		Other	3.41		Other
	100			100			100			100		

In Fig. 4, it can be seen that the chromatogram peaks at RT 11.674 minutes (method 1) and RT 8.183 minutes (method 2) are shown as eucalyptol compounds (1.8 cineol) based on the NIST 17 database library (Table 3), with the molecular formula $C_{10}H_{18}O$. The spectrum shows the molecular ion peak m/z 154 followed by m/z fragments 154, 139, 125, 108, 84, 81, 71, 58, 55, 43, with a base peak at m/z 43. Compound 1.8 cineol with an abundance of m/z 154 ($C_{10}H_{18}O$.) fragmented in the presence of (CH_3) release and formed fragments of m/z 139 $(C_9H_{15}O)^+$. Furthermore, the compound with m/z 139 releases the compound (C_4H_7) to include a fragment of m/z 84 $(C_5H_8O)^+$. Molecular ions with m/z 154 can form fragments by removing compounds (C_7H_{12}) to form fragments with m/z 58 (C_3H_6O). Molecules with m/z 58 fragmented again to form molecular ions m/z 43 (C_2H_3O), the peak of molecular ion m/z 43 was the highest in the mass spectra of the compound 1.8 cineol.

TABLE 3. RI value of each 1,8 Cineol with RI NIST library

Method	TR n-alkane (minute)	TR Targeted compound (minute)	RI Produced by experiment	RI Show in NIST library
I	C_{12} = 10,512	Cineol = 11.674	1002.96	1022
II	C_{12} = 7.524	Cineol = 8,183	1006.73	

The percentage of differences for RI value between RI experiments and RI presented in the NIST library was calculated according to Equation 3, while the result can be seen in Table 4.

$$\%\text{difference} = \left[\frac{RI_{experiment} - RI_{NIST}}{RI_{NIST}}\right] * 100\% \ldots\ldots\ldots\ldots\ldots (3)$$

TABLE 4. Percentage difference between experiment and Reference

Method	RI experiment	RI showed in NIST and other references	% Difference
I	1002.96	1022	1,86
II	1006.73		1.50

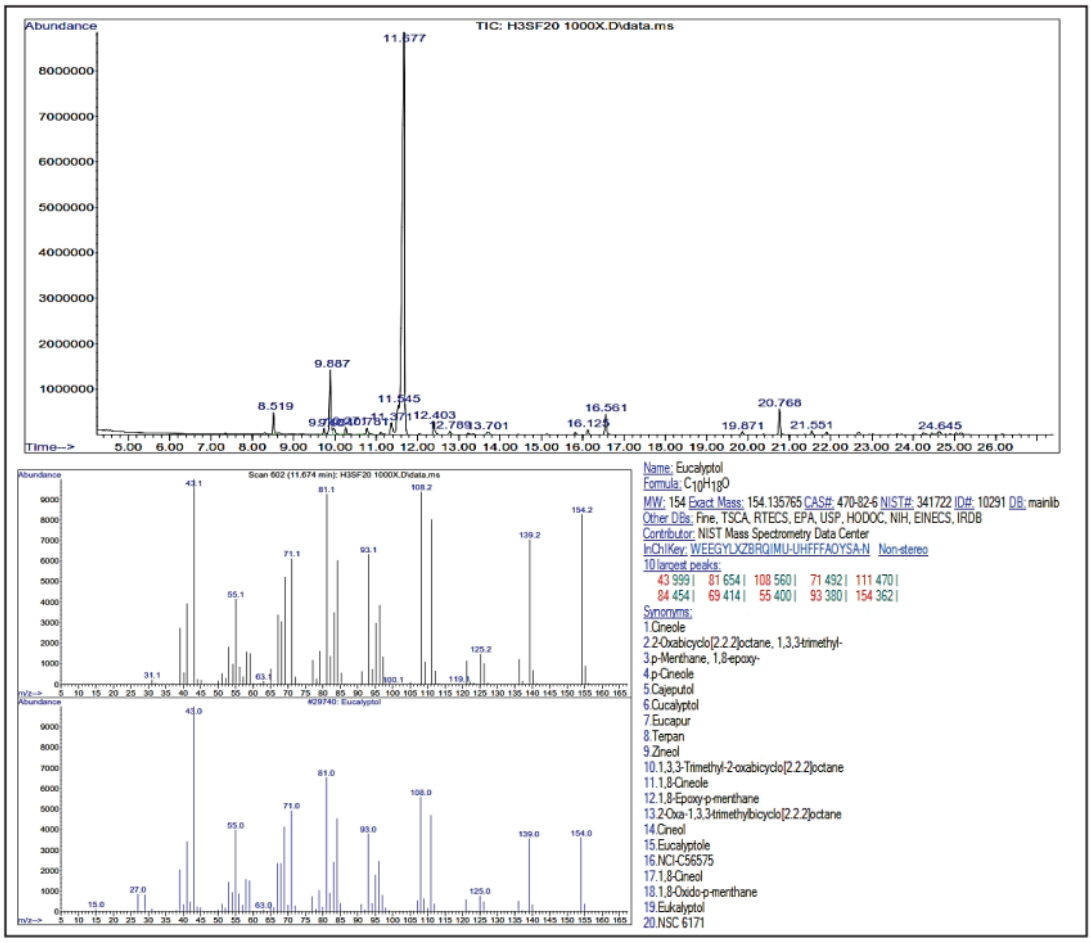

FIGURE 4. Fragmentation comparison of 1.8 cineol between cardamom samples and NIST 17 database

CONCLUSION

The results of GCMS analysis show that 1,8-cineol / eucalyptol is the principal constituent of cardamom oil. This can be seen from the fragmentation results of the highest peak using two different methods, indicating 1,8-cineol with 97% similarity to the library. In addition, the peak identification was also confirmed by calculating the Retention Index (RI). The value of the Retention Index (RI) obtained using two different analytical methods in this study agrees with RI value provided by NIST library, with only 1.5 - 1.86% differences. Between the two methods used in this study, Method 2 allows faster analysis, as demonstrated by the shorter retention time of 1,8-cineol (RT = 8.18) compared to Method 1 (RT =11.67).

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