Chemical Composition, Physico-Chemical Properties, and Antioxidant of Seed and Leaf of *Amomum cardamomum* Willd Oils

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ABSTRACT

Essential oil of cardamom seed can be used as drugs, antibacterials, anti-inflammatories, antioxidants, spices, food flavorings, and perfumes. In addition, the rhizomes and the leaves of cardamom also contain essential oils. The aims of this study were to elucidate the yield, chemical composition, physico-chemical properties, and antioxidant activities of *Amomum cardamomum* Willd oils from seeds and leaves which were obtained by water distillation and water-steam distillation methods. The results showed that *A. cardamomum* oils had wet-yield of 0.99 to 4.52% and dry-yield of 4.50 to 5.30%. The highest yield on cardamom seed oil was obtained by water distillation. The main compound contained in these *A. cardamomum* oils was 1,8-cineole (36.42 to 92.10%), whereas the highest content of 1,8-cineole, optimum physico-chemical values, and antioxidant levels were obtained from cardamom seed oil. *A. cardamomum* oil in this study possessed mild antioxidant with IC_{50} values of 2.22 to 4.92 mg/ml and IC_{90} of 17.05 to 30.25 mg/ml.
Keywords: *Amomum cardamomum*, distillation, chemical composition, physico-chemical, antioxidant.

Introduction

Cardamom is one of the essential oil-producing plants of the *Zingibereceae* family. In Indonesia, there are two types of cardamom that are known by the community, namely local cardamom/ *buhunjawa* (*Amomum cardamomum* Willd or *Amomum compactum ex Soland Maton*) and sabrang cardamom (*Elettaria cardamomum* (L) *Maton*) (Anonymous 2011). Local cardamom (*A. cardamomum* Willd) is a native Indonesian plant and endemic plant in the hilly area of West Java. This plant is now commercially cultivated as intercropping plantations in a community forest or developed on plantations and yards.

In the international market, *sabrang* cardamom is known as *true cardamom* while local cardamom is called *false cardamom* (Majo-Indo 1989). Cardamom as an export commodity is traded in the form of dried beans and essential oils. Dried cardamom is mostly exported to Singapore, Hong Kong, Japan, Middle East, and the United States. Cardamom production ranks third with a production amount of 72,760,295 kg or about 12.22% of the total production of national biopharmaceutical plants. The largest cardamom production center is Java Island with total production of 69,991,678 kg or about 96.19% of total national cardamom production (Anonymous 2014).

The cardamom essential oil is generally obtained from the seed portion by extracting by hydrodistilation. Several studies from seeds indicate the extraction of

cardamom essential oil of different types can produce different chemical components (Gopal et al. 2012). In addition to the seeds, rhizomes, and cardamom leaves also contain essential oils. Information with regard essential oils from rhizomes and leaves are still very limited. Research on the chemical content of the cardamom seed oil was performed by Mahmud (2008) for *Elettaria cardamomum*. Apart from the species, the volatile oils produced could also be affected by the conditions of the feedstock, the distillation method, and the distillation time (Guenther 1958). Several studies have shown that the method of waterdistillation and water-steam distillation results in a different yield, quality, and percent of different chemical content of the volatile oil produced (Sutiya 2006; Yuliarto et al. 2012).

A. cardamomum is generally used as a culinary ingredient and raw materials in the pharmaceutical industry. Seeds and essential oils of cardamom are used as flavoring agents in the food, medicine, and cosmetic industries. Traditionally in China and India, cardamom is used for medicines to overcome digestive problems and is also used in massage oils and lotions (Ravindran et al. 2002). Several studies have also evaluated the bioactivity of several types of cardamom. Bisht et al. (2011) described that the cardamom biocacies of the *Amulum subulatum* species as analgesic, anti-inflammatory, anti-microbial, antioxidant, anti-ulcer, cardio-adaptogen, and hypolipidemic. Sultana et al. (2010) presented the ability of cardamom seeds of *Elettaria cardamomum* as an antioxidant as other studies have evaluated the activity of cardamom oil as anti microbial (Malti et al. 2007).

The present study investigated the chemical content, physico-chemical properties, and antioxidant activity of cardamom oil obtained from local cardamom seeds and leaves (*A*. *cardamomum* Willd) extracted with water distillation and water-steam distillation. *A. cardamomum* was analyzed because of the information with regard to cardamom essential oil from *A. cardamomum* leaves is still limited. This study also elucidated antioxidant activity *A. cardamomum* oil using DPPH method (*1,1-in phenyl-2-picrylhydrazyl*).

Materials and Method

Sample, distillation, and yield

The seeds and leaves of local cardamomum (*A. cardamomum* Willd) in this study were obtained from cardamom farmers in Paitan village, Purworejo districts, Central Java. The seeds used were dried cardamom, while the leaves used were the chopped fresh leaves. The local cardamom seeds and leaves were extracted using hydrodistillation by water distillation and water-steam distillation method for approximately seven hours. The essential oil obtained was cooled and used as a test sample. The yield of this research was calculated based on the dry-yield and wet-yield. Wet-yield: the calculation of the yield obtained from the calculation of weight (volume x specific gravity) of total oil (Bm) divided by the weight of the starting material before

the distillation (Bd). Dry-yield: fresh materials were cut into small pieces, with samples of 2 g ±0.1 g in weight (BB) then were dried to a constant temperature of 103 ± 2 º C. After that, the calculation of water content of the material (KAb) was carried out. The material weight of total dry furnace used in the distillation was calculated by the following formula:

$$BT_{KT} = \frac{BTB}{[1+(\%\frac{KAb}{100})]}$$

where:

BT_{KT} : dry material weight of total furnace(g)
BT_B : wet total material weight for distillation (g)
KA_b : moisture content of the material (%)

The calculation of the yield based on the dry material weight is as follows:

$$R_K = \frac{Bm}{BTKT}\ X\ 100\%$$

where:

R_K : yield based on dry matter (%)
Bm : BJ x Vvolume of total oil produced (g)
BT_{KT} : dry total material weight for distillation (g)

GC-MS analysis

Chemical composition of seed and leaves of *A. cardamomum* was analyzed using Chromatography-Mass Spectrometry (GC-MS) QP2010S (Shimadzu Co. Ltd, Kyoto, Japan) with Agilent HP 5 MS capillary column of 30 m in length. Helium gas was used as a mobile phase with a gas flow rate of 60 ml/mins with an injection split, injection volume of 1.0 µl, injection temperature of 310 ºC. Ionization of spectrometers used EI (electron-impact ionization) at 70 eV. The result of chemical component analysis contained in *A. cardamom* oil was identified by comparing the retention time in the chromatogram with the library database contained in the instrument and compared with some literatures. The quantification of components was conducted by relative peak area method.

Physico-chemical Properties

The test for cardamom oil was performed with ISO 4733:1987 standard. Organoleptic test of cardamom oil in the form of color and odor was performed by direct test using the sense of sight and smell by comparing with commercial color of cardamom oil. The measurement of the specific gravity of oil was carried out using 2 ml pycnometer then the value was converted with a correction factor adjusted to a room temperature when the measurement was performed. The measurement of refractory oil index was done by using *hand-refractrometer* N-3000e (Atago Co. Ltd, Tokyo, Japan) and solubility test in alcohol used 70% of alcohol.

Antioxidant assay

Antioxidant was analyzed *in vitro* by DPPH method (*1,1diphenyl-2-picrilhidrazil*). A total of 1 ml of the test sample (in several concentrations of mg / ml) was dissolved in 10 ml of 0.25 mM DPPH solution (in ethanol). The solution was introduced into the

water bath at 30°C for 30 minutes and then its absorbance was measured by using a UV-Vis spectrophotometer at 515 nm wavelength. The tested controls used were only 1 ml of ethanol to which DPPH solution was added in accordance with the above mentioned methods. As a positive control, BHA (*butylated hydroxyanisole*) was used as a benchmark and tested in the same manner as the sample test. The percentage of antioxidants was calculated by the following equation:

$$\% \text{ Antioxidant} = [(Ac - As) / Ac] \times 100$$

Information: Ac=control absorbance, As =sample bsorbance.

Then the concentration value was calculated for the inhibition of oxidation (antioxidant) by 50% and 90% (IC_{50} and IC_{90}).

Statistic analysis

The data of yield, physico-chemical properties, and antioxidants of cardamom oil were analyzed using Completely Randomized Design (RAL). If there were significant different factors at test level of $\alpha = 0.05$, then post-hoc analysis was done by HSD (Honestly Significant Difference) method. The chemical components were analyzed descriptively.

Results and Discussion

Oil Yield

The results of wet-yield with the distillation of local cardamom seed *A. cardamomum* gave a higher yield value than yield value of the cardamom leaves, either by water or water-steam distillation. Values of essential oil of cardamom seeds ranged from 3.30 to 4.52%, while from leaves were 0.99-1.08% (Table 1). The result showed that the distillation method did not significantly affect the yield, whereas the material used i.e. seeds and leaves, significantly affected. The results of this study showed that the cardamom seeds contained greater volatile oil than the leaves.

The calculated yield value based on the dry weight was higher than the wet weight yield for seed oil and cardamom leaves. The dry content of *A. cardamomum* oil was 3.88 - 5.31% (Table 1). The result of variance analysis test for dry-yield showed that the factors of the material and the method gave effects to the yield. The high value of dry-yield compared to the wet- yield was due to the presence of water in the material at the time of the distillation of the material. Thus, it caused the calculation of yield still included the weight of water in the starting material. In this research, the leaf material used was in a fresh condition. Consequently, at the calculation of the wet-yield, the result of its essential oil became low whereas the calculation of dry-yield by removing the water weight, the yield would become higher. At the same time, for the seed was distilled on dry conditions with a small moisture content would make the calculation of wet- and dry-yield was not too different in their values.

Table 1. Wet-yield and dry-yield of *A. cardamomum* Oils

Sample	Wet-yield (%)	Dry-yield (%)
B1M1	4.52 ± 0.03 c	5.31 ± 0.06 d
B1M2	3.30 ± 0.06 b	3.88 ± 0.09 a
B2M1	1.08 ± 0.02 a	4.89 ± 0.16 c
B2M2	0.99 ± 0.02 a	4.50 ± 0.15 b
Average	2.47	4.65

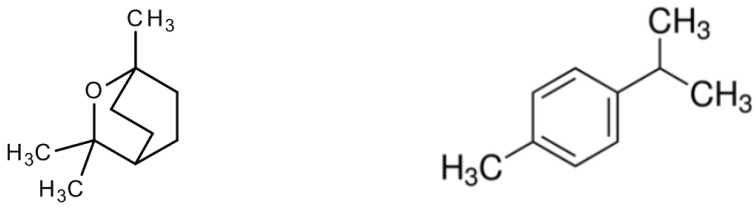
Note: B1M1= water distillation seeds, B1M2= water-steam distillation seeds,B2M1= water distillation leaves, B2M2= water-steam distillation leaves

Compared with other studies of cardamom oil from commercial *Elettaria cardamomum* seed (*sabrang*) with yield of 4.62-5.04% (Sumangat & Mulyono 2000), the dry-yield produced in this study was close to somewhat higher. Similarly, when compared to the essential oil of *E. cardamomum* from Guatemala with a yield of 2.7-3.0% (Gochev et al. 2012), local cardamom oil (*A. cardamomum*) in this study gave a higher yield value. The differences in the yield resulted in this study compared with other studies may be due to differences in the way of distillation, the material used, the basis of the calculation of yield, the difference of the growing place, and other factors.

Chemical composition

Testing of the local cardamom seed oil component (*A. cardamomum*) using Gas Chromatography - Mass Spectrometry (GC-MS) showed that the cardamom oil produced was consisted of 4-6 chemical components in the form of compounds belonging to the monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpenes (Table 2). In general, the largest components of cardamom oil from seeds and leaves with water and water-steam distillation were from oxygenated monoterpene and monoterpene groups. The main chemical components of cardamom oil in this study were *1.8-cineole* (39.42-92.10%) and *ρ-cymene* (42.16-50.77%).

GC-MS analysis showed that the cardamom oil in this study was composed of 4-6 chemical compounds with the largest main compounds of *1.8-cineole* and *ρ-cymene*. The chemical structure of the major compounds of cardamom oil composition in this study is presented in Figure 1.



1,8-Cineole *ρ-Cymene*

Figure 1. Main Compounds of *A. cardamomum* Oils

Table 2. Chemical compound of *A. cardamomum* oils in a relative peak percentage

No.	Chemical Components	Chemical Formula	B1M1	B1M2	B2M1	B2M2
1.	*β-ocimene*	$C_{10}H_{16}$	2.38	2.10	2.38	1.62
2.	*β-pinene*	$C_{10}H_{16}$	-	3.39	-	-
3.	*ρ-cymene*	$C_{10}H_{16}$	42.16	-	49.78	50.77
4.	*1,8-cineole*	$C_{10}H_{18}O$	47.22	92.10	44.54	36.42
5.	*α-terpineol*	$C_{10}H_{16}O$	-	2.41	-	-
6.	*Aromadendrene*	$C_{15}H_{24}$	8.23	-	3.30	9.09
7.	*γ-gurjunene*	$C_{15}H_{24}$	-	-	-	1.09
8	*Junipper-campor*	$C_{15}H_{26}O$	-	-	-	1.00
	Total		99.99	100.00	100.00	99.99

Note: B1M1= water distillation seeds, B1M2= water-steam distillation seeds,B2M1= water distillation leaves, B2M2= water-steam distillation leaves

Essential oils of distilled seeds by water and water-steam method contained 4 different chemical components of which water-steam distillation obtained oils provided *cineole* content of 92.10%, which was higher than other treatments. In the way of water and water-steam distillation for seeds, it obtained the same 2 compounds i.e. *β-ocimene* and *1,8-cineole* as well as 2 different chemical compounds. In the seeds by water distillation, it obtained other compounds of *ρ-cymene* and *aromadendrene*. Further, by the water-steam distillation, other compounds detected were *β-pinene* and *α-terpineol*. For the oil from the leaves by waterdistillation, it produced the same chemical constituents with the steamed leaf oil. On the other hand, by water-steam distillation method, it contained 2 more chemical compounds that were *γ-gurjunenen* and *junipper campor*. The content of *cineole* and *ρ-cymene* produced here also had almost equal percentage.

This study showed that the distillation materials and methods had an effect on the quality of the oil produced in which the chemical compounds and its content could be different. In this study, however, different distillation methods produced oil with the same main chemical components. This difference is possible in the way of water distillation as the material was directly in contact with water so that some chemical compounds underwent changes or degradation. In the way of water-steam distillation, the contact between water and materials was less and it was more optimal in the release of essential oils so that it then would generate more chemical compounds. This result is in accordance with Guenther (1987), which the condition of raw materials, distillation, and distillation time will affect the quality of oil produced.

Compared with other studies of other cardamom-oil chemicals, it was found that there were differences in the composition but there was the same primary compound of *1,8-cineole*. Sumangat and Mulyono (2000) study on the essential oil of *E. cardamomum* seeds produced the main compound of *1,8-cineole* of 44.4-45.9% and for the local cardamomum (*A. cardamomum*) was 72.10-79.40%. The main compound contained in the *E. cardamomum* species originating from Guatemala had a primary compound of *1.8-*

cineole of 29.2% (Gochev 2012), and Kumar et al. (2012) at *Amomum subulatum* gave a main component of *1,8-cineole* equal to 65.39%.

Physico-chemical properties

The local cardamom seed oil of *A. cardamomum* produced by water and water-steam distillation method generally produced clear and colorless oil, while the essential oil of *A. cardamomum* leaves had a clear yellowish color. Each of these oils had a strong and distinctive odor of cardamom (Table 3).

The study also examined the physico-chemical properties i.e. specific gravity, optical rotation, refractive index, solubility in alcohols, and acid numbers. The value of oil specific gravity in this research can be seen in Table 3 which ranged from 0.908 to 0.951. The analysis showed that the distillation method did not affect the weight of the species, but the material used had a significant effect. The average values of oil's specific gravity of cardamom seed materials were greater than those of leaf materials. By comparing to ISO standards, the oil obtained from local cardamom leaves met the standard, and the oil from the seed had a specific gravity value of the type above the standard. This was probably also due to the presence of oxygenated terpenes such as *1,8-cineole* was higher in cardamom seed oil in this study, where *1,8-cineole* was an oxidized terpene having a higher density than the unoxygenated terpenes.

Table 3. Physico-chemical of *A. cardamomum* oils

Sample	Specific Gravity	Optical Rotation (º)	Refractive index	Solubility in Alcohol	Acid Numbers	Color	Odor
B1M1	0.951 ± 0.01 a	6.43 ± 0.31 d	1.456 ± 0.00 a	1 : 1.66 ± 0.58 a	0.067 ± 0.029 a	Clear	Typical Cardamom Oil
B1M2	0.941 ± 0.02 a	5.70 ± 0.10 c	1.456 ± 0.00 a	1 : 1.66 ± 0.58 a	0.067 ± 0.029 a	Clear	Typical Cardamom Oil
B2M1	0.908 ± 0.01 a	1.40 ± 0.52 a	1.467 ± 0.01 a	1 : 2.66 ± 0.58 b	0.067 ± 0.029 a	Yellowish clear	Typical Cardamom Oil
B2M2	0.932 ± 0.02 a	2.50 ± 0.53 b	1.468 ± 0.00 a	1 : 6.33 ± 0.58 c	0.050 ± 0.087 a	Yellowish clear	Typical Cardamom Oil
ISO 4733:1987	0.919-0.938	22-41	1.462-1.468	1:2 – 1:5	Maks 6.0		

Note: B1M1= water distillation seeds, B1M2= water-steam distillation seeds,B2M1= water distillation leaves, B2M2= water-steam distillation leaves

The optical rotation level of the oil in this study was lower than the ISO standard (1.70 - 6.43 °), but when compared with the research of Sumangat and Mulyono (2000) with the local optical oil rotation values of 7-9,71°, then the values of the optical rotation were almost the same. This was probably due to cardamom oil in this study did not

contain *α-terpinyl* acetate compounds which are the main compounds in some commercial cardamomes such as *E. cardamomum.* It is known that this compound had the properties of rotating the field of polarization of a greater and positive light. The variance analysis results from the optical rotation in this study gave significantly different results in each treatment that could be due to the varied composition of the oil produced, especially on the percentage of the components.

The mean value of local cardamom oil refractive index in this study ranged from 1.456 - 1.468. Compared with the ISO standard (1.462-1.468), the value for the local cardamom seed oil (1.467-1.468) was appropriate, and the refractive index value for seed essential oil (1.456) was slightly below the standard. The results of variance analysis showed that the treatment in this study did not show a significant difference on the value of the results. It exhibited, however, a tendency of leaf material to produce the average value of the refractive index which is greater than the local cardamom seeds. This was probably due to the essential oils of the leaves containing long-chain compounds (sesquiterpenes) or more oxygen-suspended components cause the density of the essential oil medium to increase so that the incoming light will be more difficult to refract. Consequently, the refractive index of essential oils was to be greater. Essential oils with large refractive index values were better than essential oils with small refractive index values.

The levels of solubility in alcohol of local cardamom oil in this study were 1: 2 - 1: 7. This average was in accordance with ISO standards (1: 2 - 1: 5), except on the essential oils of cardamom leaves obtained by water-steam distillation (1: 7). The low solubility of oil in leaf cardamom oil by water-steam distillation was probably due to the unsaturated sesquiterpene in the oil which causes the oil was more difficult to dissolve in alcohol and requires a higher amount of alcohol to dissolve the oil. On the other side, it was found in this study that the volatile oil leaves by water-steam distillation contained the highest sesquiterpene (10.18%) compared to other treatments. The oil produced in this study can be said to be pure because the ratio of alcohols added was still below 10. It was also related to the chemical content of monoterpenes such as *β-ocimene, γ-terpinene* and others found in cardamom oil where the monoterpenes were soluble in alcohol.

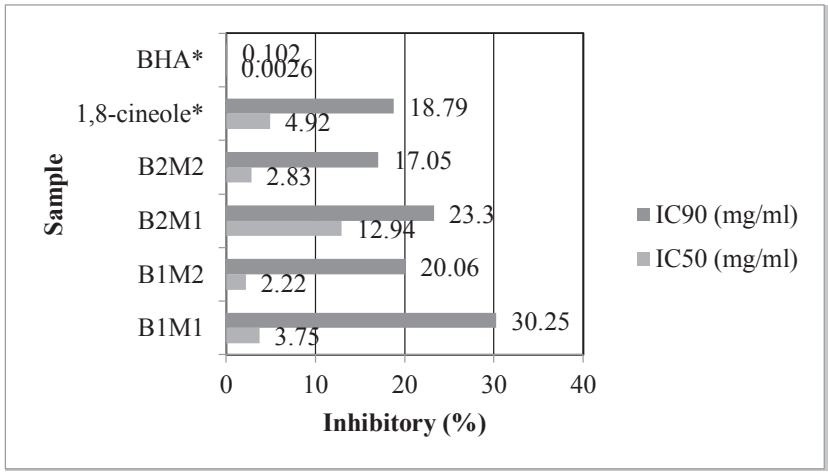
Acid number on local cardamom oil in this study showed no significant difference with the value of 0.050-0.067. This value was appropriate when compared to ISO standards (max 6). In the local cardamom, this value was small probably because there was no ester compound in the oil produced. It is because the greater the ester content and the greater the formation of acid compounds due to hydrolysis of esters. Thus, the oil produced in this study had a good quality as it had a low acid number (Table 3).

Antioxidants

The antioxidant assay in this study was performed using 5 levels of concentration (1 mg/ml, 5 mg/ml, 10 mg/ml, 15 mg/ml, and 20 mg/ml) to determine IC_{50} and IC_{90}

values. Local antioxidant test results of local cardamom oil for seeds and leaves provided higher IC_{50} and IC_{90} values for water-steam distilled oils.

A. cardamomum oil in this study had an inhibitory value against oxidation of 50% (IC_{50}) at concentrations between 2.22 mg/ml to 12.94 mg/ml and oxidation inhibition of 90% (IC_{90}) at concentrations between 17.05 mg/ml to 30.25 mg/ml, wherein cardamom seed oil and leaves obtained by water-steam distillation provided better antioxidant value (Fig. 2). When compared to 50% antioxidant inhibition value at *1,8-cineole*, the value of cardamom-oil produced in this study was better, but when compared to 90% inhibition the value of cardamom oil as an antioxidant was almost the same. However, compared with commercial antioxidants such as BHA which is a powerful antioxidant, the antioxidant activity in this study was low. The results of this study when compared with research of Winarsi et al. (2012) were almost similar in which IC_{50} of cardamom seeds was 3.6 mg/ml and from leaves was 4.05 mg/ml. It also showed the opposite results as leaf cardamom oil yielded greater inhibitory value than cardamom oil of seeds in this study. The presence of local cardamom oil antioxidant activity in this study was probably due to the presence of chemical substances such as *1,8-cineole* which had almost the same antioxidant value.



*Data sources for BHA and 1,8-cineole according to Pujiarti et al, 2012

Figure 2. IC_{50} and IC_{90} cardamom essential oil (*A. cardamomum*)

Conclusion

Local cardamom (*A. cardamomum*) oil in this study provided the largest yield in oil from seeds by water distillation. Cardamom oil obtained from seeds by water-steam distillation method provided the best physico-chemical values. The main compound contained and owned by local cardamom oil in this study was 1,*8-cineole* (36.42-92.10%). The highest value of *1,8-cineole* in cardamom seed oil with water-steam distillation was 92.10 %. The local cardamom oil in this study contained mild antioxidants in which the best antioxidant values were obtained from seeds and leaves by water-steam distillation method.

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