Chemical Compounds, Physicochemical Properties, and Antioxidant Activity of *A. cardamomum* Leaves and Rhizomes Oils on Different Distillation Time

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Abstract

Amomum cardamomum is local cardamom that grows widely and recently developed as an agroforestry crop in Indonesia. Its seeds, leaves and rhizomes are sources of essential oil. Essential oils from cardamom have many benefits for health and flavouring agent. The objectives of this study were to elucidate the yield, chemical composition, physico-chemical properties, and antioxidant activities of leaves and rhizomes oils of cardamom distillated using water-steam distillation for 4, 6, and 8 hours. The chemical composition were analyzed by GC-MS, physicochemical properties were analyzed using ISO standard and antioxidant activity were analyzed by DPPH method. The results showed that *A. cardamomum* oils yield between 0.06-0.33%. The main compound in the oils is 1,8-cineole with the highest percentage was obtained from cardamom rhizomes oil distillated for 6 hours (60.63%). The results of each sample almost have the same quality with specific gravity between 0.899 – 0.909; refractive index between 1.476-1.478; optical rotation between (+)2.05º-(+)2.38º; miscibility in 70% alcohol between 1:7-1:9; and acid number between 0.49-0.69. The leaves and rhizomes oils of *A. cardamomum* showed potent antioxidant activity with the highest antioxidant were obtained from cardamom rhizomes oil distillated for 8 hours with IC_{50}: 0.039 g/ml.

Keywords: distillation time, cardamom oils, leaf, rhizome, chemical composition, physico-chemical properties, antioxidant

Introduction

There are two types of cardamom that are grown in Indonesia namely *Amomum cardamomum* (local cardamom) and *Elettaria cardamomum* (cardamom sabrang) (Suryadinata 2008). *A. cardamomum* is native to Indonesia, endemic to the mountainous areas in western Java. This species is commonly cultivated in Western Java, Southern Sumatra, and Moluccas whereas Java and Sumatra are the major growing areas (Lim 2013).

A. cardamomum is a crop that widely cultivated in Indonesia, because of high economic value, suitable growing site, and can grow well under forest stands. Agroforestry between forest plants and cardamom began to be widely developed in Indonesia such in Ciamis, West Java. Cardamom agroforestry is profiting the farmers by 5.7 times more compared to the rainfed agriculture. In the mountainous area, agroforestry is also a favorite land management system. The implementation of cardamom agroforestry in large scale land use is quite promising for economic and ecological sustainability (Sharma *et al.* 2007). Part of cardamom plant that is generally used to produce essential oil is seed, while the leaves and rhizome cardamom has not been widely utilized. In fact, leaves and rhizome of cardamom can also produce essential oils (Winarsi 2014).

Essential oils may be extracted from different parts of the plant, such as leaves, fruit peels, seeds, bark, wood wicks, and flowers, and are usually obtained from steam or hydrodistillation (Ahn *et al.* 2018). Essential oil of cardamom can obtained by hydrodistillation with distillation time of about 6-8 hours. Cardamom plant produces an optimal seed up to the age of 10-15 years, after that the plants need to be replaced with the new plants. This condition causes the leaves and the rhizomes become waste. Previous study on cardamom provide a high yield (2.43%) from leaves oil of *Elletaria cardamomum* compared to other plants of the Zingiberaceae family (Batubara *et al.* 2016a). While research on the influence of distilation time on yield of cardamom seed oil showed that distillation times give different yield and quality (Rosjidi 1993).

Essential oils have been used in traditional medicine. The availabilities of essential oils seem to have a great potential as anti-inflammatory, anti-bacterial, anti-cancer therapeutic agents, and aromatherapies. In recent years, the essential oils and herbal extracts have attracted a great deal of scientific interest due to their potential as a source of natural antioxidants and biologically active compounds (Bozin *et al.* 2006; Tepe *et al.* 2005; Shaaban *et al.* 2011; Perricone *et al.* 2015).

Seed of cardamom known as queen of spices (Charles and Denys 2013) while cardamom essential oils have some bioactivities such as fever medicine, gout drug, and heartburn medicine. On the other hand, seeds, leaves, and cardamom rhizome also contain essential oils and compounds such as saponins, flavonoids, and polyphenols that have a potential as antioxidants (Winarsi 2014). Antioxidant activity also can be found in essential oils from *Ellettaria cardamomum* leaves (Batubara *et al.* 2016b). Antioxidant has been widely discussed for medical purposes, because its compounds can prevent the reaction caused by the presence of free radicals. To reduce the damage caused by reactive compounds, additive substances with antioxidant activity such as butylated

hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are widely used as drug compounds. However, It potentially toxic because they are made from chemicals. Therefore, natural antioxidants are in demand because they use natural ingredients, making it safer than synthetic materials. Natural antioxidant can be obtained from essential oils (Pujiarti *et al.* 2015), such as from cardamom oil (Wang *et al.* 2017).

Previous studies have analyzed some cardamom essential oils from seed. However, study of leaves and rhizomes *A. cardamomum* essential oils and hydrodistillation time are still limited. This study were conducted to elucidate the yield, chemical composition, physico-chemical properties, and antioxidant of leaves and rhizomes of *A. cardamomum* oils that were extracted from different times hydrodistillation of 4, 6, and 8 hours.

Materials and Methods

Plant Material and Extraction

Fresh leaves and rhizomes of *Amomum cardamomum* (age 11 years old) were collected from Prangkokan village in Kulonprogo district, Yogyakarta, Indonesia. The cardamom leaves and rhizomes were chopped with a length of 2 cm. For each sample, 5 kg of fresh cardamom leaves and rhizomes were extracted by hydrodistillation (water-steam distillation) for 4, 6, and 8 hours. Essential oil extraction was carried out by a hydrodistillation using 5 kg capacity. The obtained oils were kept in labeled bottles and were stored in fridge before analyzed.

Essential Oil Yield

The oil yields were determined based on dried weight of leaves or rhizomes. The 2 g fresh cardamom leaves and rhizomes were oven-dried each at 103 ± 2°C until its weight constant and water content were calculated.

GC-MS Analysis

The chemical compositions of cardamom oils were analyzed by GC-MS (Gas Chromatography-Mass Spectrometry) QP2010S with Agilent HP 5 column with length 30 m and film thickness 0.25 nm. The carrier gas was Helium and ionizing EI 70 eV. The oven column temperature was set at 70°C and the injection temperature was set to 310°C. The chemical analysis was performed on each sample. GC-MS analysis was performed with retention time 50 minutes. Quantification compounds of cardamom oils were calculated based on the relative peak area (percent area) from chromatogram and chemical components were confirmed by comparing retention time with NIST 147 data base library.

Physico-chemical Properties

Physico-chemical properties of cardamom oils was analyzed based on ISO 4733: 1981 including specific gravity, refractive index, optical rotation, miscibility in 70% alcohol, and acid numbers on each sample. The specific gravity was analyzed using pycnometer with certain temperature of 20ºC. The refractive index was analyzed using hand-refractometer. The optical rotation was analyzed using polarimeter for samples and a control (distilled water). The acid number was analyzed by NaOH 0.1 N titration.

Antioxidant Activity

Antioxidant activity was analyzed by DPPH method (1,1diphenyl-2-picrilhidrazil) based on Molyneux (2004) method with slight modification. Antioxidant activity test used 4 oil concentrations of 0.05 g/ml, 0.1 g/ml, 0.15 g/ml, and 0.2 g/ml. Tests were performed on each sample. The antioxidant percentages were analyzed by spectrometer (WPA brand) number at 515 nm wavelength. The inhibitory concentration 50% (IC_{50}) antioxidant of cardamom oils were determined by probit regression.

Statistical Analysis

All tests and analyses had been done in three replications on each sample. The results were tested by *Completely Randomized Design*/CRD. Significant differences between means were determined by Tukey HSD analysis. $P < 0.05$ was considered statistically significant.

Results and Discussion

The *A. cardamomum* leaves oils in this study have yield of between 0.26-0.33% and cardamom rhizomes oils had yield 0.06%. The yield of *A. cardamomum* oil were obtained from distillation time of 4, 6,and 8 hours were different, however the yield of leaves oils had tendency increasing by the increasing of distillation time. The highest yield of leaves oils were obtained from distillation time of 8 hours. On the other hand, distillation times 4,6, dan 8 hours gave no different yield of rhizomes oils. The yield of *A. cardamomum* leaves oils in this study lower than *Elettaria cardamomum* leaves oils (3.15%) (Batubara *et al.* 2016b), this is probably due to the different species of cardamom leaves were used. Parts of the plant also have effect on the oil produced, rhizome tissue is thicker than leaf tissue of cardamom. The hydrofusion process that occurs becomes more difficult on the rhizomes, resulting in fewer oil yields. This result accordanced with the Jaafar *et al.* (2007) study about the essential oils of the leaf and rhizome of *Etlingera elatior*, where the leaf oil has higher yield (0.0735%) than rhizome oil (0.0021%).

Table 1. Chemical composition of *A. cardamomum* essential oils

No.	Components*	Molecular Formula	Compound Group	Percentage [%]					
				Leaves			Rhizome		
				4 H	6 H	8 H	4 H	6 H	8 H
1	Alpha-Thujene	$C_{10}H_{16}$	Monoterpenes	-	-	-	5.66	12.1	13.3
2	4-Carene	$C_{10}H_{16}$	Monoterpenes	0.6	-	-	-	-	-
3	M-Cymene	$C_{10}H_{14}$	Monoterpenes	28.5	32.5	30.2	13.3	16.5	16.7
4	1,8-Cineole	$C_{10}H_{18}O$	Oxygenated Monoterpenes	55.1	52.2	50.3	51	61	51
5	Alpha-Terpinene	$C_{10}H_{16}$	Monoterpenes	-	-	-	-	-	0.56
6	Beta-Linalool	$C_{10}H_{18}O$	Oxygenated Monoterpenes	-	-	-	-	-	-
7	Linalool	$C_{10}H_{18}O$	Oxygenated Monoterpenes	-	-	-	-	3.3	3.38
8	Cyclohexane-l-ol	$C_{10}H_{16}O$	Oxygenated Monoterpenes	0.26	-	-	-	-	-
9	Alpha-Terpineol	$C_{10}H_{18}O$	Oxygenated Monoterpenes	-	-	-	-	0.57	2.56
10	Limonene oxide	$C_{10}H_{16}O$	Oxygenated Monoterpenes	-	-	-	-	-	0.52
11	Terpineol	$C_{10}H_{18}O$	Oxygenated Monoterpenes	0.18	-	-	-	-	-
12	Sabinenehydrate	$C_{10}H_{18}O$	Oxygenated Monoterpenes	-	-	-	0.9	0.96	0.81
13	Alpha-Terpineol acetate	$C_{12}H_{20}O_2$	Hydrocarbon	-	-	-	1.37	-	-
14	Cyclohexene	$C_{10}H_{18}O$	Oxygenated Monoterpenes	-	-	0.34	-	-	-
15	P-menth-1-en8-ol	$C_{10}H_{18}O$	Oxygenated Monoterpenes	0.31	-	-	-	-	-
16	Alpha-Thujenal	$C_{10}H_{14}O$	Oxygenated Monoterpenes	-	-	-	-	0.47	-
17	Azulenemethanol	$C_{15}H_{26}O$	Oxygenated Sesquiterpenes	-	-	-	-	-	0.37
18	Sabinyl acetate	$C_{12}H_{18}O_2$	Hydrocarbon	-	-	-	-	0.48	-
19	Isosafrole	$C_{10}H_{10}O_2$	Oxygenated Monoterpenes	-	-	-	1.38	0.57	-
20	Thymol	$C_{10}H_{14}O$	Oxygenated Monoterpenes	-	-	-	-	-	0.72
21	Isothujol	$C_{10}H_{18}O$	Oxygenated Monoterpenes	-	-	0.21	0.76	-	-
22	Limonene epoxide	$C_{10}H_{16}O$	Oxygenated Monoterpenes	-	-	0.16	-	-	-
23	Acetamide, N-prophyl	$C_{15}H_{11}NO$	Carboxamide	-	0.41	-	-	-	-
24	Patchoulene	$C_{15}H_{24}$	Sesquiterpenes	10.9	11.1	0.12	1.69	1.36	0.61
25	Chamigrene	$C_{15}H_{24}$	Sesquiterpenes	0.36	-	-	14	-	-
26	Octahydronapthalene	$C_{15}H_{24}$	Sesquiterpenes	-	-	-	1.05	-	-
27	Thujopsene	$C_{15}H_{24}$	Sesquiterpenes	-	0.63	0.37	-	-	0.75
28	Beta-Humulene	$C_{15}H_{24}$	Sesquiterpenes	0.17	-	-	-	-	-
29	Napthalene	$C_{15}H_{24}$	Sesquiterpenes	-	-	0.12	-	-	3.74
30	Beta-Vatirenene	$C_{15}H_{22}$	Sesquiterpenes	0.57	-	0.67	1.71	-	-
31	Cycloisolongifolene,8,9-dehydro	$C_{15}H_{22}$	Sesquiterpenes	0.29	0.84	-	-	-	-
32	Beta-Chamigrene	$C_{15}H_{24}$	Sesquiterpenes	-	-	12.8	-	-	-
33	Alpha-Bisabolene	$C_{15}H_{24}$	Sesquiterpenes	-	-	0.36	1.25	0.46	0.77
34	Cyclohexane	$C_{15}H_{24}$	Sesquiterpenes	0.23	-	-	-	-	-
35	Alpha-Panasinsen	$C_{15}H_{24}$	Sesquiterpenes	1.73	1.68	1.9	-	-	-
36	Germacrene	$C_{15}H_{24}$	Sesquiterpenes	-	-	-	-	-	2.85
37	Methanoazulen	$C_{15}H_{24}O$	Oxygenated Sesquiterpenes	-	-	0.22	-	-	-
38	Gamma-Gurjunepoxide	$C_{15}H_{24}O$	Oxygenated Sesquiterpenes	-	-	-	0.87	-	-
39	Cedrene	$C_{15}H_{24}$	Sesquiterpenes	-	-	0.21	-	0.55	-
40	Aromadendrene	$C_{15}H_{22}$	Sesquiterpenes	-	-	0.36	-	-	-

41	Lanceol	$C_{15}H_{24}O$	Oxygenated Monoterpenes	-	-	0.39	0.96	-	-
42	Spathulenol	$C_{15}H_{24}O$	Oxygenated Sesquiterpenes	0.27	-	-	-	-	-
43	Cycloprop[e]azulene	$C_{15}H_{24}$	Sesquiterpenes	-	-	1.08	-	-	-
44	Azulene	$C_{15}H_{24}$	Sesquiterpenes	0.63	-	-	-	-	-
45	Globulol	$C_{15}H_{26}O$	Oxygenated Sesquiterpenes	-	0.62	-	-	0.48	-
46	Alpha-Bisabolene epoxide	$C_{15}H_{24}O$	Oxygenated Sesquiterpenes	-	-	0.17	-	-	-
47	Ledol	$C_{15}H_{26}O$	Oxygenated Monoterpenes	-	-	-	-	0.63	-
48	Bergamotol	$C_{15}H_{24}O$	Oxygenated Sesquiterpenes	-	-	-	0.91	-	-
49	Dibutyl phthalate	$C_{16}H_{22}O_4$	Hydrocarbon	-	-	-	0.98	0.63	1.12
50	Dimethyl ester	$C_{16}H_{24}O_6$	Hydrocarbon	-	-	-	-	-	0.31
			Total	100	100	100	100	100	100

Note: * : Identification by Nist 147 library, - : not detected, H: distillation hour

GC-MS analysis showed that *A. cardamomum* oils consists of several compounds with different percentages in each sample. Chemical compounds of *A. cardamomum* essential oils showed in Table 1. The main compounds in *A. cardamomum* oils were obtained in this study are 1,8-cineole (50.30–60.95%) and m-cymene (13.29-32.54%). Previous studies also found that 1,8 cineole is the main compound of *A. cardamomum* leaf oil (Riendyani 2014). Ketaren (1985) also stated that the cardamom oil has the main compound 1,8 cineole. In this study, cardamom leaves oils were distillated for 4 hours has the highest 1,8-cineole with percentage 55.11%, while on the cardamom rhizomes oils were distillated for 6 hours oil has the highest 1,8-cineole with percentage 60.95%. Chemical compounds contained in leaves and rhizome of cardamom in this study are mostly included in the monoterpenes, oxygenated monoterpenes, and sesquiterpenes groups (Table 1).

Table 2. Physico-Chemical Properties of *A. cardamomum* Oils

Sample Oils *A. cardamomum*	Specific Gravity (20°C)	Refractive Index (20°C)	Miscibility in Alcohol 70%	Optical Rotation (°)	Acid Number
Leaves oil - distillated for 4 hours	0.903 ± 0.03a	1.476 ± 0.002a	1 : 7.33 ± 0.58a	(+) 2.37 ± 0.09a	0.50 ± 0.08a
Leaves oil -distillated for 6 hours	0.906 ± 0.02a	1.478 ± 0.001a	1 : 7.67 ± 0.58a	(+) 2.14 ± 0.13a	0.56 ± 0.08a
Leaves oil -distillated for 8 hours	0.909 ± 0.03a	1.478 ± 0.001a	1 : 8.00 ± 0.00a	(+) 2.05 ± 0.05a	0.57 ± 0.12a
Rhizomes oil- distillated for 4 hours	0.899 ± 0.00a	1.476 ± 0.002a	1 : 7.67 ± 0.58a	(+) 2.38 ± 0.02a	0.49 ± 0.09a
Rhizomes oil- distillated for 6 hours	0.901 ± 0.01a	1.477 ± 0.002a	1 : 8.00 ± 0.00a	(+) 2.28 ± 0.13a	0.59 ± 0.07a
Rhizomes oil- distillated for 8 hours	0.902 ± 0.01a	1.478 ± 0.001a	1 : 8.33 ± 0.58a	(+) 2.24 ± 0.17a	0.69 ± 0.02a
ISO 4733:1981*	0.191 – 0.938	1.462 – 1.468	1:2 – 1:5	22 - 41	Max. 6

(*Source : ISO 4733:1981)

Note : identical letters (a, b, etc.) mean no significant difference between mean in same column at P<0.05

Physicochemical properties of each sample were analyzed in this study almost had the same qualities and values. Statistical analysis showed no significant difference for each sample. The physicochemical properties of *A. Cardamomum* essential oils from this study are showed in Table 2. The results showed that the *A. Cardamomum* leaves oil had specific gravity value of between 0.903-0.909 while the *A. Cardamomum* rhizomes oils had specific gravity value of between 0.899-0.902. The highest specific gravity were *A.cardamomum* oils distillated for 8 hours, both cardamom leaves oils and cardamom rhizomes oils. The value of specific gravity of *A. cardamomum* oils in this study probably influenced by the present of 1,8-cineol and m-cymene compounds with specific gravity 0.922 and 0.861, respectively (Haynes 2014). Refractive index of *A. cardamomum* leaves and rhizomes olis had value of 1.476-1.478. Cardamom leaves oil which had the highest refractive index value is cardamom oil distilled for 6 hours, while the cardamom rhizomes oil having the highest refractive index is cardamom oil obtained for 8 hours distillation. *A. cardamomum* leaves oils had optical rotation value in average between (+)2.05º-(+)2.37º. While the cardamom rhizomes oils had optical rotation value in average between (+)2.24º-(+)2.38º. The optical rotation value of cardamom oils obtained in this study is lower than the standard by ISO 4733:1981. It probably due to this study used *A. cardamomum* while ISO 4733: 1981 is the standard for cardamom essential oil of *Ellettaria cardamomum*. This study used ISO standard because there is no standard for local cardamom of *A. cardamomum*. The results of the miscibility in 70% alcohol showed that the miscibility of *A. cardamomum* oils in alcohol were generally similar to the

ratio of 1: 7 to 1: 9 with an average miscibility of 1: 8. Miscibility of oil is influenced by the rapidity of oil solubility and the oils quality. If the oil mostly contained by oxygenated components, it will easily to be dissolved in alcohol (Guenther 1987). According to Ketaren (1985), the longer the amount of carbon chain, the more difficult the oil to be dissolved. The *A. cardamomum* leaves oils had an average acid number of 0.50-0.57 while *A. cardamomum* rhizomes oils had an average acid number of 0.49-0.69. The acid number in this study increased with the length of distillation time. The longer distillation time, water and oil contact is also longer and heat causes ester hydrolysis process to increase. Alcohol compounds with high molecules will be oxidized to aldehydes, carboxylic acids, and ketones.

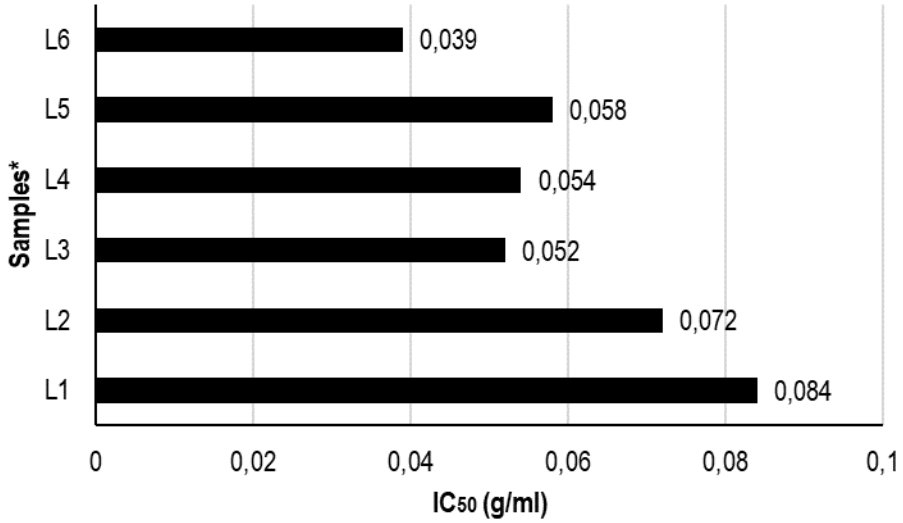

Figure 1. IC_{50} of *A. cardamomum* Leaves and Rhizomes Essential Oils (L1: Leaves oil-distillated for 4 hours, L2: Leaves oil-distillated for 6 hours, L3: Leaves oil-distillated for 8 hours, L4: Rhizomes oil-distillated for 4 hours, L5: Rhizomes oil-distillated for 6 hours, L6: Rhizomes oil-distillated for 6 hours)

This study also tested the antioxidant activity of essential oils which was analyzed by DPPH method. The result showed that the antioxidant activities of *A. cardamomum* oils have tendency increase by increasing the concentration. The concentration of 0.05 g/ml, 0.1 g/ml, 0.15 g/ml, 0.2 g/ml of *A. cardamomum* oils in this study have inhibitory concentration between 40.53 - 57.34%, 52.10 - 65.90%, 59.86 - 78.64%, 71.26 - 86.5%, respectively. Previous study also gave value of antioxidant activity increase by increasing concentration of essential oils (Pujiarti *et al.* 2015). Overall, *A. cardamomum* oils in this study have mild antioxidant activity. IC_{50} values in each of cardamom oil are presented in Figure 1. The lower IC_{50} value had better antioxidant activity, because in small percentages. The antioxidant compound can prevent about 50% radical activity. The highest IC_{50} value of antioxidant activity was found in cardamom leaves oils distillated for 4 hours, while the lowest IC_{50} value of antioxidant activity was found in cardamom rhizomes distillated for 8 hours. The results of chemical analysis of cardamom oil obtained in this study showed that most of the chemical components in *A. cardamomum* oils consists of terpenoid groups from the monoterpenes group, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes with the largest percentage is the oxygenated monoterpenes. Alkaloids and terpenoids from medicinal plants extraction have antioxidant activity when are tested with DPPH (Awouafack *et al.* 2013). Antioxidant activity of leaves and rhizomes oils in this study probably caused by the terpenoid contained in these oils such as m-cymene, patchoulene, and others terpenoid compounds. This study also found that average of antioxidant activity of cardamom leaves oils smaller than cardamom rhizomes oils. This is probably due to rhizomes oils have more terpenoid compound such as alpha thujene, linalool, and alpha-terpineol where are not found in leaves oils.

Conclusions

Leaves oils of *A. cardamomum* have higher yield than rhizomes oils. Distillation time had an effect on chemical compound of leaves and rhizomes oils of *A. cardamomum*, in which the chemical components of the oils were varied with the main compound was 1,8 cineole. However, distillation times had no effect on oils physico-chemical properties. *A. cardamomum* leaves and rhizomes oils obtained from hydrodistillation 4,6, and 8 hours had same qulities. Leaves and rhizomes oils of *A. cardamomum* posses mild antioxidant, while the highest antioxidant

activity obtained from rhizomes oils were distillated for 8 hours.

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