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Profile Volatile Compounds in Essential Oils on Different Parts of Cardamom with Antioxidant Activity

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Abstract: Amomum compactum Soland Ex. Maton from the Zingiberaceae, known as Java cardamom in Indonesia, is a valuable medicinal plant because of its bioactivity. This study aims to identify and evaluate the bioactive compounds and antioxidant activity of Java cardamom parts (leaves, stems, rhizomes, fruits (pods and seeds)) to explore their bioactivity values. GC-MS analysis was used to identify the bioactive compounds of Java cardamom parts in essential oils. Antioxidant activity was carried out by two methods: DPPH and FRAP. GC-MS analysis of four parts of the Java cardamom obtained 47 compounds as monoterpenes (33), sesquiterpenes (9), hydrocarbons (1), fatty alcohols (1), fatty acids (1), fatty acid esters (1), and diterpenoids (1). 1.8-Cineol is the most dominant secondary metabolite and is found in every part of Java cardamom essential oil, with the highest content produced in Java cardamom steam essential oil (50.78%), followed by Java cardamom fruits essential oil (45.59%). Furthermore, the activity of DPPH and FRAP ranged from 19.07 (leaves) – 27.38 (stems) and 93.43 (stems) – 115.99 (fruits) mol TEAC/g FW. The maximum antioxidant activity is produced in Java cardamom fruit essential oil. Thus, it can be used as a source of producing metabolites as antioxidants in the pharmaceutical industry.

Keywords: antioxidant activity; essential oil; Java cardamom; profiling metabolite; volatile compounds.

1. Introduction

Cardamom (*Amomum compactum* Soland Ex. Maton) is an aromatic herbal plant used for a long time as traditional medicine and cultivated in several Asian countries such as Sri Lanka, India, Nepal, Indonesia, Guatemala, and Tanzania [1, 2]. Cardamom is known as the 'queen of spices' because it is one of the spices with the third-highest economic value after vanilla and saffron [3]. Java cardamom is a type of cardamom originating from Indonesia, which is famous for its distinctive taste, aroma, and benefits and contains high bioactive compounds [4]. Java cardamom is a member of the genus *Amomum*, the second-largest genus after *Alpinia* from the *Zingiberaceae* and the order *Zingiberales* [5, 6].

Cardamom is traditionally used as a spice, aromatherapy, health drink, and traditional medicine for dried fruits and its essential oil [7]. Essential oils are aromatic oils derived from various parts of plants (flowers, seeds, leaves, stems, pods and seeds, rhizomes, and plant roots). Essential oils contain various chemical components [8], composed of a complex

structure of natural polar and non-polar compounds, most of which are terpenoid compounds and their derivatives. Types of molecules such as alpha-hydrocarbons, acids, alcohols, aldehydes, non-cyclic esters, or lactones, nitrogen and sulfur-containing compounds, coumarins, and phenylpropanoid homologs may also be present in the structure of essential oils [9, 10].

Cardamom essential oil is an herbal oil with medicinal properties and is used to treat various diseases [10]. In addition, essential oils are also applied in the perfumery, food, cosmetic, and pharmaceutical industries [11]. Several studies reported that different parts of the cardamom plant, such as fruits (pods and seeds) [2], seeds [12], rhizomes [13], and leaves [14], have been used in the treatment of several diseases [15]. The bioactivity of cardamom are reported to act as anti-bacterial [16], antioxidant [15], antimutagenic [17], anti-inflammatory [12], antihypertensive [18], anti-carcinogenic [19] and anticancer [20]. The bioactivity is related to the content of various phytochemical compounds found in cardamom plants, especially phenolic compounds, terpenoids, flavonoids, alkaloids, and anthocyanins [12]. Cardamom essential oil contains α-terpinyl acetate and 1,8-Cineole as the main chemical components that give cardamom essential oil a distinctive aroma, but in different varieties and cultivation areas, α-terpineol and linalool are the highest chemical compounds in cardamom essential oil. [21].

Free radicals are reactive oxygen compounds with unpaired electrons, thus causing these compounds to be very reactive in finding partners by attacking and binding electrons to molecules around them [22]. These free radicals can cause oxidative stress due to an imbalance between ROS and the anti-oxidative defense system that causes various diseases such as cancer, cardiovascular disease, neurodegenerative diseases, rheumatoid arthritis, atherosclerosis, hypertension, and AIDS. Antioxidants can inhibit or delay oxidative processes by preventing the initiation and propagation of oxidizing chain reactions [23].

Gas chromatography-mass spectrometry (GC-MS) is one of the most suitable for the determination of essential oil components [7] and identifying different metabolites of plant extracts [24-26]. The chemical composition of the essential oil of leaves, fruits, and stems of the Large cardamom (*E. cardamomum*) through GC-MS analysis has been reported, but reports on the composition of the essential oil of different parts of the Java cardamom plant (*A. compactum* Soland ex. Maton) such as leaves, stems, rhizomes, pods, and seeds as well as the antioxidant activity of each part of the Java cardamom plant is very limited. Therefore, this study was conducted to investigate and evaluate the volatile profile and antioxidant activity of essential oils in different parts of the Java cardamom plant (*A. compactum* Soland ex. Maton) and classify the parts of the cardamom plant using chemometric analysis. The results obtained can show the maximum part of the Java cardamom plant and has the potential as an antioxidant.

2. Materials and Methods

2.1. Material.

The materials used in this study were ethanol pro analysis purchased from Merck KGaA (Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH) powder purchased from Himedia (India), Trolox purchased from Merck KGaA (Germany), powder *2,4,6-tripyridyl-s-triazine* (TPTZ) was purchased from Sisco Research Laboratories Pvt. Ltd (India), aqua dest, hydrogen chloride (HCl) was purchased from Merck KGaA (Germany), ferric chloride ($FeCl_3$) was purchased from Sisco Research Laboratories Pvt. Ltd (India), and acetate buffer.

2.2. Plant material and sample preparation.

The leaves, stems, rhizomes, and fruits (pods and seeds) fresh of the Java cardamom (*Amomum compactum* Soland ex. Maton) from local farmers in Purwabakti Village, Pamijahan District Bogor Regency, West Java, Indonesia. Samples were extracted using hydrodistillation (water-steam distillation) according to Pujiarti and Kusumadewi [27] with modifications. Cardamom leaves, stems, and rhizomes were cut to a size of ± 2 cm. Fresh weight for each sample, i.e., leaves (10.5 kg), stems (10 kg), rhizomes (8.5 kg), and fruits (pods and seeds) (5.5 kg), were extracted using hydrodistillation (water-steam distillation) for 6 hours calculated from the first drop of distillate out. The first drop of essential oil from each sample was about 1 hour after the sample was distilled. The essential oil was obtained and stored in labeled bottles. The yield of essential oil was calculated as volume based on sample weight using the following formula: % yield (%, v/w) = volume of oil obtained (mL)/weight of the sample (g) x 100 [28].

2.3. GC-MS analysis.

The chemical composition of cardamom essential oil was analyzed using GC-MS (Gas Chromatography-Mass Spectrometry) based on Nurcholis *et al*. [26] with modifications. Metabolite profile analysis was performed using GC-MS (Gas Chromatography-Mass Spectrometry) Agilent Technologies 5977 equipped with an HP-5MS 5% PhenylMethylSilox (internal diameter 30 m x 250 µm, film thickness 0.25 µm, and maximum temperature 325°C). The carrier gas flow rate (He) is 1 mL min^{-1}. A sample of 1 L was injected into GC-MS with an injection temperature of 280°C. The GC-MS temperature was set for operating conditions, namely with an initial temperature of 50°C (holding 0 minutes) and then increasing it at an increasing rate of 10°C min^{-1} to a final temperature of 280°C (holding 9 minutes) with a total time of 32 minutes. The mass spectrophotometer is operated at 70eV, and the scanned mass spectrum ranges from 35-650 amu. Each sample was analyzed once without replication. Identification, including name, chemical structure, and molecular weight of each cardamom essential oil, was calculated based on the chromatogram's relative peak area (percent area) and confirmed chemical components by comparing retention times with Willey 9 library database and PubChem data.

2.4. Antioxidant analysis.

Samples of essential oil from each part of the Java cardamom made a stock solution of 1000 ppm essential oil by dissolving 0.1 mL of essential oil and ethanol pro analysis solvent in a 100 mL volumetric flask. The total antioxidant capacity determined the volatile oil solution from each part of the Java cardamom plant by two *in vitro* methods. Antioxidant testing in the form of 2,2-diphenyl picrylhydrazyl (DPPH) to evaluate free radical scavenging activity and ferric reducing antioxidant power (FRAP) to determine the reducing power of Fe^{3+} complexes to Fe^{2+}.

DPPH radical activity was measured using a nano-spectrophotometer based on Nurcholis *et al*. [29] with modifications. Briefly, 100 µL of 500 ppm essential oil solution per cardamom plant part was added with 100 µL of 125 M DPPH solution (in ethanol pro analysis) into a 96-well microplate (Biologix Europe GmbH). Then homogenized and incubated for 30 minutes at darkroom and room temperature. The absorbance of the volatile oil solution of each

part of the Java cardamom was measured using a nano-spectrophotometer (SPECTROstar Nano BMG LABTECH) at a wavelength of 515 nm. The final unit is expressed in mol TE (Trolox equivalent)/g fresh weight.

The activity of ferric reducing antioxidant power (FRAP) was measured using a nano-spectrophotometer according to Calvindi *et al*. [30] with a modification. Briefly, as much as 10 µL of 1000 ppm essential oil solution per cardamom plant part was added with 300 µL of FRAP reagent (made by mixing acetate buffer pH 3.6 with 10 M TPTZ solution (in 40 M HCl) and 20 µM $FeCl_3$ (in distilled water) in the ratio v/v/v 10:1:1) was placed in a 96-well microplate (Biologix Europe GmbH). Then homogenized and incubated for 30 minutes at darkroom and room temperature. The absorbance of the volatile oil solution of each part of the cardamom plant was measured using a nano-spectrophotometer at a wavelength of 593 nm. The final unit is expressed in mol TE (Trolox equivalent)/g fresh weight.

2.5. Data analysis.

Statistical analysis of antioxidant data was performed using analysis of variance (ANOVA) at a significance level of α = 5% and continued with Tukey's test using IBM SPSS Version 25.0 program. Significant differences between the part of Java cardamom on antioxidant activity were carried out by PCA (principal component analysis) multivariate analysis and HCA (hierarchical cluster analysis)-heatmap dendrogram using https://www.metaboanalyst.ca/. Graphs of the figure were using GraphPad Prism for Windows (GraphPad Software Inc., San Diego, California, USA) Version 8.0.1.

3. Results and Discussion

3.1. Extraction yield.

Java cardamom essential oil produced using hydrodistillation (water-steam distillation) for 6 hours is pale yellow-dark yellow. Cardamom fruits (pods and seeds) essential oil is pale yellow; this is in line with research conducted by Joshi *et al*. [31], which produces Java cardamom seeds essential oil, which is pale yellow, while the essential oil of Java cardamom rhizomes and stems is yellow, and Java cardamom leaves essential oil which is dark yellow. The percentage of essential oil yield of each part of the Java cardamom produced is different; this is because the fresh weight of each part of the Java cardamom plant used is different. After all, it experiences a loss of weight. Table 1 shows the results of the % yield of essential oils from each part of the Java cardamom plant.

Table 1. Hasil % rendemen pada tiap bagian tanaman kapulaga Jawa (*A. compactum* Soland ex. Maton).

Plant parts	Fresh weight (Kg)	Oil obtained (mL)	Yield (% v/w)
Leaves	10.5	110	1.1
Stems	10	4	0.038
Rhizomes	8.5	25	0.454
Fruits (Pods and seeds)	5.5	88.5	1.041

[1] Note: % v/w; % volume/weight

The oil produced by hydrodistillation gives a strong, distinctive odor to each part of the plant; cardamom fruits (pods and seeds) essential oil gives a more concentrated odor when compared to the essential oils of the leaves, stems, and rhizomes of Java cardamom. The highest

yield of Java cardamom essential oil was produced in the fruits (pods and seeds) of Java cardamom by 1.041% (5.5 kg, %v/w), and the lowest essential oil was produced in the stems of Java cardamom, which was 0.038% (10 kg, %v/w) based on fresh weight. The resulting yield is smaller than the research conducted by Jena *et al*. [11], was reported that the average leaves and stems essential oil yields on *E. cardamomum* were 0.45% and 0.23% (500 g, %w/w), while the cardamom seed essential oil yields reported by Tambunan [32] which is 0.76% (500 g, %v/w) based on fresh weight. The weight and condition of the sample, both fresh and dry, will affect the yield of essential oils. Oil content is influenced by maturity, climatic conditions, and harvest time [31]. Essential oils are compounds that evaporate quickly at room temperature without undergoing decomposition and are generally in the form of liquids obtained from plant parts such as roots, bark, stems, leaves, fruit, seeds, and flowers by distillation using steam and soluble in organic solvents and insoluble in water [33].

3.2. Metabolite compositions.

The essential oils of each part of the Java cardamom in the form of leaves, stems, rhizomes, and fruits (pods and seeds) were analyzed for their compound content using GC-MS analysis. Forty-seven metabolites from 4 parts of the Java cardamom were identified in the essential oil. Phytochemical compounds were identified based on retention time, peak area, and the molecular formula (Table 2). Of four parts of the Java cardamom plant, the compounds identified can be categorized into different groups (Figure 1), namely monoterpenes (33), sesquiterpenes (9), hydrocarbons (1), fatty alcohols (1), fatty acids (1), fatty acid ester (1), and diterpenoid (1) with a total of 47 compounds which were arranged with the initial peak width and initial threshold of 0.1 and 24 respectively. Details of volatile compounds identified from essential oil are presented in Table 2.

Table 2. Volatile compounds identified from essential oil on different parts of Java cardamom.

No	Compounds	Group compounds	MF	MW (g/mol)	RT	% Area			
						Leaves	Stems	Rhizomes	Fruits
1	α-Thujene	Monoterpenes	$C_{10}H_{16}$	136.23	4.208			1.27	0.71
2	1R-α-Pinene	Monoterpenes	$C_{10}H_{16}$	136.23	4.306	1.49	2.02		
3	α-Pinene	Monoterpenes	$C_{10}H_{16}$	136.23	4.313			4.45	4.47
4	Camphene (CAS)	Monoterpenes	$C_{10}H_{16}$	136.23	4.521		1.19	1.49	
5	Sabinene	Monoterpenes	$C_{10}H_{16}$	136.23	4.848	1.9	2.49	2.45	
6	(-)- β-Pinene	Monoterpenes	$C_{10}H_{16}$	136.23	4.910			2.63	
7	2-β-Pinene	Monoterpenes	$C_{10}H_{16}$	136.23	4.917				10.1
8	β-Myrcene	Monoterpenes	$C_{10}H_{16}$	136.23	5.063			5.14	2.99
9	α-phellandrene	Monoterpenes	$C_{10}H_{16}$	136.23	5.292		2.78	15.56	7.17
10	1,8-Cineole	Monoterpenes	$C_{10}H_{18}O$	154.25	5.897	40.53	50.78	40.77	45.59
11	γ-terpinene	Monoterpenes	$C_{10}H_{16}$	136.23	6.092	0.5	0.67	1.92	3.97
12	*trans*-Sabinene hydrate	Monoterpenes	$C_{10}H_{18}O$	154.25	6.244				2.69
13	α-Terpinolene	Monoterpenes	$C_{10}H_{16}$	136.23	6.495			2.02	
14	2-Nonanol (CAS)	Fatty alcohols	$C_9H_{20}O$	144.25	6.661	1.8	2.28		
15	(+-)-Linalool	Monoterpenes	$C_{10}H_{18}O$	154.25	6.675			4.71	3.94
16	trans-p-Mentha-2,8-dienol	Monoterpenes	$C_{10}H_{16}O$	152.23	7.023	3.32	2.55		
17	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-, *trans*-	Monoterpenes	$C_{10}H_{16}O$	152.23	7.245	1.82	1.82		
18	*trans*-Pinocarveol	Monoterpenes	$C_{10}H_{16}O$	152.23	7.328	3.44	3.94		
19	Camphor (CAS)	Monoterpenes	$C_{10}H_{16}O$	152.23	7.370			0.91	
20	Cyclopentane, 1,2-dimethyl-3-(1-methylethenyl)-	Monoterpenes	$C_{10}H_{18}$	138.25	7.523		0.85		
21	(+)-Pinocarvone	Monoterpenes	$C_{10}H_{14}O$	150.22	7.641	2.66	3.57		

No	Compounds	Group compounds	MF	MW (g/mol)	RT	% Area			
						Leaves	Stems	Rhizomes	Fruits
22	delta-Terpineol	Monoterpenes	$C_{10}H_{18}O$	154.25	7.718	2.14	2.09	1.42	2.12
23	4-Terpineol	Monoterpenes	$C_{10}H_{18}O$	154.25	7.850	1.48	2.05	2.17	1.8
24	Cyclohexanol, 2-methylene-5-(1-methylethenyl)-	Monoterpenes	$C_{10}H_{16}O$	152.23	8.023	4.22	6.33		
25	α-Terpineol	Monoterpenes	$C_{10}H_{18}O$	154.25	8.058			6.43	
26	Terpineol	Monoterpenes	$C_{10}H_{18}O$	154.25	8.079				9.23
27	(-)-Myrtenol	Monoterpenes	$C_{10}H_{16}O$	152.23	8.135	3.61	5.24		
28	α-Phellandrene epoxide	Monoterpenes	$C_{10}H_{16}O$	152.23	8.211			1.03	
29	Carveol	Monoterpenes	$C_{10}H_{16}O$	152.23	8.440	1.46	1.47		
30	*cis*-Carveol	Monoterpenes	$C_{10}H_{16}O$	152.23	8.586	2.72	3.46		
31	(+)-Carvone	Monoterpenes	$C_{10}H_{14}O$	150.22	8.788	1.14	1.67		
32	α-Copaene	Sesquiterpenes	$C_{15}H_{24}$	204.35	10.560	0.77		0.55	0.74
33	β-Selinene (CAS)	Sesquiterpenes	$C_{15}H_{24}$	204.35	12.019	0.87			2.36
34	(-)-β-Bisabolene	Sesquiterpenes	$C_{15}H_{24}$	204.35	12.200			1.58	1.07
35	β-Sesquiphellandrene	Sesquiterpenes	$C_{15}H_{24}$	204.35	12.401			0.99	1.04
36	Teresantalol (CAS)	Monoterpenes	$C_{10}H_{16}O$	152.23	12.401	3.04			
37	trans-γ-Bisabolene	Sesquiterpenes	$C_{15}H_{24}$	204.35	12.484			0.83	
38	4,8-Dimethyl-2-(2-methyl-1-propenyl)-1-oxaspiro [4.5] dec-7-ene	Hydrocarbons	$C_{15}H_{24}O$	220.35	12.832	2.82			
39	Nerolidol	Sesquiterpenes	$C_{15}H_{26}O$	222.36	12.853			0.97	
40	p-Mentha-1,5,8-triene (CAS)	Monoterpenes	$C_{10}H_{14}$	134.22	13.117	1.38			
41	α-Terpinene	Monoterpenes	$C_{10}H_{16}$	136.23	13.228	2.6			
42	*trans*-calamenene	Sesquiterpenes	$C_{15}H_{22}$	202.34	13.291	3.47			
43	Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-, (S)-	Sesquiterpenes	$C_{15}H_{24}$	204.35	13.513	4.09			
44	Acetic acid, 3-(2,2-dimethyl-6-methylene-cyclohexylidene)-1-methyl-butyl ester	Fatty acid	$C_{16}H_{26}O_2$	250.38	13.833	1.53			
45	ar-Curcumene	Sesquiterpenes	$C_{15}H_{22}$	202.3352	14.333	0.86			
46	Hexadecanoic acid, 1-methylethyl ester (CAS)	Fatty acid ester	$C_{19}H_{38}O_2$	298.29	17.668		1.99		
47	Phytol	Diterpenoid	$C_{20}H_{40}O$	296.53	18.530	2.24	0.74		

[2] Note: MF: molecule formula; MW: molecule weight (g/mol); and RT: retention time (min)

In detail, the compounds identified from the essential oil of four parts of the Java cardamom consist of leaves (27), stems (21), rhizomes (21), and fruits (pods and seeds) (16) compounds which represent 97.9%, 99.98%, 99.29%, and 99.99% of the total leaves, stems, rhizomes, and fruits (pods and seeds) essential oils, respectively. The results of the analysis showed that the compound 1.8-Cineole is the main component of the essential oil of four parts of the Java cardamom, namely the leaves (40.53%), stems (50.78%), rhizomes (40.77%), and fruits (pods and seeds) (45.59%). This is in line with the research conducted by Jena *et al*. [11] reported 1.8-Cineole (20.66%) as the main component of cardamom leaves essential oil (*E. cardamomum*), but α-terpinyl acetate (19.75%) was reported as the main component of cardamom stems essential oil. In comparison, essential oil *E. cardamomum* Tambe *et al*. [34] reported the compound 1.8-Cineole (33.64%) as the main component. Based on previous research, there are differences between the types and amounts of chemical compounds in

essential oils in different plant species due to biodiversity between species, changes in climate and seasons, as well as graphic conditions, plant growth stage, harvest period, and the type of distillation used when refining essential oils [35, 10]. In this study, the content of the compound 1.8-Cineole in the essential oil of both cardamom leaves and fruits (pods and seeds) was higher than in previous studies. This higher concentration difference could occur due to differences in varieties and environmental effects [36].

The other main terpenoid components of each Java cardamom essential oil, namely the Java cardamom leaves essential oil are Cyclohexanol,2-methylene-5-(1-methylethenyl)- (4.22%), Cyclohexene,6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-, (S)- (4.09%), (-)-Myrtenol (3.61%), trans-Calamenene (3.47%), trans-Pinocarveol (3.44%), trans-p-Mentha-2,8-dienol (3.32%), and Teresantalol (CAS) (3.04%). In Java cardamom stems essential oils, namely Cyclohexanol,2-methylene-5-(1-methylethenyl) -(6.33%), (-)-Myrtenol (5.24%), *trans*-Pinocarveol (3.94%), (+)-Pinocarvone (3.57%), *cis*-Carveol (3.46%), and α-phellandrene (2.78%). Meanwhile, the other main terpenoid components in Java cardamom rhizomes essential oil are α-Phellandrene (15.56%), α-Terpineol (6.43%), β-Myrcene (5.14%), (+-)-Linalool (4.71%), α-Pinene (4.45%), (-)- β-Pinene (2.63%), and Sabinene (2.45%), and in Java cardamom fruits (pods and seeds) essential oils are 2-β-Pinene (10.1%), Terpineol (9.23%), α-phellandrene (7.17%), α-Pinene (4.47%), (+-)-Linalool (3.94%), and β-Myrcene (2.99%). The most dominant secondary metabolite in the essential oil of the four parts of the Java cardamom is 1.8-Cineole, with the highest production produced by Java cardamom stem essential oil. In addition, other metabolites produced from the four parts of the Java cardamom are γ-terpinene, delta-Terpineol, and 4-Terpineol, with the highest production being produced by essential oils of fruits (pods and seeds), leaves, and rhizomes of Java cardamom, respectively. The compound 1.8-Cineol is a natural monoterpene known as eucalyptol which has anti-inflammatory activity [37], antioxidant, and anticancer [38]. In addition, α-Pinene compounds contained in essential oils of rhizomes and fruits (pods and seeds) and α-phellandrene compounds contained in essential oils of Java cardamom stems, rhizomes, and fruits (pods and seeds) have anti-inflammatory [39] and antifungal [40].

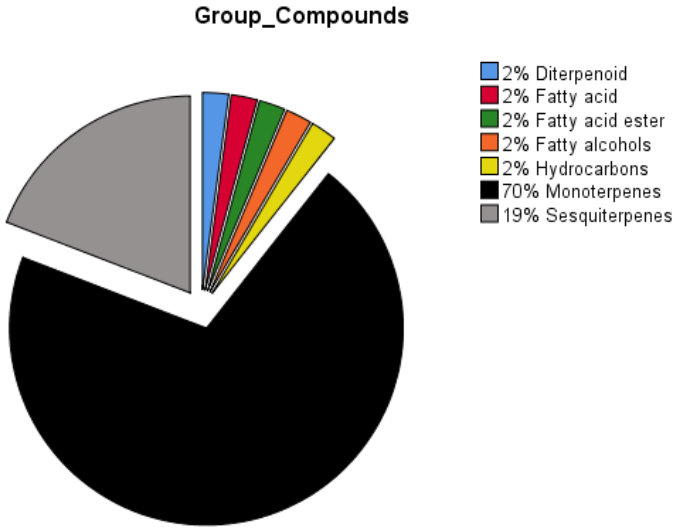

Figure 1. Pie Diagram displays the percentage of compound groups found on different parts of Java cardamom.

In the essential oil of four parts of the Java cardamom plant, monoterpenes were the dominant class, each for leaves (70%), stems (90%), rhizomes (76%), and fruits (pods and seeds) (75%), followed by sesquiterpenes (19%), hydrocarbons (4%), fatty alcohols (4%), and diterpenoids (4%) in Java cardamom leaves essential oil. Similarly, in the essential oils of Java

cardamom stems, namely fatty alcohols (5%) and diterpenoids (5%), while the rhizomes and fruits (pods and seeds) essential oils were followed by sesquiterpenes (24%) and sesquiterpenes (25%). This is in line with research conducted by Abdullah *et al*. [41] reported higher concentrations of monoterpenes than sesquiterpenes in the essential oil of *E. cardamomum* from a local market in Pakistan. Snoussi *et al*. [42] also reported the same thing, the difference in the concentration of monoterpenes and sesquiterpenes in *E. cardamomum* obtained from the local Tunisian. A total of 4 compounds were found in four parts of the plant in Java cardamom essential oil, such as 1.8-Cineole, γ-Terpinene, delta-Terpineol, and 4-Terpineol. Ashokkumar *et al*. [3] also reported the same thing, that the compound 1.8-Cineole is a compound found in every accession of green cardamom plants. While Sabinene compounds are found in essential oils in 3 parts of the Java cardamom plant, such as in the leaves, stems, and rhizomes, α-phellandrene compounds are found in Java cardamom essential oils in the stems, rhizomes, fruits (pods and seeds) and α-Copaene compounds are found in Java cardamom essential oils in leaves, rhizomes, and fruits (pods and seeds). Qualitative differences in composition were also observed between the leaves, stems, rhizomes, pods, and Java cardamom essential oil seeds. Previous studies reported that the main components in black cardamom essential oil were 1,8-Cineole (36.66%), β-Pinene (8.55%), α-Terpineol (8.44%), 1R-α-Pinene (5.10%), and Limonene (4.51%) [43], while Jabbar and Ghorbaniparvar [7] reported that the main components in green cardamom essential oil were 1,8-Cineol (47.18%), *alpha*-Terpinyl Acetate (14.33%), Linalool (6.28%), Terpineol (4.94%), and l-4-Terpineol (2.48%). Shrestha [44] reported that the main components in *Amomum subulatum* Roxb fruits essential oil were α-Terpineol (27.85%), Terpine-4-ol (11.19%), Pinocarvone (8.02%), Nerolidol (6.90%), and Pinocarveol (6.32%). Therefore, based on previous research, it can be seen that based on GC-MS examination, the type of species, source of raw material, and the growing area will determine the amount and quality of metabolites in cardamom essential oil. In addition, there are differences in the volatile profiling of essential oils in aromatic and medicinal plants depending on various environmental factors such as temperature, rainfall, humidity, plant nutrition, genetic variation, and geographical location [11,45,46].

3.3. Antioxidant activity.

The antioxidant activity aims to measure the total antioxidant capacity of different parts, such as the leaves, stems, rhizomes, and fruit of java cardamom. The antioxidant activity of essential oils in four parts of the Java cardamom was measured using the DPPH and FRAP methods.

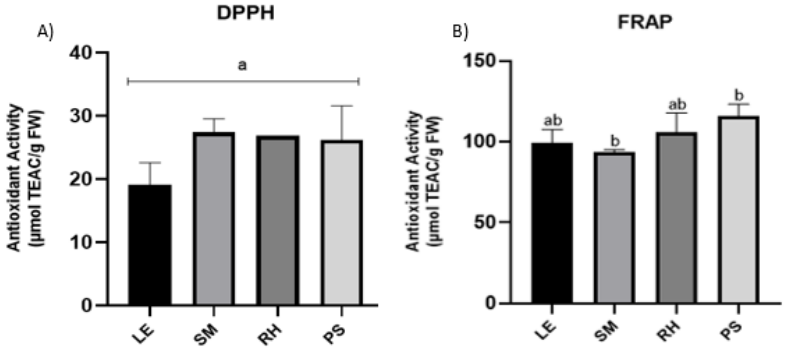

Figure 2. Antioxidant activities on a different part of Java cardamom A) DPPH and B) FRAP. Each value is presented as the mean of three replicates ± standard deviation (SD). The mean value in each column marked with different letters differs significantly at $p < 0.05$ and Tukey test results 5%. TEAC, Trolox equivalent antioxidant capacity; LE, Leaves; SM, Steams; RH, Rhizomes; PS, Pods, and Seeds.

The antioxidant activity of the four parts of the Java cardamom in the essential oil is presented in Figure 2. In this study, the antioxidant activity was measured using two methods because the antioxidant activity depends on the antioxidant mechanism of the metabolites in the extract [30,47]. Thus, different methods are needed to evaluate the nature of the antioxidant capacity. In this study, DPPH and FRAP were used to measure the antioxidant capacity of the essential oils, four parts of Java cardamom. Antioxidant testing in the form of 2,2-diphenyl picrylhydrazyl (DPPH) to evaluate free radical scavenging activity and ferric reducing antioxidant power (FRAP) to determine the reducing power of Fe^{3+} complex compounds to Fe^{2+}.

Based on the antioxidant activity obtained by DPPH (Figure 2A) and FRAP (Figure 2B), the antioxidant potential of the essential oils of the four parts of the Java cardamom tested by the FRAP method showed higher antioxidant activity than those tested by the DPPH method. These results indicate that Java cardamom essential oil has more great antioxidant properties in its reduction capacity compared to free radical scavenging activity; this is related to the different reaction mechanisms that occur in Java cardamom essential oil, which is Java cardamom essential oil more dominant using a single electron transfer mechanism (SET) compared to atomic hydrogen transfer (HAT). This is in line with the research conducted by Nurcholis *et al*. [15] was reported that the antioxidant potential of cardamom fruits accession extract using the CUPRAC method was higher than the DPPH method. This DPPH value is also consistent with research conducted by Alam *et al*. [48]. The antioxidant activity of DPPH in the essential oil of four parts of the Java cardamom showed no significant results ($p > 0.05$). The highest DPPH antioxidant activity was found in Java cardamom stems essential oil of 27.38 mol TEAC/g FW, while the lowest DPPH antioxidant activity was found in Java cardamom leaves essential oil of 19.07 mol TEAC/g FW. This is in line with research conducted by Alam *et al*. [48] was reported that the antioxidant activity of green cardamom leaves essential oil was lower than the standard antioxidant compounds. While Jena *et al*. [11] reported different results, like the antioxidant activity of cardamom leaves (*E. cardamomum*) essential oil was better than that of cardamom (*E. cardamomum*) stems essential oil. In contrast to the antioxidant activity of DPPH, the antioxidant activity of FRAP in the essential oils of the four parts of Java cardamom showed significant results ($p < 0.05$). The highest FRAP antioxidant activity was found in Java cardamom fruits (pods and seeds) essential oil of 115.99 mol TEAC/g FW, while the lowest FRAP antioxidant activity was found in Java cardamom stems essential oil of 93.43 mol TEAC/g FW. The results obtained showed that the antioxidant activity of FRAP was higher than in previous studies using methanol cardamom fruits [49]. The chemical complexity of the various essential oils causes differences in the antioxidant capacity of each part of the Java cardamom.

The difference between the two methods used in this test is that the DPPH method tests antioxidant activity that uses a chemical reaction. Antioxidant compounds will react with DPPH through a hydrogen atom donor mechanism so that it will cause a change in DPPH color from dark purple to yellow; the degree of color change, that indicates the potential of antioxidant compounds in their ability to donate hydrogen atoms [22], while the FRAP method is an antioxidant test in plants that aims to determine the reducing power of Fe3+ complex compounds to Fe2+ complex compounds [50]. The two methods of antioxidant testing were carried out because different methods of measuring antioxidant activity will refer to the observation of different antioxidant mechanisms of action due to the influence of the chemical

structure of antioxidants from free radicals and the Physico-chemical properties of different sample preparations [51].

Several previous studies reported that 1.8-Cineole [38], camphene [52], α-pinene [53], and caryophyllene oxide [54] are the main compounds that have the potential to scavenge free radicals. In addition, α-Terpinyl Acetate, Linalyl Acetat [48], β-Pinene [55], α-Terpineol, and Terpinen-4-ol [56] also have potential as antioxidants. 1.8-Cineole, camphene, α-Pinene, β-Pinene, and α-terpineol compounds contained in Java cardamom essential oil with different levels in the four parts of the Java cardamom plant, causing antioxidant activity of four parts of the Java cardamom plant in the different oil essentials. 1.8-Cineole and α-Pinene compounds are known to protect hydrogen peroxide in inducing oxidative stress in pheochromocytoma cells in mice [11, 57]. Camphene compounds are known to reduce lipid peroxidation induced by AAPH (2.22-Azobis(2-methylpropionamidine) dihydrochloride) [58]. Several previous studies have reported that cardamom has the potential as a good antioxidant and the potential to fight oxidative stress [48].

3.4. Multivariate analysis.

In this study, multivariate analysis, namely chemometrics, was carried out using PCA (principal component analysis) and HCA (hierarchical cluster analysis)-heatmap dendrogram analysis of 47 aromatic compounds from four parts of the Java cardamom in essential oils (Figure 3). The chemometric analysis aims to analyze quantification and increase understanding of the properties and quality of data instruments [26,59], as well as to determine chemical components in plants that have potential as drugs [26, 60-62].

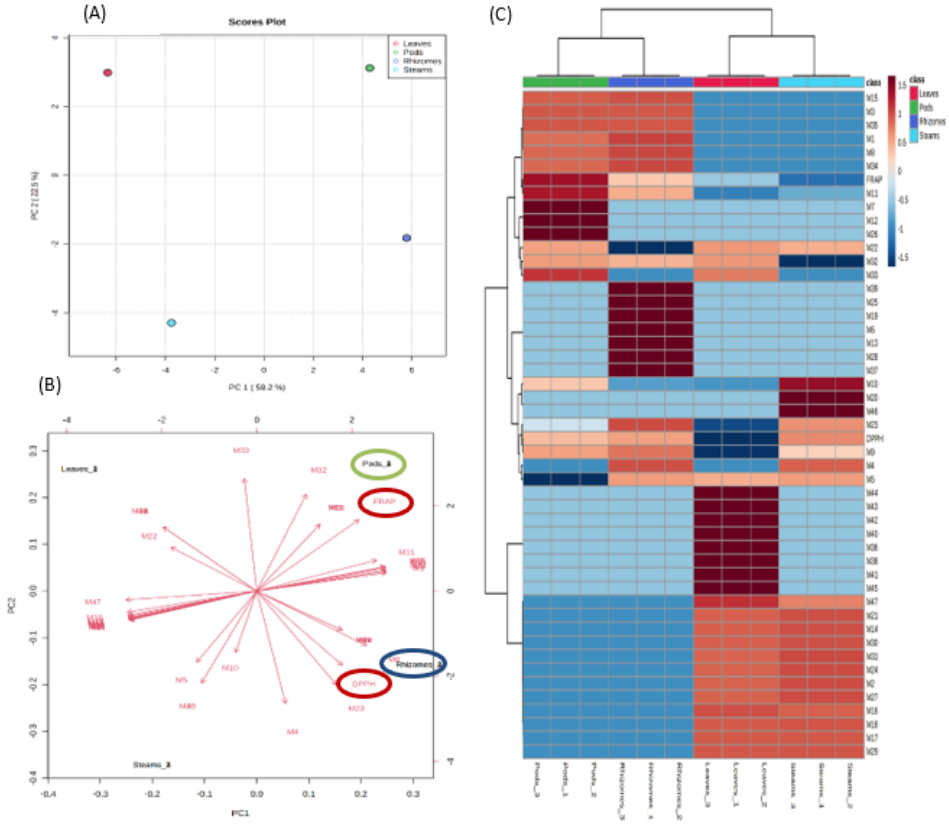

Figure 3. (A) Score plot; **(B)** loading plot; and **(C)** HCA-heatmap dendrogram of PCA on different parts of Java cardamom using the metabolites (M1-M47, see in Table 1) and antioxidant activities DPPH and FRAP lines matrix as input variables. The darker red, orange, and darker blue presented higher, moderate, and lower metabolite contents and antioxidant activities, respectively. (for an explanation of parameter symbols, see Table 1).

PCA analysis based on the first two principal components (shown in Figure 3) accounted for 81.7% of the data variation described. The individual score plots (Figure 3A) show the sample distributions for the first two principal components (PC1 and PC2). The loading plots (Figure 3B) show the distributions of 47 aromatic compounds and antioxidant activity for the first two principal components (PC1 and PC2), which represent significant differences from the first two major components (PC1 and PC2). Four parts (leaves, stems, rhizomes, and fruits (pods and seeds)) of the Java cardamom in the studied essential oil. PCA analysis allows for identifying patterns that show similarities and differences in the data for the four parts of the Java cardamom plant in the essential oil studied [63-65]. In addition, PCA analysis aims to evaluate the phytochemical content and bioactivity of the four parts of the Java cardamom plant in essential oils. The results of PCA analysis showed that fruits (pods and seeds) and rhizomes parameters were dominant on PC1, while leaves and stems were dominant on PC2. Biplot analysis of phytochemicals and antioxidant activity was made from the comparison of PC1 and PC2 components showing two groups of Java cardamom parts (Figure 3B). The antioxidant activity of DPPH and FRAP was more dominant in group 1, consisting of the fruits (pods and seeds) and rhizomes of Java cardamom with high secondary metabolite content in γ-terpinene (M11), 4-Terpineol (M23), α-Phellandrene (M9), Camphene (CAS) (M4), α-Thujene (M1), (+-)-Linalool (M15), β-Sesquiphellandrene (M35), and (-)-β-Bisabolene (M34). Meanwhile, group 2 consists of leaves and stems which contain high secondary metabolites in Phytol (M47), Hexadecanoic Acid, 1-Methylethyl Ester (CAS) (M16), Sabinene (M5), delta-Terpineol (M22), (+)-Pinocarvone (M21), 2-Nonanol (CAS) (M14), *cis*-Carveol (M30), Cyclohexanol, 2-methylene-5-(1-methylethenyl)- (M24), 1R-α-Pinene (M2), (-)-Myrtenol (M27), *trans*-Pinocarveol (M18), 2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethenyl)-,*trans*- (M17), and Carveol (M29).

HCA (hierarchical cluster analysis)-heatmap dendrogram analysis was performed to compare with the results from PCA [66, 65]. HCA analysis aims to study and identify the relationship between parts of the Java cardamom with aromatic compounds and the strongest antioxidant activity [67, 65]. Based on the data shown, the HCA analysis resulted in 2 groups (shown in Figure 3C). Group 1 represents two parts of the Java cardamom, namely the fruits (pods and seeds) and rhizomes, these two parts of the Java cardamom plant are related to secondary metabolites and antioxidant activity. The data shown shows that both parts of the plant contain high secondary metabolites in (+-)-Linalool (M15), α-Pinene (M3), β-Sesquiphellandrene (M35), α-Thujene (M1), β-Myrcene (M8), (-)-β-Bisabolene (M34), γ-Terpinene (M11), and α-Phellandrene (M9). Antioxidant activity of DPPH and FRAP were also found in both parts of the Java cardamom, and antioxidant activity of DPPH and FRAP in each part of the Java cardamom plant, namely fruits (pods and seeds) (26.19 mol TEAC/g FW; 115.99 mol TEAC/g FW) and rhizomes (26.85 mol TEAC/g FW; 105.99 mol TEAC/g FW), this indicates a relationship between the maximum secondary metabolites and antioxidant activity with DPPH antioxidant activity in the rhizomes being higher than that in the fruits (pods and seeds). In contrast, the antioxidant activity of FRAP in the fruits (pods and seeds) is higher than that in the rhizomes. Some secondary metabolites are high in one part (fruits) of the Java cardamom, such as 2-β-Pinene (M7), trans-Sabinene Hydrate (M12), Terpineol (M26), delta-Terpineol (M22), α-Copaene (M32), β-Selinene (CAS) (M33), and 1,8-Cineole (M10). At the same time, the Java cardamom rhizomes contain high secondary metabolites Nerolidol (M39), α-Terpineol (M25), Camphor (CAS) (M19), (-)- β-Pinene (M6), α-Terpinolene (M13), α-Phellandrene Epoxide (M28), *trans*-γ-Bisabolene (M37), 4-Terpineol (M23), Camphene

(CAS) (M4), and Sabinene (M5). Previous studies have reported that these compounds from several medicinal plants can be useful as antioxidants. Wang *et al*. [57] reported that α-pinene was the strongest free radical scavenger of DPPH (IC50 value = 12.57 ± 0.18 mg/mL) and had high reducing power (213.7 ± 5.27 g/mL of L-ascorbic acid equivalents). β-Sesquiphellandrene compound in *Zingiber officinale* (ginger) rhizomes was identified as the most active antibacterial component and showed antioxidant activity of DPPH [68]. Meanwhile, α-Thujene and camphene [69], (+-)-Linalool [70, 61], β-Myrcene [72], (-)-β-Bisabolene [73], γ-Terpinene [74], α-Phellandrene [75], α-Pinene [76] have been reported to have antioxidant activity *in vitro* FRAP and DPPH. The results show that these phytochemical parameters can be used as selection parameters for the development of cardamom varieties in addition to antioxidant activity through breeding programs.

Group 2 consists of 2 parts of the Java cardamom, namely the leaves and stems; both parts of this plant contain high secondary metabolites in delta-Terpineol (M22), Sabinene (M5), Phytol (M47), (+)-Pinocarvone (M21), 2-Nonanol (CAS) (M14), *cis*-Carveol (M30), (+)-Carvone (M31), Cyclohexanol,2-methylene-5-(1-methylethenyl)- (M24), 1R-α-Pinene (M2), (-)-Myrtenol (M27), *trans*-p-Mentha-2,8-dienol (M16), *trans*-Pinocarveol (M18), 2-Cyclohexen-1-ol,1-methyl-4-(1-methylethenyl)-,*trans*- (M17), and Carveol (M29). In addition to the above metabolites, the Java cardamom leaves contain high secondary metabolites in Acetic acid,3-(2,2-dimethyl-6-methylene-cyclohexylidene)-1-methyl-butyl Ester (M44), Cyclohexene,6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-,(S)- (M43), *trans*-Calamenene (M42), p-Mentha-1,5,8-triene (CAS) (M40), Teresantalol (CAS) (M36), 4,8-Dimethyl-2-(2-methyl-1-propenyl)-1-oxaspiro[4.5]dec-7-ene (M38), α-Terpinene (M41), ar-Curcumene (M45), α-Copaene (M32), and β-Selinene (CAS) (M33), but were low in the stems of Java cardamom. While in the stems, the secondary metabolite content is high in 1,8-Cineole (M10), Cyclopentane, 1,2-dimethyl-3-(1-methylethenyl)- (M20), Hexadecanoic Acid, 1-methylethyl Ester (CAS) (M46), 4-Terpineol (M23), α-Phellandrene (M9), and Camphene (CAS) (M4). Interestingly, the Java cardamom stems have a higher DPPH antioxidant activity than the fruits (pods and seeds) and rhizomes parts (27.38 mol TEAC/g FW); this may be due to the higher content of 1.8-Cineol in the stems compared to the other three parts. Previous studies reported that the compounds 1.8-Cineol [77,78] and 4-Terpineol [57] have antioxidant activity.

4. Conclusions

The results showed that Java cardamom essential oil contained a variable pattern of monoterpenes, sesquiterpenes, diterpenes, and other compounds. 1.8-Cineol is the main compound of the studied Java cardamom essential oil. The compounds γ-terpinene, delta-Terpineol, and 4-Terpineol were found in every part of the Java cardamom plant. The chemometric analysis concluded that (+-)-Linalool, α-Pinene, β-Sesquiphellandrene, α-Thujene, β-Myrcene, (-)-β-Bisabolene, γ-Terpinene, α-Phellandrene, 1,8-Cineole, Camphene (CAS), and 4-Terpineol are secondary metabolites responsible for antioxidant activity in Java cardamom essential oil. Therefore, the fruits (pods and seeds) part can be used as a source of secondary metabolites potential as antioxidants.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Garg, G.; Sharma, S.; Dua, A.; Mahajan, R. Antibacterial potential of polyphenol rich methanol extract of cardamom (*Amomum subulatum*). *Journal Innovative Biology* **2016**, *3*, 271-275.
2. Abu-Taweel, G.M. Cardamom (*Elettaria cardamomum*) perinatal exposure effects on the development, behavior and biochemical parameters in mice offspring. *Saudi Journal of Biological Sciences* **2018**, *25*, 186-193, http://dx.doi.org/10.1016/j.sjbs.2017.08.012.
3. Ashokkumar, K.; Vellaikumar, S.; Murugan, M.; Dhanya, M.K.; Ariharasutharsan, G.; Aiswarya, S.; Akilan, M.; Warkentin, T.D.; Karthikeyan, A. Essential oil profile diversity in cardamom accessions from southern India. *Frontiers in Sustainable Food Systems* **2021**, *5*, 1-8, https://doi.org/3389/fsufs.2021.639619.
4. Raissa.; Amalia, W.C.; Ayurini, M.; Khumaini, K.; Ratri, P.J. The Optimization of Essential Oil Extraction from Java Cardamom. *Journal of Tropical Pharmacy and Chemistry* **2020,** *5*, 125-129, https://doi.org/-10.25026/jtpc.v5i2.257.
5. Setyawan, A.D.; Wiryanto, Suranto, Bermawie, N.; Sudarmono. Comparisons of isozyme diversity in local Java cardamom (*Amomum compactum*) and true cardamom (*Elettaria cardamomum*). *Nusantara Bioscience* **2014**, *6*, 94-101, https://doi.org/10.13057/nusbiosci/n060114.
6. Hartady, T.; Balia, R.L.; Syamsunarno, M.R.A.A.; Jasni, S.; Prioseoeryanto, B.P. Bioactivity of *Amomum Compactum* Soland Ex Maton (Java Cardamom) as a Natural Antibacterial. *Systematic Reviews in Pharmacy* **2020**, *11*, 384-387, https://doi.org/10.31838/srp.2020.9.55.
7. Jabbar, M.; Ghorbaniparvar, H. Use of gc-ms combined with resolution methods to characterize and to compare the essential oil components of green and bleached cardamom. *Journal Research in Chemistry and Environment* **2015**, *5*, 76-85, https://ijrce.org/index.php/ijrce/article/view/307.
8. Kurniawan, E.; Sari, N.; Sulhatun. Ekstraksi sereh wangi menjadi minyak atsiri. *Jurnal Teknologi Kimia Unimal* **2020**, *10*, 43-53, https://doi.org/10.29103/jtku.v9i2.4398.
9. Talebi, S.M.; Yarmohammadi, M.; Nohooji, M.G. Essential oil analysis of eight 'Nepeta' taxa in Iran. *Mediterranean Botany* **2020**, *41*, 43-53, https://doi.org/10.5209/mbot.64567.
10. Ashrafi, B.; Rashidipour, M.; Gholami, E.; Sattari, E.; Marzban, A.; Kheirandish, F.; Khaksarian, M.; Taherikalani, M.; Soroush, S. Investigation of the phytochemicals and bioactivity potential of essential oil from *Nepeta curvidens* Boiss. & Balansa. *South African Journal of Botany* **2020**, *135*, 1-8, https://doi.org/10-.1016/j.sajb.2020.08.015.
11. Jena, S.; Ray, A.; Sahoo, A.; Champati, B.B.; Padhiari, B.M.; Dash, B.; Nayak, S.; Panda, P.C. Chemical Composition and Antioxidant Activities of Essential oil from Leaf and Stem of Elettaria cardamomum from Eastern India. Journal of Essential Oil-Bearing Plants **2021**, 24, 1-9, https://doi.org/10.1080/0972060X.202-1.1937335.
12. Garza, G.R.C.; Luevano, J.H.E.; Rodrigurze, A.F.B.; Montes, A.C.; Hernandez, R.A.P.; Delgado, A.J.M.; Villarreal, S.M.L.; Rodriguez, J.R.; Casas, R.M.; Velazquez, U.C.; Luis, O.E.R. Benefits of Cardamom (*Elettaria cardamomum* (L.) Maton) and Turmeric (*Curcuma longa* L.) Extracts for Their Applications as Natural Anti-Inflammatory Adjuvants. *Journal MDPI* **2021**, *10*, 2-17, https://doi.org/10.3390/plants1009-1908.

13. Winarsih, H.; Nuraeni, I.; Roslenina, A.; Andreas, A. Sensory and antioxidant evaluation of functional drinks based on Cardamom rhizomes (*Amomum cardamomum* willd.). *Journal Food Research* **2021**, *4*, 2169-2175, https://doi.org/10.26656/fr.2017.4(6).265.
14. Winarsi, H.; Sasongko, N.D.; Purwanto, A.; Nuraeni, I. Effect of cardamom leaves extract as antidiabetic, weight lost and hypocholesterolemic to alloxan-induced sprague dawley diabetic rats. *Int. Food Res. J.* **2014**, *21*, 2253–2261.
15. Nurcholis, W.; Putri, D.S.; Husnawati, Aisyah, S.I.; Priosoeryanto, B.P. Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of *Amomum compactum* fruits. *Annals of Agricultural Sciences* **2021**, *66*, 58-62, https://doi.org/10.1016/j.aoas.2021.04.001.
16. Mierza, V.; Sudewi. Antibacterial activity mouthwash preparation ethanol extract of cardamom (*Amomum compactum* Sol. Ex Maton) for growth of bacteria Staphylococcus Aureus and Streptococcus Mutans. *Journal of Pharmaceuutical and Sciences* **2020**, *3*, 51-57, https://doi.org/10.36490/journal-jps.com.v3i1.45.
17. Saeed, A.; Sultana, B.; Anwar, F.; Mushtag, M.; Alkharfy, K.M.; Gilani, A.H. Antioxidant and antimutagenic potential of seeds and pods of green cardamom (*Elettaria cardamomum*). *International Journal of Pharmacology* **2014**, *10*, 461-469, https://doi.org/10.3923/ijp.2014.461.469.
18. Kanthlal, S.K.; Arya, V.S.; Bindhu, P.P.;' Vijayakumar, M.; Rema.; Shree, A.B.; Uma, D.P. Aqueous extract of large cardamom inhibits vascular damage, oxidative stress, and metabolic changes in fructose-fed hypertensive rats. *Clinical and Experimental Hypertension Journal* **2021**, *43*, 622-632, https://doi.org/10.1080/1064-1963.2021.1925682.
19. Singh, R.; Kaushik, R.; Jaglan, V. Antibacterial and antioxidant activity of green cardamom and rosemary extract in food products: A brief review. *The Pharma Innovation Journal* **2018**, *7*, 568-573, https://www.thepharmajournal.com/archives/2018/vol7issue6/PartI/7-6-102-124.pdf.
20. Ashokkumar, K.; Murugan, M.; Dhanya, M.; Warkentin, T. Botany, traditional uses, phytochemistry and biological activities of cardamom [*Elettaria cardamomum* (L.) Maton] - a critical review. *J. Ethnopharmacol* **2019**, *246*, 112244, https://doi.org/10.1016/j.jep.2019.112244.
21. Singletary, K. Cardamom potential health benefits. *Food Science* **2022**, *57*, 38-49, https://doi.org/10.1097/NT.00000-00000000507.
22. Asra, R.; Azni, N.R.; Rusdi, Nessa. Uji antioksidan ekstrak etanol fraksi heksan, fraksi etil asetat dan fraksi air daun kapulaga (*Elettaria Cardamomun* (L.) Maton*). Journal Pharmaceutical and Sciences* **2019**, *2*, 30-37, https://doi.org/10.36490/jo-urnal-jps.com.v2i1.17.
23. Shah, P.; Modi, H.A. Comparative study of DPPH, ABTS and FRAP assays for determination of antioxidant activity. *IJRASET* **2015,** *3*, 636-641, retrieved from ion at: https://www.researchgate.net/publication/30.
24. Ghimire, B.K.; Yoo, J.H.; Yu, C.Y.; Chung, I.M. GC-MS analysis of volatile compounds of *Perilla frutescens Britton* var. *Japonica* accessions: Morphological and seasonal variability. *Asian Pac J Trop Med* **2017,** *10*, 643-651, https://doi.org/10.1016/j.apjtm.2017.07.004.
25. Ukwubile, C.A.; Ahmed, A.; Katsayal, U.A.; Ya'u, J.; Mejida, S. GC-MS analysis of bioactive compounds from *Melastomastrum capitatum* (Vahl) Fern. leaf methanol extract: An anticancer plant. *Sci African* **2019**, *3*, e00059, https://doi.org/10.1016/j.sciaf.2019.e00059.
26. Nurcholis, W.; Khumaida, N.; Bintang, M.; Syukur, M. GC-MS analysis of rhizome ethanol extracts from *Curcuma aeruginosa* accessions and their efficiency activities as anticancer agent. *Biodiversitas* **2021**, *22*, 1179-1186, https://doi.org/10.13057/biodiv/d220313.
27. Pujiarti, R.; Kusumadewi, A. Chemical compounds, physicochemical properties, and antioxidant activity of cardamomum leaves and rhizomes oils on different distillation time. *Wood Research Journal* **2020**, *11*, 35-40, https://doi.org/10.51850/wrj.2020.11.1.35-40.
28. Ridwan, M.A.; Ahmad, I.; Gusni, S. Analisis lama penyulingan terhadap rendemen minyak atsiri kulit jeruk pangkep (*Citrus maxima*) dengan metode basah dan kering.*Prosiding Seminar Hasil Penelitian (SNP2M)* **2017**, pp.151-155, http://jurnal.poliupg.ac.id/index.php/snp2m/article/download/315/318.
29. Nurcholis, W.; Khumaida, N.; Syukur, M.; Bintang, M. Variability of total phenolic and flavonoid content and antioxidant activity among 20 *Curcuma aeruginosa* Roxb. Accessions of indonesia. *Asian Journal of Biochemistry* **2016**, *11*, 142-148, https://doi.org/10.3923/ajb.2016.142.148.
30. Calvindi, J.; Syukur, M.; Nurcholis, W. Investigation of biochemical characters and antioxidant properties of different winged bean (*Psophocarpus tetragonolobus*) genotypes grown in Indonesia. *Biodiversitas* **2020**, *21*, 2420-2424, https://doi.org/10.13057/biodiv/d210612.

31. Joshi, R.; Sharma, P.; Sharma, V.; Prasad, R.; Sud, R.K.; Gulati, A. Analysis of the essential oil of large cardamom (*Amomum subulatum* Roxb.) growing in different agro-climatic zones of Himachal Pradesh, India. *J. Sci. Food Agric* **2012**, *93*, 1303-1309, https://doi.org/10.1002/jsfa.5886.
32. Tambunan, L.R. Isolasi dan identifikasi komposisi kimia minyak atsiri dari biji kapulaga (*Amomum cardamomum* Willd). *Jurnal Kimia Riset* **2017,** *2*, 57-60, https://doi.org/https://doi.org/10.20473/jkr.v2i1.4023 .
33. Yuliana, D.A.; Zurohaina, S.N.; Aswan, A.; Febriana, I. Proses pengambilan minyak atsiri dari tanaman nilam (*Pogestemon cablin* Benth) menggunakan metode microwave hydrodixtillation. *Jurnal Kinetika*, **2020**, *11*, 34-39, https://jurnal.polsri.ac.id/index.php/kimia/index.
34. Tambe, M.E.A.; Gotmare, S. Chemical characterization of three cardamom oils (*Elettaria cardamomum*) by GCMS. *World Journal of Pharmacy and Pharmaceutical Sciences* **2019**, *8*, 876-885, https://doi.org/10.20959/wjpps-201911-14879.
35. Shakeri, A.; Khakdan, F.; Soheili, V.; Sahebkar, A.; Shaddel, R.; Asili, J. Volatile composition, antimicrobial, cytotoxic and antioxidant evaluation of the essential oil from Nepeta sintenisii Bornm. *Industrial Crops and Products* **2016**, *84*, 224–229, https://doi.org/10.1016/j.indcrop.2015.12.030.
36. Ashokkumar, K.; Murugan, M.; Dhanya, M.; Warkentin, T. Botany, traditional uses, phytochemistry and biological activities of cardamom [*Elettaria cardamomum* (L.) Maton] - a critical review, J. Ethnopharmacol **2020**, *246*, 112244, https://doi.org/10.1016/j.jep.2019.112244.
37. Juergens, U.R. Anti-inflammatory Properties of the Monoterpene 1.8-Cineole: Current Evidence for Co-medication in Inflammatory Airway Diseases. Drug Research **2014**, *64*, 638-646, http://dx.doi.org/10-.1055/s-0034-1372609.
38. Cai, Z.M.; Peng, J.Q.; Chen, Y.; Tao, L.; Zhang, Y.Y.; Fu, L.Y.; Long, Q.D.; Shen, X.C. 1,8-Cineole: a review of source, biological activities, and application. *Journal of Asian Natural Products Research* **2020**, 1-17, https://doi.org/10.1080/10286020.2020.18394.
39. Oropeza, J.S.; Estrada, M.J.; Torres, A.P.; Rodriguez, A.E.C.; Milan, R.B.; Monroy, M.A.R.; Yanes, K.J.; Martinez, M.M.C. Wound Healing Activity of α-Pinene and α-Phellandren. *J. MDPI* **2021**, 26, 1-14, https://doi.org/10.3390/molecules26092488.
40. Zhang, J.H.; Sun, H.L.; Chen, S.Y.; Zheng, L.; Wang, T.T. Anti-fungal activity, mechanism studies on α-Phellandrene and Nonanal against *Penicillium cyclopium*. *Botanical Studies* **2017**, *58*, 1-9, https://doi.org/10.1186/s40-529-017-0168-8.
41. Abdullah, A.; Butt, M.S.; Shahid, M.; Asghar, A.; Shahid, M.; Huang, Q. Evaluating the antimicrobial potential of green cardamom essential oil focusing on quorum sensing inhibition of chromobac-terium violaceum. *J Food Sci Technol* **2017**, *54*, 2306–2315, https://doi.org/10.1007/s13197-017-2668-7.
42. Snoussi, M.; Noumi, E.; Dehmani, A.; Emira, N.; Guido, F.; Mahjoub, A.; Madiha, A.; Abdulbasit, A. Chemical composition and antimicrobial activities of Elettaria Cardamomum L. (Manton) essential oil a high activity against a wide range of food borne and medically important bacteria and fungi. *J Chem Bio Phy Sci* **2016**, *6*, 248–259.
43. Jabbar, M.; Ghorbaniparvar, H. Determination of volatile components in black cardamom with gas chromatography-mass spectrometry and chemometric resolution. *International Journal of Engineering Research & Technology (IJERT)* **2014**, *3*, 1280-1286.
44. Shrestha, R.L. GC-MS analysis, antibacterial and antioxidant study of *Amomum subulatum* Roxb. *Journal of Medicinal Plants Studies* **2017**, *5*, 126-129, https://www.plantsjournal.com/archives/2017/vol5issue6/PartB/5-6-21-999.pdf.
45. Sahoo, S.; Singh, S.; Dash, M.; Jena, S.; Sahoo, A.; Nayak, S.; Kar, B. Electron ionization based detection of volatile constituents of aerial parts of *Eclipta prostrata* (Linn.) by one dimensional Gas Chromatography and Mass Spectrometry. *J. Essent. Oil Bear Plants* **2020**, 23, 559-566, https://doi.org/10.1080/0972060X.2020.1776-644.
46. Jena, S.; Ray, A.; Sahoo, A.; Kar, B.; Panda, P.C.; Nayak, S.Chemical constituents of leaf essential oil of Curcuma angustifolia Roxb. growing in Eastern India. J. Essent. Oil Bear.Plants **2016**, 19, 1527-1531, https://doi.org/10.1080/0972060X.2016.1250677.
47. Mercado-Mercado, G.; de la Rosa, L.A.; Alvarez-Parrilla, E. Effect of pectin on the interactions among phenolic compounds determined by antioxidant capacity. *J Mol Struct* **2020**, *1199*, 126967, https://doi.org/10.1016/j.molstruc.2019.126967.

48. Alam, A.; Jawaid, T.; Alam, P. *In vitro* antioxidant and anti-inflammatory activities of green cardamom essential oil and *in silico* molecular docking of its major bioactives. *Journal of Taibah University for Science* **2021**, *15*, 757-768, https://doi.org/10.1080/16583655.2021.2002550.
49. Chimbetete, N.; Verghese, M.; Sunkara, R.; Walker, L.T. Phytochemical content, radical scavenging ability & enzyme inhibiting activities of selected spices (cinnamon, cardamom and cloves). *Food and Nutrition Sciences* **2019**, *10*, 266-275, https://doi.org/10.4236/fns.2019.103020.
50. Maesaroh, K.; Kurnia, D.; Andhori, J.A. Perbandingan metode uji aktivitas antioksidan dpph, FRAP dan FIC terhadap asam askorbat, asam galat dan kuersetin. *Chimica et Natura Acta Journal* **2018**, *6*, 93-100, https://doi.org/-10.24198/cna.v6.n2.19049.
51. Maryam, S.; Baits, M.; Nadia, A. Pengukuran aktivitas antioksidan ekstrak etanol daun kelor (*Moringa oleifera* Lam.) menggunakan metode FRAP (ferric reducing antioxidant power). *Jurnal Fitofarmaka Indonesia* **2015,** *2*, 115-118, https://dx.doi.org/-10.33096/jffi.v2i2.181.
52. Hachlafi, N.E.; Aanniz T.; Menyly, N.E.; Baaboua, A.E.; Omari, N.L.; Balahbib, A.; Shariati, M.A.; Zengin, G.; Benbrahim, K.F.; Bouyahya, A. *In vitro* and *in vivo* biological investigations of camphene and its mechanism insights: a review. *Food Review International* **2021,** 1-28, https://doi.org/10.1080/87559129.2021.1936007
53. Miri S. Phytochemistry, antioxidant, and lipidperoxidation inhibition of the essential oils of *Lavandula officinalis* l. in iran. *International Jpurnal of Food Properties* **2018**, *21*, 2550-2556, https://doi.org/10.1080/-10942912.2015.1027921.
54. Di-Giacomo, S.; Abete, L.; Cocchiola, R.; Mazzanti, G.; Eufemi, M.; Di-Sotto, A. Caryophyllane sesquiterpenes inhibit DNA-damage bytobacco smoke in bacterial and mammalian cells. *Food Chem. Toxicol* **2018**, *111*, 393-404, https://doi.org/10.1016/j.fct.2017.11.018.
55. Salehi, B.; Upadhyay, S.; Erdogan, O.I.; Jugran, A.K.; Jayaweera, S.L.D.; *et al*. Therapeutic potential of α- and β-pinene: a miracle gift of nature. *Biomolecules* **2019**, *9*, 738, https://doi.org/10.3390/biom9110738.
56. Bicas, J.L.; Neri-Numa, I.A.; Ruiz, A.L.T.G.; De-Carvalho, J.E.; Pastore, G.M. Evaluation of the antioxidant and antiproliferative potential of bioflavors. *food chem toxicol* **2011**, *49*, 1610–1615, https://doi.org/10.1016/j.fct.20-11.04.012.
57. Wang, C.Y.; Chen, Y.W.; Hou, C.Y. Antioxidant and antibacterial activity of seven predominant terpenoids. *Int. J. Food Prop* **2019**, *22*, 230-238., https://doi.org/10.1080/10942912.2019.1582541.
58. Quintans-Júnior, L.; Moreira, J.C.; Pasquali, M.A.; Rabie, S.; Pires, A.S.; Schröder, R.; Rabelo, T.K.; Santos, J.; Lima, P.S.; Cavalcanti, S.C.; Aaújo, A.A. Antinociceptive activity and redox profile of the monoterpenes (+)-camphene, p-cymene, and geranyl acetate in experimental models. *ISRN toxicol* **2013**, ID:459530, https://doi.org/10.1155/2013/459530.
59. Batubara, I.; Komariah, K.; Sandrawati, A.; Nurcholis, W. Genotype selection for phytochemical content and pharmacological activities in ethanol extracts of fifteen types of *Orthosiphon aristatus* (Blume) Miq. leaves using chemometric analysis. *Sci Rep* **2020,** *10*, 20945, https://doi.org/10.1038/s41598-020-77991-2.
60. Abbasi, S.; Benvidi, A.; Gharaghani, S.; Rezaeinasab, M. Chemometric studies of thymol binding with bovine serum albumin: A developing strategy for the successful investigation of pharmacological activity. *Bioelectrochemistry* **2018**, *124*, 172-184, https://doi.org/10.1016/j.bioelechem.2018.07.017.
61. Windarsih, A.; Rohman, A.; Swasono, R.T. Application of 1H-NMR based metabolite fingerprinting and chemometrics for authentication of *Curcuma longa* adulterated with *C. heyneana*. *J. Appl. Res. Med. Aromat Plants* **2019**, *13*, 100203, https://doi.org/10.1016/j.jarmap.2019.100203.
62. Guo, L.; Gong, M.; Wu, S.; Qiu, F.; Ma, L. Identification and quantification of the quality markers and anti-migraine active components in Chuanxiong Rhizoma and Cyperi Rhizoma herbal pair based on chemometric analysis between chemical constituents and pharmacological effects. *J. Ethnopharmacol* **2020**, *246*, 112228, https://doi.org/10.1016/j.jep.2019.112228.
63. Kwarteng, A.O.; Abogoom, J.; Adu-Amoah, R.; Nyadanu, D.; Nyam, C.K.; Ghunney, T.; Awuah, E.; Ziyaaba, J.Z.; Ogunsanya, J.O.; Orhin, E.E.; Asiedu, D.D. Phenomic characterization of twenty-four accessions of spider plant (*Cleome gynandra* L.) the Upper East region of Ghana. *Sci Hortic (Amsterdam)* **2018**, *235*, 124-131, https://doi.org/10.1016/j.scienta.2018.02.046.
64. Mirto, A.; Iannuzzi, F.; Carillo, P.; Ciarmiello, L.F.; Woodrow, P.; Fuggi, A. Metabolic characterization and antioxidant activity in sweet cherry (*Prunus avium* L.) Campania accessions: Metabolic characterization of sweet cherry accessions. *Food Chem* **2018**, *240*, 559-566, https://doi.org/10.1016/j.foodchem.201-7.07.162.

65. Khumaida, N.; Syukur, M.; Bintang, M.; Nurcholis, W. Phenolic and flavonoid content in ethanol extract and agro-morphological diversity of *Curcuma aeruginosa* accessions growing in West Java, Indonesia. *Biodiversitas* **2019**, *20*, 656-663, https://doi.org/10.13057/biodiv/d200306.
66. Péroumal, A.; Adenet, S.; Rochefort, K.; Fahrasmane, L.; Aurore, G. Variability of traits and bioactive compounds in the fruit and pulp of six mamey apple (*Mammea americana* L.) accessions. *Food Chem*. **2017**, *234*, 269-275, http://dx.doi.org/10.1016/j.foodchem.2017.04.145.
67. Bakić, I.V.; Rakonjac, V.S.; Čolić, S.D.; Akšić, M.M.F.; Nikolić, D.T.; Radović, A.R.; Rahović, D.D. 2017. Agro-morphological characterisation and evaluation of a Serbian vineyard peach [*Prunus persica* (L.) Batsch] germplasm collection. *Sci Hortic (Amsterdam)* **2017**, *225*, 668-675, https://doi.org/10.1016/j.scienta.2017.07.036.
68. Pandini, J.A.; Pinto, F.G.S.; Scur, M.C.; Santana, C.B.; Costa, W.F.; Temponi, L.;G. Chemical compotion, antimicrobial and antioxidant potential of the essential oil of *Guarea kunthiana* A.Juss. *Brazilian Journal of Biology* **2018,** *78*, 53-60, http://dx.doi.org/10.1590/1519-6984.04116.
69. Radoukova, T.; Zheljazkov, V.D.; Semerdjieva, I.; Dincheva, I.; Stoyanova, A.; Kacaniova, M.; Markovic, T.; Radanovic, D.; Astatkie, T.; Salamon, I. Differences in essential oil yield, composition, and bioactivity of three juniper species from Eastern Europe. *Industrial Crops and Product* **2018**, *124*, 643-652, https://doi.org/10.1016/j.indcrop.2018.08.012.
70. Seol, G.H.; Kang, P.; Lee, H.S.; Soel, G.H. Antioxidant activity of linalool in patients with carpal tunnel syndrome. *BMC Neurology* **2016**, 16, 1-6, https://doi.org/10.1186/s12883-016-05.
71. Al-Ansari, M.; Andeejani, A.M.I.; Alnahmi, E.; Al-Malki, R.H.; Masood, A.; Vijayaraghavan, P.; Rahman, A.A.; Choi, K.C. Insecticidal, antimicrobial and antioxidant activities of essential oil from *Lavandula latifolia* L. and its deterrent effects on *Euphoria leucographa*. *Elsevier Industrial Crops & Products* **2021**, *170*, 113740l, https://doi.org/10.1016/j.indcrop.2021.113740.
72. Surendran, S.; Qassadi, F.;Geyan, S.; Lilley, D.; Heinrich, M. Myrcene—What Are the Potential Health Benefits of This Flavouring and Aroma Agent?. Front Nutr **2021**, *8*, 699666, https://doi.org/10.3389/fnut.2021.699666.
73. Kazemi, M.; Rostami, H. Chemical composition, antimicrobial and antioxidant activities of the essential oil of *Psammogetoncanescens*. *Natural Product Research* **2014**, *29*, 277-280, https://doi.org/10.1080/14786419.2014.95-1357.
74. Guo, Y,.; Baschieri, A.; Amorati, R.; Valgimigli, L. Synergic antioxidant activity of γ-terpinene with phenols and polyphenols enabled by hydroperoxyl radicals. *Food Chemistry* **2021**, *345*, 128468. https://doi.org/10.1-016/j.foodchem.2020.128468.
75. Scherer, M.M.D.C.; Marques, F.M.; Figueira, M.M.; Peisino, M.C.O.; *et al*. Wound healing activity of terpinolene and α-phellandrene by attenuating inflammation and oxidative stress *in vitro*. *J. Tissue Viability* **2019**, *28*, 94-99, https://doi.org/10.1016/j.jtv.2019.02.003.
76. Xanthis, V.; Fitsiou, E.; Voulgaridou, G.P.; Bogadakis, A.; Chlichlia, K.; Galanis, A.; Pappa, A. Antioxidant and cytoprotective potential of the essential oil *pistacia lentiscus* var . *chia* and its major components Myrcene and α-Pinene. *Antioxidant (Basel)* **2021**, *18*, https://doi.org/10.3390/antiox10010127.
77. Noumi, V.D.; Nguimbou, R.M.; Tsague, M.V.; Deli, M.; Rup-Jacques, S.; Amadou, D.; Baudelaire, E.N.; Sokeng, S.; Njintang, N.Y. Phytochemical profile and *in vitro* antioxidant properties of essential oils from powder fractions of eucalyptus camaldulensis leaves. *American Journal of Plant Sciences* **2021**, *12*, 329-346, https://doi.org/10.4236/ajps.2021.123021.
78. Chandorkar, N.; Tambe, S., Amin, P.; Mandankar, C. A systematic and comprehensive review on current understanding of the pharmacological actions, molecular mechanisms, and clinical implications of the genus *Eucalyptus*. *Elesevier Phytomedicine Plus* **2021**, *1*, 100089, https://doi.org/10.1016/j.phyplu.202-1.100089.