Coriandrum sativum l. (apiaceae) and *elettaria cardamomum* (l.) maton (zingiberaceae) for antioxidant and antimicrobial protection

Coriandrum sativum l. (apiaceae) and *elettaria cardamomum* (l.) maton (zingiberaceae) for antioxidant and antimicrobial protection

Windri Handayani[1], Yasman[1], Retno Yunilawati[2], Vivi Fauzia[3], Cuk Imawan[3*]

[1]Department of Biology, FMIPA Universitas Indonesia, Kampus UI Depok Depok 16424, Indonesia
[2]Center for Chemical and Packaging, Ministry of Industry, Jakarta, 13710
[3]Department of Physics, FMIPA Universitas Indonesia, Kampus UI Depok, Depok 16424, Indonesia

*cuk.imawan@sci.ui.ac.id

Abstract. Spice plants are known for their compounds that are useful as foods flavoring, food preservatives, and medicines. This due to the presence of secondary metabolite compounds in plants such as terpenoids, flavonoids, phenols, and saponins. These compounds are known to be potential to inhibit microorganism's growth causing decay in food and oxidation. The use of these sources for applications in the food sector is relatively safer and environmentally friendly than the use of antibiotics in general. This study was conducted to determine the antimicrobial and antioxidant activities from *Coriandrum sativum* L. (coriander) and *Elettaria cardamomum* (L.) Maton (cardamom). The essential oil extract from these plants was tested for phytochemical content qualitatively for terpenoid screening and by using Gas Chromatography-Mass Spectroscopy (GC/MS). Furthermore, the antioxidant activity from the oil extracts was tested by DPPH method. Meanwhile, their ability to inhibit gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* were tested by paper disc method. The phytochemical characterization showed a positive result of terpenoid and GC/MS result showed dominant of monoterpenes compounds, such as α-pinene and β-pinene. The DPPH results revealed that the essential oils have different antioxidant and antimicrobial potential, whereas Coriander tends to have a higher antimicrobial activity, while Cardamom superior in antioxidant activity. These results will become the basis for the development of potential essential oil with the best antimicrobial activity for food active packaging materials.

1. Introduction

Aromatic plant extracts very potentially for food safety applications and food preservation [1,2,3]. These plants contain secondary metabolites which play a role in fulfilling demand in food safety. We can use active plant compound to be an alternative source to reduce synthetic chemicals that at risk of causing poisoning, carcinogenic and difficulty to degrade, resulting in pollution for the environment [3,4]. One of the roles of phytochemical compounds is their ability to inhibit microorganism's growth [4] and as antioxidant properties [5]. This activity opens the opportunity for the development of alternative natural resources material, to overcome the problem of antibiotic resistance [6].

In general, compounds with antimicrobial activity from aromatic plants, obtained by extracting the essential oils. Essential oils are compounds that are difficult to dissolve in water and have a distinctive

aroma [7]. These extracts contain a mixture of very complex compounds from several aromatic compounds in an individual. Herbal plants and spices commonly used as food ingredients consist of more essential oils with volatile properties which have the antimicrobial ability [5,6].

Aside from antimicrobial potential, phytochemical compounds also have antioxidant properties [8]. These compounds are capable of binding to free radicals which can lead to a more stable molecule. Free radicals can be defined as a species of molecules that lose one electron from their free electron pair. The molecule contains unpaired electrons in atomic orbitals which are unstable and very reactive. Free radicals can change lipids, proteins, and DNA so that they can trigger several diseases in humans [6], and causing food decay [9]. The abilities from certain compounds as an antioxidant and antimicrobial agent have a great prospect to be developed in food application as the components of food active packaging.

In this research, *Coriandrum sativum* L. (Apiaceae) and *Elettaria cardamomum* (L.) Maton (Zingiberaceae) know as herbs and spices containing aromatic compounds. This study aims to see the strength of antioxidants and antimicrobials activities from both plants. The information from this research will be used to support the development of food active packaging.

2. Material and methods

2.1. Plant extract and phytochemical screening

The extract used as the sample were commercial essential oil that obtains from essential oils and aromatic chemical company in Indonesia, both extracted using steam distillation. *Coriandrum sativum* L. (coriander) essential oil was extracted from the seeds. Meanwhile, *Elettaria cardamomum* (L.) Maton essential oil (cardamom) was extracted from the rhizome part.

2.1.1. Terpenoid content detection. The terpenoid detection from extract using Salkowski test: 1 ml extract mixed with 1 ml chloroform and 1.5 ml concentrated H_2SO_4, then carefully added to form a layer. A reddish-brown coloration formed to show a positive result of terpenoids presence [10].

2.1.2. GC/MS Characterization. Characterization and analysis from the extract were using GC/MS and performed using Gas Chromatograph (GC) Agilent 6890 series with capillary column HP-5MS, 30 m x 0.25 mm id x 0.25 μm film thickness. Helium gas (65 kPa) was used as the carrier gas at constant pressure, and an injection volume of 1 μL was employed (split ratio of 25:1); The oven temperature was programmed from 60-240°C, with an increase of 3°C/min until it reaches 250°C. Components were identified based on a comparison of relative retention time and mass spectrum [11].

2.2. *Antioxidant activity*

Antioxidant activity was determined using 1,1- diphenyl-2-picryl hydrazine (DPPH) as free radical [12] that interact with the essential oil samples. The samples diluted with methanol to certain concentration (0, 10, 20, 30, 40, 50 μg/mL). The Ratio of extract and DPPH 0.1 mM was 1:1 (v/v) and the samples with different concentration tested by mixed the solution then homogenized with vortex and kept it in the dark, at room at temperature for 30 minutes. Absorbance from the mixed solution then measured with Spectrophotometer UV-Vis at 517 nm [Thermo Genesys S10]. Ascorbic acid measured as a standard compound. The ability of the sample to scavenge DPPH radical determined from [8]:

$$\% \text{ Inhibition} = \frac{Control\ OD - Sample\ OD}{Control\ OD}\ x\ 100 \quad (1)$$

After that IC50 value from each extract were calculated, by plotting their regression to get the equation from the graph trendline (Y=1.3607x+45.238 for ascorbic acid; Y=0.4955x+1.5207 for coriander; Y=1.2393x+13.396 for cardamom). The IC50 values can used to describe the effects of a drug on a molecular target. After the IC50 values known, the antioxidant activity index (AAI), based on Scherer and Godoy (2009) [13]. DPPH concentration in reaction mixture is 39,232 μg/ml. Samples were

classified in 3 groups, which are poor antioxidant activity (AAI < 0.5), moderate (0.5 < AAI < 1.0), strong (1.0 < AAI < 2.0) and very strong (AAI > 2.0). AAI calculated as follows:

$$\text{AAI} = \frac{\text{DPPH concentration in reaction mixture (µg/mL)}}{\text{IC50 (µg/mL)}} \quad (2)$$

2.3. Antimicrobial activity

Paper disc diffusion method used to determine the antimicrobial activities. This test using type strain of *Staphylococcus aureus* NBRC 100910 and *Escherichia coli* NBRC 3301. The Muller Hinton Agar medium was prepared by pouring 10 ml of molten media into sterile Petri plates (d=90 mm) and allowed to solidify for 5 minutes. After that, in a tube, 10 µl of bacteria culture 10^{-6} CFU/mL added with 10 ml of medium and mixed gently with the inoculate before poured on the top of molten media before and allowed to dry for 5 minutes. The negative control (sterile distilled water), positive control (tetracyclin 15 µg/mL), a sample with concentration 500 and 1000 µg/mL loaded on 6 mm disc, whereas the volume for each disc was 10 µl. The loaded disc placed on the surface of the medium then incubates at 32°C for 18 hours. After the end of incubation, a clear zone formed around the disc measured. Each experiment done in triplicate, and the activity index measured [14].

$$\text{Activity Index} = \frac{Clear\ zone\ diameter\ (mm) - paper\ disc\ diameter(mm)}{paper\ disc\ diameter(mm)} \quad (3)$$

3. Result and discussion

The results obtained from the terpenoid qualitative test showed that the two samples contained terpenoid compounds. In the coriander, the test result confirmed the formation of a very thick brick red color, while the cardamom sample showed a brown color with a slight red brick color. The color intensity can qualitatively show the presence of the compound in the extract (Table 1). Meanwhile, based on the results of the DPPH test to determine the antioxidant strength from the sample showed that extracts from coriander had a lower inhibition percentage than cardamom. When the extract concentration reached 50 µg/ml showed that the percentage of inhibition in coriander reached 24.7% and cardamom reached 70.8%. From the graph in figure 1. plotting of the regression equation calculated based on the graph. IC50 values calculated by entering 50% as the Y-axis. The results show that cardamom has a smaller IC50 value (29.5 µg/ml) than coriander (97.84 µg/ml). IC50 values at <100 µg/ml indicate that the extract is active. The smaller the IC50 value, the sample tend to have good antioxidant activity [15,16]. After the IC50 value is known, the antioxidant activity index (AAI) can be measured. The results show the AAI coriander value below 0.1 in the poor category, while cardamom has a strong AAI value.

Based on the GC/MS results, the chromatogram profile detected 7 peaks in the coriander and 8 peaks in cardamom (Figure 2). The detected compounds identified by the GC/MS analysis and used the result with that quality above 95% (Table 2). Based on this, coriander is known to contain 4 compounds, namely α-pinene, camphene, l-Limonene, and Camphor. Meanwhile, cardamoms contain α-pinene, camphene, l-Limonene, β-pinene, and 1,8-Cineole. These compounds are monoterpenes, terpenoid compounds [8,17]. Based on the results of GC/MS, β-pinene and 1,8-Cineole compounds only detected in the cardamom chromatogram profile, and camphor only detected in coriander. Essential oils are a mixture of complex volatile, lipophilic and odiferous compounds from plant secondary metabolism. Consists of monoterpenes, sesquiterpenes and oxygenated derivatives (ketones, alcohols, esters, phenols, aldehydes, and oxides) [17].

Table 1. Terpenoid, IC50 value and antioxidant activity index content from *C. sativum* and *E. cardamomum* essential oil

Samples	Terpenoid	IC50 Values (µg/mL)	AAI	Category based on AAI
Ascorbic acid	n/a	3.49	11.24	very strong
Coriander	++	97.84	0.40	poor
Cardamom	+	29.53	1.33	strong

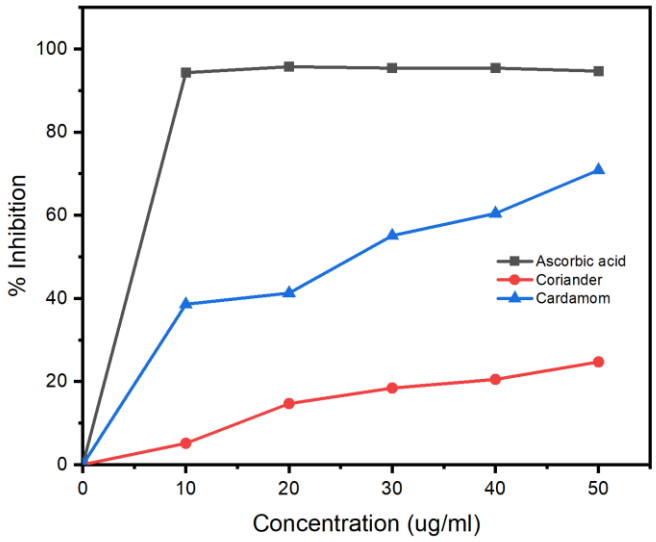

Figure 1. Antioxidant activity of *C. sativum* and *E. cardamomum* essential oil with ascorbic acid as a comparison.

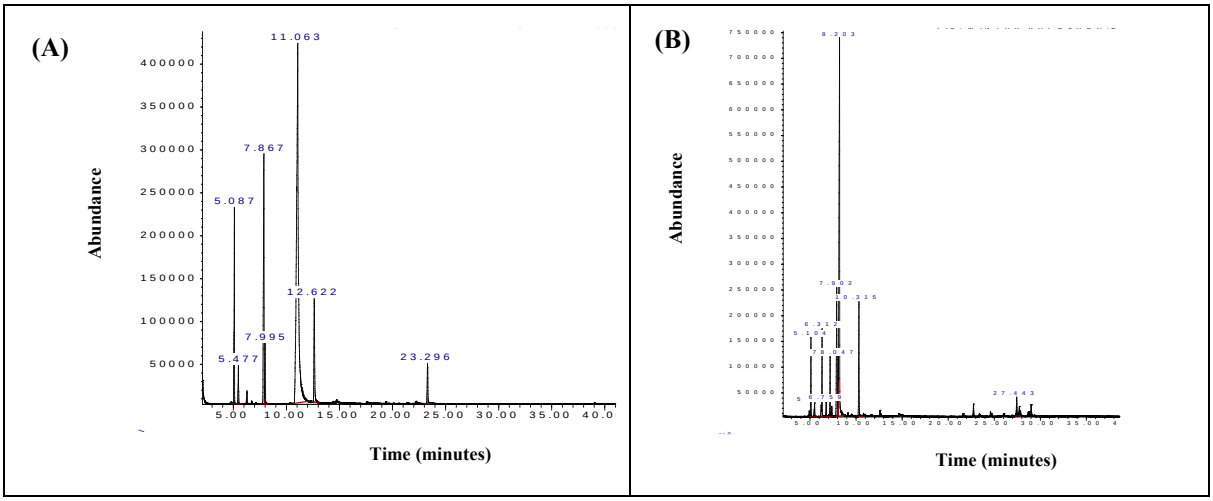

Figure 2. GC/MS chromatogram profile from *C. sativum* and *E. cardamomum* essential oil

Table 2. GC/MS analysis from *C. sativum* and *E. cardamomum* essential oil

No	Compounds	Molecular formula	*C. sativum*		*E. cardamomum*	
			Retention time	Relative percentage area (%)	Retention time	Relative percentage area (%)
1	α-pinene	$C_{10}H_{16}$	5.089	8.25	5.105	5.26
2	Camphene	$C_{10}H_{16}$	5.476	1.67	5.497	1.22
3	l-Limonene	$C_{10}H_{16}$	7.994	2.85	8.049	5.78
4	Camphor	$C_{10}H_{16}O$	12.622	6.79	n/a	n/a

5	β-pinene	$C_{10}H_{16}$	n/a	n/a	6.310	7.13
6	1,8-Cineole	$C_{10}H_{18}O$	n/a	n/a	8.203	48.98

The results of the antimicrobial test (Figure 3) showed that the clear zone formed in the positive control (tetracycline 15 µg/ml), coriander at a concentration of 500 µg/mL and 1000 µg/ml both in gram-positive *S. aureus* bacteria and gram-negative bacteria *E. coli*. Meanwhile, in cardamom, the diameter of the clear zone is relatively very low from 0 to 0.94±0.096 mm. In the coriander, clear zone formed in gram-positive and gram-negative bacteria nearly alike. The 1000 µg/ml concentration clear zone diameter superior to 500 µg/ml (Table 3). The result also shows that the concentration of coriander essential oils begins to have antioxidant activity starting at a concentration of 500 µg/ml. There are several chemical and physical factors that can affect the amount and composition of EO from aromatic plants [3,5]. Environmental conditions where the plant grow, physiology and extraction methods can also influence this [6].

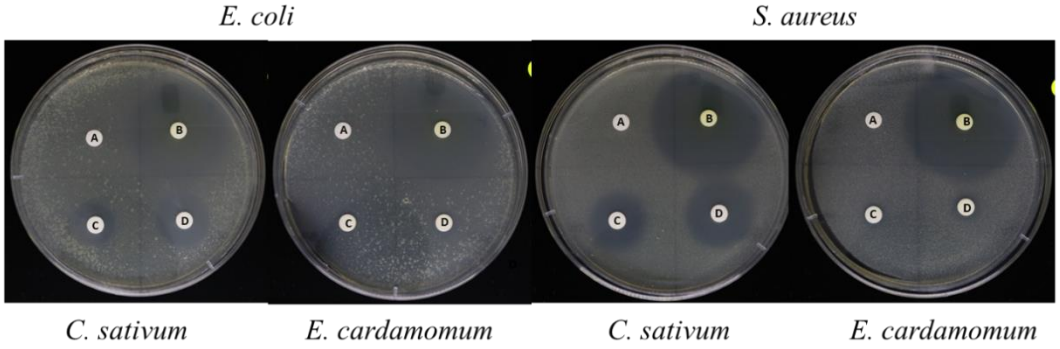

Figure 3. Antibacterial activities from *Coriandrum sativum* (A) and *Elettaria cardamomum* (B) against gram negative bacteria *E. coli* and *S. aureus*; A= negative control; B= positive control (tetracycline 15 µg/ml) ; C=sample 500 µg/ml; D=sample 1000 µg/ml.

Table 3. Inhibition zone from antibacterial activities from *Coriandrum sativum* (A) and *Elettaria cardamomum* (B) against gram negative bacteria *E. coli* and *S. aureus*.

Samples	*E. coli* (mm)		*S. aureus* (mm)	
	500 µg/ul	1000 µg/ul	500 µg/ul	1000 µg/ul
C. sativum	2.00±0.60	2.50±0.20	1.89±0.10	2.78±0.40
E. cardamomum	0	0.70±0.01	0.33±0.09	0.94±0.096

The antioxidants and antimicrobials activities can be associated with essential oil compound from coriander and cardamom. Both extracts contain l-Limonene that was effective against *S. aureus*, *L. monocytogenes*, *S. enterica*, *S. bayanus* and several others [3]. Meanwhile, pinene has antimicrobial activity against *C. albicans*, *C. neoformans*, *R. oryzae* and methicillin-resistant *S. aureus* (MRSA) [17]. However, the antimicrobial activity cannot be confirmed based only from one compound activity. The bioactivities from the essential oil might occur due to synergy activities of several compounds [8]. The action of EO can be related to the ability of molecules from these compounds to penetrate the bacterial membrane from outside to the inside of the cell. The bacterial growth inhibition indicates inhibitory activity from cell function and its lipophilic properties which cause leakage of the internal cell contents. These leaks can damage the cell membrane system in the cytoplasm, as well as cellular energy generation systems like Adenosine triphosphate (ATP) synthesis. This activity disrupts the proton motive force in the cell [2,6]. Gram-positive organisms appear to be far more susceptible to EO than Gram-negative bacteria.

4. Conclusion

In this research, the essential oil from Coriander has a good antimicrobial activity based on the result of the activity index, in gram-negative bacteria *E. coli* and gram-positive bacteria *S. aureus.* Meanwhile, cardamom has poor antimicrobial activity and tend to be active in gram-positive bacteria. For antioxidant activities, cardamom has a strong antioxidant activity compared to coriander. Therefore, *Coriandrum sativum* L. (Apiaceae) essential oil can be explored for their antimicrobial activities, while *Elettaria cardamomum* (L.) Maton (Zingiberaceae) potential as an antioxidant source. Those activities are affected by how the extraction prosses that can lead to the compound content in each extract, where in this case the content very dominant in monoterpene group.

Acknowledgment

This work supported by Penelitian Strategis Nasional Institusi (PSNI) 2018 first year Grant by Minister of Research, Technology and Higher Education of Republic of Indonesia No: 544/UN2.R3.1/HKP05.00/2018, thank you for the financial support. We also thanked Center of Excellence Biology Resources-Genome Study (CoE IBR-GS) for the facilities and equipment to support this research.

References

[1] L. Atarés & A.Chiralt. 2016. Trends in Food Science & Technology **48** 51-62
[2] R. Ribeiro-Santos, et.al. 2017. Trends in Food Science & Technology **61** 132-140.
[3] J. R. Calo, et.al. 2015. Food Control **54** 111-119.
[4] M.M. Cowan.1991. Clinical Microbiology Reviews, 564–582
[5] A. Duarte, et.al. 2016. Food Control **61** 115-122
[6] V. Lobo, et.al. 2010. Pharmacognosy Reviews. **4**(8), 118-126.
[7] F. Solórzano-Santos and M. G. Miranda-Novales. 2012. Current Opinion in Biotechnology 23 136–141
[8] B. Teixeira, et.al. 2013. Industrial Crops and Products **43** 587–595
[9] A.M. Khaneghaha, S.M.B.Hashemi, & S. 2018. Limboc. Food and Bioproducts Processing **111** 1-19
[10] R. Sheel, K. Nisha, & J. Kumar. 2014. IOSR Journal of Applied Chemistry, **7** (1), 10-13.
[11] R.Yunilawati, Ermawati , & R. Lamria, I.R. 2016. Prosiding Seminar Hasil Riset dan Standardisasi **VI** 45-54
[12] R. K. Sahu, M. Kar, and R. Routray, 2013. Journal of Medicinal Plant Studies, 21-27
[13] G. Scherer & H. T. Godoy. 2009. Food Chemistry **112** 654–658
[14] D. Nandita, et.al. 2012. Journal of Pharmacy Research **5** (8), 4201—4203.
[15] S. Sultana, et.al. Pakistan Journal of Biological Sciences **13**(7) 340-343
[16] N. Artanti, et.al. 2006. Journal of Applied Sciences **6** (8), 1659-1663
[17] A.C.R. da Silva, et.al. 2012. Molecules **17** 6305-6316.
[18] J.C. Goldbeck, et.al. 2014. LWT, Food Science and Technology **59** 813-819