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

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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 monoterpene 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 H2SO4, 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 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]:

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\text{% Inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100
\]  

(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:

$$AAI = \frac{DPPH \text{ concentration in reaction mixture (} \mu g/mL)}{IC50 \ (\mu g/mL)}$$

(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 10⁶ 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].

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

(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 in coriander (Figure 1). 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 higher 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].

| 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                 |

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

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

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

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

Environmental conditions where the plant grow, physiology and extraction methods can also influence this [6].

| Samples      | E. coli (mm) | S. aureus (mm) |
|--------------|--------------|----------------|
| C. sativum   | 2.00±0.60    | 1.89±0.10      |
| E. cardamomum| 0            | 0.33±0.09      |

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.

The antioxidants and antimicrobials activities can be associated with essential oil compound from coriander and cardamom. Both extracts contain β-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 processes that can lead to the compound content in each extract, where in this case the content very dominant in monoterpene group.

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