The Antibacterial Effect from Combining Cinnamon, Patchouli and Coriander Essential Oils

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

[1]Departemen Biologi, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Indonesia, Kampus Depok, Indonesia 16424
[2]Badan Penelitian dan Pengembangan Industri, Kementerian Perindustrian
[3]Departemen Fisika, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Indonesia, Kampus Depok, Indonesia 16424

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Abstract: Essential oils are dynamic organic liquids that work in synergy with each other. In general, essential oils work better when mixed with other essential oils. Every essential oil has many compounds and known for their benefits like healing properties and aromatic compound. When mixing essential oils between one oil and another, they can compensate for their strengths and weaknesses of each other. In this research, Cinnamon, Patchouli and Coriander essential oil combined to strengthen their antimicrobial activities. Variation was done by combining between 3 types of essential oils at different combination ratios 1:1 and 1:2 (v/v). Furthermore, the oil was tested on Gram-positive bacteria Staphylococcus aureus and Gram-negative bacteria Escherichia coli using the paper disk method and vapour test. From the results obtained it is known the strength of the antibacterial activity when the oil is in direct contact with microorganisms and the strength of volatile compounds of essential oils in inhibiting antimicrobial activity. The essential oils were also characterized using Gas Chromatography Mass Spectroscopy (GC-MS) to determine the levels and presence of compounds suspected of having antimicrobial activity. The results show the weakest antimicrobial activity EO combination were when using patchouli and coriander. Meanwhile, the strongest when testing paper disks and the vapour test is a combination of cinnamon and patchouli, cinnamon and coriander, cinnamon, patchouli and coriander.

1 INTRODUCTION

Nowadays, the development of active packaging by utilizing natural ingredients are quite engaging studies (Calo, et.al., 2015). Aromatic plants and their extracts have the potential to be applied in the field of food safety and food preservation. A part of phytochemical compounds there are group of essential oils that generally contain a very complex mixture of several types of aromatic phytochemical compounds, which had potential to be develop in this application (Chen et.al. 2018; Marques et.al., 2019).

Essential oils are dynamic organic liquids that work together in synergy. In general, essential oils work better when they mixed with other essential oils. Each essential oil contains many compounds and is known for its benefits, such as healing properties and aromatic compounds. When mixing essential oils between one oil and another, they can compensate for each other's strengths and weaknesses. Antagonism activity observed when the effects of one or both compounds are less when they are applied together than when applied individually. Synergism observed when the effects of the combined substances are higher than the sum of the individual effects (Davidson and Parish, 1989; Park et.al., 2018).

Certain phytochemical compounds derived from plants are known to have a role in inhibiting the growth and survival of microorganism (Guedes et.al., 2018; Merino et.al., 2019). However, besides their benefits, the used of essential oil as a food preservative have some disadvantages because of the strong smell that will affect the aroma and food flavour. Therefore, it is necessary to choose the type, composition, and dosage of a constant essential oil.

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Developing effective synergistic EO combinations could be an approach for improving their antimicrobial efficacies and food application potentials. More works are needed in this area as studies to date have reported inconsistent results in EO combinations (synergistic, additive and antagonistic) (Park et.al., 2018). In this research, 3 types of EO from Cinnamon, Patchouli, and Coriander tested for their antimicrobial activity during direct contact and in vapour phase. We tested the EO composition both single and combination against Gram-positive bacteria and Gram-negative bacteria to study their synergistic bioactivity for their potential as active label packaging mixture application.

2 MATERIALS & METHODS

2.1 Materials

Essential oil Cinnamon (Ci) (*Cinnamomum burmanii*), Coriander (Co) (*Coriandrum sativum*) and Patchouli (Co) (*Pogostemon cablin*) used in this experiment obtained from a local essential oils company in Indonesia Nusaroma. The bacteria used in this work were *Escherichia coli* NBRC 3301 strain and *Staphylococcus aureus* NBRC 100910 collection from UICC CoE IBR-GS, FMIPA UI represented Gram-negative bacteria and Gram-positive bacteria. The Muller Hinton Agar (Difco) was used for the culture of the bacterial medium.

2.2 Methods

2.2.1 Experimental Design

In this study, we tried to improve the antibacterial activity from 3 different essential oil (EO) which were cinnamon, patchouli, and coriander by combining in certain ratio. The combination of 2 essential oil with ratio 1:2 and 1:1 (v/v), respectively (Table 1). The essential oil used without further dilution.

Table 1: The ratio combination from 3 kinds of essential oil in this research

	Cinnamon	Patchouli	Coriander
Cinnamon		1:1 ; 1:2	1:1 ; 1:2
Patchouli	1:2		1:1 ; 1:2
Coriander	1:2	1:2	

2.2.2 EO Gas Chromatography Characteristic

Characterization and analysis from the EO based on their ratio combination 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 following the same method used in Handayani (2019).

2.2.3 Direct Contact Agar Diffusion Test

Paper disc diffusion method used to determine the antimicrobial activities by direct contact with the EO. 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 (Tetracycline 7 µg/mL), the essential oil then 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 32o C for 24 hours. After the end of incubation, a clear zone formed around the disc measured. Each experiment done in triplicate.

2.2.4 Vapour Phase Antibacterial Test

The antimicrobial activities from volatile compound from the EO were tested in vapour phase agar diffusion test. The vapour phase method follows the method used by Wang (2016) and Zaika (1988). We used the same bacteria and medium for preparation the paper disk diffusion assay. The EO loaded on to 6 mm disc and put under the paper disk cover and incubate incubated in reverse position.

3 RESULTS & DISCUSSION

3.1 GC/MS Characterization

GC/MS characterization was carried out to determine the compound content from each EO. Figure 1 shows the results of the chromatogram from 4 type of EO. The GC/MS showed that the types of compounds in each EO are relatively different and varied (Table 2). In Ci there are 2 major compounds detected, they are Cinnamaldehyde and Copaene. Meanwhile, the compounds contained in Po are α-Guaiene, Azulene, and 4-Aromadendrene. Furthermore, the Co containing Benzene, 1-methyl-2-(1-methylethyl) and 3-Carene. The abundance of major compounds also known from the chromatogram results and match with the know EO compounds from Cinnamon (Yang *et al.*, 2019), Patchouli (van Beek & Joulain, 2018) and Coriander (Laribi *et al.*, 2015). Furthermore, the EO combined to see its bioactivity in inhibiting bacterial growth both during direct contact and through vapor phase from the aromatic compounds.

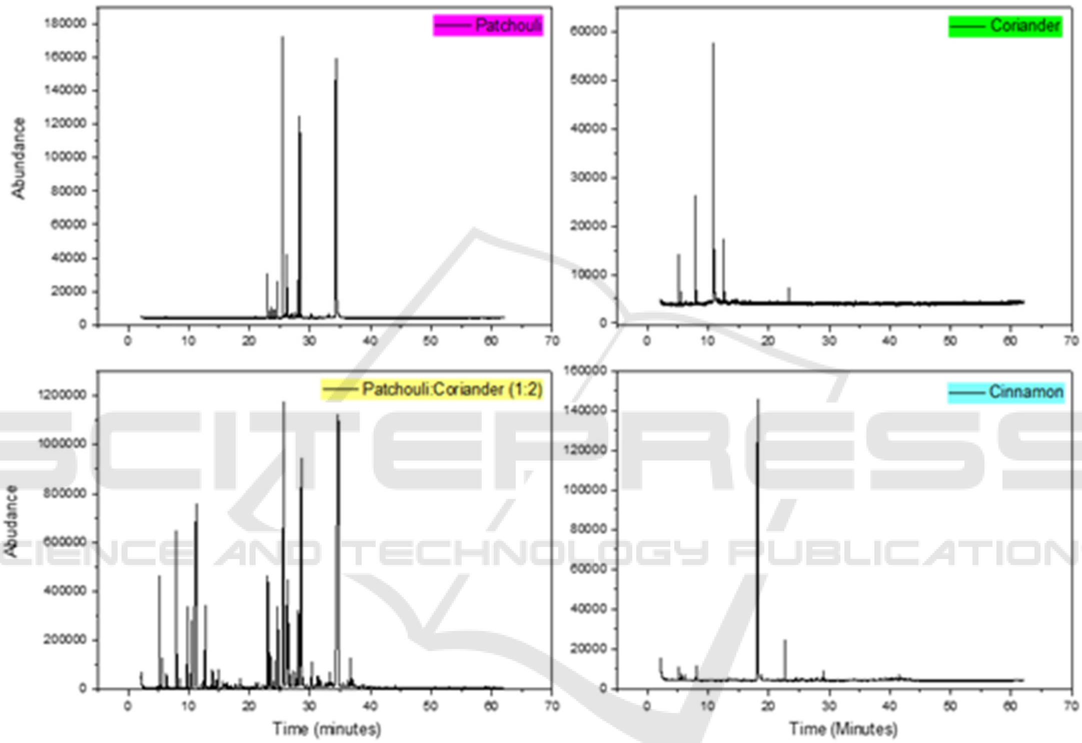

Figure 1: GC/MS chromatogram profile from Patchouli, Coriander, Cinnamon, and combination from Patchouli and Coriander EO

Figure 2. shows the antimicrobial activity of each type of EO using Patchouli (Po), Cinnamon (Ci), and Coriander (Co) in a single mixture. These results show there are formation of a clear zone from all the treatments. Among the three types of EO, Ci had the highest inhibitory activity against both *E. coli* and *S. aureus*, followed by Co. Meanwhile, Po had no activity against *E. coli* only has activity against *S. aureus*. This shows that there was an activity that selective to gram-positive or negative bacteria.

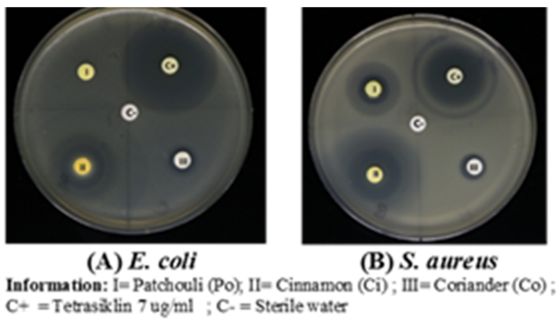

Figure 2: Antimicrobial activity from each Single EO.

Table 2: GC/MS analysis from Cinnamon, Patchouli and Coriander EO.

Cinnamon		
PK	RT	Library/ID
1	18.1664	Cinnamaldehyde
2	22.7314	Copaene
Patchouli		
PK	RT	Library/ID
1	22.94	β-Patchoulene
2	24.5992	Caryophyllene
3	25.483	α-Guaiene
4	26.0791	α-Patchoulene
5	28.3137	Azulene
6	34.2794	4-Aromadendrene
Coriander		
PK	RT	Library/ID
1	5.1052	γ-Terpinene
2	7.8733	Benzene, 1-methyl-2-(1-methylethyl)
3	10.8416	3-Carene
4	12.5842	Camphore

3.2 Antibacterial with Direct Contact Assay

Furthermore, the results of combined EO mixture tested for their antimicrobial activity. The results obtained can be seen in Figure 3 which shows the antimicrobial activity from Po/Co. The results obtained indicate an increase in antimicrobial activity than single EO mixture. Antimicrobial activity in Po/Co combination (2: 1) has the best activity. While the ratio of 1:1 has relatively the same antimicrobial activity with the single mixture, and the Po/Co ratio (1: 2) has the weakest antimicrobial activity.

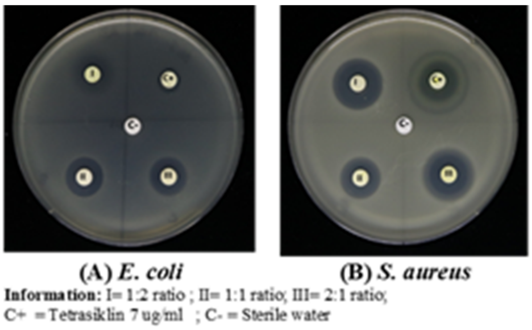

Figure 3: Antimicrobial activity from Patchouli (Po): Coriander (Co) combination.

Meanwhile, Figure 4 and 5 show that the combination from each ratio shows inhibitory activity which tends to be the same in all ratios. The strongest activity dominated went the mixture contained EO from Cinnamon. Figure 4 shows the comparison of the clear zone diameter sizes of each treatment in the direct contact test. These results indicate that in the single form the EO from Ci has the strongest activity. While Patchouli only has positive activity on *S. aureus* and Coriander has activity on *E. coli* and *S. aureus* so that when combined with Po/Ci or Co the antibacterial activity increased. The Ci/Po combination showed a better activity compared to other EO combinations in this study.

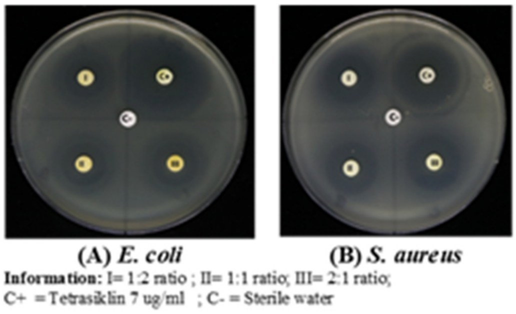

Figure 4: Antimicrobial activity from Cinnamon (Ci): Coriander (Co) combination with different ratio.

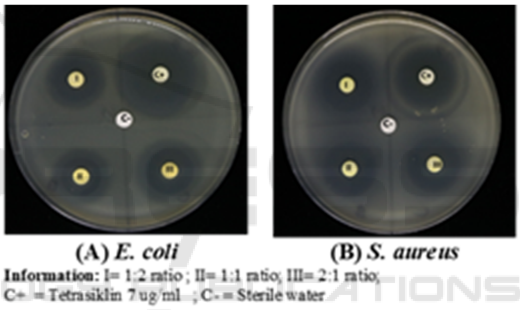

Figure 5: Antimicrobial activity from Cinnamon (Ci): Patchouli (Po) combination with different ratio.

This is also seen in Figure 6. which shows that a single EO Ci tends to have the strongest activity compared to Po and Co, where the diameter of the clear zone formed can reach 45 mm. The EO from Ci had almost equal activity to gram negative and positive. Co tend to have stronger activity for Gram-negative bacteria than Po, meanwhile the Po tend to be weakest activity. Meanwhile, Po had stronger activity against gram positive bacteria than Co. With the combination from Po/Co (2:1) the activity against *E. coli* increasing this result show possibility compliment action of synergistic activity with the mixture from those 2 EO by direct contact. Based on Figure 1, when we combined the EO from Co/Po the chromatogram detected increasing peak number from the previous one, which indicate the increasing of compound number that contained from both Co and Po EO.

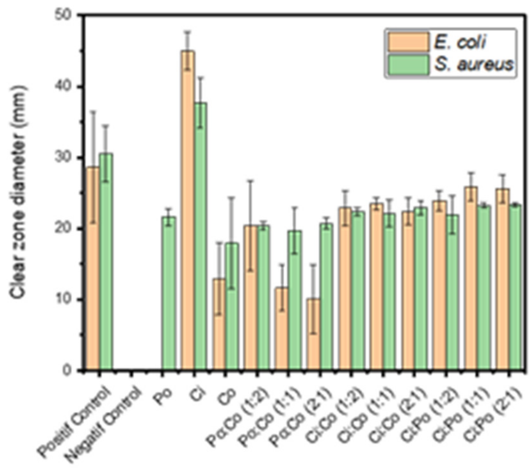

Figure 6: The diameter of inhibition zone from each treatment with direct contact.

3.3 Antibacterial Vapor Test

In addition, beside the antimicrobial test through direct contact, the vapor phase from the EO were also tested for its antimicrobial activity. Of the three EOs used, Ci showed better inhibitory activity on *E. coli* than *S. aureus* (Figure 7). Meanwhile, the EO vapor test from Co and Po, there were no clear zone observed from the two test bacteria (Table 3).

Table 3: Antimicrobial activity from vapour phase test of single EO

	Patchouli (Po)	Cinnamon (Ci)	Coriander (Co)
E. coli	-	+++	-
S. aureus	-	++	-

Figure 8. Shows the inhibition zone of the combination of Ci with other EO did not show an increasing in the antimicrobial activity. The activity tends to decrease, which predicted as the result from the decreasing in the abundance of aromatic compounds after combined with the ratio treatment per 10 µL. These results indicate that the vapor from Ci EO has strong antimicrobial activity. While other EOs have volatile compounds that do not have antimicrobial activity. Cinnamaldehyde also known as cinnamic aldehyde is known to be an aromatic compound contained in the EO that gives cinnamon its flavor and odor. Cinnamaldehyde is the major component comprising 85% in the essential oil and the purity of cinnamaldehyde in use is high (> 98%). Both oil and pure cinnamaldehyde are equally effective in inhibiting the growth of various microorganisms such as Gram-positive and Gram-negative (*E. coli*) bacteria, and fungi including yeasts, filamentous molds and dermatophytes (Ashakirin, 2017). Other research by Ács et. al (2018) highlight that Gram-negative strains were more sensitive to EO vapours.

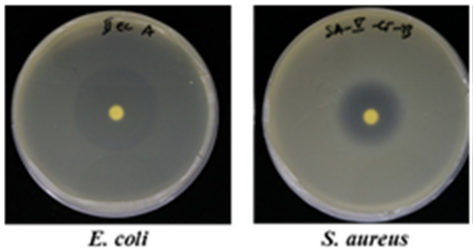

Figure 7: Clear zone formed from vapour phase test of Cinnamon EO for antibacterial inhibition.

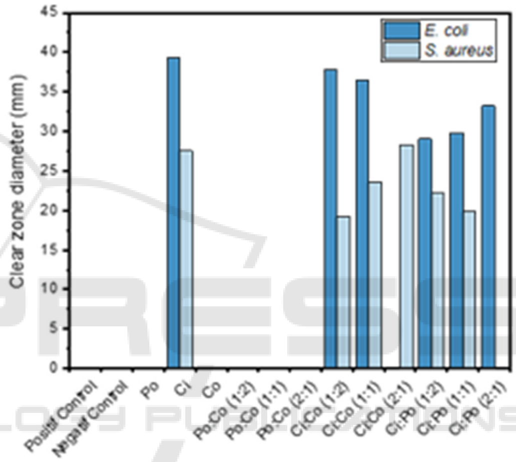

Figure 8: The inhibition zone from each treatment with vapour phase against *E. coli* and *S. aureus*.

4 CONCLUSIONS

The results showed that EO, with the most potent activity in direct contact using paper disks and vapour tests, were Ci/Po and Ci/Co. Meanwhile, the weakest antibacterial activity with EO combination was when using patchouli and coriander. The combination of Po/Co improves their strength against both bacteria than when they were as single EO. The factors that were affecting EO's antimicrobial activity in this study are the abundance and the EO species, which depend on a specific active compound in each plant species — combining the essential oil need to be more selective, to improve antimicrobial activity and determine the synergistic and antagonistic effect from the compound.

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