Antimicrobial Effect of Concord Paper Containing with Lemongrass Oil against *Escherichia coli* and *Staphylococcus aureus*

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Abstract: The use of an antimicrobial label in food packaging as a form of active packaging is an interesting to investigate. This label can be used to extend the shelf life of food. Lemongrass oil is one of essential oil that is potential used as an antimicrobial agent. In this study, the antimicrobial effect of label made from concord paper which incorporated with lemongrass oil was prepared and tested against the bacteria *Escherichia coli* and *Staphylococcus aureus* using disk inhibition zone method. This antimicrobial label was tested using FTIR to investigate the interaction between essential oil and the matrix. The lemongrass oil was tested using Gas Chromatography-Mass Spectrometry to determine the levels and presence of compounds suspected of having antimicrobial activity. The labels have antibacterial activity against *E. coli* with the diameter of inhibition zone maximum about 47.85 mm but not active toward the *S. aureus*. From the results of the antibacterial test can be seen that the use of antibacterial label is promising when used for food safety with a prolonged shelf life.

1 INTRODUCTION

Contamination of food can occur during the process of harvesting, food processing, packaging and distribution. Packaging is one of the effective ways to protect food from contamination from the outside environment such as air, dust, physical, chemical and biological impacts such as microbes that cause food spoilage. Conventional packaging which is widely used today, cannot actively control the reactions that occur in food (Mousavi et al., 2018). One of the packaging technologies that have been developed to maintain the quality of food and extend the shelf life of food is to use active packaging. The use of antimicrobial labels on active packaging is now an interesting technology for research.

With the aim to reduce the use of additional chemical substances in food, one way is to use natural ingredients to inhibit the growth of microbes that cause food spoilage that does not have a negative effect on human health (Chiralt and Atar, 2016). Essential Oil is one of the antimicrobial agents derived from plant extracts that have antimicrobial properties. However, this essential has a strong enough odour that it is rarely used to be added directly to food because it will damage the taste of the food itself. Because that reason, it is interested to combine essential oils into a matrix to reduce the strong odour as antimicrobial label.

Several studies have been carried out by combining essential oil such as oregano with cassava starch/chitosan with oregano essential oil (Pelissari et al., 2009), alginate with lemongrass oil (Chiralt and Atar, 2016), and coated paper with *Cuminum cyminum* L. and *Prubus mahaleb* L. in terms of improving antimicrobial properties (Ezel and Dal, 2018). In this research, lemongrass oil is combined into a paper matrix. The paper used is Concord paper. Concord paper which has another name namely Japanese linen paper is textured paper. By combining lemongrass oil into the concord, it is hoped that it can make an effective antimicrobial label which effective against *Eschericha coli* and *Staphylococcus aureus* which can be used to extend the shelf life of food.

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2 MATERIALS AND METHOD

2.1 Materials

Lemongrass oil was used in this experiment obtained from Nusaroma, a local essential oils company in Indonesia. The matrix used in this study is concord paper with a gramatur of 220 gr / mm^2 produced by PT. Parisindo Pratama.

2.2 Preparation of Antimicrobial Labels

The antimicrobial label was prepared by dropping of 25 µL lemongrass oil using a micro pipette onto the surface of the concord paper with a size of 1 cm x 3 cm, then allowed at room temperature for 5 minutes.

2.3 Characterization

2.3.1 Lemongrass Oil Characterization

Characterization Lemongrass Oil using GC- MS. Lemongrass oil compounds were identified by gas chromatography with a mass spectrometer detector (GCMS) Agilent 6890 series with capillary column HP-5MS, 30 m x 0.25 mm id x 0.25 µm film thickness. Helium gas was used as the carrier gas at constant pressure of 65 kPa. The lemongrass oil was injected with a volume of 1 µL in split ratio of 1:25. The increasing of oven temperature was programmed from 60-240°C with step of 3°C per minute until reaching 240°C.

Antimicrobial Activities Assay of Lemongrass Oil: Direct Contact Agar Diffusion Tests. The antimicrobial activities determined by the paper disc diffusion method using type strain of *Staphylococcus aureus* NBRC 100910 and *Escherichia coli* NBRC 3301 in The Mueller Hinton Agar. 10 ml of molten media poured 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 15 µg/mL), lemongrass oil with concentration 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 35° C for 18 hours. After the end of incubation, a clear zone formed around the disc was measured.

2.3.2 Label Characterization

Antimicrobial Activities Assay of Labels. The antimicrobial activities of labels were tested in vapour phase agar diffusion test, because in its application as label antimicrobial will used vapour phase. The vapour phase method follows the method used by (Wang et.al, 2016).

Labels are cut in a circle with a diameter of 0.6 cm and then placed in a petri dish to test antimicrobial activity. The vapour phase agar diffusion test was technically similar to the direct contact diffusion test. However, the filter discs were placed at the top in centre of the inner side of the Petri dish cover. The dishes were then sealed using laboratory parafilm to avoid evaporation of the test compounds, followed by incubation at 32° C for 24h. The diameter of the inhibition zone was recorded.

Efficacy Test of Label on the Product. The efficacy of the antimicrobial label was evaluated by placing the label with a size of 1x3 cm above the surface of a plastic package containing chicken meat (10 g) purchased from the local market in Depok. Then the chicken meat is kept at room temperature for 5 days to see the visual changes found in the chicken meat.

Fourier Transform-Infra Red (FTIR) Analysis. The spectra of the antimicrobial label (control paper and paper that had been dropped with lemongrass oil) were test using Fourier Transform Infrared (FTIR) using a double beam spectrophotometer (Thermo Nicolet iS5) to determine functional groups. FTIR analysis was carried out on blank label before and after it was used to store chicken breast filled.

UV-Vis Analysis. UV- vis spectrophotometer was used to measure the reflectance of the antimicrobial label (control paper and paper that had been dropped with lemongrass oil) with a size of 1x3 cm. The brand of UV-Vis apparatus is Shimadzu UV-2450.

3 RESULT AND DISCUSSION

3.1 Chemical Compounds of the Lemongrass Oil

Characterization using GC-MS showed the chromatogram profile detected 6 peaks in lemongrass oil (Figure 1) which indicated there were 6 compounds in lemongrass oil. The compounds were identified based on comparison of mass spectrum

with reference data from the database (Wiley 7). Based on this, lemongrass oil was known contain 6 compounds, namely neral (beta-citral), geraniol, geranial (alpha-citral), geranyl acetate, beta-caryophyllene and gamma cadinene (Table 1) with the main compounds being citral and geraniol. These results appropriated with previous finding reported in literature, citral and geraniol has been described as the main compounds of lemongrass oil (Ganjewala, 2009).

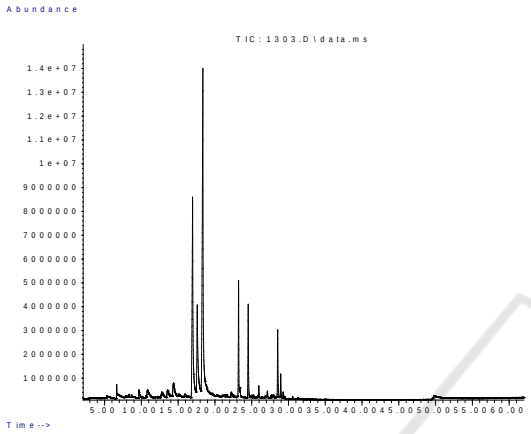

Figure 1: GC-MS chromatogram of the lemongrass oil.

Table 1: Chemical compound identified of lemongrass oil with GC-MS.

No	Retention time	Identified compound	Molecular formula	Relative percentage area (%)
1	17.101	Neral (beta-citral)	$C_{10}H_{16}O$	29.00
2	17.753	Geraniol	$C_{10}H_{18}O$	10.80
3	18.524	Geranial (Alpha citral)	$C_{10}H_{16}O$	44.21
4	23.302	Geranyl acetate	$C_{12}H_{20}O_2$	6.50
5	24.588	Beta-caryophyllene	$C_{15}H_{24}$	5.67
6	28.589	Gamma-cadinene	$C_{15}H_{24}$	3.83

Citral (3,7 dimethyl-2-6-octadienal) is an unsaturated aldehyde, the most common flavour in citrus oil and widely used in food and beverages. Citral is the mixture of two isomers geometric, neral (beta-citral) and geranial (alpha-citral) which are monoterpene aldehyde. Citral has an activity antibacterial against Gram-positive bacteria and Gram-negative bacteria, both on oil form and vapour form (Argyropoulou et al., 2007) It is revealed the presence of C = O bond for aldehyde from indicates the presence of citral compounds. Antimicrobial activity of cinnamaldehyde was found against E. coli and staphylococcus aureus. Citral that have aldehyde function group plays a role in disrupting bacterial cell membranes (Firmino et al., 2018)

3.2 Antimicrobial Activities of Lemongrass Oil and the Label

The bacteria is one of indicator used for examination of spoilage to meat products (Pranoto et al., 2005). Meat and processed products that are perishable food because they are very vulnerable to contamination by microorganisms. Spoilage meat can contain pathogenic bacteria such as *S. Aureus* and *E. coli*. Therefore, in this research an Antimicrobial activity of lemongrass oil against test was carried out against *E. coli* and *S. aureus* (Figure 2). The inhibitory activity is measured based on the clear zone that occurs around the label. The measurement of the clear zone diameter is calculated including the diameter of the label. The diameter produced will be greater than the diameter of the label if a clear zone is detected. If no clear zone is formed around the label, then it is assumed that there is no inhibitory region and the diameter is declared zero.

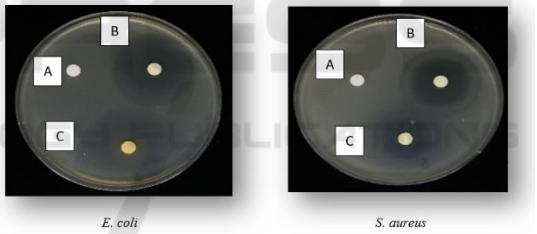

Figure 2: Antimicrobial activities of lemongrass oil using paper disk method against Gram-positive bacteria *S. aureus* and Gram negative bacteria *E. coli*; A = negative control; B=positive control; C=sample.

In Figure 2 it can be seen that the negative control in the form of sterile distilled water does not form a clear zone which means it does not show an inhibitory effect on E. coli and S. aureus bacteria. Inhibition diameter can be seen in Table 2. For positive control in the form of antibiotic tetracycline, a clear zone with a diameter of 1.9 cm can be seen for *E. coli* bacteria and 3.1 cm for *A. aureus bacteria*. As for the lemongrass oil, a clear zone with a diameter of 4.7 cm is formed for *E. coli* bacteria and 2.5 cm for *A. aureus bacteria*. The diameter of the clear zone formed in lemongrass oil against *E. coli* bacteria (gram -) is greater than that of *S. aureus* (gram +), which means that lemongrass oil is more effective against *E. coli* bacteria (gram -). This is because Gram positive has

a cell wall structure that is different from gram negative bacteria. In addition, gram-positive bacteria have cell walls composed of a thicker layer of peptidoglycan (20 to 80 nanometres), while gram-negative bacteria have a thinner layer of peptidoglycan.

Table 2: Diameter of inhibition zone of lemongrass oil.

S. aureus(cm)			E.coli (cm)		
Sample	Control (-)	Control (+)	Sample	Control (-)	Control (+)
2,5	-	3,1	4,7	-	1,9

Besides seeing the antimicrobial activity of Lemongrass oil, it was also carry out an antimicrobial test from the label. For the label antimicrobial test, the vapour method is used to match the application used. The antimicrobial activity of the label can be seen in Figure 3.

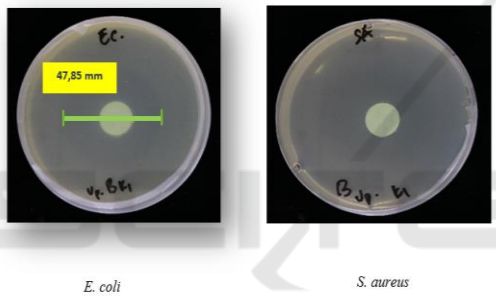

Figure 3: Antimicrobial activities of antimicrobial labels with lemongrass oil concentration 10% using vapour method against Gram-positive bacteria *S. aureus* and Gram-negative bacteria.

From Figure 3 it can be seen that the label provides antimicrobial effect on *E. coli* (gram negative bacteria) but not on *S. aureus* (gram positive bacteria). It can be seen from a clear zone or the diameter of the inhibition zone of *E. coli* is around 47.85 cm, while the *S.aureus* bacteria do not form a clear zone, then it is assumed that there is no inhibitory region and the diameter is zero, this is possible because the antimicrobial testing of the label was use the vapour method. The effectiveness of lemongrass oil on *E.coli* is also similar as that of other researchers (Faleiro, 2019) and (Naik et al., 2010). Other research which states that lemongrass essential oil also has antimicrobial properties against other bacteria such as *A. baumannii* (Adukwu et al., 2016).

3.3 Efficacy Test of Label on the Product

The efficacy of the antimicrobial label was evaluated within 5 days using chicken breast filled. From Figure 4 it can be seen that there is a change in the colour, texture and odour of the chicken breast filled. On the fifth day, Figure 4 (A) is chicken breast filled without using a label. Figure 4 (A) shows the colour of chicken breast filled is paler compared to Figure 4 (B). In addition to colour observation, the texture of chicken breast filled in Figure 4 (A) is also soggier when compared to Figure 4 (B), this indicates that the label application can maintain the freshness of chicken breast filled. In addition to investigating the colour and texture, an investigation was also conducted on odours. In this experiment, the odour of lemongrass oil still affected the odour of the food in the packaging.

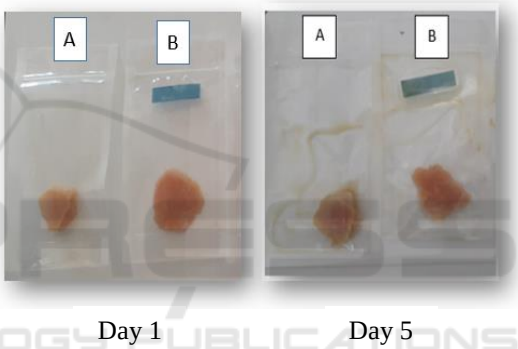

Figure 4: Label application on the chicken breast filled, (a) label without application, (b) label with application.

3.4 FTIR Analysis of Label

Functional group analysis is performed to determine changes in functional groups that occur during efficacy tests on the labels. The performance test of labels on chicken breast was carried out for five days. During this time, functional group analyses are carried out using FTIR. Figure 5 displays the spectra of concord paper and label.

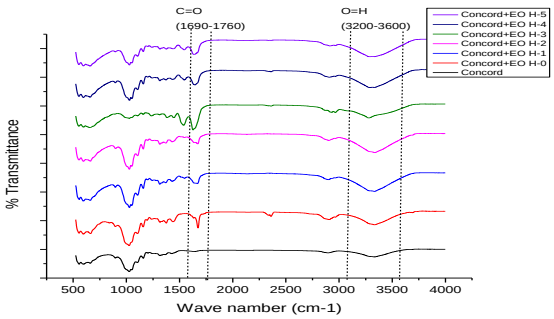

Figure 5: FT-IR Spectra.

Fingerprint for lemongrass oil is mostly in the range of 1800-600 cm-1 (Li, 2013). In the IR spectra, it is shown that the absorbance band at 1690-1760 cm^{-1} revealed the presence of C=O bond for aldehyde from indicates the presence of citral compounds (Adinew 2014). Besides that, it is shown that the absorbance band at 3200-3600 cm-1 revealed the presence of O=H, which indicates the presence of compounds geraniol. From the Figure, the C=O intensity of citral is decreasing. It is because citral has to be released from the label and the presence of this citral compound was strengthened by GC MS results and the result of antimicrobial assay.

3.5 UV-VIS Analysis

UV-Vis analysis was carried out to see the Reflect ants from the label before and after the addition of essential oil. From Figure 6 there is a change in the % reflectance intensity of the label. The color change occurred from blue to green can be seen in Figure 7. The green color change occurred after the addition of lemongrass oil followed by a decrease in the intensity value of % Reflectance at wavelength around 600-650 nm.

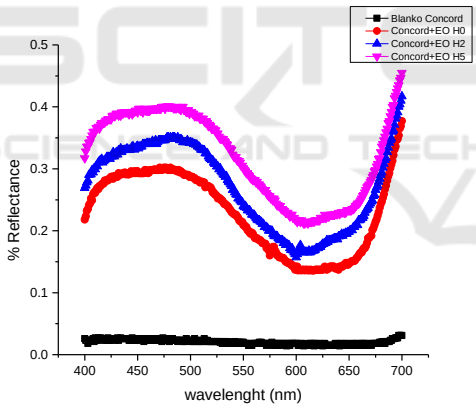

Figure 6: Reflectance spectra of antimicrobial labels.

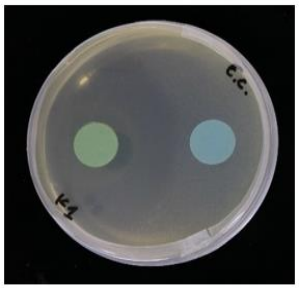

Figure 7: Discoloration of label.

4 CONCLUSIONS

In this study, it can be concluded that labels made from Concorde paper added with lemongrass oil have the potential to become antimicrobial label. The labels have antibacterial activity against *E. coli* with the diameter of inhibition zone maximum about 47,85 mm but not active toward the *S. aureus*. However, the application will depend on the type of food where flavour is not a problem.

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