

# Synthesis of Antimicrobial and Emulsifier Compounds through Hydroxyl Group Esterification of Oxidized Ricinoleic Acid

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**Abstract:** In this study, ester synthesis from commercial oxidized ricinoleic acid using various carboxylic acid was conducted. The double bond in ricinoleic acid was oxidized using  $\text{KMnO}_4$  in alkaline condition to form two hydroxyl groups. Oxidized ricinoleic acid was then esterified chemically using palmitic acid, decanoic acid, and butyric acid by  $\text{ZnCl}_2$  as catalyst with the molar ratio of oxidized ricinoleic acid to carboxylic acid was 3:1. Esters produced were characterized using FTIR examined as emulsifier and the antimicrobial activity. The results showed that each ester product gave absorption band  $\text{C}=\text{O}$  ester at the range of  $1600\text{ cm}^{-1}$ - $1720\text{ cm}^{-1}$ . The highest conversion percentage of esterification was obtained by oxidized ricinoleic acid-palmitic acid esters with the value of 75%. Simple emulsifier test was performed for each ester and the result showed that esters were able to maintain an emulsion form approximately 24 hours with water-in-oil emulsion (w/o) type. The antimicrobial activity test of esters gave positive results in the presence of inhibition zone to the growth of *Propionibacterium acnes* and *Staphylococcus epidermidis*. The highest antimicrobial activity against *P. acnes* and *S. epidermidis* was produced by oxidized ricinoleic acid-decanoic acid esters.

## 1 INTRODUCTION

*Ricinus communis L.* is one of industrial plant that has bright prospects to be developed in Indonesia. *Ricinus communis L.* seeds contain 46% oil, which are known as castor oil. Castor oil content is dominated by ricinoleic acid (89,5% of total fatty acids). Ricinoleic acid ( $\text{C}_{18}$ ) is an unsaturated fatty acid with one double bond at carbon-9 ( $\text{C}_9$ ) and hydroxyl group in the side chain (R) position of carbon-12 ( $\text{C}_{12}$ ). Other than double bonds and hydroxyl groups, ricinoleic acid has carboxylic groups which make this fatty acid can be reacted through various reactions to get its derivative product. One form of its derivative product is oxidized ricinoleic acid esters (Kajikawa et al., 2016).

The double bond in ricinoleic acid can be oxidized by using a strong oxidizer to produce diol. The addition of diol groups can increase polarity of ricinoleic acid which makes this compound to reduce surface tension, because it can combine polar and nonpolar phase. The hydroxyl groups of oxidized ricinoleic acid can be esterified by carboxylic acids to form oxidized ricinoleic acid

esters which can be used as an emulsifier due to its properties.

Previous study showed that derivatives of fatty acid in the form of long chain esters could be used to be skin barrier (Pérez et al., 2016). In this study, oxidized ricinoleic acid esters were synthesized by esterification reaction with palmitic acid, decanoic acid, and butyric acid using  $\text{ZnCl}_2$  as catalyst. Esterification products were then characterized using FT-IR and examined its ability as emulsifier and its antimicrobial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis*.

## 2 EXPERIMENT

### 2.1 Materials

Materials used in this study were commercial ricinoleic acid, sodium hydroxide, potassium permanganate, chloroform, Wijs solution, potassium iodide, sodium thiosulphate, starch, palmitic acid, decanoic acid, butyric acid, zinc chloride, *n*-hexane, eosin, phenolphthalein, methanol, antibiotic clindamycin, nutrient broth, nutrient agar,

*Propionibacterium acnes* and *Staphylococcus epidermidis* from Universitas Indonesia.

## 2.2 Methods

### 2.2.1 Oxidation of Commercial Ricinoleic Acid

Oxidation was carried out by mixing commercial ricinoleic acid, NaOH 2 M, and KMnO<sub>4</sub> 0.5 M. This mixture was stirred using *magnetic stirrer* at temperature 25<sup>0</sup>C-27<sup>0</sup>C for 30 minutes. After 30 minutes, there would be two phases. Organic phase was then separated and filtered.

### 2.2.2 Determination of Iodine Value

Oxidized ricinoleic acid was mixed with chloroform and Wijs solution, then left for 30 minutes in dark place. After 30 minutes, KI 15% and distilled water were added. The mixture was then titrated using sodium thiosulphate 0.1 N until the colour of mixture turn to yellowish. Starch was added and the mixture was titrated again by sodium thiosulfate until the colour turned clear (Goud et al., 2006).

### 2.2.3 Esterification

Esterification was carried out by reacting oxidized ricinoleic acid in *n*-hexane as solvent with palmitic acid on reflux system at temperature 60°C for 6 hours using ZnCl<sub>2</sub> as catalyst. ZnCl<sub>2</sub> used in this reaction used in this reaction was 0.3 % of the total mass substrate (w/w substrate). The mol ratio of oxidized ricinoleic acid to palmitic acid used in this reaction was 3:1 (mol/mol) (Gonçalves et al., 2011). The same steps were performed using decanoic acid and butyric acid. Ester products were then purified by extraction using *n*-hexane and methanol.

### 2.2.4 Determination of Conversion Percentage

The conversion percentage was determined by titrating the organic phase that has been obtained from extraction in *n*-hexane using 0.1 N NaOH and phenolphthalein as indicator (Handayani et al., 2012).

### 2.2.5 FT-IR Analysis

Commercial ricinoleic acid, oxidized commercial ricinoleic acid, and all esters were characterized using FTIR.

### 2.2.6 Simple Emulsifier Test and Determination of Emulsion Type

Emulsifier test was performed by mixing 0.1 g esters, oil, and water according to Table 1. The mixtures were shaken, and the emulsion stability was observed up to 24 hours. The emulsion type determination was observed under microscope using eosin as indicator.

Table 1: Variation of Oil and Water Composition in the Making of Emulsion.

Tube Number	Oil in Water					Tube Number	Water in Oil				
	1	2	3	4	5		1	2	3	4	5
Water (ml)	2	2	2	2	2	Water (drops)	2	4	6	8	10
Oil (drops)	2	4	6	8	10	Oil (ml)	2	2	2	2	2

### 2.2.7 Antimicrobial Activity Assay

Disc diffusion method was performed to determine antimicrobial activity. An aliquot of 200 µL bacteria suspension with density 1x10<sup>8</sup> cells/mL was aseptically mixed by 20 mL nutrient agar in a sterile petri dish. The sterile paper disc (± 6 mm in diameter) was placed on top of the medium and dropped by 4 µL sample to be tested. The medium was then incubated at 37°C for 24 hours. The clear zone around the paper discs were measured. The bacteria that used in this research were *Staphylococcus epidermidis* and *Propionibacterium acnes*.

## 3 RESULT AND DISCUSSIONS

### 3.1 Oxidation of Commercial Ricinoleic Acid

The double bond on commercial ricinoleic acid were oxidized through dihydroxylation reaction, which would produce two hydroxyl groups. In this study, oxidation was performed in an alkaline condition. At cold temperatures with low concentrations of oxidizing agents, alkenes tend to form glycols. At the end of reaction, MnO<sub>2</sub> was produced. The MnO<sub>2</sub> could be removed by filtering oxidation product.

### 3.2 Determination of Iodine Value

Oxidation product was identified through determination of iodine value to testify that oxidation has successfully carried out. The iodine

value of oxidized ricinoleic acid before oxidation was 45.30 mg/g and after oxidation it decreased to 17.29 mg/g. The decreased in iodine number proved that ricinoleic acid was successfully oxidized.

### 3.3 Esterification

In this study, esterification reaction was carried out on the OH group of oxidized ricinoleic acid with palmitic acid, decanoic acid, and butyric acid using  $ZnCl_2$  catalyst. The ester products expected to be produced were monoesters. The mole of oxidized ricinoleic acid is higher than fatty acid. This referred to Le Chatelier's principle. The increased of reactants amount will produce more products.

### 3.4 Determination of Conversion Percentage

Esters that formed were purified by extraction using *n*-hexane and methanol. The *n*-hexane layer was at the top. This layer consisted of non-oxidized and non-esterified compounds, while methanol layer is at the bottom consisted of esters. The conversion percentage is shown in Figure 1. The highest conversion percentage was obtained by oxidized ricinoleic acid-palmitic acid esters with the value of 75%. At the beginning of reaction, conversion percentage of ester products will decrease along with the chain lengthening of fatty acid used. This is because ester products from short-chain fatty acid are more soluble in organic solvents, such as *n*-hexane. However, when the reaction is completed, conversion percentage will remain the same (Macierzanka & Szeląg, 2004).

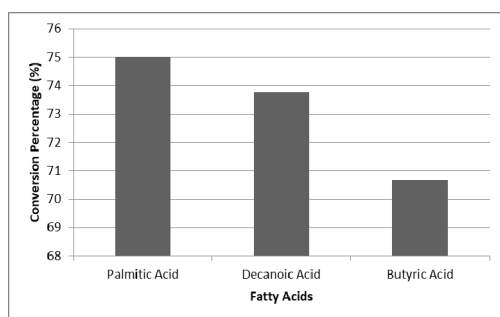


Figure 1: The Curve of Fatty Acid Variation vs Conversion Percentage.

### 3.5 FT-IR Analysis

The formed product was characterized using FTIR to determine the success of the hydrolysis, oxidation, and esterification reaction. IR spectrum of ricinoleic

acid, oxidation products, and ester products are shown in Figure 2.

The FTIR spectrum of ricinoleic acid showed the absorption peak of group  $C=O$  carboxylic acid at wave number  $1710.93\text{ cm}^{-1}$ . In addition, there was also the absorption peak of the  $-OH$  at wave numbers  $3408.36\text{ cm}^{-1}$ .

Oxidized ricinoleic acid which was successfully oxidized has the addition of two  $-OH$  groups to the fatty acid carbon chain. The  $-OH$  group's intensity increased indicated the oxidation reaction has succeeded. Oxidation products had an absorption peak of  $-OH$  at wave number  $3426.69\text{ cm}^{-1}$ . In this study, there was still  $C=C$  group with a small intensity which indicated that not all double bonds were oxidized.

IR spectra of esterification products showed absorption peak of  $C-O$  ester group in the range of wave numbers of  $1300-1000\text{ cm}^{-1}$  which indicated the ester has formed. The  $-OH$  group's intensity decreased since esterification has occurred.

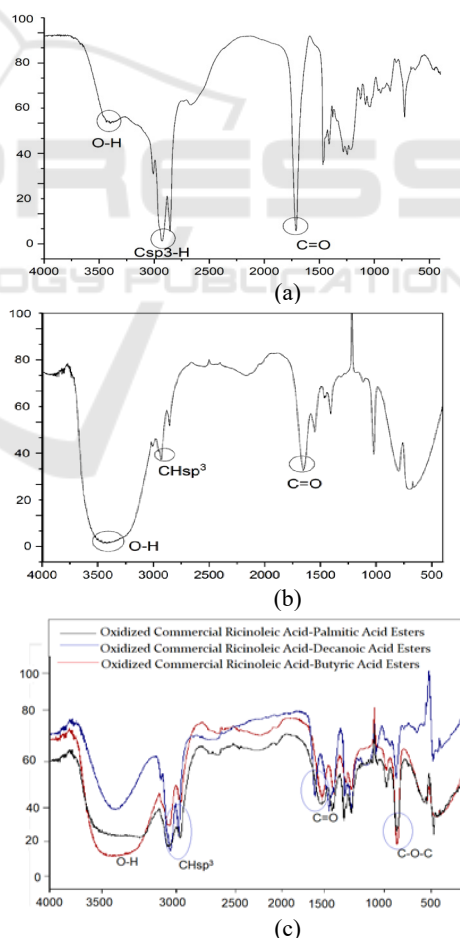


Figure 2: FTIR Spectra (a) Commercial Ricinoleic Acid, (b) Oxidized Ricinoleic Acid, (c) Esters.

### 3.6 Simple Emulsifier Test and Determination of Emulsion Type

The simple emulsifier test showed that commercial ricinoleic acid, oxidized ricinoleic acid, and esters can act as emulsifier up to 24 hours. All products had ability to form water in oil (w/o) type of emulsion (Figure 3). Figure 3 showed that clear zone surrounded red droplets. Red droplets formed were water phase with eosin dye which dissolved because they have same polarity, while the surrounding clear solution was oil.

The ability of a compound as an emulsifier is determined based on the presence of a hydrophobic group and a hydrophilic group. The hydrophilic group will bind to water, while the hydrophobic group will bind to oil. If the ratio of water and oil composition is balanced, the emulsion formed will be stable.

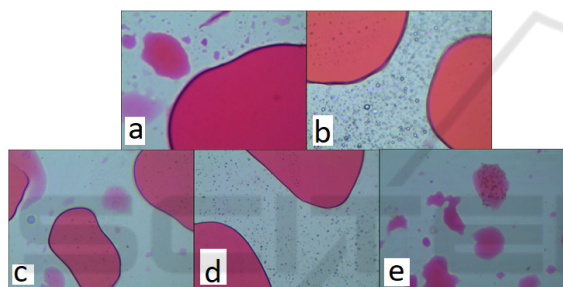


Figure 3: Emulsion Types (a) Commercial Ricinoleic Acid, (b) Oxidized Ricinoleic Acid, (c) Oxidized ricinoleic acid-palmitic acid ester, (d) Oxidized ricinoleic acid-decanoic acid ester, (e) Oxidized ricinoleic acid-butyric acid ester.

### 3.7 Antimicrobial Activity Assay

In this study, bacteria that used were *Staphylococcus epidermidis* and *Propionibacterium acnes*. These two bacteria are acne causing bacteria. Table 2 shows the effectiveness classification of antimicrobial substances (Greenwood, 1995), while Table 3 shows antimicrobial activity of all products. The positive control used in this study was clindamycin 0.5%. Clindamycin is an antibiotic commonly used for acne-causing bacteria. The negative controls used were n-hexane and methanol as they were used as solvents for dilution. The purpose of using negative control was to compare that the solvent used did not affect antimicrobial activity (Natheer et al., 2012).

Based on Table 3, it was known that all esters showed antimicrobial activity, while the positive control showed no antimicrobial activity. Oxidized

ricinoleic acid-decanoic acid esters showed the most effective antimicrobial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis*.

Table 2: Classification of effectiveness of antimicrobial substances (Greenwood, 1995).

Inhibit Zone Diameter	Response of growth barriers
> 20mm	Strong
16-20 mm	Medium
10-15 mm	Weak
< 10 mm	Not effective

Table 3: Diameter of the Inhibitory Zone of Various Compunds against *P. acnes* and *S. epidermidis*.

Sample	Inhibitory Zone (mm)		Effectiveness	
	<i>P. acnes</i>	<i>S. epidermidis</i>	<i>P. acnes</i>	<i>S. epidermidis</i>
Commercial Ricinoleic Acid	11	7	Weak	Not effective
Oxidized Ricinoleic Acid	20	22	Medium	Strong
Oxidized Ricinoleic Acid-Palmitic Acid Ester	13	12	Weak	Weak
Oxidized Ricinoleic Acid-Decanoic Acid Ester	22	29	Strong	Strong
Oxidized Ricinoleic Acid-Butyric Acid Ester	15	20	Weak	Medium
Palmitic Acid	-	-	Not effective	Not effective
Decanoic Acid	10	15	Weak	Weak
Butyric Acid	27	25	Strong	Strong
Clyndamycin	10	10	Weak	Weak
n-hexane	-	-	Not effective	Not effective
Methanol	-	-	Not effective	Not effective

Antimicrobial activity is influenced by the form of esters. Monoesters are more active as antimicrobial agents than diesters and triesters because the longer carbon chain the more nonpolar

molecule. So that its solubility in water is low (Kabara, 1984).

## 4 CONCLUSIONS

Synthesis of oxidized ricinoleic acid esters using the  $ZnCl_2$  as catalyst have been successfully performed by showing the characteristics of the C-O ester group at FTIR spectra. Esters could act as emulsifiers with a type of water-in-oil emulsion and have antimicrobial activity against *Propionibacterium acnes* and *Staphylococcus epidermidis*. Oxidized ricinoleic acid-decanoic acid esters showed the highest inhibition zone against both bacteria.

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