Study of Enzymatic Synthesis of Glycol – Castor Oil Fatty Acid and Glycol – Palmitic Acid Esters as Emulsifier and Antimicrobial Compounds Using *Candida rugosa* Lipase EC. 3.1.1.3

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Keywords: Antimicrobial.sal, castor oil, emulsifier, fatty acid glycol ester, lipase, palmitic acid.

Abstract: This study was conducted to synthesize glycol – castor oil fatty acid and glycol – palmitic acid esters using *Candida rugosa* lipase as biocatalyst. The ester products was expected to have emulsifier and antimicrobial properties. Esterification was conducted by reacting fatty acid and glycol at 37 ^{0}C for 18 hours. The variation of fatty acid mol ratio to glycol used were 1:1, 1:2, 1:3, and 1:4. The ester product was characterized using FTIR and the conversion percentage was determined by titrimetric method. Emulsifier test also performed to determine the ability of ester product as emulsifier. Antimicrobial assay were also conducted using disc diffusion method against *Propionibacterium acne* and *Staphylococcus epidermidis.* FTIR spectra for glycol – castor oil fatty acid and glycol – palmitic esters showed the absorption of C=O functional groups at wave numbers 1732.27 and 1741.88 cm^{-1}, respectively. The highest conversion percentage value for glycol – castor oil fatty acid and glycol – palmitic ester were 85% and 82%, respectively. The emulsifier test showed both glycol – fatty acid ester have properties as emulsifiers. Antimicrobial assay showed that glycol – castor oil fatty acid ester has activity as antimicrobial against both bacteria. However, glycol – palmitic ester has no activity as an antimicrobial agent.

1 INTRODUCTION

Castor plant (*Ricinus Communis* L.) is a flowering species in the spurge family; euphorbuaceae and belong to the genus Ricinus. This plant contains 83 chemical compounds including alkaloid, terpenoid, flavanoid, benzoic acid derivatives, coumarin, tocopherol, and fatty acids such as risinoleic acid (Ribeiro, 2016). In general, all parts of the castor plant can be utilized. However, the most widely used is the oil, because it has several functions as a drug for skin rash, cosmetics, and biodiesel (Salihu, 2012). Spesifically, most content of castor oil is ricinoleic acid (87%) (Swern, 1979). Ricinoleic acid can be obtained from castor oil by way of hydrolysis.

One of oleochemical production using fatty acid is esterification reaction of glycol and fatty acid. The reaction between carboxylic acid and alcohol with or without the aid of catalyst, which will produce ester compound known as esterification reaction. This reaction is very slow and takes several times to reach equilibrium when it is not using a catalyst. Therefore a catalyst is needed to accelerate the rate of esterification reaction (Mandake, 2013).

In previous study, esterification of glucose fatty acid from coconut oil hydrolysis was performed using *Candida rugosa* lipase as a catalyst (Hudiyono, 2012). In addition, the utilization of castor oil has been made as a formulation of hand and body cream products and cosmetic application (Perez, 2016). Then, castor oil was also reported to be very effective for the treatment of skin problems such as dry skin, burnt skin and stretch marks (Salihu, 2012). Furthermore, oil from castor seeds was reported to have antibacterial activity against gram-positive and negative bacteria (Rahmati, 2015).

In this study, the synthesis of emulsifier and antibacterial compounds has been conducted through esterification between glycol and fatty acid from castor oil hydrolysis and palmitic acid. The esterification was catalyzed by *Candida rugosa* lipase. The emulsifier test and antimicrobial assay were performed to observe the stability of the ester product to have properties as emulsifier and able to

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inhibit the activity of *Propionibacterium acne* and *Staphylococcus epidermidis* on human skin.

2 MATERIALS AND METHODS

2.1 Materials

Materials used in this study were *Candida rugosa* Lipase (2.45 U/mg) that obtained from Sigma-Aldrich, castor oil, ethylene glycol, palmitic acid, ethanol, potassium hydroxide, hydrogen chloride, , aquades, n-hexane, sodium hydroxide phosphate buffer pH 8, phenolphthalein, eosin, clindamycin, DMSO 10%, nutrient agar, nutrient broth, *Propionibacterium acne* and *Staphylococcus epidermidis.*

2.2 Methods

2.2.1 Hydrolysis of Castor Oil

Hydrolysis of castor oil was performed by mixing 100 g of castor oil and 100 mL potassium hydroxide in ethanol and then heated for 1 hour at 70±2 ^{0}C. After heated, 55 mL of Hydrogen Chloride was added while stirring for 1 hour. Then, the product must be waited for 24 hours. After 24 hours it will form 2 phase.

2.2.2 Esterification

Esterification was started by mixing ethylene glycol, castor oil fatty acid, n-hexane, and *Candida rugosa* lipase as a catalyst. The variation of mol ratio for glycol to fatty acid that used were 1:1, 1:2, 1:3, and 1:4 (mol/mol). The ratio of solvent and substrate used was 1:1 (v/v substrate), while the enzyme used was 5% of the total substrate (w/w substrate). The mixture then was incubated on a horizontal incubator shaker at 200 rpm, for 18 hours at 37 ^{0}C temperature. The mixture was the heated at 80^{0}C to terminated the reaction. After that, the mixture was centrifuged at 3400 rpm for 15 minutes. The same process was conducted for palmitic acid.

2.2.3 Conversion Percentage Value Determination

The value of determination conversion was determined using titration method. The remaining fatty acids in the organic phase was titrated by 0.1 N sodium hydroxide and indicator that use was phenolphthalein.

2.2.4 FTIR Characterization for Esterification Product

FTIR Characterization was conducted for esterification product, glycol, fatty acids obtained from hydrolysis and palmitic acid.

2.2.5 Emulsifier Test and Determination of Emulsion Type

The emulsifier test was performed by mixing 0.1 g of ester product, water, and oil according to Table 1. Then, the mixture was shaken by vortex for 30 second and the emulsion stability was observed.

Table 1: Variation of water and oil for emulsifier test.

	Water (mL)					Oil (drops)				
Var. 1	1	1	1	1	1	2	4	6	8	10
	Water (drops)					Oil (mL)				
Var. 2	2	4	6	8	10	1	1	1	1	1

Determination of emulsion type test was performed by mixing 1 drop of emulsion with eosin in preparation glass. Then, the mixture was observed under a microscope to determine the type of emulsion; oil in water or water in oil emulsion.

2.2.6 Antimicrobial Assay Using Disc Diffusion Method

Antimicrobial assay were performed using disc diffusion method. Suspension of 200 µL *P. acnes* bacteria with a cell density of 1 x 10^8 cell/m L was aseptically mixed with 20 mL nutrient agar in a sterile petri dishes. The media then was left to harden. The sterile disc paper (6 mm in diameter) was placed on each section of media and dropped by 4 µL of sample. After that, the media was incubated for 24 hours at 37 ^{0}C. Clindamycin 0.5% was used as positive control while DMSO as negative control. The clear zone around the disc paper was measured. The same test was performed using *S. epidermidis.*

3. RESULT AND DISCUSSION

3.1 Hydrolysis of Castor Oil

The catalyst used in this reaction is a strong base; potassium hydroxide, because the reaction that occur can take place quickly and commercially. In addition, the use of a strong base aims to break ester bonds in

castor oil. The castor oil fatty acids that obtained from this process were used for esterification. Afterwards, the yield percentage obtained from castor oil hydrolysis was 83.6%.

3.2 Determination of Conversion Percentage

Esterification product formed emulsion system. To break the emulsion, the mixture is needed to be centrifuged. Thereafter, three phases were formed and the upper phase was used for determination of conversion percentage.

The relation between mol variations of fatty acid with conversion percentage found on Figure 1. As the mol of glycol increase, the conversion percentage increased. In accordance with *Le Chatelier* principle which states that the addition of one excess reactants in equilibrium reaction will cause a reaction shift leading to the formation of the product. The highest value of conversion percentage was obtained at ratio 1:4 with conversion percentage were 84.7% for castor oil fatty acid and 81.9% for palmitic acid.

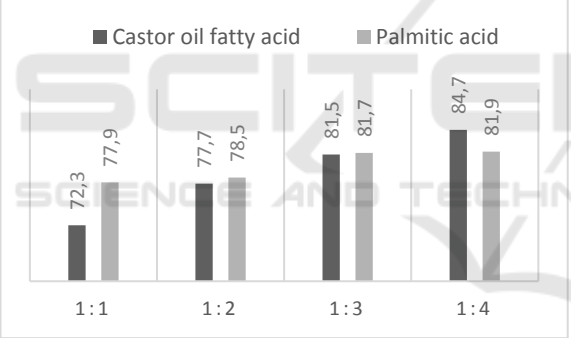

Figure 1: Relation between mol variations of fatty acid versus conversion percentage value.

3.3 Characterization of Esterification Product using FTIR

The IR spectra of fatty acid from castor oil hydrolysis, palmitic acid, glycol – castor oil fatty acid and glycol - palmitic esters are shown in Figure 2 and Figure 3.

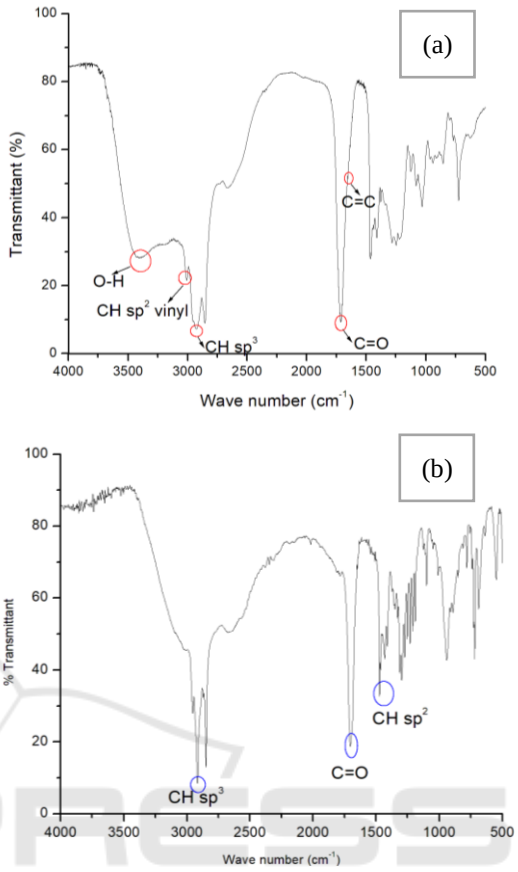

Figure 2: FTIR spectra (a) fatty acids from castor oil hydrolysis, (b) palmitic acid

Figure 2(a) shows the FTIR spectra of castor oil fatty acid. There are several typical functional groups appeared in the spectra. The absorption peak at wave number 3009.73 cm^{-1} for CH sp^2 which indicates that the compound have double bonds. Then, at the wave number 1712.86 cm^{-1} there is an absorption of C=O carboxylate in the range 1700-1725 cm^{-1} (Silverstein, 2005). Furthermore, Figure 2(b) shows FTIR spectrum of palmitic acid. The absorption at the wave numbers 1701.28 cm^{-1} for C=O carboxylic acid and 1471.75 cm^{-1} for CH sp^2. Both spectrum indicate that absorption of typical functional groups for fatty acid, i.e. C=O carboxylic acids.

Figure 3 (a) and (b) shows FTIR spectra for glycol – castor oil fatty acid and glycol – palmitic acid ester, respectively. Both spectrum showed the absorption of C=O ester functional groups at the range 1735-1750 cm^{-1}. The wave number 1735.64 cm^{-1} is the absorption of C=O ester functional group for glycol - castor oil fatty acid ester. While the wave number 1738.16 cm^{-1} for the glycol – palmitic ester.

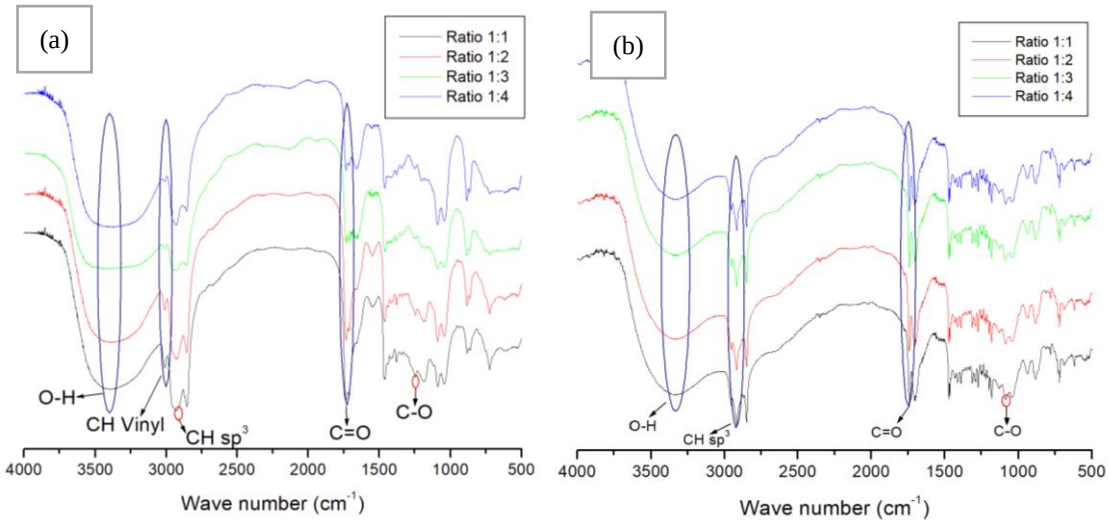

Figure 3: FTIR spectra (a) glycol – castor oil fatty acid ester, (b) glycol – palmitic acid ester.

3.4 Simple Emulsifier Test

The result of the emulsion test on the ester product showed that glycol – castor oil fatty acid emulsion was better than glycol – palmitate ester. After 36 hours observation glycol – castor oil fatty acid emulsion still looks turbid, indicating that the ester product has properties as emulsifier. Similarly for glycol – palmitic acid ester emulsion, after 24 hours the mixture still looks turbid. However, the ability of glycol-palmitic acid ester was not good as glycol – castor oil fatty acid that can last for 36 hours. In comparison, the emulsion properties of glycol – castor oil fatty acid ester more stable than glycol – palmitic acid ester (Figure 4).

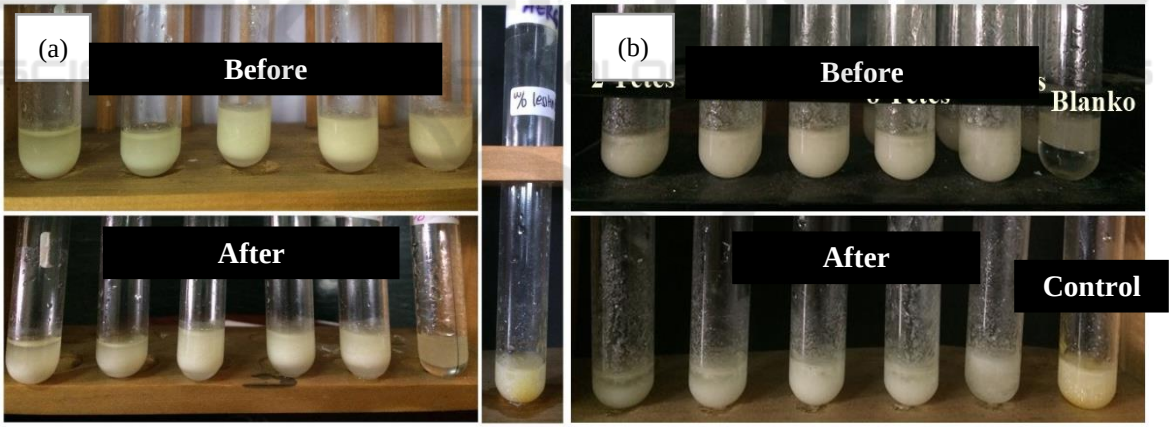

Figure 4: Emulsion formed using (a) glycol – castor oil fatty acid ester (after 36 hours), (b) glycol – palmitic ester (after 24 hours).

Microscopic observations for the determination type of emulsions are shown in Fig. 5. Both glycol – castor oil fatty acid and glycol – palmitic esters show water in oil emulsion type because red droplets are produced in yellow medium.

The emulsion type produced depends on the emulsifier properties and the Hydrophillic to Liophillic Balance (HLB) value. Emulsifiers with low HLB values (3-6) can produce water in oil emulsion. Whereas high HLB vallue (8-18) produces an oil in water emulsions (Luna *et al*, 2013). However, in this study HLB value were not determined.

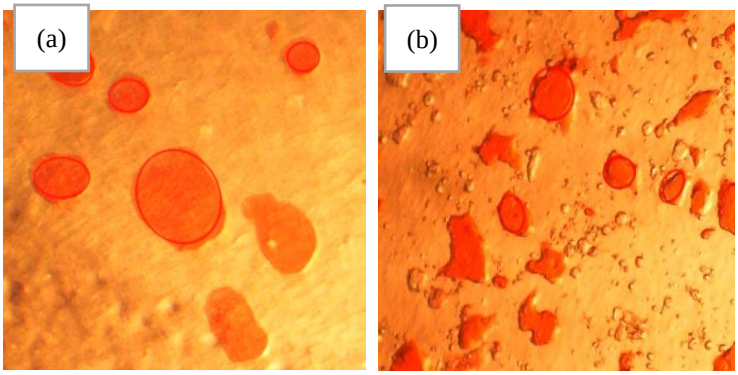

Figure 5: The observe emulsion type using microscope (a) glycol – castor oil fatty acid, (b) glycol – palmitic esters.

3.5 Antimicrobial Assay

Propionibacterium acne and *Staphylococcus epidermidis* bacteria were used in this study. The principle of the disc diffusion method is to measure the zone of bacterial growth resistance that occurs due to the diffusion of antimicrobial substances in solid media. The inhibition zone is a clear region around the disc. The area of the inhibition zone is directly proportional to the antimicrobial activity. The stronger antimicrobial power the more prevelant the inhibition area (Chouhan *et al*, 2017).

The antimicrobial assay showed that glycol – palmitic acid were all ineffective as antimicrobials. While for the glycol – castor oil fatty acid ester showed the antimicrobial activity. The highest inhibition zone was obtained at 80% concentration against *P. acnes* bacteria with clear zone diameter 12 mm and classified as weak activity. Then for *Staphylococcus epidermidis*, the highest inhibition zone was obtained at 60% with clear zone diameter 10 mm and classified as weak activity. Table 2 shows the antimicrobial activity of esterification product, ethylene glycol, n-hexane, and DMSO against *P. acne* and *S. epidermidis* bacteria. While Table 3 shows the classification of effectiveness antimicrobial compounds.

Table 2: Antimicrobial activity against *P. acnes* and *S. epidermidis*

Sample	Concentration (%)	Inhibition Zone			
		P. acnes	Classification	*S.epidermidis*	Classification
Glycol – palmitic ester	20	-	No Activity	9	Ineffective
	40	-	No Activity	9	Ineffective
	60	-	No Activity	-	No Activity
	80	-	No Activity	-	No Activity
Glycol – Castor oil Fatty acid Ester	20	11	Weak	8	Ineffective
	40	10	Weak	9	Ineffective
	60	8	Ineffective	10	Weak
	80	12	Weak	9	Ineffective
Palmitic Acid	50	-	No Activity	-	No Activity
Castor oil fatty acid	50	12	Weak	14	Weak
	100	13	Weak	15	Weak
Castor oil	100	-	No Activity	-	No Activity
Ethylene Glycol	100	-	No Activity	-	No Activity
n-hexane	100	-	No Activity	-	No Activity

Clindamycin	0.5	12	Weak	14	Weak
DMSO	100	-	No Activity	-	No Activity

Table 3. Antibacterial effectiveness classification

Inhibit zone diameter	Response of growth barrier
>20 mm	Strong
16-20 mm	Medium
10-15 mm	Weak
<10 mm	Ineffective

[Source: Greenwood, 1996]

From Table 2, it can be seen that 500 ppm clindamycin has inhibitory activity against both bacteria; *P. acnes* and *S. epidermidis* for 12 mm and 14 mm, respectively. Negative control that used in this research was DMSO which is dropped on sterile disc paper. The purpose of using DMSO as negative control was to compare that the solvent used did not affect the results of antibacterial test. Therefore, the negative control used was 100% DMSO. It can be seen from Table 2 that the inhibitory zone results in negative control for both bacteria were 0 mm. these results indicate that the use of DMSO as solvent did not affect the antibacterial test results. Furthermore, the result of inhibition zone for both bacteria goes into the weak classification. So it can be concluded that glycol – castor oil fatty acid has the same inhibitory power as clindamycin. As known that clindamycin is an antibiotic usually used for drug acne (Handayani, *et al*, 2015). This indicates that glycol – castor oil fatty acid ester can potentially be an antimicrobial agent.

4 CONCLUSIONS

The glycol – castor oil fatty acid and glycol – palmitic acid esters were successfully synthesized enzymatically using *Candida rugosa* lipase that indicated by characteristic of –C=O ester group at FTIR spectra. Both ester products have properties as emulsifiers for water in oil (w/o) emulsion type, but only glycol-castor oil fatty acid ester has potential to be an antimicrobial agent.

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REFERENCES

Chouhan, S., Sharma, K., Guleria, S., 2017. Antimicrobial Activity of Some Essential Oils – Present Status and Future Perspectives. *Medicines*, 4, 58.

Greenwood., 1995. *Antibiotic susceptibility (sensitivity) test, antimicrobial and chemotherapy*. USA: Mc Graw Hill Company.

Handayani, S., Julia E., Raydhi M. P,, Hudiyono, S., 2015. Esterification Palmitic Acid with Glucose and Fructose Using Immobilized Candida rugosa Lipase E.C. 3.1.1.3 on Fe_3O_4-Chitosan Nanoparticles in Varied Solvent. *The 4th International Conference of The Indonesian Chemical Society* 2015 (ICICS 2015).

Handayani, S., Rahayu, P., Mandala, K., Lugianto., J., Bakri, R., Hudiyono, S., 2015. Synthesis of Glucose-Palm Oil Fatty Acid Esters, a Food Emulsifier, catalyzed by Immobilized Candida rugosa Lipase EC 3.1.1.3 on Fe3O4-Chitosan Nanoparticles. *International Symposium on Engineering and Applied Science (ISEAS)*.

Hudiyono S., Handayani S., Susilo B., 2012. Esterification of Glucose Fatty Acids of Coconut Oil Catalyzed by Candida rugosa Lipase EC 3.1.1.3 Immobilized on an Indonesia's Natural Zeolite Matrix. *World Applied Sciences Journal* 19 (8):1105-1111.

Luna, Prima; Andarwulan, Nuri., 2013. Potensi Produk Monoasilgliserol sebagai Emulsifier Nabati. *Buletin Teknologi Pascapanen Pertanian* Vol 9 (2), 2013 : 108-116

Mandake, M.B. Anekar, S.V. Walke, S.M., 2013. Kinetic Study of Catalyzed and Uncatalyzed Esterification Reaction of Acetic Acid With Ethanol, India: Kore Institute Of Engineering and Technology.

Perez, Bianca., 2016. Ultralong Fatty Acyl Derivatves as Occlusive Structure Lipid for Cosmetics Applications: Syinthesis and Characterization. *ACS Sustainable Chem. Eng*. 2016, 4, 7137-7146.

Rahmati, *et al.*, 2015. Antimicrobial Activity of Castor Oil Plant (Ricinus communis) Seeds Extract Against Gram Positive Bacteria, Gram Negative Bacteria and Yeast. *International Journal of Molecular Medicine and Advance Sciences* (1):912,2015. Medwell Journals.

Ribeiro, Paulo; Castro, Renato; Fernandez, Luzimar., 2016. Chemical Constituents of the oilseeds crop Ricinus

Communis and their pharmacological Activities: A review. *Industrial Crops and Product 91* (2016) 358-376.

Salihu et al., 2012. Castor Oil Plant (Ricinus communis L): Botany, Ecology, and Uses. *International Journal of Science and Research (IJSR) ISSN* (Online):2319-7064.

Silverstein, et al., 2005. *Spectrometric Identification of Organic Compounds.* 4th ed.New York: John Wiley and Sons, 1981. QD272.S6 S55.

Swern, D., 1979. *Balley's Industrial Oil and Fat Products*. Ed ke-4. Vol e-1. New York: J.Wiley.