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ANTIBACTERIAL AND PHYSICOCHEMICAL PROPERTIES OF PALM AND OLIVE-BASED WAX ESTERS

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Abstract

Palm-based and olive-based wax esters which are oleyl palmitate and oleyl oleate have been successfully synthesized using an esterification reaction. The products were verified using Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy to determine the presence of ester bond in their molecular structure. The synthesized wax esters were further analysed for their antibacterial activity against both Gram-positive and Gram-negative bacteria. In screening of an antibacterial activity, the agar diffusion test was employed. Minimum inhibition concentration (MIC) value was determined by microdilution method. The results showed that both Gram-positive bacteria, which are *S. aureus* and *B. subtilis* had high sensitivity toward oleyl oleate and oleyl palmitate. MBC (minimum bactericidal concentration) was determined to evaluate the antibacterial properties of both synthesized wax esters towards Gram-positive bacteria. Two antibacterial activities including bactericidal effects (killed the bacteria), or bacteriostatic effect (inhibit the bacterial growth) were determined by MBC value. Both wax esters possess a bactericidal effect towards all the Gram-positive bacteria studied. Finally, the physicochemical properties such as SPF value, peroxide value, saponification value and iodine value of both wax esters have been successfully determined and the data obtained suggest that the synthesized wax esters can be used as main ingredients in cosmetics formulation.

Keywords: Olive-based wax ester; palm-based wax ester; esterification; antibacterial; physicochemical

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Introduction

In cosmetic industry, wax ester has been a main raw component in formulating cosmetics. Wax ester is a long-chain fatty acid and alcohol which contains a chain of 12 or more carbons that possess wetting behaviour without greasy feeling when applied on the skin surfaces. Due to these properties, it has an immense potential application in cosmetics and pharmaceutical formulations (Mat Hadzir et al., 2013). Wax esters are valuable chemicals obtained from petroleum, chemical synthesis, or natural sources. However, wax esters that are made from various sources have different application. Petroleum and chemical synthesis-derived wax esters are applied to the candles, lubricants, coatings, printing inks, rubber and plastic processing, whereas the bio-based wax ester are used in the pharmaceuticals and personal care products such as cosmetics, hair care, skin care, and other allied products (Song et al., 2021).

Analysis of jojoba oil revealed that it contains this unique wax ester, similar to sperm whale oil that can maintain its viscosity at very high temperatures. This makes jojoba oil gain worldwide interest in industries such as pharmaceutical and cosmetics as it can be used as a lubricant and an ingredient for medicines (Khairi, 2019). One of the most important criteria of plant-based wax ester that makes it great to be used as an ingredient in cosmetic products is antibacterial properties (Chermahini and Majid, 2013). Wax ester was proven to prevent the activity and growth of fungi and bacteria (both Gram-positive and Gram-negative bacteria) while maintaining great emulsion characteristic (Kim et al., 2020).

The physicochemical properties such as SPF value, peroxide value, saponification value and iodine values of vegetable wax ester are important, and it is proven that vegetable wax ester can substitute for costly wax that usually used in cosmetics, foods and pharmaceuticals due to its familiar physicochemical properties (Sonal et al., 2012).

The natural source wax esters are limited and expensive due to its high demand in the market, scientists worldwide have begun to synthesize this compound by optimizing the chemical reaction to obtain wax ester using cheaper materials and faster process. Wax ester can be synthesized via two main methods which are chemical and enzymatic reactions. In this study, vegetable-based wax esters were synthesized via simple esterification method between palm-based and olive-based fatty acids with oleyl alcohol in the presence of hexane. Although much research has been conducted to produce wax esters, it is difficult to find information about their antibacterial and physicochemical properties, in which these are important for cosmetic formulator in industry. So, in this research, the antibacterial properties of the synthesized wax esters were assessed by determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the sample on bacteria used. On the other hand, the physicochemical properties such as peroxide value, saponification value, SPF value and iodine value of the wax esters was also determined via a standard method.

Thus, based on the problem stated above, the main objectives of this study are to synthesize wax esters from olive-based and palm-based fatty acids via esterification reaction, to evaluate the antibacterial properties of synthesized wax esters by using dilution techniques such as Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) and to study the physicochemical characteristics of synthesized wax esters such as SPF value, peroxide value, saponification value and iodine value. The focus of this study is to synthesize wax esters which are oleyl oleate and oleyl palmitate from olive-based fatty acid and palm-based fatty acid with oleic acid in organic solvent. Then, the synthesized products were assessed on their antibacterial and physicochemical properties. To evaluate their antibacterial properties, a dilution method was applied involving Gram-positive bacteria and Gram-negative bacteria to obtain Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the samples. Meanwhile, to assess the physicochemical properties of both wax esters, four parameters were studied which are peroxide value, saponification value, SPF value and iodine value.

Methods

Synthesis of Olive-Based and Palm-Based Wax Esters

Palm-based wax ester (oleyl palmitate) has been obtained through an esterification reaction between palmitic acid and oleyl alcohol. For the first step, palmitic acid was mixed with of oleyl alcohol in a 250 mL universal bottle with a molar ratio of 1:2 respectively. n-Hexane acted as a solvent in the oleyl alcohol-palmitic acid mixture. The mixture was incubated in a water bath shaker (150 rpm) at 50°C reaction temperature with 5 hours of reaction time. For the synthesis of olive-based wax ester, the only difference was the palmitic acid as reactant had been substituted with the same amount of oleic acid.

Antibacterial Assay

The bacteria used for this study were *Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli*. The bacteria were cultured at 37°C on Mueller-Hinton broth (MHB) medium overnight. The optimal density (OD) of bacterial growth is then determined at 600 nm by Bio Photometer (Eppendorf AG, Hamburg, Germany). The bacterial inoculum was diluted to the concentration of 10^7 cell/mL to be used in the antibacterial test.

The agar diffusion test was conducted by referring to Smith-Palmer et al. (1998). By using sterilized cotton swabs, the bacteria cultures at a concentration of 107 cell/mL were spread on the Mueller-Hinton agar. All experiments were performed in duplicate. To create the test wells, each of the agar plates was poked with corkborer that is 6 mm size in diameter. 10 µL of each different concentration of test samples will be loaded into each test well. For positive control, the Streptomycin Sulphate solution at a

concentration of 10 mg/mL was loaded into their respective test wells. The lowest concentration of tested for which an inhibition zone observed at the inoculation points of samples after incubation at 37°C for overnight was recorded. The diameter of the inhibition zone observed was measured and expressed in millimeters.

The bacteria that demonstrated a strong inhibition zone in the agar diffusion method were chosen for MIC determination using the standard broth dilution assay. The procedure is carried out in a 96-well microtiter plate. (Costar, USA). A 50 µL of prepared bacterial inoculum before was added into every well in the microtiter plate containing 50 µL of the test sample solutions (palm and olive-based wax ester). The microtiter plate was then closed with a lid and then incubated overnight at 37°C. The bacterial viability following the treatment with test substances was tested using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution. MTT solution (at a concentration of 0.3 mg/mL) is prepared by dissolving about 0.3 mg MTT powder in 1 mL of sterilized distilled water. About 40 µL of MTT was added into each test well and the microtiter plate was further incubated for 5 min at 37°C. The lowest concentration without visible formation of formazon blue was defined as MICs.

SPF Value

For the SPF value assay, 1 g of all samples was weighed, and all the samples was transferred to a 100 mL volumetric flask and the solution was diluted with ethanol. The process was continued with the ultrasonification process for 5 minutes and then was filtered through cotton with rejecting the first 10 mL for UV-Visible test, the absorption spectra of samples in solution was determined around 290-450 nm. Then, ethanol in 1cm quartz cell was used as a blank. The absorption data was obtained in the range of 290 to 320 nm every 5 nm and 3 absorption was made at each point. The Mansur equation below was used to determine the SPF value.

$$\text{Mansur eq} = CF \times \Sigma_{290}^{320} \times EE \times I \times Abs \qquad (1)$$

Where, CF = correction factor
EE = an erythemal effect spectrum
I = the solar intensity spectrum
Abs = absorbance of the sample

Peroxide Value

A 250 mL conical flask was filled with 5.0 g of the sample. The sample was then dissolved with 10 mL chloroform, which was swirled. The mixture was then combined with 15 mL of acetic acid in 1 mL of potassium iodide solution. The mixture was then placed in a dark place for 5 minutes. The procedure was continued by incorporating 30 mL of distilled water, followed by the addition of 1 mL of starch indicator. The solution was subsequently titrated with 0.05 molarity of sodium thiosulphate, until the blue colour of the indicator was no longer visible. The peroxide value was determined using equation as below.

$$\text{Peroxide value} = [(B-S) \times N \times 1000]/W \qquad (2)$$

Where, S = volume of titrant (mL) for the sample
B = volume of titrant (mL) for blank
N = normality of sodium thiosulphate solution (mmol/mL)
W = mass (g) of the sample (wax ester)

Saponification Value

A quantity of 2.0 grams of each sample was accurately weighed and placed into a 250 mL conical flask. The mixture was subsequently refluxed for a duration of 60 minutes, with the addition of 25 mL of ethanolic potassium hydroxide. Following that, 1 mL of phenolphthalein solution was added to the mixture, which was then titrated with 0.5 N of hydrochloric acid, HCl, until the pink colour of the

indicator disappeared. The saponification value was then computed using equation as below.

$$\text{Saponification value} = [(B-S) \times N \times 56.1]/W \quad (3)$$

Where, B = volume of titrant (mL) for the blank
S = volume of titrant (mL) for the sample
N = normality of hydrochloric acid HCl (mmol/mL)
W = mass (g) of sample (wax ester)

Iodine Value

In a 500 mL stoppered flask, 2.0 grams of each sample were accurately weighed, and 10 mL of chloroform was added. Subsequently, 25 mL of Wij's solution was precisely pipetted into the flask, the flask was stopped, and the mixture was swirled to ensure adequate mixing. The flask was then kept at room temperature for a period of 30 minutes in a dark place. The procedure was continued by incorporating 20 mL of potassium iodide, followed by 100 mL of freshly boiled and cooled distilled water. The mixture was subsequently titrated with 0.05 molarity of sodium thiosulphate solutions until the yellow colour was almost no longer visible. 1 mL of a starch indicator was added, and the titration was subsequently repeated until the blue colour of the indicator was no longer visible. A blank determination was carried out under the same conditions, and the iodine value was determined using equation as below.

$$\text{Iodine Value} = [(B-S) \times N \times 126.9]/W \quad (4)$$

Where, B = volume of titrant (mL) for the blank
S = volume of titrant (mL) for the sample
N = normality of sodium thiosulphate (mmol/mL)
W = mass of sample.

Verification of Wax Ester

Thin Layer Chromatography was used as a predeterminant step before the samples were verified by FTIR and NMR. In this testing, a sintered glass column was used. Then, a slurry of silica gel in hexane was added in to make a 10 cm high column. Sample solution in hexane was pipette into the column. Hexane, ethyl acetate, and acetic acid were used as the solvent systems to elute the sample and the ratio would be (8.5:20:0.5, V/V). 1 drop of sample was collected and tested with Thin Layer Chromatography (TLC) to purify the sample by its fraction. The purity of oleyl oleate and oleyl palmitate was then scraped from TLC paper before being carried out for further analysis. The presence of oleyl oleate and oleyl palmitate was identified by comparing the wax ester with the authentic standard.

The product of reaction was analysed by using Fourier Transform Infrared Spectroscopy (Perkin Elmer, model spectrometer 100) to determine the functional group. Attenuated Total Reflectance (ATR) Infrared spectroscopy analysis was used as the sample of wax ester is liquid and the absorption peak were analysed by OPUS software. The sample ester was then analysed by Nuclear Magnetic Resonance (NMR) using DMSO as the solvent to determine the structure of the synthesized product. The chemical shift of the compound was observed to determine the compound using spectrum of H-NMR and C-NMR.

Result and Discussion

Synthesis of Olive-Based and Palm-Based Wax Esters

In this study, olive-based wax esters which is oleyl oleate were synthesized using oleic acid and oleyl alcohol while palm-based wax ester which is oleyl palmitate was synthesized using palmitic acid and oleyl alcohol by esterification reaction. Figure 1 and Figure 2 show the chemical reaction pathway to produce both wax esters. It is expected that wax esters products that have been synthesized will produce a high percentage yield (Mat Radzi et al., 2015). The reaction was performed in the presence of hexane

as a solvent to dissolve the reactant as well as shift the equilibrium to the product of the process.

The esterification process is superior compared to other synthetic processes such as transesterification because the reaction is simple with only water molecules formed as a by-product. In simplest sense, esterification may be thought of as the reverse for hydrolysis process. The process involved of the carboxylic acid (in this case oleic acid and palmitic acid) as acyl donor and being reacted with oleyl alcohol as nucleophile to form an ester.

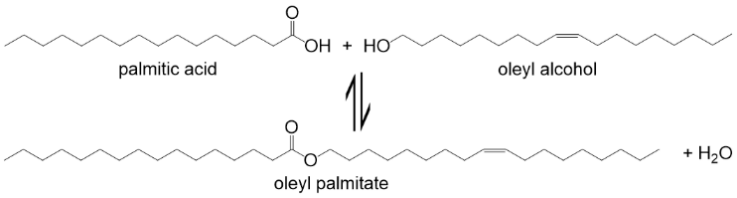

Figure. 1 Chemical reaction pathway to produce oleyl palmitate

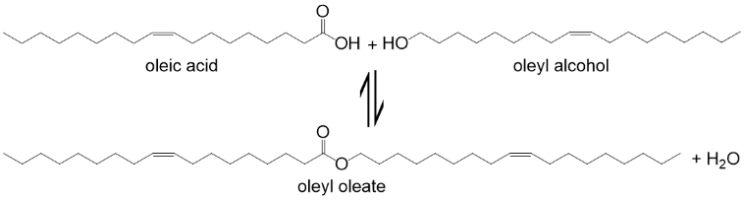

Figure. 2 Chemical reaction pathway to produce oleyl oleate

Antibacterial Activities of Synthesized Olive-Based and Palm Wax Esters via Agar-Well Diffusion Method

The antibacterial activity can be analysed using agar-well diffusion method by determining of clear zone or inhibition zone formation on the agar plate as shown in Figure 3.

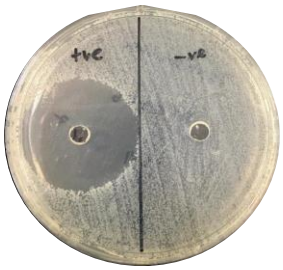

Figure 3. Formation of inhibition zone at positive control and no clear zone at negative control

In this study, four selected bacteria consisting of *Escherichia coli*, *Salmonella typhimurium, Bacillus subtilis* and *Staphylococcus aureus* from Gram-positive and Gram-negative bacteria had been tested with palm-based and olive-based wax esters. The bacteria were incubated overnight at 37°C and then diluted into various concentrations using Muller Hinton Broth to obtain bacteria with less than 0.030 optical density. The bacteria concentration for *Bacillus subtilis*, *Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli* were determined to be 0.030, 0.014, 0.019 and 0.028 respectively. Antibacterial activity for oleyl palmitate and oleyl oleate was summarized in Table 1. The various sizes of inhibition zone had been observed according to the used and strains of bacteria tested.

Table 1. Diameter of Inhibition Zone for Antibacterial Test of Oleyl Palmitate and Oleyl Oleate via Agar-Well Diffusion Assay

Bacteria	Diameter of Inhibition Zone (mm)		
	Oleyl palmitate	Oleyl oleate	Streptomycin sulfate
Bacillus subtilis	11.0±0.5	15.0±0.5	30.0±0.5
Staphylococcus aureus	10.0±0.5	13.0±0.5	29.0±0.5
Salmonella typhimurium	5.0±0.5	6.0±0.5	25.0±0.5
Escherichia coli	3.0±0.5	4.0±0.5	27.0±0.5

Based on the result, both oleyl oleate and oleyl palmitate show observable clear zones on the agar plate when tested with both *Bacillus subtilis* and *Staphylococcus aureus* from Gram-positive and *Salmonella typhimurium* from Gram-negative bacteria but no clear observable zone with *Escherichia coli* from Gram-negative bacteria strain.

In this study, it was observed that Gram-positive bacteria were more susceptible to all the samples tested as compared to Gram-negative bacteria. The finding that Gram-positive bacteria (*B. subtilis and S. aureus*) were more susceptible to the esters tested is in accordance with previous studies (Mat Radzi et al., 2015). The weaker antibacterial activity observed against Gram-negative bacteria can be attributed to the presence of an outer membrane composed primarily of lipopolysaccharide, which forms a hydrophilic permeability barrier that provides protection against toxic agents (Carole et al., 2020). Gram-negative organisms have been found to be less susceptible than Gram-positive bacteria due to the presence of an outer membrane surrounding the cell wall, which restricts the diffusion of hydrophobic compounds through its lipopolysaccharide cover (Carole et al., 2020).

Minimum Inhibitory Concentration (MIC) Value

The microdilution method was used to dilute synthesized oleyl palmitate and oleyl oleate using Muller-Hinton broth to 80%, 60%, 40% and 20% concentration. The determination of minimum inhibitory concentration (MIC) values was evaluated on all types of bacterial strains used before. The wax esters with different concentrations were mixed with bacteria sample in 96-will microtiter plate and then tested with 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT, thiazolyl blue). If there was any observable formazan blue formed, it showed that the bacterial growth occurred in the wells (Figure 4).

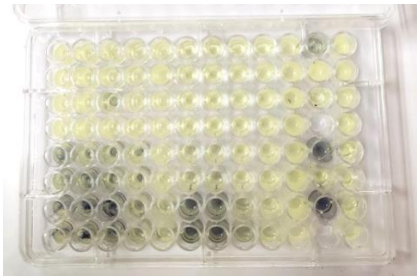

Figure 4. 96-well microtiter plate loaded with test samples and bacteria

The dark cloudy mixture in the wells indicates bacterial growth while the clear mixture indicates no growth of bacteria. The minimum inhibitory concentration (MIC) values for both wax esters towards *Bacillus subtilis* and *Staphylococcus aureus* are shown in Table 2. The MIC values of the Gram-negative bacteria cannot be obtained. This is because wax esters sample at the stock solution from the synthesized products is unsuccessful to inhibit the growth of both bacteria. The results turn out that the MIC value for both wax esters on both Gram-positive bacteria are 20%. This happened due to the lowest concentration prepared for this test is 20% and even at that concentration, the microorganism growth was inhibited. Further studies need to be done to find exactly where the MIC value is, but this study has successfully narrowed the MIC value to ≤20%.

Table 2. The Minimum Inhibitory Concentration (MIC) Values of Oleyl Palmitate and Oleyl Oleate on Tested Bacteria

Wax ester sample	MIC value (%)	
	Bacillus subtilis	***Staphylococcus aureus***
Oleyl palmitate	20	20
Oleyl oleate	20	20

Minimum Bactericidal Concentration (MBC) Value

The determination of minimum bactericidal concentration (MBC) values was evaluated only on the Gram-positive bacteria strain. MBC is defined as the endpoint of the lowest concentration of antibacterial agent that kills more than 99.90% of the initial bacterial population where no visible growth of the bacteria observed on the agar plates (Petrus et al., 2011). Table 3 shows the MBC value of oleyl oleate and oleyl palmitate on Gram-positive bacteria.

Table 3. The Minimum Bactericidal Concentration (MBC) Values of Oleyl Palmitate and Oleyl Oleate on Tested Bacteria

Wax ester sample	MBC value (%)	
	Bacillus subtilis	***Staphylococcus aureus***
Oleyl palmitate	100	80
Oleyl oleate	40	40

In this experiment, oleyl palmitate and oleyl oleate at different concentration showed either bactericidal (bacteria-killing) or bacteriostatic (bacteria-inhibiting) effects. Although there is no blue pigment formation after tested with MTT solution, bacteria can still grow when transferred to agar plate. Figure 5 shows what happens when bacteria were killed or inhibited and then be transferred on agar plate.

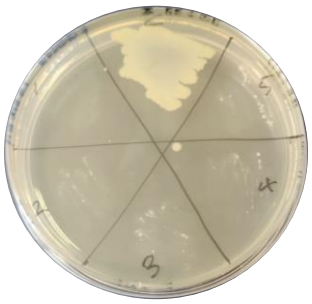

Figure 5. An agar plate showing the growth of bacteria after been mixed with different concentration of wax ester

Bactericidal concentration shows the lowest concentration at which bacteria fail to grow in broth, while bacteriostatic concentration can be described as the lowest concentration at which bacteria fail to grow in broth but grow when the broth is plated onto agar. The MBC/MIC value of oleyl palmitate and oleyl oleate to both Gram-positive bacteria are shown in Table 4 and Table 5.

Table 4. MIC And MBC Values from Microdilution Test snd MBC/MIC Ratio for Oleyl Palmitate

Test bacteria	MIC (v/v)	MBC (v/v)	MBC/MIC
Bacillus subtilis	0.2	1	5
Staphylococcus aureus	0.2	0.8	4

Table 5. MIC And MBC Values from Microdilution Test and MBC/MIC Ratio for Oleyl Oleate

Test bacteria	MIC (v/v)	MBC (v/v)	MBC/MIC
Bacillus subtilis	0.2	0.4	2
Staphylococcus aureus	0.2	0.4	2

Physicochemical Properties

The parameters that had been determined are SPF value, peroxide value, saponification value and iodine value. Table 6 shows the summarized physicochemical properties for both wax esters.

Table 6. Summarize Physicochemical Properties For Oleyl Oleate And Oleyl Palmitate

Physicochemical properties	Wax ester	
	Oleyl Palmitate	Oleyl Oleate
SPF value	14.0	12.0
Peroxide value	17.0	18.0
Saponification value	86.8	89.0
Iodine value	78.5	83.4

SPF Value

The effectiveness of a substance's sun protection on the human skin has long been determined using the sun protection factor (SPF) value. Sunscreens products that have high SPF value will provide higher protection against the sun rays (Latha et al., 2013). The sunscreens products that are classified as low sun protections products with having SPF value from 2 to 12. Products that have SPF value from 12 to under 30 are considered as normal sun protection products while SPF value above 30 are considered as high sun protection products (Gregoris et al., 2011).

The SPF value can be measured in several methods and one of them is by measuring the absorbance using UV-Vis Spectroscopy and calculated the value via Mansur equation (Dutra et al., 2004). This in vitro method is considered economic in many cosmetic formulations before proceeding to the in vivo test. In this study, the SPF value of oleyl palmitate and oleyl oleate were determined to be 14.0 and 12.0 respectively which considered in the range of moderate sun protection products. Oleyl oleate has a higher SPF value due to the presence of the unsaturated carbon in its compound while oleyl palmitate is saturated compound.

Peroxide Value

To determine the peroxide value of ester compound, there are several methods for evaluation of anti-oxidative action of a sample. The assessment for peroxide value in ester compound used a method by determining the early oxidative changes in the ester though the formation of primary oxidation products. Peroxide value can be defined as the milliequivalents (mEq) of peroxide per kilogram of the sample. The highest quality products possess zero peroxide value (Becker et al., 2004). In this study, the peroxide value for the synthesized palm and olive-based wax ester was determined to be 17.0 and 18.0 respectively. This proved that wax esters have high quality lipids. Therefore, both wax esters are proven to possess a good quality of ester and it is effective for cosmetic formulations and could increase the shelf life (Abdul Rahman et al., 2015).

Saponification Value

Saponification value was defined as the number of weight (g) of potassium hydroxide (KOH) require to saponify 1 g of ester (Issariyakul et al., 2007). Hence, a higher amount of KOH was used to saponify the ester binds in the molecule. The value of saponification of wax ester predicted to have a high mean molecular weight of triacylglycerols (Abdul Rahman et al., 2015). High saponification value corresponds to low molecular weight of the samples (Gunawan et al., 2005). The author also reported the high value of saponification in ester showed that the ester contained a short chain of fatty acids.

In the experiment, the saponification value of oleyl palmitate and oleyl oleate was determined at the value of 86.8 and 89.0 respectively. This proves that both wax esters have low value of saponification as the molecular weight of the ester of oleyl palmitate and oleyl oleate are calculated to be at 506.9 $gmol^{-1}$ and 532.9 $gmol^{-1}$ respectively which are considered as high in value. Long chain fatty acids were found to have low saponification value due to the low number of carboxylic functional groups per unit mass of the fats as compared to short chain fatty acids.

Iodine Value

The iodine value assessed in this experiment was successfully determined using the method mentioned before. Iodine value is important to distinguish the degree of unsaturation of fatty acid in the samples (Ekop et al., 2009). In this experiment, the iodine value of oleyl palmitate and oleyl oleate was determined to be at 78.5 and 83.4 respectively. The higher the iodine value indicates the higher amounts of unsaturation fatty acids in the sample (Keng et al., 2009) . Higher iodine value in ester was assumed to provide a greater liquidity property to the compound itself (Abdul Rahman et al., 2015). It is also proven that the iodine value increases due to the increase in the amount of double bonds (Gopinath et al., 2009). Hence, with the value determined for both wax esters, it was concluded that oleyl oleate possesses higher iodine value compared to oleyl palmitate due to higher double bond amount in oleyl oleate.

Verification of Wax Esters Using FTIR

In this study, the generated FTIR spectrum of oleyl oleate and oleyl oleate was analysed. Figure 6 and Figure 7 show the FTIR absorption spectra of oleyl oleate and oleyl palmitate respectively while Table 7 and Table 8 show the summary of FTIR absorption spectra of oleyl oleate and oleyl palmitate respectively. According to the spectrum for both samples, it shows that that there are esters group in both sample due to the medium stretching in 1712.08 cm^{-1} region for oleyl oleate and in 1712.02 cm^{-1} region for oleyl palmitate.

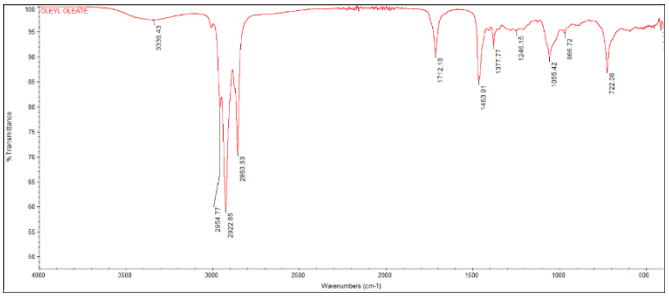

Figure. 6 FTIR absorption spectra of oleyl oleate

Table 7. The Summary of FTIR Absorption Spectra of Oleyl Oleate

Type of Vibration	Wavenumber (cm^{-1})	Intensity
-CH-aliphatic	2922.65	Small
C=O ester bond	1712.18	Medium
C-O stretching	1246.15 – 1055.42	Weak
-CH_2-bend	1463.91	Medium
-CH_3-bend	1377.77	Weak

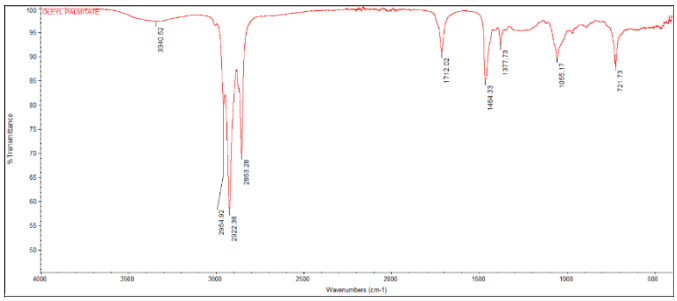

Figure 7. FTIR absorption spectra of oleyl palmitate

Table 8. The Summary of FTIR Absorption Spectra of Oleyl Palmitate

Type of Vibration	Wavenumber (cm^{-1})	Intensity
-CH-aliphatic	2922.36	Small
C=O ester bond	1712.02	Medium
C-O stretching	1055.17	Weak
$-CH_2$-bend	1463.33	Medium
$-CH_3$-bend	1377.73	Weak

Verification of Wax Esters Using NMR

Figures 8 shows the spectrum of ^{1}H-NMR of oleyl oleate indicates that 3-hydrogen triplet at 0.9 ppm indicating terminal methyl group. Based on the ^{1}H-NMR chemical shift table in Figure 8, 2 hydrogen methylene signals were observed at 1.2 – 1.4 ppm. While at 1.6 ppm, methane hydrogen was observed which had a larger chemical shift than the previous methylene and methyl group. At 2.1 – 2.5 ppm, it was noticed that there were singlet and triplet 3-hydrogen, that indicated all hydrogen on a carbon next to a carbonyl group gave absorption in the same magnetic field.

Furthermore, the presence of the $-CH_2$-COO signal at about 2.26 ppm for 1H chemical shift is the most important feature indicate that the esterification reactions were successful. This result is similar to as reported in the production of oleic acid-based wax ester using acidic homogeneous catalysts [21]. A large chemical shift was due to deshielding effect of the electronegative oxygen atom that attached to the same carbon.

While for the ^{13}C-NMR spectrum for oleyl oleate in Figure 8 shows that at peak 14.1 ppm indicated the presence of aliphatic saturated carbon. There were abundant peaks in the region between 22.76 – 64.59 ppm, which indicated the present of aliphatic saturated and unsaturated carbon with no electronegative elements. While at the region 130.6 ppm, there was peak that indicated the presence of unsaturated carbon in sample. An ester group was observed at the peak 173.2 ppm, which confirmed that the sample was an ester. The main signals present in ^{1}H-NMR ^{13}C-NMR functional groups of oleyl oleate are shown in Table 9 and Table 10.

The NMR spectrum for oleyl palmitate in Figure 9 also show that there is ester group in the compound. This is due there is a peak observed at 173 ppm. The main signals present in ^{1}H-NMR ^{13}C-NMR functional groups of oleyl palmitate are shown in Table 11 and Table 12. Due to both compounds which are oleyl oleate and oleyl palmitate having a nearly identical structure and functional group, the NMR spectrum does not appear very different when comparing the NMR spectrum of both wax esters.

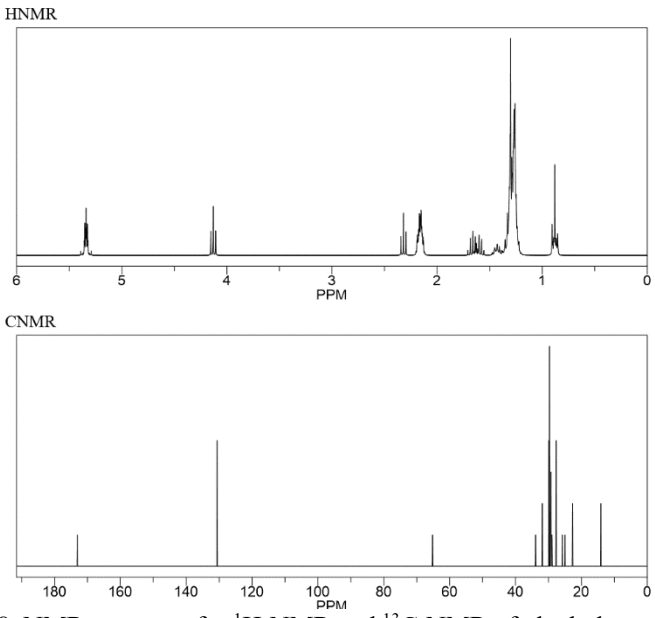

Figure 8. NMR spectrum for ^{1}H-NMR and ^{13}C-NMR of oleyl oleate

Table 9. Signals Present in ^{1}H-NMR Functional Groups of Oleyl Oleate

Assignment	**Chemical Shifts, ppm**
-CH=CH-	5.32
$-CH_2-COO$	2.26
CH_4	1.6
CH_2	1.2-1.4
CH_3	0.9

Table 10. Signals Present in ^{13}C-NMR Functional Groups of Oleyl Oleate

Assignment	**Chemical Shifts, ppm**
C=O	173.2
C=C	130.6
C-O	64.59
$CH_2-COO-CH_2$	32.82 22.76-29.98
CH_3	14.12

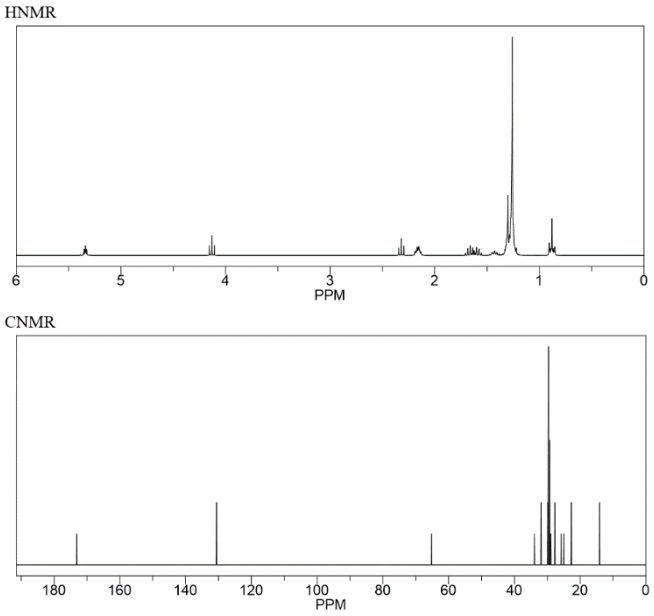

Figure 9. NMR spectrum for ^{1}H-NMR and ^{13}C-NMR of oleyl palmitate

Table 11. Signals Present in ^{1}H-NMR Functional Groups of Oleyl Palmitate

Assignment	**Chemical Shifts, ppm**
-CH=CH-	5.32
$-CH_2-COO$	2.26
CH_4	1.6
CH_2	1.2-1.4
CH_3	0.9

Table 12. Signals Present in ^{13}C-NMR Functional Groups of Oleyl Palmitate

Assignment	**Chemical Shifts, ppm**
C=O	173.2
C=C	130.6
C-O	64.59
$CH_2-COO-CH_2$	22.76-29.98
CH_3	14.12

Conclusion

Synthesis of palm-based and olive-based wax esters using conventional methods were accomplished using oleyl alcohol, oleic acid, and palmitic acid. From the antibacterial study, the results show that the oleyl palmitate and oleyl oleate have antibacterial activity against four selected bacteria which are *Escherichia coli*, *Salmonella typhimurium, Bacillus subtilis* and *Staphylococcus aureus*. Oleyl oleate and oleyl palmitate with different concentrations give an inhibition towards all the bacteria tested even in only small inhibition zone was observed in agar diffusion essay. Through the three-assay conducted, it can be concluded that oleyl oleate has a higher level of antibacterial activity compared to oleyl palmitate. The physicochemical properties such as SPF value, peroxide value, iodine value and saponification value of oleyl oleate and oleyl palmitate were also determined in this study. The physicochemical assay done suggests that the synthesized wax esters can be used as main ingredients in cosmetics formulation. Lastly, by analysing the FTIR and NMR spectrum, the existence of the ester group on both wax esters was proven by the absorption peaks at 1712 cm^{-1} for FTIR and ^{13}C-NMR chemical shift for an ester group was observed at the peak 173.2 ppm for NMR.

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Author Contribution

Mazlan, M.F. – Data collection; Mat Radzi, S. – Supervision, review and editing.; Abd Rahman, N. J – Synthesis of ester; Mohd Rehan, M – Antimicrobial study; Mohd Amin, N. – Verification of product.

Conflict of Interest

Authors declare no conflict of interest

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