BIOLOGICAL AND PHYSIOCHEMICAL STUDIES OF OLEYL PALMITATE AS AN ACTIVE AGENT IN COSMETIC FORMULATION

NURUL FADHILAH ABDUL HALIM [1], SALINA MAT RADZI [1*], MARYAM MOHD REHAN [1], NOR AMALINA MOHD AMIN [1] AND NURUL JANNAH ABDUL RAHMAN [2]

[1] Faculty of Science and Technology, Universiti Sains Islam Malaysia
71800 Nilai, Negeri Sembilan, MALAYSIA

[2] Tamhidi Centre, Universiti Sains Islam Malaysia
71800 Nilai, Negeri Sembilan, MALAYSIA

**Corresponding author: salina@usim.edu.my*

ABSTRACT: Oleyl palmitate, a wax ester extensively utilized in several industries, particularly in cosmetic formulation, is difficult to source naturally due to its elevated cost and restricted availability. In this study, oleyl palmitate was synthesized through an esterification reaction using oleyl alcohol and palmitic acid. Characterization of the synthesized wax ester was performed using Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy to verify the existence of ester bonds in its molecular structure. The antibacterial activity of oleyl palmitate was evaluated against both Gram-positive and Gram-negative bacteria using the agar diffusion test. The Minimum Inhibition Concentration (MIC) value was ascertained through the microdilution method. Two biological activities, bactericidal effects (killing bacteria) and bacteriostatic effects (inhibiting bacterial growth), were determined by Minimum Bactericidal Concentration (MBC) value. Synthesized oleyl palmitate exhibited a bactericidal effect against all Gram-positive bacteria studied, including *Bacillus subtilis* and *Staphylococcus aureus*. Additionally, physicochemical properties such as SPF values (15.0), peroxide values (18.0 mEq O_2/kg), saponification values (90.0 mg KOH/g), and iodine values (87.0 g I_2/100 g) were determined. The data obtained suggest that the synthesized oleyl palmitate holds potential as an active ingredient in cosmetic formulations.

KEY WORDS: *Oleyl Palmitate, Biological, Physicochemical, Wax Ester, Cosmetic Formulation*

1. INTRODUCTION

Wax esters, derived from various botanical and zoological sources, are esters with a chain length of 12 carbons or more, created by combining fatty acids and alcohols. They serve diverse functions in nature, including protection, water resistance, and energy storage. Examples include their role in forming a protective layer on plant surfaces and as lubricants in animal sebum [23][24]. These compounds, also known as fat esters, find applications in industries such as cosmetics, food, and medicine due to their beneficial properties like non-greasiness and emollience [1].The synthetic production of wax esters like oleyl palmitate

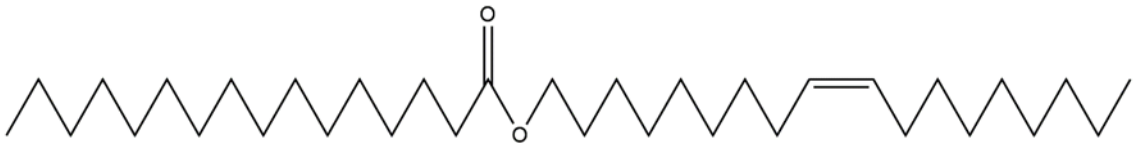
has become necessary due to cost and sourcing challenges of natural wax esters. Chemical and enzymatic methods are used to create synthetic wax esters with qualities similar to their natural counterparts [2], offering advantages like controlled composition and enhanced stability, making them preferred for cosmetic use [23][24].

This study focuses on synthesizing oleyl palmitate through esterification, evaluating its biological properties through Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) experiments on specific bacteria. The produced wax ester will be evaluated based on physicochemical parameters, including peroxide value, saponification value, SPF value, and iodine value.

The objectives aimed to create oleyl palmitate, a wax ester formed by reacting palmitic acid and oleyl alcohol in hexane using esterification. The resulting compounds were then analyzed for both their biological and physicochemical attributes. Biological characteristics were examined by diluting the samples with Gram-positive and Gram-negative bacteria such as Bacillus subtilis and Escherichia coli to ascertain the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Additionally, the physicochemical characteristics of oleyl palmitate, including peroxide value, saponification value, SPF value, and iodine value, will be investigated.

1.1. Oleyl Palmitate

Wax esters are extended chains of esters formed by combining fatty acids and alcohols. Wax esters' unique qualities have made them widely used in a variety of sectors. Their great emollient properties make them popular in cosmetics, and they are used in a variety of personal care products [3]. Throughout this research, the focus will be on oleyl palmitate, a specific type of wax ester compound (Scheme 1).



Scheme 1: Composition of Oleyl Palmitate

The fatty acid ester oleyl palmitate is made up of two components: oleyl alcohol and palmitic acid. Oleyl alcohol, an unsaturated fatty alcohol with 18 carbons and a double bond between the ninth and tenth, is generated from oleic acid, which can be found in a variety of vegetable oils. Oleyl alcohol is represented by the chemical formula $CH_3(CH_2)_7CH=CH(CH_2)_7CH_2OH$.

Palmitic acid, a saturated fatty acid with 16 carbons, is found in variety of dietary fats, both plant and animal, including palm and coconut oils. It has the chemical formula $C_{16}H_{32}O_2$ and is made up of a straight carbon chain with a carboxyl group (COOH) at one end. The absence of double bonds in the carbon chain indicates total saturation with hydrogen atoms [4].

1.2. Characterization of Oleyl Palmitate

To validate the identification, purity, and quality of oleyl palmitate, a multitude of methods for evaluation are commonly utilized. Common approaches include Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy. The simultaneous use of these processes enables an exhaustive investigation of oleyl palmitate, ensuring precise identification, high purity, and quality in cosmetic formulations. It is vital of note out the validation procedures employed may vary depending on the specifications provided by the manufacturer and the limitations of regulation.

1.3. Antimicrobial Properties

In the context of cosmetic formulations, antimicrobial characteristics refer to a substance's capacity to inhibit the growth and spread of microorganisms such as bacteria, fungus, and yeast. Antimicrobial compounds are added to cosmetic items to prevent microbiological contamination, promote product stability, and maintain customer safety.

Costumer apprehensions regarding the safeness of synthetic compounds, including concerns about toxicity, antibacterial efficacy, and potential carcinogenic characteristics, have triggered substantial discussions on their safety. Consequently, there is an increasing preference among consumers towards natural-based products as opposed to artificial, chemical-based cosmetics. This concern has elevated consumer awareness regarding safer options and fostered a deeper comprehension of the safety considerations associated with synthetic chemical cosmetics [5].

Antimicrobial tests such as Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MCB) were used to determine whether the material in this investigation, oleyl palmitate, possesses antimicrobial characteristics. The minimum inhibitory concentration (MIC) is the lowest concentration (expressed in mg/L or g/L) of an antimicrobial agent required to prevent visible microbial growth in vitro, whereas the minimum bactericidal concentration (MBC) is the lowest concentration of antibiotics required to eliminate 99.9% of the inoculum.

1.4. Physicochemical Properties

The inquiry of the relationships between an element's chemical structure and physical qualities is known as physicochemical analysis. The work conducted here is crucial for understanding the intrinsic properties of elements and their ability to interact with certain reagents. Physicochemical tests like as SPF, peroxide, saponification, and iodine value are used to validate the efficacy of cosmetic compositions [6].

1.4.1. SPF value

The term "SPF" (Sun Protection Factor) relates to a product's effectiveness in protecting the skin from the harmful outcomes of UV radiation. The SPF value protects against UVB (ultraviolet B) rays, which leads to sunburn and skin damage. Increased SPF ratings indicate higher degrees of defense. The SPF value is determined using particular methodologies, such as in vitro or in vivo tests [7].

1.4.2. Peroxide value

The peroxide value (POV) measures reactive oxygen levels in milliequivalents (mEq) of free iodine per kilogram of fat. This value can be acquired by titrating the iodine released from potassium iodide with a sodium thiosulphate solution [8]. The peroxide value is especially important in cosmetic compositions that include oils such as carrier oils, plant oils, or oils obtained from organic sources. It sheds light on the durability and freshness of these oils, suggesting probable levels of oxidation. Elevated peroxide readings indicate increased oxidation and product deterioration.

1.4.3. Saponification value

Saponification is the hydrolysis of an ester in alkaline solution. The saponification value represents the chain length of all fatty acids. It is evaluated by the amount of potassium hydroxide required to neutralize the fatty acids produced by completely hydrolyzing 1 g of the sample in oil or fat. Notably, long-chain fatty acids, which include fewer carboxylic functional groups, have a low saponification value [9].

1.4.4. Iodine value

The iodine value indicates the quantity of unsaturation or double bonds found in fats and oils. It is also used as one of the criteria for determining the quality of olein. This figure represents the weight proportion of absorbed halogens, specifically iodine, under test conditions. The definition entails calculating the amount of iodine (in grams) absorbed by the fat (in 100 grams) over the test period. The iodine value is regarded as a crucial constant that is easily calculated for fats and oils [10].

2. MATERIALS AND METHODOLOGY

Chemicals

Ethanol, hexane, toluene, chloroform, dimethyl sulfoxide (DMSO), palmitic acid, oleyl alcohol, sodium hydroxide, streptomycin sulfate powder, Wij's solution, hydrochloric acid, sodium thiosulphate, starch indicator, potassium iodide, phenolphthalein solution, potassium hydroxide, acetic acid and 3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide (MTT) solution.

Instruments

Fourier transform-infrared (FTIR) and nuclear magnetic resonance (NMR).

Microorganisms

Bacillus subtilis and *Staphylococcus aureus* represented the Gram-positive bacteria. *Salmonella typhimurium* and *Escherichia coli* were the Gram-negative bacteria.

2.1. Methodology

The methodology was divided into four main components: the production of oleyl palmitate, the characterization of oleyl palmitate, the examination of antimicrobial properties, and the analysis of physicochemical characteristics, which illustrated in the layout of experiment is shown below (Fig. 1).

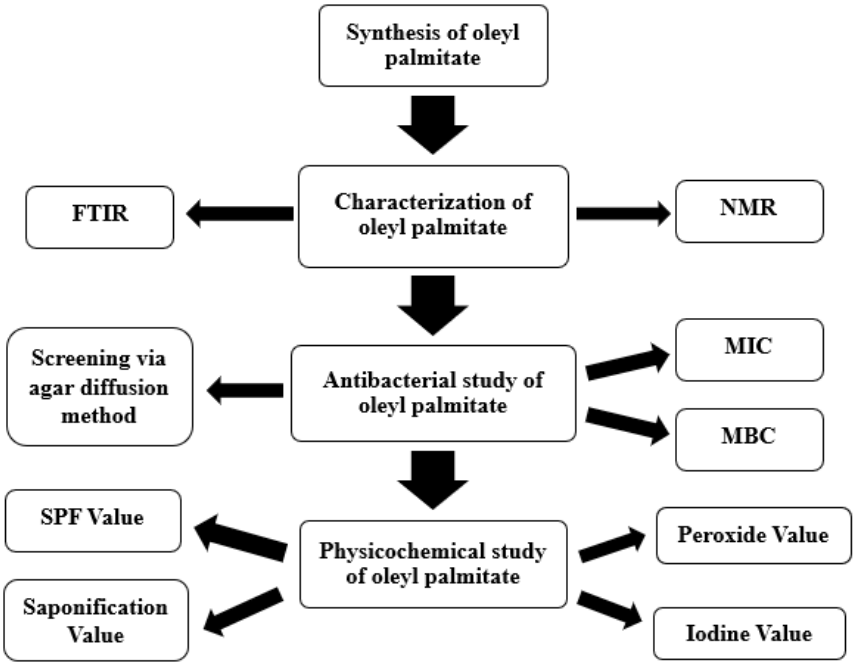


Fig. 1. Experimental Design

2.1.1. Synthesis of Oleyl Palmitate

Oleyl palmitate was synthesized by reacting palmitic acid with oleyl alcohol. In the initial stage, palmitic acid and oleyl alcohol were combined in a 250 mL universal bottle with n-hexane as the solvent, maintaining a molar ratio of 1:2. The mixture underwent a 5-hour incubation at a reaction temperature of 50°C in a water bath shaker set at 150 rpm [11].

2.1.2. Characterization of Oleyl Palmitate

During the experimental process, a sample of oleyl palmitate obtained from the reaction was subjected to thin-layer chromatography (TLC) analysis. Subsequently, the sample underwent scrutiny using spectroscopic techniques including Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR). Before employing FTIR and NMR for verification, TLC was utilized as an initial step. For FTIR analysis to identify functional groups, the resulting product was examined using Fourier Transform Infrared Spectroscopy (Perkin Elmer, model spectrometer 100, England) with attenuated total reflectance (ATR) sample preparation. The ATR method involves placing a small liquid

droplet directly onto a clean ATR crystal, minimizing the sample handling for efficient analysis, especially suitable for viscous samples [26].

In NMR analysis, the compound's structure was determined by analyzing the ester sample in DMSO solvent. H-NMR and C-NMR spectra were used to measure chemical shifts for compound identification. Proper sample preparation is crucial, typically requiring 5-25 mg of material for ^{1}H-NMR spectra and 50-100 mg for ^{13}C-NMR spectra. Deuterated solvents like DMSO should be used, along with clean NMR tubes and caps. Additionally, an internal standard and proper labeling of the sample are recommended for accurate analysis [25].

2.1.3. Antimicrobial Study of Oleyl Palmitate

The effectiveness of the sample against microorganisms was assessed through an antimicrobial assay utilizing the agar diffusion method [12]. The MIC of the sample was established through the dilution method [13], and its MBC was also determined [13]. Microorganisms employed in this research were *Bacillus subtilis, Staphylococcus aureus, Salmonella typhimurium* and *Escherichia coli.* This is because, these organisms are well-characterized and widely used in laboratory settings, allowing for standardized experimental conditions and reproducibility.

2.1.3.1. Agar Diffusion Method

To perform the agar diffusion test, guidance from Smith-Palmer et al. (1998) was followed. Bacterial cultures at a concentration of 10^7 cells/mL were spread onto Mueller-Hinton agar (MHA) using sterilized cotton swabs. Each experiment was conducted twice. Test wells, each 6 mm in diameter, were created on every agar plate using a cork borer. Subsequently, each test well received 10 µL of varied test sample concentrations. Streptomycin Sulphate solution (10 mg/mL) was used as a positive control in each test well. The lowest dose at which an inhibitory zone was observed at the sample inoculation sites following an overnight incubation at 37°C was recorded. The diameter of the inhibitory zone was measured and reported in millimeters. [14].

2.1.3.2.Minimum Inhibitory Concentration (MIC) value

Bacteria that showed a strong inhibition zone in the agar diffusion method were chosen for the standard broth dilution experiment to determine MIC. This process was carried out using a 96-well microtiter plate (Costar, USA). To each well of the microtiter plate containing 50 mL of the test sample solutions (oleyl palmitate), 50 µL of a previously produced bacterial inoculum was added. The microtiter plate was then covered with a lid and incubated at 37°C overnight [14].

The bacteria's vitality upon exposure to the test compounds was tested using a solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). To make the MTT solution (at a concentration of 0.3 mg/mL), dissolve roughly 0.3 mg of MTT powder in 1 mL of sterilized distilled water. Each test well received approximately 40 µL of MTT, and the microtiter plate was incubated at 37°C for an additional 5 minutes. The lowest concentration that did not cause apparent formazon blue formation was defined as the MIC. All experiments were repeated twice [14].

2.1.3.3.Minimum Bactericidal Concentration (MBC) value

The Minimum Bactericidal Concentration (MBC) value was calculated using Smith-Palmer et al.'s (1998) technique. To use this method, 50 µL of a prepared bacterial inoculum was combined with 50 µL of each type of test sample in a 96-well microtiter plate. The microtiter plates were incubated at 37°C overnight. Each sample from the test wells was then swabbed into the Mueller-Hinton agar (MHA) medium. Bacterial growth was evaluated during an overnight incubation at 37°C. The lowest concentration of each test sample that showed no bacterial growth was designated as the Minimum Bactericidal Concentration (MBC) [14].

2.1.4. Physicochemical Study of Oleyl Palmitate

Four parameters were chosen for the physicochemical analysis of oleyl palmitate: SPF value, peroxide value, saponification value, and iodine value.

2.1.4.1. SPF value

The samples, each weighing 1 g, were transferred to 100 mL volumetric flasks for SPF value analysis. Subsequently, the solution was diluted with ethanol, ultrasonified for 5 minutes, and filtered through cotton, with the first 10 mL removed for UV-Visible testing. The absorption spectra of the samples in solution were measured in the 290-450 nm region. Ethanol in a 1cm quartz cell served as a blank, and absorptions were recorded every 5 nm, accumulating data in the 290-320 nm range [15]. The Mansur equation (Eq.1) was employed to calculate the SPF value [16].

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

Where, CF = correction factor

EE = an erythemal effect spectrum

I = the solar intensity spectrum

Abs = absorbance of the sample

2.1.4.2. Peroxide value

In a 250 mL conical flask, 5.0 g of material was dissolved in 10 mL of chloroform and stirred. The mixture was then mixed with 15 mL of acetic acid and 1 mL potassium iodide solution. After 5 minutes in a dark atmosphere, the process continued by adding 30 mL of distilled water and 1 mL of starch indicator. The solution was titrated with 0.05

molarity sodium thiosulphate until the indicator's blue hue faded [17]. Eq. 2 was applied to determine the peroxide value [17].

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

Where, S = volume of titrant (mL) for the sample

B = volume of titrant (mL) for blank

N = normality of sodium thiosulphate solution (mmol/mL)

1000 = conversion of the unit (g/kg)

W = mass (g) of the sample (oleyl palmitate)

2.1.4.3. Saponification value

Each sample, weighed precisely at 2.0 g, was placed in a 250 mL conical flask. Following this, 25 mL of ethanolic potassium hydroxide was added, and the mixture was refluxed for 60 minutes. Then, 1 mL of phenolphthalein solution was added, and the mixture was titrated with 0.5 N of hydrochloric acid (HCl) until the indicator's pink tint faded [18]. Eq. 3 was then applied to calculate the saponification value [19].

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

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)

56.1 = the molecular weight of potassium hydroxide KOH (g/mol)

W = mass (g) of sample (oleyl palmitate)

2.1.4.4. Iodine value

Each sample was precisely weighed at 2.0 g before being placed in a 500 mL stoppered flask with 10 mL of chloroform. Then, 25 mL of Wij's solution was gently pipetted into the flask, which was capped and spun to ensure thorough mixing. The flask was placed at room temperature in a dimly lit setting for 30 minutes. The method continued with the addition of 20 mL of potassium iodide, followed by 100 mL of freshly cooked and cooled distilled water. The combination was titrated with 0.05 M sodium thiosulphate solutions until the yellow tint was almost undetectable. After adding 1 mL of a starch indicator, the titration was repeated until the indicator's blue hue disappeared. A blank decision was carried out under similar settings [18]. Eq. 4 was employed to determine the iodine value [20].

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

Where, B = volume of titrant (mL) for the blank

S = volume of titrant (mL) for the sample

N = normality of sodium thiosulphate (mmol/mL)

126.9 = the molecular weight of iodine (g/mol)

W = mass of sample

3. RESULTS AND DISCUSSION

3.1.Synthesis of Oleyl Palmitate

The esterification reaction mixtures of oleyl alcohol and palmitic acid were incubated in a water bath shaker (150 rpm) at 50°C reaction temperature and with 5 hours of reaction time in the presence of n-hexane that acts as solvent to help shift the reaction towards the products. The esterification between palmitic acid and oleyl alcohol to produce oleyl palmitate is shown in Figure 2. In this reaction, the hydroxyl group (OH) from the carboxylic acid (palmitic acid) and the hydrogen atom from the hydroxyl group (OH) of oleyl alcohol combine to form water (H_2O), a by-product of the reaction. This condensation reaction facilitates the formation of the ester bond (C-O) between the carboxylic group of palmitic acid and the hydroxyl group of oleyl alcohol. The result is the formation of oleyl palmitate, a fatty ester, and water as the reaction proceeds towards equilibrium.

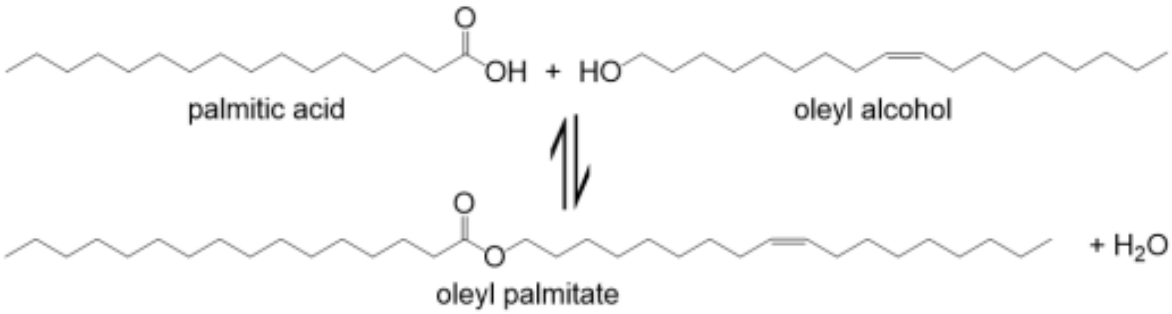


Fig. 2. Chemical Reaction Pathway to Produce Oleyl Palmitate

3.2.Characterization of Oleyl Palmitate

Before synthesized oleyl palmitate was characterized using FTIR and NMR, it will undergo thin layer chromatography (TLC). The purpose of TLC to separate synthesized oleyl palmitate from other components in a mixture, used to assess the purity of oleyl palmitate and employed to isolate specific fractions containing oleyl palmitate for further analysis by NMR spectroscopy. For TLC results, the separation of mixtures was observed by exposing them to UV light at 254 nm. The chromatogram was characterized by the

retention factor (Rf) of each analyte, which is the fractional distance where the analyte spot to solvent by moving along the plate. The distance for each substance spot is measured from the initial point of the substances, whereas the development distance refers to the distance to the center of the substance spot.

In Fig. 3, the FTIR spectrum of oleyl palmitate displays characteristic absorption peaks corresponding to its functional groups, confirming its structure as a fatty ester. The strong peaks at 2921.67 cm^{-1} and 2854 cm^{-1} are indicative of aliphatic C-H stretching vibrations, typical of long-chain hydrocarbons. A prominent peak at 1711.24 cm^{-1} corresponds to the ester carbonyl (C=O) stretch, a defining feature of esters. The presence of unsaturation in the oleyl chain is evident from the C=C stretching peak around 1672.48 cm^{-1}. Additional peaks at 1465.09 cm^{-1} and 1377.73 cm^{-1} correspond to CH_2 and CH_3 bending vibrations, respectively, while the band around 1243 cm^{-1} indicates the C-O stretching of the ester group. The overall spectrum is consistent with the molecular structure of oleyl palmitate, confirming its identity as a long-chain ester with unsaturated and saturated fatty acid components.

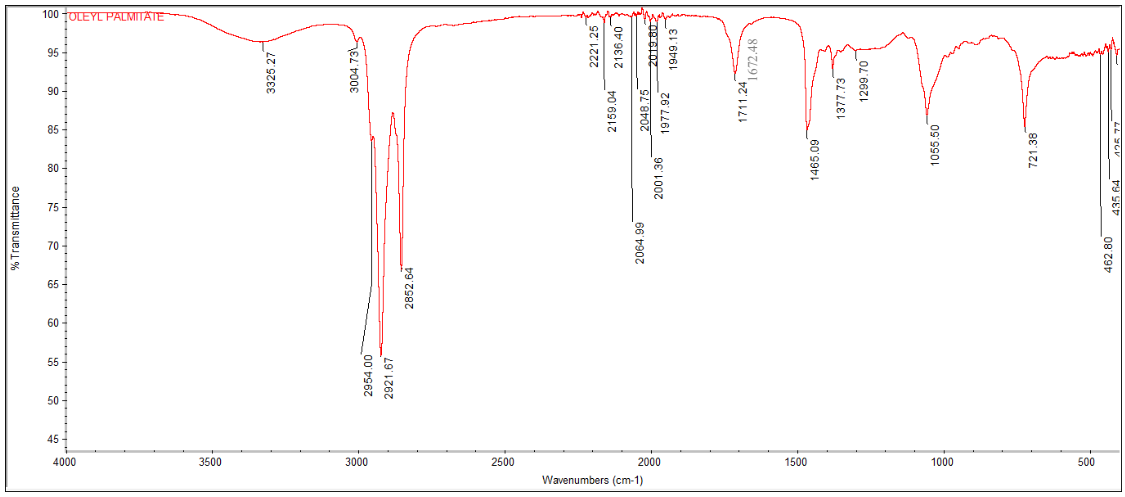


Fig. 3. FTIR Absorption Spectra of Oleyl Palmitate.

Fig. 4 illustrates the ^{1}H-NMR wavelength and ^{13}C-NMR wavelength of oleyl palmitate. The NMR analysis of oleyl palmitate provides a clear confirmation of its molecular structure as shown in Table 4 and 5. The ^{1}H-NMR spectrum shows characteristic peaks for key functional groups, including olefinic protons (-CH=CH-) at 5.32 ppm, indicating the presence of unsaturation, as well as methylene protons adjacent to the ester group (-CH_2-COO) at 2.26 ppm, confirming esterification. Additional signals at 1.6 ppm, 1.2-1.4 ppm, and 0.9 ppm correspond to protons within the aliphatic chains, typical of long-chain fatty esters. The ^{13}C-NMR spectrum further supports these findings, with a carbonyl carbon signal at 173.2 ppm confirming the ester bond (C=O), and a peak at 130.6 ppm indicating the presence of a double bond (C=C). Other signals for carbons within the aliphatic chains (CH_2 and CH_3) fall within the expected ranges. Together, these results confirm the successful synthesis of oleyl palmitate, with its characteristic ester bonds, unsaturation, and long carbon chains, consistent with its intended structure for cosmetic applications.

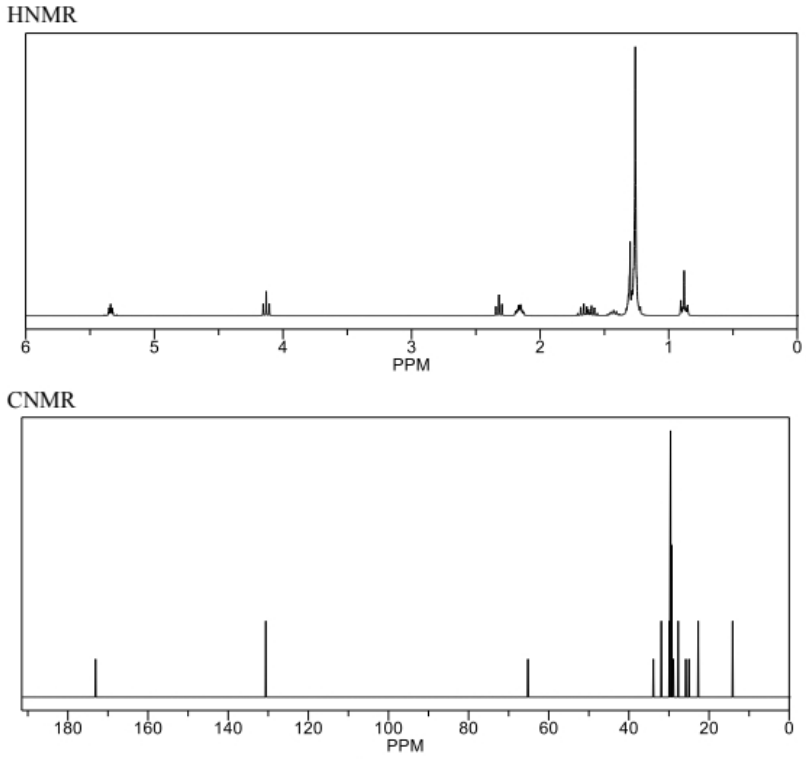


Fig. 4. NMR Spectrum for ^{1}H-NMR and ^{12}C-NMR of Oleyl Palmitate.

Table 4: The Primary Signals Observed in ^{1}H-NMR Functional Groups of Oleyl Palmitate

Assignment	Chemical Shift, 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 5: The Primary Signals Observed 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

3.3.Antimicrobial Assays of Oleyl Palmitate

The antibacterial investigation included use of agar-well diffusion and dilution procedures. The dilution approach included two separate analyses: MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration).

According to the outcomes of agar-well diffusion method, when examined against Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, oleyl palmitate generated distinct clear zones on the agar plate. In contrast, there were no observable clear zones when the same compound was tested towards Gram-negative bacteria *Salmonella typhimurium* and *Escherichia coli* as shown in Figure 5 below.

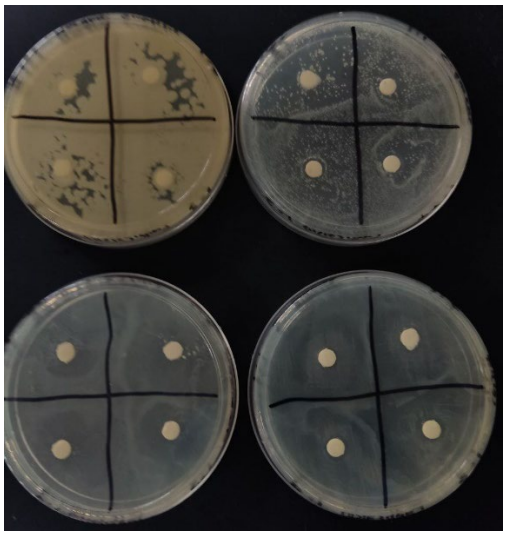


Fig. 5. Formation of Clear Zones Showed the Antimicrobial Activity of the Test Samples

In the dilution method, MIC is identified as the lowest concentration (maximum dilution) of the antimicrobial agent that prevents turbidity growth, indicating that the agent is bacteriostatic, allowing some bacteria to remain alive [21]. The result of this technique in Table 6 shows that the MIC value for oleyl palmitate on *Bacillus subtilis* is at the second concentration (1:2 v/v) while on *Staphylococcus aureus* is at the third concentration (1:3 v/v). For Gram-negative bacteria which are *S. typhimurium* and *E. coli*, the MIC value for *S. typhimurium* cannot be obtained while the MIC value for *E. coli* is at the first concentration which is 1:1 v/v. The MIC value for *S. typhimurium* cannot be obtained might be because of the presence of other microbes could inhibit the growth of *S. typhimurium* and affect the accuracy of MIC determination.

Table 6: The Minimum Inhibitory Concentration (MIC) Values of Oleyl Palmitate on Tested Bacteria.

Concentration of test samples (v/v)	Test Bacteria			
	Bacillus subtilis	*Staphylococcus aureus*	*Salmonella typhimurium*	*Escherichia coli*
1:1	-	-	+	-
1:2	-	-	+	+
1:3	+	-	+	+
1:4	+	+	+	+
Streptomycin sulfate	-	-	-	-

+: Presence of bacteria growth, -: Absence of the bacteria growth

Whereas, Gram-positive and Gram-negative bacterial strains were examined for Minimum Bactericidal Concentration (MBC). MBC is identified as the lowest concentration of an antimicrobial agent that eliminates more than 99.90% of the initial bacterial population, leaving none visible bacterial growing on agar plates [22]. Table 7 presents the MBC values of oleyl palmitate against the tested bacteria. Streptomycin sulfate is an aminoglycoside antibiotic that primarily inhibits protein synthesis in bacteria. Streptomycin sulfate is used in MIC testing to find the lowest concentration of antibiotic that suppresses the test organism's visible growth.

Table 7: The Minimum Bactericidal Concentration (MBC) Values of Oleyl Palmitate on Tested Bacteria.

Concentration of test samples (v/v)	Test Bacteria			
	Bacillus subtilis	*Staphylococcus aureus*	*Salmonella typhimurium*	*Escherichia coli*
1:1	-	-	+	+
1:2	+	-	+	+
1:3	+	+	+	+
1:4	+	+	+	+
Streptomycin sulfate	-	-	-	-

+: Presence of bacteria growth, -: Absence of the bacteria growth

3.4.Physicochemical Study of Oleyl Palmitate

The physicochemical properties of oleyl palmitate are summarized in Table 8, reflecting key indicators of its stability and functionality. The Sun Protection Factor (SPF) value of 15.0 suggests that oleyl palmitate offers moderate protection against ultraviolet radiation, making it a suitable ingredient for sunscreen formulations. The peroxide value of 18.0 indicates the extent of lipid oxidation, implying moderate oxidative stability, which is essential for maintaining the quality of the product over time. The saponification value of 90.0 indicates the amount of potassium hydroxide needed to saponify the ester, which reflects the molecular weight and probable application of oleyl palmitate in soap and cosmetic manufacture. Finally, the iodine value of 87.0 reveals the degree of unsaturation in the molecule, which influences its reactivity and shelf life. Together, these properties provide a comprehensive profile of oleyl palmitate's suitability for applications for personal care and cosmetic products.

Table 8: Summarize Physicochemical Properties for Oleyl Palmitate.

Physicochemical Properties	Oleyl Palmitate
SPF Value	15.0
Peroxide Value	18.0
Saponification Value	90.0
Iodine Value	87.0

4. CONCLUSION

The study on the synthesis of oleyl palmitate, followed by its verification through FTIR and NMR analysis, and subsequent biological and physicochemical evaluations, leads to several key conclusions. The typical peaks found in the FTIR and NMR spectra proved the effective synthesis of oleyl palmitate, which correspond to the expected functional groups, such as the ester carbonyl (C=O) and unsaturated aliphatic chains. Physicochemical properties, including SPF, peroxide, saponification, and iodine values, demonstrate that oleyl palmitate possesses moderate UV protection, acceptable oxidative stability, and appropriate reactivity for potential use in cosmetic and personal care applications. Biological studies further affirm its suitability for such applications, potentially offering skin benefits without significant toxicity. Overall, oleyl palmitate is established as a viable candidate for use in various industrial and cosmetic formulations due to its desirable chemical structure, stability, and functional properties.

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