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CYTOTOXIC AND ANTI-ACNE ACTIVITY OF SUGAR FATTY ACID ESTERS (SFAE) FROM CPO AND PALM OIL HYDROLYSATE

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ABSTRACT

This study aimed to determine the potential of sugar fatty acid esters (SFAE) derived from crude palm oil (CPO) and palm oil hydrolysates as cytotoxic and anti-acne agents. The influence of structural variations among different saccharides on the SFAE properties was also investigated. Three types of saccharides were tested in this study; D-mannitol (a sugar alcohol), D-mannose (an aldose) and D-fructose (a ketose). SFAEs were prepared enzymatically by reacting fatty acids and saccharides using the low-cost liquid lipase Eversa® Transform 2.0 at 5% (w/w) concentration. Thin layer chromatography (TLC) analysis, fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) spectra, emulsification properties, conversion rate and degree of esterification indicated the formation of SFAE. Some SFAE exhibited strong cytotoxic and antibacterial activities. SFAE of palmitic acid-mannitol and SFAE of stearic acid-fructose showed the highest cytotoxic activity against MCF-7 cells, with an IC_{50} value of 29 ppm. The SFAE of palm oil hydrolysate exhibited stronger cytotoxic activity than the SFAE of CPO hydrolysate. Additionally, the SFAE from CPO hydrolysate and D-mannitol exhibited the strongest antibacterial activity, achieving 100% inhibition against Cutibacterium acnes *at 0.9 mg/mL. These findings highlight the potential of SFAE as a promising raw materials for the development of health and cosmetic products.*

Keywords: CPO and palm oil hydrolysate, cytotoxic and anti-acne activities, enzymatic esterification, Eversa® Transform 2.0, sugar fatty acid ester.

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INTRODUCTION

Indonesia and Malaysia are the two largest palm oil producers in the world, accounting for most of global production (U.S. Department of Agriculture, 2024). These two countries dominate the market because of their favourable climates for palm oil cultivation and large-scale plantations. However, by the end of 2022, the European Union issued a new regulation on deforestation-free products that tightened the rules for importing crude palm oil (CPO) into its territory. Therefore, downstreaming palm oil and CPO into derivative products (oleochemicals) is crucial in increasing their sales value and benefits. The most common oleochemical products were fatty acid hydrolysates, glycerin, soap noodles and oleic acid. Oleochemical products in the form of sugar fatty acid esters (SFAE) have not been widely studied. One of the companies operating in this field is Ryoto™ Sugar Ester, which is based in Japan. The main application of sucrose-fatty acid esters from Ryoto™ is as an emulsifier in the food sector.

The application of SFAE as an emulsifier in the food industry showed that these compounds are safe for consumption (Sardar *et al*., 2024). The emulsification properties of SFAE have also been applied to the pharmaceutical, detergent and cosmetics industries (Qi *et al*., 2024). The non-ionic

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surfactant SFAE has also been used to produce niosomes for quercetin encapsulation (Elmowafy *et al*., 2020). Niosome-encapsulated quercetin showed a higher hepatoprotective activity than free quercetin. Emulsification properties have also been reported to be associated with biological activities, such as antibacterial (Falk, 2019; Pirog *et al*., 2019) and anticancer (Chiewpattanakul *et al*., 2010; Walvekar *et al*., 2022). Emulsifiers or surfactants can damage the membranes of both microbes and cancer cells. The cytotoxic activity of SFAE has been described by Teng *et al*. (2021), Snoch *et al*. (2021), Chen *et al*. (2023) and Wang *et al*. (2024), while the antimicrobial activity of SFAE has been reported by Matin *et al*. (2020; 2022), Teng *et al*. (2021), and Yang *et al*. (2024). The role of SFAE in suppressing inflammation caused by acne bacteria was even reported by a US patent in 2005 (Pauly & Grisoni, 2005). However, research on the bioactivity comparison of SFAE from CPO hydrolysate and palm oil hydrolysate for anticancer and anti-acne has never been reported. Research into developing SFAE as a safe active compound is expected to be an alternative to anticancer and anti-acne medication. Anticancer and anti-acne medicines with minimal side effects are urgently needed because recent research by Vitiello *et al*. (2024) even shows that anticancer drugs can change the ocular surface of cancer chemotherapy patients. Wiśniewska *et al*. (2023) mentioned that salicylic acid, a well-known anti-acne cosmetic ingredient, causes several side effects such as hyperpigmentation, allergic reaction and swelling.

SFAE can be prepared either chemically or enzymatically. The chemical synthesis of SFAE has been described using a base-catalysed transesterification reaction between triglycerides or fatty acid methyl esters and sugars (Teng *et al*., 2021). In contrast, lipases have been widely reported for the enzymatic synthesis of SFAE. Surprisingly, proteases also exhibited the ability to catalyse the formation of SFAE (Bernal *et al*., 2018). Commercially available lipases that are widely used for the synthesis of SFAE include immobilised lipase B derived from *Candida antarctica* (Chen *et al*., 2023; Hollenbach *et al*., 2020; Nieto *et al*., 2021; Shin *et al*., 2019; Snoch *et al*., 2021) and immobilised lipase derived from *C. rugosa* (El-Baz *et al*., 2021). Ferdjani and Selaimia (2024) prepared SFAE using lipase from maize seed. The utilisation of lipase Eversa® Transform 2.0 as a catalyst for synthesising SFAE has never been studied. Eversa® Transform 2.0 is a low-cost liquid lipase for biodiesel production (Chang *et al*., 2021; Farobie *et al*., 2021). Monteiro *et al*. (2024) reported the utilisation of Eversa® Transform 2.0 to produce fatty ester for biolubricants. This study prepared several SFAEs from CPO and palm oil hydrolysates using Eversa® Transform 2.0 lipase as the catalyst. Commercially available pure palmitic acid, stearic acid and oleic acid were also employed to prepare SFAE as a comparison. Three types of saccharides were used in this study, namely, D-mannitol (a sugar alcohol), D-mannose (an aldose) and D-fructose (a ketose), to better understand the relationship between the structural moiety, chemical reactivity and bioactivity. The feasibility of SFAE application in the health and cosmetics sector was explored by screening the synthesised SFAE for anticancer (cytotoxic) activity against MCF-7 cells and antimicrobial activity against *Cutibacterium acnes*. The MCF-7 cell line is a model for studying breast cancer, whereas *C. acnes* is associated with *Acne vulgaris*. Cancer is among the top ten causes of death worldwide, and breast cancer is among the top five deadly cancers (Sung *et al*., 2021).

MATERIALS AND METHODS

Fifteen SFAEs were included in this study. Fatty acids were obtained by the hydrolysis of CPO and palm oil. The palm oil was purchased from a local supermarket. Commercially pure (≥ 98.0%) palmitic acid (Merck), stearic acid (Pudak Scientific) and oleic acid were used in this study. D-Mannitol was obtained from Sigma-Aldrich, D-fructose from Sigma-Aldrich and D-mannose from HiMedia. The commercial lipase Eversa® Transform 2.0 from Novozyme was used as the catalyst in this research. Thin layer chromatography (TLC) analysis was conducted on TLC aluminium sheets coated with silica gel 60 F_{254} using 2′, 7′-dichlorofluorescein (Sigma-Aldrich) as the staining agent. All chemicals were used without further purification.

Hydrolysis of CPO and Palm Oil

CPO and palm oil were hydrolysed separately under alkaline conditions. CPO and palm oil were separately mixed with a 1M NaOH solution in alcohol. The reaction mixture was refluxed for 1 hr and monitored using TLC. A solution of 1M HCl was added to the reaction mixture to neutralise the product. The product was extracted twice using *n*-hexane, dried using anhydrous Na_2SO_4 and concentrated to obtain CPO and palm oil hydrolysates. The fatty acid composition of CPO and palm oil was analysed using GC-MS. The acid and iodine numbers were also measured using the standard titrimetric method.

Synthesis of SFAE

Prior to the reaction, the reactants were dissolved in a suitable solvent. Fatty acids were dissolved separately in *n*-hexane, whereas the saccharides were dissolved in a small amount of water. Fatty acids were obtained by the hydrolysis of CPO,

hydrolysis of palm oil and commercially available fatty acids. Saccharides solution was added to the fatty acid solution followed by 5% (*w*/*w*) lipase Eversa® Transform 2.0. The mole ratio of saccharide to fatty acid was 1:6. The mixture was incubated at 40°C for 48 hr and stirred at 500 rpm. The reaction mixture was heated at 100°C to deactivate the enzyme and stop the reaction. The product was separated from the reaction mixture by centrifugation. The product was washed with water to separate the enzyme and hexane to remove unreacted fatty acid. The amount of unreacted fatty acid was measured by acid-base titration using NaOH solution in alcohol. Conversion rate and degree of esterification were calculated by using the Equations (1) and (2):

$$\text{Conversion rate} = \frac{\text{Mole of initial fatty acid} - \text{Mole of unreacted fatty acid}}{\text{mole of initial fatty acid}} \times 100\% \quad (1)$$

$$\text{Degree of esterification} = \frac{\text{Mole of initial fatty acid} - \text{Mole of unreacted fatty acid}}{\text{Mole of saccharide}} \quad (2)$$

All SFAE was analysed by using fourier transform infrared spectroscopy (FTIR) and only a representative of SFAE was analysed by using nuclear magnetic resonance (NMR) to confirm the formation of SFAE. FTIR spectra were analysed on potassium bromide (KBr) using a Shimadzu 2450 Prestige. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in CD_3OD on a Bruker Avance Neo 500 (500 MHz) spectrophotometer.

Emulsification Properties

The emulsification properties of SFAE were evaluated by adding 50 mg of SFAE to a water-oil mixture. A water-in-oil (*w*/*o*) emulsion was prepared by mixing 0.3 mL of water and 1 mL of oil, while a mixture of 0.3 mL of oil and 1 mL of water was used as a model for an *o*/*w* emulsion. The mixture containing SFAE was vortexed. The stability of the emulsions was observed for 24 hr. Phase separation was used as the parameter for emulsion stability. A light microscope and water-soluble dye (methylene blue) were used to facilitate observation of the emulsion. The emulsification properties of the starting material (saccharides and fatty acids) were also observed. A mixture of water and oil without a sample was used as the negative control.

Cytotoxic Activity Against MCF-7

MCF-7 cell suspension was added to 96-well plates at 5,000 cells/100 µL of medium. Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum was used as the medium. The medium was enriched with 100 U/mL of penicillin and 100 µg/mL of streptomycin to prevent microbial contamination. The cells were incubated for 24 hr at 37°C before the addition of a series concentration of SFAE samples in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the medium was 1% (*v*/*v*). Therefore, 1% DMSO was used as the negative control. After 48 hr of incubation, 10 µL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) (5 mg/mL) was added to each well. The plates were then incubated for another 4 hr. Ethanol was added to dissolve the formazan, and the sample absorption was read at 595 nm. The experiment was conducted in triplicate.

Antimicrobial Activity against *C. acnes*

Antibacterial activity against *C. acnes* was determined using the broth dilution method in reinforced clostridial medium (RCM). A 250 mL *C. acnes* suspension was mixed with 5 mL RCM media and a 250 mL sample at room temperature. The DMSO was used as the SFAE solvent and the negative control. A 1,000 ppm salicylic acid was used as a positive control (the final concentration was 45 ppm). Antibacterial activity was determined by measuring optical density at 600 nm (OD600). The experiment was conducted in duplicate.

RESULTS AND DISCUSSION

Fatty Acid Composition of CPO and Palm Oil

The fatty acid composition of palm oil and CPO was analysed using GC-MS (*Table 1*). CPO and palm oil showed similar fatty acid compositions. Oleic acid and palmitic acid were the major fatty acid constituents of CPO and palm oil, respectively. However, the oleic acid content of palm oil was slightly higher than that of CPO. Moreover, unsaturated medium-chain fatty acids (C8 and C10) were only detected in CPO but not in palm oil. Chompoo *et al*. (2019) reported that the degumming process during the conversion of CPO to palm oil increased the total amount of unsaturated fatty acids and reduced the total amount of saturated fatty acids.

Hydrolysates of CPO and Palm Oil

Hydrolysis of both CPO and palm oil was conducted under alkaline conditions. Acidic conditions were avoided to prevent changes in the C=C configuration of the unsaturated fatty acids in CPO and palm oil. The hydrolysate of CPO had a lower R_f value (R_f = 0.57, hexane-ethyl acetate 3:1)

on TLC than the starting material (R_f = 0.86, hexane-ethyl acetate 3:1), indicating the conversion of triglycerides to fatty acids. The TLC analysis of palm oil and its hydrolysate also showed a similar trend. The CPO hydrolysate was an orange solid, whereas the palm oil hydrolysate was a pale-yellow solid. Izuddin *et al*. (2022) stated that CPO is rich in β-carotene. Thus, the orange colour of CPO hydrolysate exhibited that the β-carotene could not be separated from the fatty acids. However, the presence of β-carotene in CPO hydrolysates did not interfere with the SFAE synthesis through the esterification reactions due to the absence of hydroxyl and carboxyl groups in its structure.

The formation of fatty acids from the hydrolysis of CPO and palm oil was also demonstrated by a significant increase in the acid number after hydrolysis (*Table 2*). However, the iodine numbers before and after hydrolysis were within similar ranges. It can be concluded that hydrolysis under alkaline conditions did not influence the unsaturated fatty acid content in CPO and palm oil. The electron-rich nature of the C=C bond is not reactive towards the nucleophilic property of the hydroxide anion from the alkaline hydrolysis reaction. The difference in chemical composition between CPO and palm oil hydrolysates affected the acid and iodine number values. CPO hydrolysate contained fatty acids and other nonpolar compounds (Goon *et al*., 2019) that cannot be separated during hydrolysis.

Synthesis of SFAE

The enzymatic reaction for preparing SFAE was conducted at 40°C according to Chang *et al*. (2021) and Farobie *et al*. (2021). Farobie *et al*. (2021) stated that 40°C was the optimum temperature for enzymatic esterification using Eversa® Transform 2.0 lipase. The esterification reaction in this research was carried out in an organic phase with as little water as possible to promote the esterification and prevent the hydrolysis of the product. The water was used only to dissolve the sugar. The amount of water was calculated depending on the solubility of each saccharide. Therefore, molecular sieves were not used in the present study. Water adsorption by the molecular sieve separated the sugar from the reaction mixture. The esterification reaction catalysed by lipase occurs at the interface between the organic phase and water (Khan *et al*., 2017). Under hydrophobic conditions, the subdomain covering the lipase active site shifted, and the active site was accessible to the substrate. Hexane, which has a log *P* value of 3.5, was chosen as the hydrophobic solvent in this study. Kumar *et al*. (2016) stated that solvents with log *P* values above 2 did not interfere with the essential water in enzymes. Thus, hexane did not influence the active conformation of lipase.

The formation of SFAE was indicated by the appearance of a white layer between the water and hexane layers (*Figure 1*). The SFAE obtained in this research was sparingly insoluble in water or hexane at room temperature. The solubility test of sucrose fatty acid esters in water conducted by Nagai *et al*. (2017) showed that nine out of ten samples precipitated in water. Sucrose-lauric acid ester was the only sucrose ester that formed a transparent homogeneous solution (Nagai *et al*., 2017). The orange colour of the hexane layer in the SFAE of CPO hydrolysate (*Figure 1a*) denoted the presence of β-carotene. The β-carotene and SFAE of CPO hydrolysate can be separated easily at this stage.

TABLE 1. FATTY ACID COMPOSITION OF CPO AND PALM OIL SAMPLES

Fatty acids	Percentage (%)	
	CPO	Palm oil
Caprylic acid (C8:0)	0.02	-
Capric acid (C10:0)	0.02	-
Lauric acid (C12:0)	0.18	0.21
Myristic acid (C14:0)	0.94	0.93
Palmitic acid (C16:0)	39.60	36.00
Stearic acid (C18:0)	4.59	4.08
Oleic acid (C18:1; 9*c*)	40.60	44.20

TABLE 2. ACID NUMBER AND IODINE NUMBER BEFORE AND AFTER HYDROLYSIS OF CPO AND PALM OIL SAMPLES

Quality parameter	CPO		Palm oil	
	Before	After	Before	After
Acid number (mg NaOH/g sample)	28.6	204.8	5.3	183.1
Iodine number (mg I/g sample)	54.7	n.d	58.4	51.5

Note: n.d. - not determined.

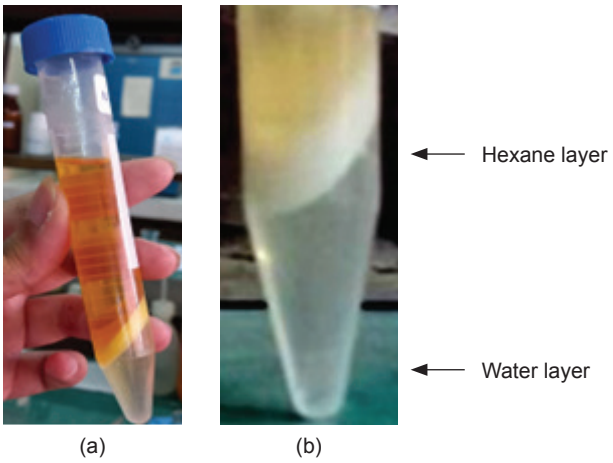

Figure 1. Formation of SFAE from (a) CPO hydrolysate and (b) palm oil hydrolysate.

Conversion Rate and Degree of Esterification

The esterification between carboxylic acids and alcohols is a reversible reaction. This study used an excessive amount of fatty acids to shift the equilibrium towards the formation of SFAE. The amount of reacted fatty acids was used to calculate the conversion rate and the degree of esterification (*Table 3*). The conversion rate and degree of esterification vary for each SFAE. In general, the Eversa® Transform 2.0 lipase used in this study preferred oleic acid, followed by stearic acid and palmitic acid. Depending on the source, lipases exhibit selectivity toward certain fatty acids (Halldorsson *et al*., 2004).

TABLE 3. CONVERSION RATE AND DEGREE OF ESTERIFICATION DURING THE SYNTHESIS OF SFAE

Sample's name	Conversion rate (%)	Degree of esterification
SFAE palmitic acid-mannitol	11.70	0.8
SFAE palmitic acid-mannose	21.40	1.3
SFAE palmitic acid-fructose	27.30	1.6
SFAE stearic acid-mannitol	30.30	1.8
SFAE stearic acid-mannose	20.60	1.2
SFAE stearic acid-fructose	13.90	0.8
SFAE oleic acid-mannitol	39.73	2.4
SFAE oleic acid-mannose	19.11	1.1
SFAE oleic acid-fructose	20.67	1.2
SFAE CPO-mannitol	74.80	4.4
SFAE CPO-mannose	58.10	2.0
SFAE CPO-fructose	61.00	3.7
SFAE palm oil-mannitol	22.40	1.3
SFAE palm oil-mannose	34.30	1.4
SFAE palm oil-fructose	21.80	2.0

Note: SFAE - sugar fatty acid esters; CPO - crude palm oil

Most of the SFAE obtained in this study had a low degree of esterification. Zheng *et al*. (2015) reported that the products of enzymatic esterification commonly had a low degree of substitution. Lipase enzymes are more selective towards primary hydroxyl groups because of their low steric hindrance (Zago *et al*., 2021). Based on their structures, D-mannitol and D-fructose have two primary hydroxyl groups, whereas D-mannose only has one. Therefore, the degree of esterification of D-mannitol and D-fructose SFAE should be higher than that of D-mannose. SFAE of CPO hydrolysate, SFAE of oleic acid and SFAE of stearic acid showed the highest degree of esterification in D-mannitol. The hydroxyl groups of D-mannitol were assumed to be more reactive than those of D-fructose because D-mannitol does not form furanose or pyranose structures in solution. Meanwhile, the SFAE of palm oil hydrolysate and the SFAE of palmitic acid exhibited different behaviours, with D-fructose possessing the highest degree of esterification. Although the fatty acid compositions of CPO hydrolysate and palm oil hydrolysate were similar, the SFAE of CPO hydrolysate displayed a higher degree of esterification than SFAE-palm oil. The non-fatty acid components of the CPO hydrolysate may promote lipase activation. Sterol, vitamin E, dolichol and polyprenol are components of CPO (Goon *et al*., 2019) that may be responsible for lipase activation. Research by Delome *et al*. (2011) proved that the presence of surfactant can increase the activity of lipase by changing the three-dimensional structure of the enzyme and improving the accessibility of the substrate to the active site.

FTIR and NMR Spectra of SFAE

The formation of SFAE was confirmed by using FTIR and NMR spectra. The representative data of the FTIR spectra can be seen in *Figure 2*. The FTIR spectrum of the fatty acid (palmitic acid) showed a typical very broad hydroxyl peak at 3,400-2,500 cm^{-1} and a sharp carbonyl peak absorption of carboxylic acid at 1,703 cm^{-1}. On the other hand, the hydroxyl group of the D-fructose and D-mannitol appeared as a broad peak at 3,400-3,000 cm^{-1}. The absence of a very broad hydroxyl peak of the carboxylic group in the SFAE indicated the formation of an ester group between fatty acid (palmitic acid) and saccharides. Moreover, the shifting of the carbonyl peak to a higher frequency (1,734 cm^{-1}) also proved the presence of an ester group of SFAE.

The ^{13}C-NMR spectrum (*Figure 3a*) of SFAE palmitic acid-fructose exhibited the presence of carbonyl groups of esters at 175 and 176 ppm. The formation of an ester between palmitic acid and fructose can also be seen from the ^{1}H-NMR spectrum (*Figure 3b*). Based on the peak integral

value, it can be concluded that the degree of esterification of SFAE palmitic acid-fructose was more than one. The number of protons from the palmitoyl was about twice the number of protons from the D-fructose. However, the peak of fructose's acidic protons (the hydroxyl groups) cannot be seen clearly because the acidic protons are able to exchange with deuterated methanol as the analysis solvent. This data was suitable with the degree of esterification of SFAE palmitic acid-fructose.

Emulsification Properties of SFAE

The surfactant properties of the starting materials (fatty acids and saccharides) and SFAE obtained in this research were examined by emulsification test in water-in-oil (*w*/*o*) and oil-in-water (*o*/*w*) systems. Emulsion stability was determined based on visual observation. The saccharides could not stabilise both *w*/*o* and *o*/*w* mixture. The *o*/*w* mixture with the addition of fatty acids gave a milky system that underwent phase separation after 15 min. The milky system consisting of a *w*/*o* system and fatty acids was stable for up to 30 min. Fameau *et al*. (2012) described that the fatty acids can reduce the surface tension.

The addition of SFAE prolonged the phase separation of the *w*/*o* and *o*/*w* system. All SFAE were more suitable for *w*/*o* emulsions than *o*/*w* emulsion systems. The phase separation was not

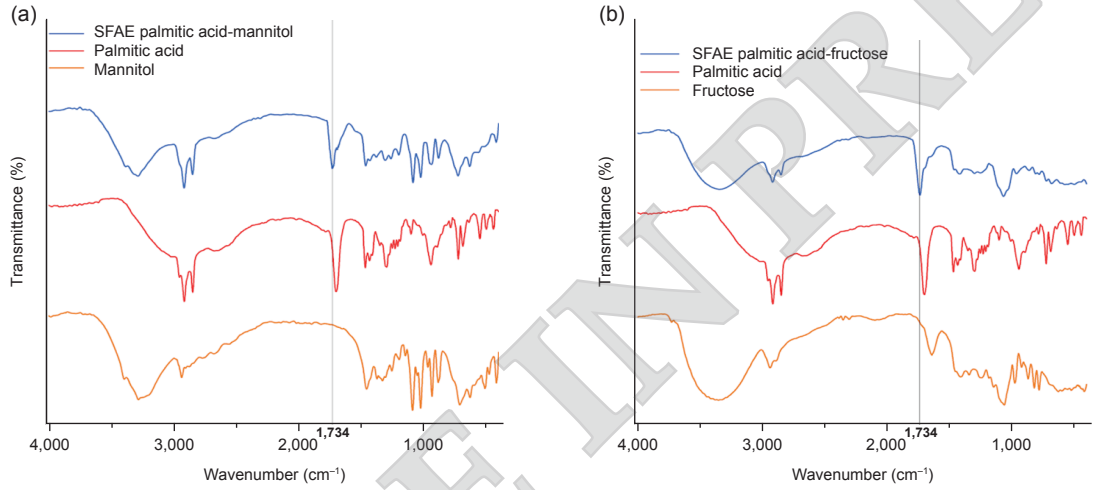

Figure 2. FTIR spectra of (a) SFAE palmitic acid-fructose and (b) SFAE palmitic acid-mannitol compared to its starting material.

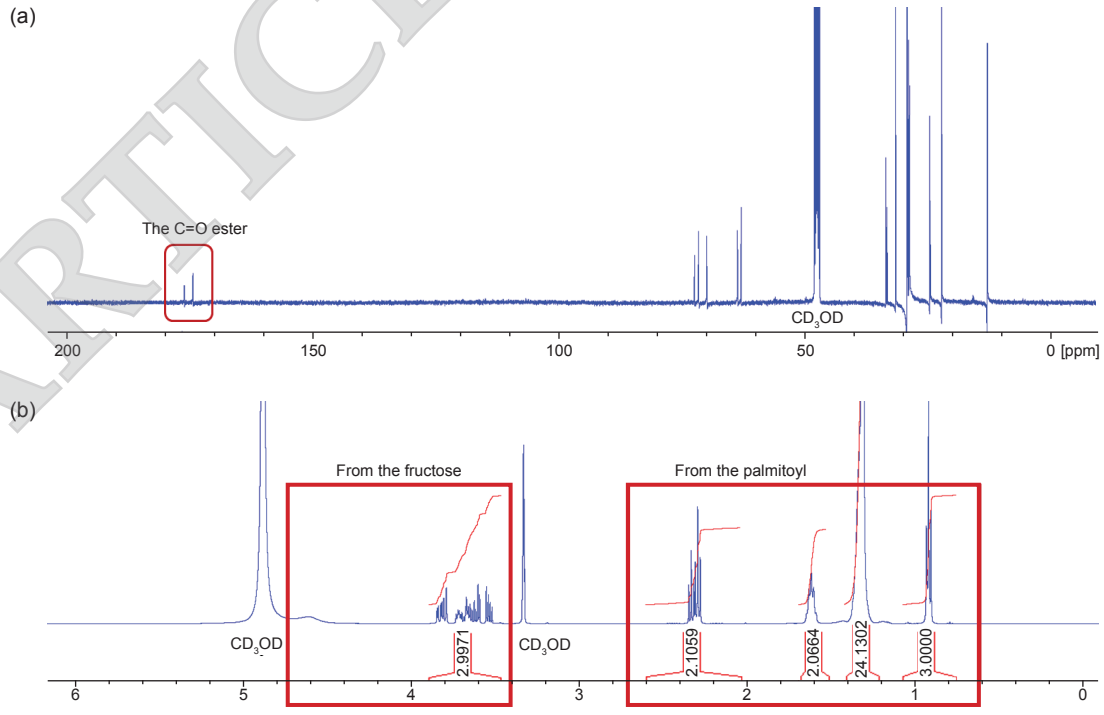

Figure 3. (a) ^{13}C-NMR spectrum and (b) ^{1}H-NMR spectrum of SFAE palmitic acid-fructose.

observed on the *w/o* emulsion system containing SFAE after 24 hr. On the other hand, phase separation of the *o/w* system was noticed after 4 hr. SFAE possesses emulsifying properties because of the polar residues from sugar and nonpolar residues from fatty acids (Teng *et al*., 2021). Representative data are presented in *Figure 4a* and *4b*. *Figure 4c* shows the microscopic observation of the *w/o* emulsion. Blue indicates the water phase.

Cytotoxic Activity Against MCF-7

The cytotoxic activity of SFAE was determined using an MTT assay based on mitochondrial enzyme activity in viable MCF-7 breast cancer cells (Perinelli *et al*., 2016). The cytotoxic activity of SFAE varied, with IC_{50} values between 29 and 200 ppm (*Table 4*). The SFAE of saturated fatty acids (palmitic and stearic acid) was more cytotoxic than the SFAE of oleic acid. These data are consistent with our previous research on the cytotoxicity of lipoamide derivatives. Lipoamides from saturated fatty acids exhibited the lowest IC_{50} value compared to lipoamides from unsaturated fatty acids (Wukirsari *et al*., 2024). The presence of the C=C bond is disadvantageous for cytotoxicity.

The cytotoxicity SFAE of D-mannose was lower than the SFAE of D-fructose and D-mannitol. The hydrophilic part SFAE of D-mannose (a pyranose ring) is bigger than D-fructose (a furanose ring) and D-mannitol (a linear chain). Among the SFAEs of D-fructose and D-mannitol synthesised from the same fatty acids, in general, the cytotoxic activity was inversely proportional to the degree of esterification. The SFAE of CPO hydrolysate with a higher degree of esterification also possessed lower cytotoxicity than the SFAE of palm oil hydrolysate. It seemed that the large size of SFAEs was unfavourable for SFAE-cell membrane interaction. According to Bunchongprasert and Shao (2020), the bulky size of the hydrophilic parts of fatty acid ester increased the intermolecular interactions of micelles and limited the SFAE interactions with the phospholipid bilayer membrane of cancer cells. The disruption of cell membrane integrity is important for the cytotoxic activity of SFAE (Bunchongprasert & Shao, 2020). Okabe *et al*. (1999) stated that SFAE inhibited cancer cell proliferation by suppressing the secretion of TNF-α.

TABLE 4. CYTOTOXIC ACTIVITY OS SUGAR-FATTY ACID ESTERS (SFAE) AGAINST MCF-7 CELL LINE

Sample's name	IC_{50} (ppm)
SFAE palmitic acid-mannitol	29.5 ± 3.6
SFAE palmitic acid-mannose	95.9 ± 5.4
SFAE palmitic acid-fructose	78.4 ± 6.0
SFAE stearic acid-mannitol	54.3 ± 2.2
SFAE stearic acid-mannose	104.4 ± 7.2
SFAE stearic acid-fructose	29.7 ± 2.0
SFAE oleic acid-mannitol	127.4 ± 3.6
SFAE oleic acid-mannose	>200
SFAE oleic acid-fructose	125.2 ± 1.1
SFAE CPO-mannitol	131 ± 28
SFAE CPO-mannose	107 ± 11
SFAE CPO-fructose	66.0 ± 5.5
SFAE palm oil-mannitol	65.5 ± 1.0
SFAE palm oil-mannose	102.8 ± 3.0
SFAE palm oil-fructose	65.5 ± 1.0

Note: SFAE - sugar fatty acid esters; CPO - crude palm oil

Antimicrobial activity Against *C. acnes*

Using the broth dilution method, SFAE applications in the cosmetic sector were investigated by determining their antibacterial activity against the acne-causing bacterium *C. acnes*. The assay was conducted at a 0.9 mg/mL concentration for all samples. Bacterial growth was directly proportional to the optical density value. Therefore, samples with lower optical densities displayed higher antimicrobial activity. CPO hydrolysate, palm oil hydrolysate and SFAE showed antibacterial activity against *C. acnes* (*Table 5*). The sugar moiety of SFAE

(a) (b) (c)

Figure 4. Emulsification of SFAE properties in the (a) w/o and (b) o/w emulsion systems. (c) Observation of the w/o emulsion under a microscope; the blue colour indicates water dyed with methylene blue.

exhibited variable effects on antibacterial activity against *C. acnes*. Based on its cell wall composition, *C. acnes* is classified as a Gram-positive bacteria (Mayslich *et al*., 2021). Zhao *et al*. (2015) mentioned that Gram-positive bacteria were more sensitive towards SFAE than Gram-negative bacteria. Many researchers have also reported the antibacterial activity of fatty acids against *Propionibacterium acnes* (*C. acnes*) (Casillas-Vargas *et al*., 2021; Desbois & Lawlor, 2013; Kim *et al*., 2021). Several mechanisms for the antibacterial activity of fatty acids have been reported. Unsaturated fatty acids have been reported to prevent bacterial growth by interfering with DNA replication, cell wall formation and protein synthesis (Desbois & Lawlor, 2013). The disruption of bacterial cell membrane permeability by SFAE has been proposed by Zhao *et al*. (2015). Zhao *et al*. (2015) observed the leakage of bacterial cell components such as protein and reducing sugars after being treated with SFAE. Disruption of the microbe membrane is associated with the emulsification properties of SFAE (Falk, 2019; Pirog *et al*., 2019). The emulsification properties are influenced by the degree of esterification of SFAE and its HLB value (Chen *et al*., 2023). Therefore, the CPO and palm oil hydrolysate and also SFAE are promising candidate as active ingredient in anti-acne agents. The addition of SFAE as an ingredient in anti-acne products will provide dual benefits, *i.e*., as an antibacterial and emulsifier agent. The strong antimicrobial activity of SFAE was also stated by Yang *et al*. (2024).

TABLE 5. ANTIMICROBIAL ACTIVITY AGAINST *Cutibacterium acnes*

Sample's name	Inhibition (%)
CPO Hydrolysate	86 ± 17
SFAE CPO-mannitol	100 ± 0
SFAE CPO-mannose	23
SFAE CPO-fructose	35 ± 12
Palm oil hydrolysate	100 ± 0
SFAE palm oil-mannitol	35 ± 9
SFAE palm oil-mannose	39 ± 9
SFAE palm oil-fructose	81 ± 6
Salicylic acid (45 mg/mL)	25 ± 1.7

Note: SFAE - sugar fatty acid esters; CPO - crude palm oil

CONCLUSION

In this study, SFAE were synthesised through enzymatic esterification using the lipase enzyme Eversa® Transform 2.0. The potent cytotoxic effect was attributed to the saturated fatty constituents (acyl group) of the SFAE and SFAE having a low degree of esterification. SFAE of palmitic acid-mannitol and SFAE of stearic acid-fructose showed the highest cytotoxic activity against MCF-7 breast cancer cells, with an IC_{50} value of 29 ppm. The SFAE of palm oil hydrolysate with a lower degree of esterification exhibited stronger cytotoxic activity than the SFAE of CPO hydrolysate. In contrast, SFAE from CPO hydrolysate and D-mannitol showed the strongest antibacterial activity achieving 100% inhibition against *C. acnes* at 0.9 mg/mL.

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