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Evaluation of Cytotoxicity and Antibacterial Properties of Bio-Based Surfactants Synthesized from Palm-Based Oleochemicals

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Personal protective equipment such as glove is widely used to protect the hands against chemicals or infectious agents. Surfactants in the glove act to reduce the surface tension and stabilise the rubber particle. However, some of the surfactants may exert toxicity to skin cells. Hence, a series of novel palm oil-based surfactants was synthesised to replace the conventional surfactants in the glove. This study aims to determine the cytotoxicity of this series, namely PS1, PS2, PS3 and PS4. The surfactants were synthesised from different palm-based oleochemicals. The typical ester bond (intense C=O stretch at 1740 cm^{-1}) in the backbone of these surfactants were identified from the Fourier transform infrared (FTIR) spectra, indicating the successful synthesis of these surfactants. Gel permeation chromatography (GPC) results indicate that these surfactants are polydispersed, with the number average molecular weight (M_n) ranged around 2000. These surfactants showed critical micelle concentration (CMC) values ranging from 2.215×10^{-3} to 3.075×10^{-3} mM/L with surface tension recorded between 24 and 36 mM/m. 2,5-Diphenyl-2H-tetrazolium bromide (MTT) assay was used to investigate the cytotoxicity of these surfactants against HaCat keratinocytes. Agar dilution susceptibility testing technique was employed to determine the antibacterial activity of the surfactants. All surfactants were non-toxic towards HaCat keratinocytes at 1.56 mg/mL. However, they exhibited dose-dependent toxicity, especially at higher concentrations (25 and 50 mg/mL). Results showed that PS1, PS2 and PS3 slightly reduced the growth of *Enterococcus faecalis* ATCC 29212 and *Streptococcus mutans* ATCC 25175 but not Gram-negative bacteria. However, none of the tested bacteria was susceptible to PS4. In conclusion, these surfactants tested exhibited moderate toxicity on skin cells and possessed mild antibacterial activities.

Keywords: Cytotoxicity; antibacterial; bio-based surfactants; palm-based oleochemicals

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Different types of glove including natural rubber latex gloves are commonly worn to protect our hands when contact with infectious materials, radioactive materials, or hazardous chemicals [1]. Latex gloves exhibited more toxicity and immediate allergenicity than non-latex gloves [2]. Surfactants are commonly used as wetting agents in the manufacturing of rubber gloves [3]. They are added into coagulant solution to reduce surface tension of the dispersion to ensure even coating on the former during dipping. Besides, surfactants are also used as stabilizers in natural rubber latex (NRL) [4]. For example, potassium laurate which can be found in carboxylate soaps acts as a key stabilizer and protective ingredient for NRL [5]. Sodium dodecyl sulphate (SDS) acts as protein denaturants to produce deproteinized NRL. However, these surfactants might raise some toxicity concerns with their limited bio-degradability, causing environmental pollution.

Surfactants will form foam and froth in the wastewater system owing to their surface-active properties. Surfactants in the environment can promote the solubility of toxic organic compounds in soil, enhancing the mobility of toxic agents and further contaminating the aquatic environment [6]. Surfactants are also capable of penetrating the cell membrane and thus cause toxicity to living organisms [7].

Presently, the synthetic surfactants are more widely applied in the industry compared to the natural surfactants. Natural surfactants are derived from animal or vegetable fats. Traditionally, people make soap from

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olive, palm kernel, coconut oil, or avocado oils with sodium hydroxide, or in ancient times, wood ash. Biosurfactants are produced by a variety of micro-organisms from various natural substances including waste materials. Synthetic surfactants are surfactants that produced by chemical synthesis, and mostly using petrochemicals as main source. The synthetic process is commonly conducted under very energy-intensive conditions with high usage of fossil fuels. The natural surfactants are more favourable as the synthetic surfactants will lead to environmental issues [8, 9]. For example, alkylphenol ethoxylates (APEs) are made from alkylphenols and ethylene oxide polymer chains which both derived from petrochemicals. The petroleum-based surfactants have many good characteristics such as good at detergency and wetting. However, the disadvantages of using petroleum-based surfactants include they will contribute to the depletion of a non-renewable resource and are highly polluting. They are slightly biodegradable and will release toxic chemicals when they decompose. Petroleum hydro-carbon compounds bind to soil components, and they are difficult to be removed or degraded. The surfactants in the detergents can disrupt lipid membranes that protect cells, and cause irritation to skin, eyes, and respiratory systems. Thus, the natural surfactants are more favourable than the synthetic surfactants.

Bio-based surfactants are usually produced by chemical synthesis, integrating fats, sugars or amino acids from renewable sources or agro-industrial waste materials [8, 9]. Bio-based surfactants have many advantages such as lower toxicity, higher bio-degradability, and effective at extreme temperatures or pH values. They also exhibit biocompatibility and digestibility. They can be economically produced and show better environmental compatibility. Palm oil has been widely used as a feedstock for surfactants used in industrial applications and consumer products. Palm oil is an eco-friendly compound and harmless to the human. The palm kernel oil (PKO) is non-cytotoxic on HepG2 cells even at high exposure level [10]. The protease and pepsin-pancreatin hydrolysates from PKO protein were non-cytotoxic on HepG2 cells even at high exposure levels as determined using WST-1 cell proliferation assay. Thus, palm oil-based surfactants are commonly found in most of cleansing, laundering, and personal care products [11]. These surfactants were also reported to demonstrate promising potential for other applications. Lee *et al.* has reported anionic and non-ionic surfactants were successfully synthesized from palm oleic acids for coating applications [12]. These surfactants exhibited promising anti-foaming, wetting and plasticizing properties, which could avoid forming defects in surface coatings, aqueous coating formulations and impart softness and flexibility in the films. In a study conducted by Afida *et al.*, the tested palm-based methyl ester sulphonates (MES) homologues were readily biodegradable with percentage of bio-degradation achieved for C12, C14 and C16 MES was 73% within 6 days, 66% within 8 days and 63% within 16 days, respectively and all the results surpassed the pass level of 60% [11]. Thus, palm-based surfactants are more environmentally friendly and can be used as an alternative to petroleum-based surfactant. However, the cytotoxicity of these palm oil-based surfactants generally has not been determined.

The production of fatty alcohols through hydrogenation of methyl esters from palm oil was described by Echeverri *et al*. [13]. Hydrogenation of palm oil methyl esters with Ru-Sn/Al_2O_3 catalysts produced a mixture of saturated alcohols containing 16 to 18 carbon atoms, oleyl alcohol and heavy esters. In addition, Lim *et al*. successfully synthesized palm-based lauryl alcohol ethoxylate surfactant through ethoxylation reaction of lauryl alcohol with ethylene oxide using potassium hydroxide as the catalyst [14]. In a study conducted by Moore *et al*., a homologous series of non-ionic alcohol ethoxylate surfactants with the same head group size but varying number of carbon atoms in their tail group from 10 to 16 was found to exert bacteriostatic activity against *Staphylococcus aureus* and *Escherichia coli* [15]. It was reported in another study that non-ionic alcohol ethoxylate surfactants significantly increased the membrane fluidity of *Proteus mirabilis* and *S. aureus* but only showed moderate biocidal activity towards both bacterial strains. The results reveal that no correlation could be made between the extent of membrane fluidization by the surfactant and the biocidal activity [16]. Hence, it is clearly indicating that different surfactants display distinct antibacterial activity, either inhibiting/slowing their growth or killing them. This might be influenced by many factors, such as the chemical structure of the surfactants, the electrostatic properties of the surfactant solution or the bacterial cell wall characteristics and so on. The glove that coated with bioactive compounds with bactericidal effect to eliminate those bacteria could be one of a very important key for the personal protective equipment, especially the medical glove. It is not just to protect the user's hands, but these bactericidal properties of the glove also prevent the spread of pathogenic bacteria when cross-contamination risk is present due to the misuse of the glove by healthcare workers.

Hence, the objective of this study is to investigate the cytotoxicity and antimicrobial properties of bio-surfactants which are synthesized from palm-based oleochemicals. The compounds used in this study were PS1, PS2, PS3 and PS4. For cytotoxicity study, these biosurfactants were tested on human keratinocytes (HaCaT cells) since any future presence of them in gloves would directly affect keratinocytes of the skin epidermis. A reference set of experiment was also carried out using a commercial surfactant, SDS. 2,5-Diphenyl-2H-tetrazolium bromide (MTT) assay was carried out on the treated cells. The cytotoxicity results of both biosurfactants were compared with the results of the reference commercial surfactant, SDS, and the biosurfactants showed higher biocompatibility,

potentially making better alternatives to replace SDS in the future. On the other hand, the antibacterial activity of biosurfactants was screened on the targeted bacteria of Gram-positive and Gram-negative. Agar dilution susceptibility testing technique was employed to determine the antibacterial activity of the bio-surfactants. In general, preliminary results indicated that biosurfactants could slightly reduce the growth of certain tested Gram-positive bacteria.

EXPERIMENTAL

Chemicals and Materials

Four anionic biosurfactant samples, namely PS1, PS2, PS3 and PS4 were formulated and gifted by the Malaysian Rubber Board. Dulbecco's Modified Eagle's Medium (DMEM), penicillin-streptomycin, and TrypLE™ were purchased from GIBCO (Grand Island, NY, USA). Phosphate buffered saline (PBS) was purchased from VWR Life Science (Radnor, PA, USA). Sodium chloride, Tween-20 and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (Burlington, MA, USA). Fetal bovine serum (FBS) was purchased from GIBCO (3 Fountain Drive, Paisley, UK). Sodium dodecyl sulphate (SDS) was purchased from Fisher Bioreagents (China). Tryptic soy agar (TSA), tryptic soy broth (TSB), Mueller Hinton agar (MHA) and glycerol were purchased from Thermo Scientific (Waltham, MA, USA). All chemical reagents were reagent grade and were used without further purification.

Surfactant Synthesis and Characterization

All biosurfactants were synthesized through the similar polyesterification route as reported elsewhere [12], with minor differences in palm-based oleochemicals. The surfactants carried anionic charges and bio-compatible as they were reacted with palm-based oleochemicals and complete bio-sourced monomers. Table 1 summarizes the oleochemicals used to produce the biosurfactants.

Fourier Transform Infrared Spectroscopy Analysis

Samples were subjected to Fourier transform infrared (FTIR) analysis using a Thermo Scientific FTIR NICOLET 6700 series FTIR spectrometer. FTIR spectra were recorded with 128 scans from 4,000 to 600 cm^{-1} at a resolution of 1 cm^{-1} at ambient temperature.

Gel Permeation Chromatography Measurement

Samples were dissolved in tetrahydrofuran (THF) at 0.2% w/v and filtered through polytetrafluoroethylene filter with pore size of 0.45 µm. About 100 µL of the sample solution was injected into a gel permeation chromatography (GPC) instrument with THF was used as the mobile phase and a flow rate of 0.8 mL/min was employed. The GPC machine used was Malvern GPC Viscotek using 3 CLM3005-T5000, Organic GPC/SEC columns with calibration was performed against polyisoprene standards. The results were recorded as number-average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersity index (PDI).

Surface Tension Measurement

Surface tensions measurements were carried out using a Manual Kruss Easydrop DSA 25E, Hamburg, Germany. Samples were prepared in a concentration ranged from 10^{-3} to 10^{1} and measured with a time interval of two minutes. Surface tension measurements were performed using pendant drop method based on the camera images and calculated by analyzing the shape of the hanging drop using Laplace equation and ADVANCE software. Each concentration of the sample was tested in triplicates at 25 ± 3 °C. The values of the critical micelle concentration (CMC) were determined from the curve of surface tension versus concentration of surfactant solutions.

HaCaT Cell Culture

HaCat cells were first cultured in T25 flasks that were supplemented with 6 mL of complete DMEM, which contained 10% FBS, and 1% penicillin-streptomycin. For sub-culturing, old medium was discarded, and 1 mL of PBS was added to the flask to wash the cells, and then discarded. Around 600 µL of TrypLe™ was added to the flask and incubated at 37 °C, 5% CO_2 for 8-10 minutes. Two milliliters of complete DMEM were added to the flask, and the cells were then transferred to a 15 mL tube. The tube was then centrifuged at 1500 rpm, for 5 minutes. The supernatant was removed from the tube, and 1 mL PBS was added to the tube, and centrifuged again under same condition, to wash the TrypLe™ from the cells. The PBS supernatant was then removed, and complete DMEM was added to the cell pellet in the tube, which was then resuspended. To sub-culture the cells, 100 µL cells from the tube

Table 1. Palm-based oleochemicals used in synthesizing palm-based biosurfactants.

Samples	Palm-based oleochemicals
PS1	Palm kernel oil and palm-based fatty alcohols
PS2	Palm kernel olein and palm-based fatty alcohols
PS3	Palm kernel olein and distilled palm-based fatty acids ranging from C8 to C20
PS4	Palm kernel olein and palm-based caprylic-capric acid blend containing medium-chain fatty acids (MCFAs) which are saturated fatty acids with 6–12 carbon atoms

was added into a new T25 flask that contained 6 mL of new complete DMEM, and then left to incubate at 37 °C, 5% CO_2 for future use.

MTT Assay

HaCat cells (1 x 10^5 cells/mL) were seeded onto 96 well plates and MTT assay was performed according to Koh *et al*. [17]. Different working concentrations of surfactants or SDS ranging from 1.56 to 50 mg/mL were prepared by using medium without FBS. Then, medium in each well was replaced with the newly prepared medium containing surfactant and left for incubation for 24, 48 and 72 hours. The three incubation periods represent effect of tested compounds at short, intermediate and prolonged time points, respectively. Once the 24-, 48-, and 72-hour incubations were completed, all the wells with cells in the 96-well plate were added with 20 µL MTT solution, which was prepared with 5 mg/mL MTT powder in PBS. The plates were then incubated at 37 °C, 5% CO_2 for 4 hours. The MTT containing medium was then discarded carefully from each well, observing purple formazan crystals. These crystals were dissolved by adding 50 µL of DMSO solution. The plates were then read using an Infinite 200 Pro microplate reader, at a wavelength of 570 nm, with a reference wavelength of 630 nm. The results are expressed as a percentage of cell viability using the following formula: Optical density (OD) sample/OD control x 100%. Untreated cells served as the control in this study.

Agar Dilution Method

The agar dilution tube method was carried out according to Clinical Laboratory Standards Institute (CLSI) guideline with some minor modifications [18]. This is a modified agar dilution method for the testing of antibacterial activities. In CLSI, the agar dilution method is usually used to determine the minimum inhibitory concentration of the antimicrobial agents. In this study, however, it was used to test the effect of biosurfactants against the initial inoculum spotted on the agar (10^4 cfu), followed by calculation of the survivability of tested bacteria. Initially, the bio-surfactant samples were dissolved accordingly in 9% aqueous DMSO with 5% v/v of Tween-20 to obtain 7.30% v/v of PS1, 6.08% v/v of PS2, 24.19% v/v of PS3 and 26.51% v/v of PS4 accordingly. The concentrations of the surfactants used in this study were different because different surfactants have different solubilities, and the concentrations used were the maximum level of concentration that could be achieved for each surfactant. A total of 2 mL of each solution was added to 18 mL of molten and tempered MHA. The mixtures were mixed immediately and vigorously to give final concentrations of PS1, PS2 PS3 and PS4 with their 10-fold diluted factor. Similarly, the final concentration of DMSO and Tween-20 in each tube was 0.9% and 0.5% v/v, respectively. The growth control tube contained only MHA with the presence of DMSO and Tween-20, but without any surfactant samples. Before that, ten different types of bacteria (*E. coli* ATCC 25922, *S. aureus* ATCC 29213, *S. aureus* ATCC 43300, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Acinetobacter baumannii* ATCC 15149, *Proteus vulgaris* ATCC 9920, *Streptococcus mutans* ATCC 25175 and *Enterococcus faecalis* ATCC 29212) were cultured in the Mueller Hinton broth (MHB) until its exponential phase at 37 °C and its cells density was adjusted to 10^8 cfu/mL (equal to 0.5 McFarland). Then, the bacterial suspensions were further diluted to 10^7 cfu/mL. A total of 2 µL of the bacterial suspension was dropped on the surface of agar in each tube followed by incubation at 37 °C for 20 hours. The tested tubes were then re-suspended thoroughly with fresh MHB and the number of bacteria in the suspension was determined by standard plate count approach after the incubation. The bacterial density in growth control tubes were quantified by the same method. The survivability of bacteria was calculated based on the density of viable bacteria ($\log_{10}$ transformed) at the 20-hour time point relative to the initiate viable bacterial density and expressed in percentage.

Statistical Analysis

For statistical analysis, Student's t-test was performed for each surfactant, and p value < 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Physicochemical Analysis of Palm-based Biosurfactants

The FTIR spectra of all palm-based biosurfactants are shown in Figure 1. All spectra were found to be identical, with the characteristic band of carbonyl groups in ester linkages was prominent in all figures and appeared as a strong band around 1742 cm^{-1}. This signifies all the palm-based biosurfactants are successfully synthesized into polyesters. In general, these biosurfactants are expected to possess the same distinctive chemical properties. Having the same functional groups will make these biosurfactants undergo the same or similar characteristic chemical reactions.

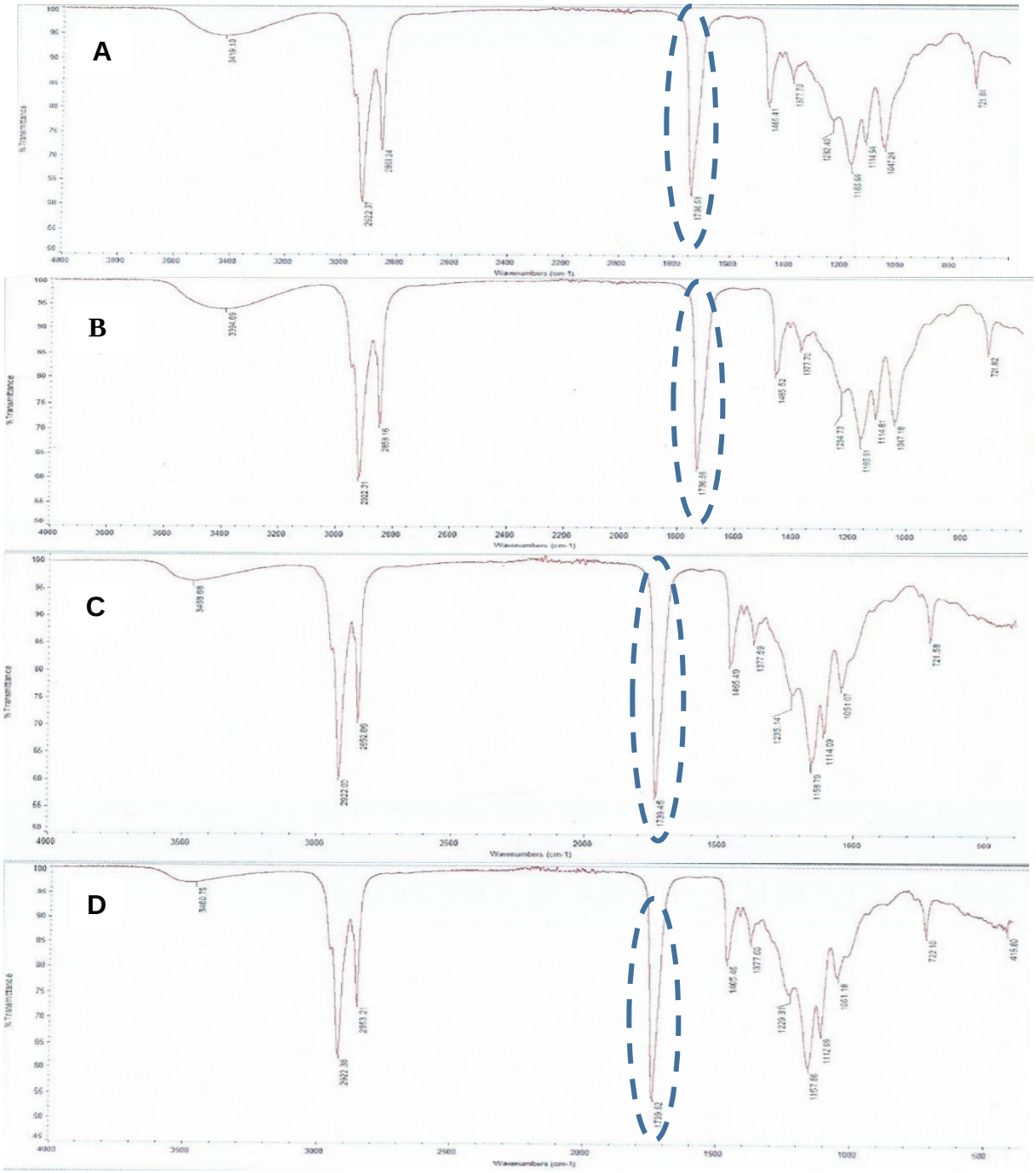

Figure 1. FTIR spectra of palm-based biosurfactants: (A) PS1, (B) PS2, (C) PS3 and (D) PS4.

Table 2. The gel permeation chromatography (GPC) results of palm-based biosurfactants.

Samples	GPC analysis		
	Number average molecular weight (M_n)	Weight average molar mass (M_w)	Polydispersity index (PDI)
PS1	678	913	1.346
PS2	658	852	1.294
PS3	897	1211	1.350
PS4	887	1173	1.322

Table 2 show the GPC results of all palm-based biosurfactants. From this table, the molecular weights of all biosurfactants were rather similar and rather low, with M_n and M_w ranged around 650 and 1200, respectively. The results also indicated that all surfactants were polydispersed, with PDI more than 1. It was expected that synthetic polymers exhibit polydispersity and different size distribution. This implied that heterogeneity occurs in these biosurfactants in the presence of polymer chains of unequal length.

One of the criteria of surface activity of surfactants is the CMC. Surfactant molecules have the tendency to form aggregates at CMC. Practically, the CMC is determined with a tensiometer by measuring the surface tension of a concentration series of studied biosurfactants. The CMC values were determined as the molar concentration at the intersection of two linear parts of the relationship $\gamma = f(\log C)$ above and below the discontinuity. Table 3 listed the surface tension and CMC values of all biosurfactants. The CMC graphs of all palm-based biosurfactants are sketched and illustrated as Figure 2. In the present study, all biosurfactants exhibited CMC values ranging from 2.215×10^{-3} to 3.075×10^{-3} mM/L with surface tension recorded between 24 and 36 mM/m. This indicates that these biosurfactants can form micelles when there is an increased entropy that causes the hydrophobic regions of the surfactants to be removed from water and formed ordered micelle structures. CMC is an important characteristic to justify the surface active properties of these biosurfactants.

Cytotoxic Activity of Palm-based Biosurfactants Against Human Keratinocytes

HaCat cell was used in this study as a preliminary test for the cytotoxicity because keratinocytes are the outermost layer of the skin. It will be first affected by the toxic substances through the direct contact between the skin and the glove [19]. According to ISO 10993-5, the cell viability percentage more than 80% considered as non-cytotoxicity; ranged between 60-80% as weak; 40-60% as moderate and less than 40% considered as strong cytotoxicity [20].

Table 3. Surface tension and critical micelle concentration (CMC) of palm-based biosurfactants.

Sample	CMC (mM/L)	Surface tension (γ, mM/m)
PS1	2.125×10^{-3}	24.67
PS2	3.075×10^{-3}	27.71
PS3	2.023×10^{-3}	36.29
PS4	2.413×10^{-3}	35.64

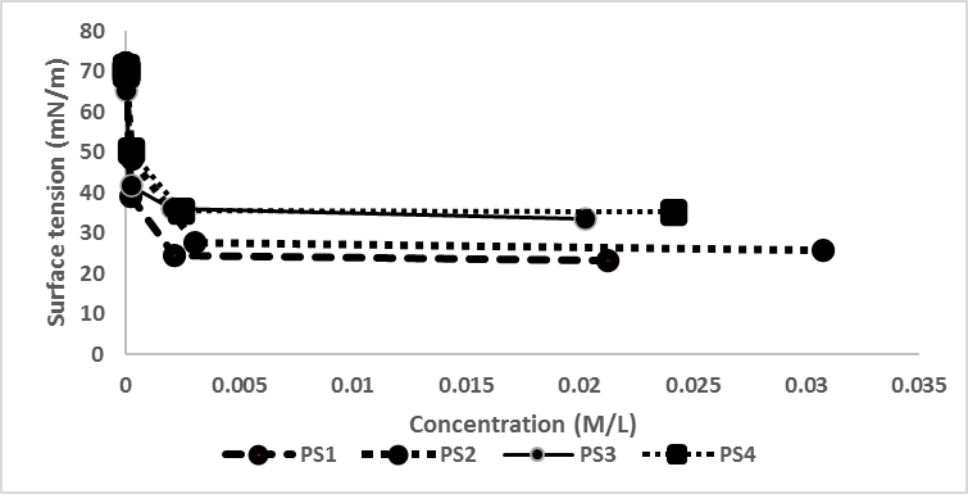

Figure 2. The critical micelle concentration (CMC) curves of palm-based biosurfactants.

Figure 3 illustrates the effect of palm-based biosurfactants on the viability of HaCat cell after 24, 48 and 72 hours treatment. SDS was used as the commercial surfactant reference. From Figure 3(A), it is clearly seen that the cell viability decreased significantly as the concentration of the PS1 increased. In fact, PS1 induced cell death significantly from 3.125 until 12.5 mg/mL. Thus, PS1 exhibited dose-dependent toxicity but not the time dependent as the cell which treated for 72 hours had higher viability compared to 24 and 48 hours of treatment. In general, the longer the time incubation, the lower the cell viability. However, in this case, cells could have re-proliferated after initial death, causing higher cell number at 72 hours. All the treated groups had the significant difference with the control group except 1.56 mg/mL of PS1.

From Figure 3(B), the decrease of the cell viability can be clearly observed as both the concentration of the PS2 and incubation time increased. Therefore, PS2 exhibited both dose- and time-dependent toxicity. In fact, the cell viability was more than 100% when 1.56 mg/mL of PS2 was treated for 24 and 48 hours. This might be caused by the presence of essential and non-essential amino acids in the surfactant. In a study by Chang *et al.*, oil palm kernel protein hydrolysates were found not cytotoxic to HepG2 liver cells but promoted and prolonged the HepG2 cell viability [10]. Toxicity of PS2 at 12.5 mg/mL after 24 hours treatment, 6.25 and 12.5 mg/mL after 48 hours treatment, as well as 3.125, 6.25 and 12.5 mg/mL after 72 hours treatment was significantly different from the control group. In general, it was found that PS2 was less cytotoxic compared to PS1 that might be due to the presence of more unsaturated fatty acids in palm kernel olein. From a study by Vlasta *et al.*, unsaturated fatty acids such as palmitoleic and oleic acids were able to inhibit the cell death induced by the saturated fatty acid such as palmitic and stearic acid in human pancreatic β-cell line NES2Y [21].

From Figures 3(C) and 3(D), it can be observed that the cell viability for both biosurfactants, PS3 and PS4, were above 80% until the concentration reached 3.125 mg/mL at 72 hours, stipulating dose-dependent cytotoxicity of both bio-surfactants. This might be due to the presence of palm-based fatty acids in both biosurfactants, particularly the presence of intracellular medium chain fatty acids (MCFAs). At higher concentrations, significant reduction in cell viability was observed, specifically at 6.25 mg/mL for PS3 and 25 mg/mL for PS4. Borrull *et al.* reported that the intracellular MCFAs such as C8 and C10 chains, which were mostly found in PS4, were able to promote the cell death [22]. Conversely, PS3 contained oleic acids which have higher proliferative nature and better blockade of proapoptotic activity. This implied that PS3 was more biocompatible than PS4. The presence of oleic acids could also be the reason that cell viability exceeded 100% at lower concentrations.

From Figure 3(E), the decrease of HaCat cell viability can be clearly observed as the concentration of SDS increased. However, SDS exhibited dose-dependent but not the time-dependent toxicity. All the treated groups showed significant differences from the control group. In fact, SDS was strongly cytotoxic to the HaCat cell as the cell viability was lower than 40% at various concentrations tested in this study. This was in agreement with Inácio *et al.* study, the anionic surfactants SDS induced cytotoxicity to all the tested cell types at a very low concentration and this was around its CMC values. This might be related to the destabilization or destruction of the cell membranes [23]. In short, palm-based biosurfactants are safer and comparatively the non-cytotoxic alternatives over SDS.

Antimicrobial Activity of Palm-based Biosurfactants

Many investigational studies have been done by researchers to evaluate the antibacterial property of palm oil and PKO. For instance, an investigation carried out by Akpomie *et al.*, revealed that *P. aeruginosa, S. aureus, E. coli* and *Corynebacterium* species were susceptible to PKO [24]. The high susceptibility of the bacteria strains might be attributed to the phytochemicals such as flavonoid, tannin, terpenoid and alkaloid detected in PKO. According to a study conducted by Ugbogu *et al.*, various PKO preparations including laboratory-prepared PKO with and without steeping, mechanically extracted PKO and commercially bought PKO showed inhibitory effects against *Streptococcus* species and *S. aureus* [25]. The lauric acid content was found to be the highest in the commercially bought PKO, followed by mechanically extracted PKO and the lowest in the laboratory-prepared PKO. Commercially bought PKO which contains the highest content of lauric acid showed the greatest inhibitory effect against both bacterial species. Thus, the antibacterial agent presents in PKO could be lauric acid. However, this inhibitory agent was not highly effective against the drug-resistant bacteria. Floriana *et. al.* reported that enzymatically hydrolyzed PKO exhibited greater antibacterial activity against *Salmonella typhi*, *S. aureus* and *E. coli* as compared to the unhydrolyzed PKO; and *S. aureus* was the most susceptible to both hydrolyzed and unhydrolyzed PKO among all bacterial strains. The antibacterial activity increased with the increase of hydrolyzed PKO concentration as more lauric acid and monolaurin that possess antibacterial properties were produced [26].

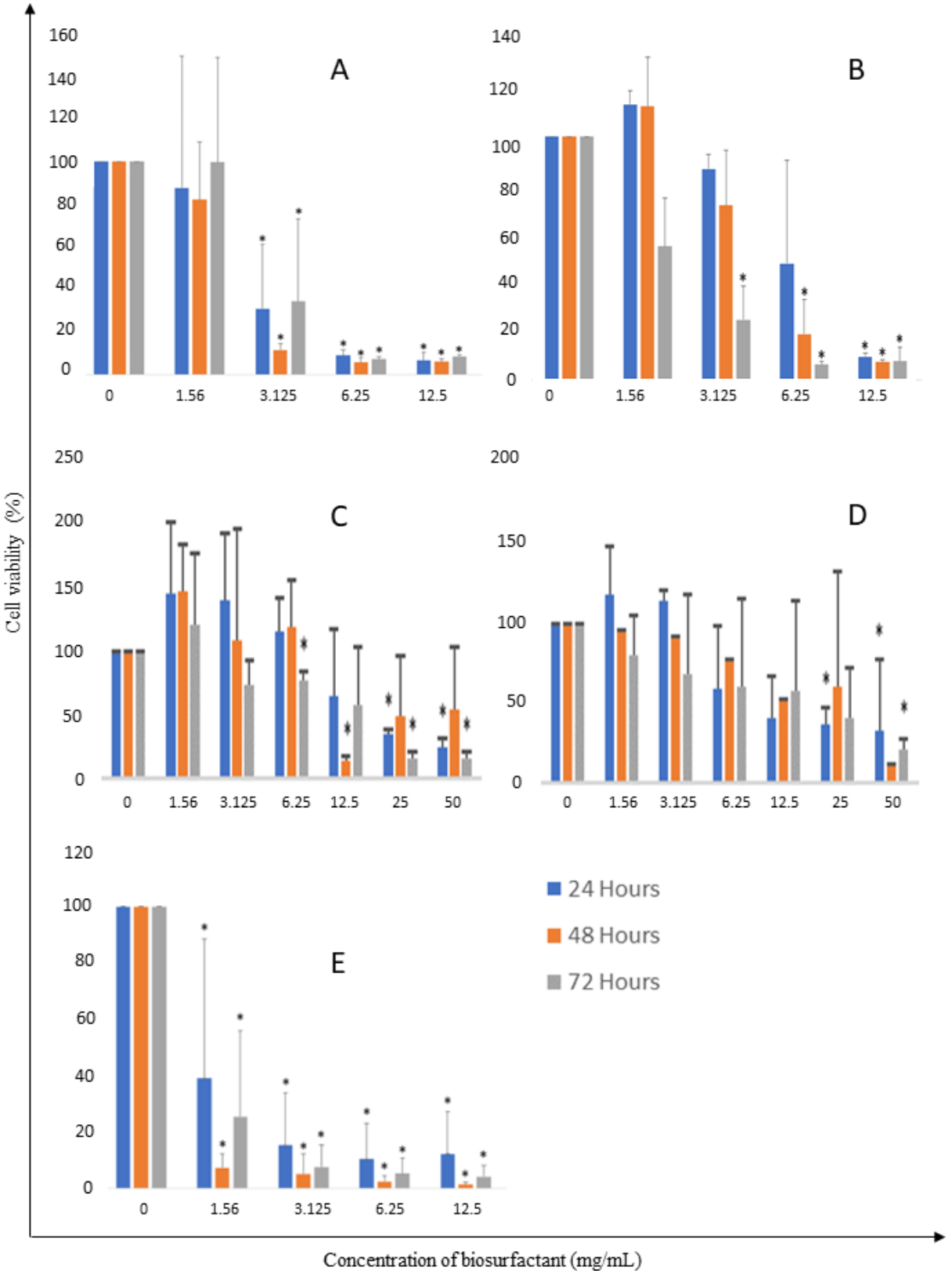

Figure 3. Effect of palm-based biosurfactants (A) PS1, (B) PS2, (C) PS3 and (D) PS4, and reference commercial surfactants (E) SDS on the viability of HaCat cell after 24, 48 and 72 hours treatment. Cell viability was presented as the percentage of the absorbance with 100% representing the group without treatment. The data was presented in mean ± SD (n=3). The symbol (*) in the graph denotes there is a significant difference between the control and the treatment groups at the p value < 0.05.

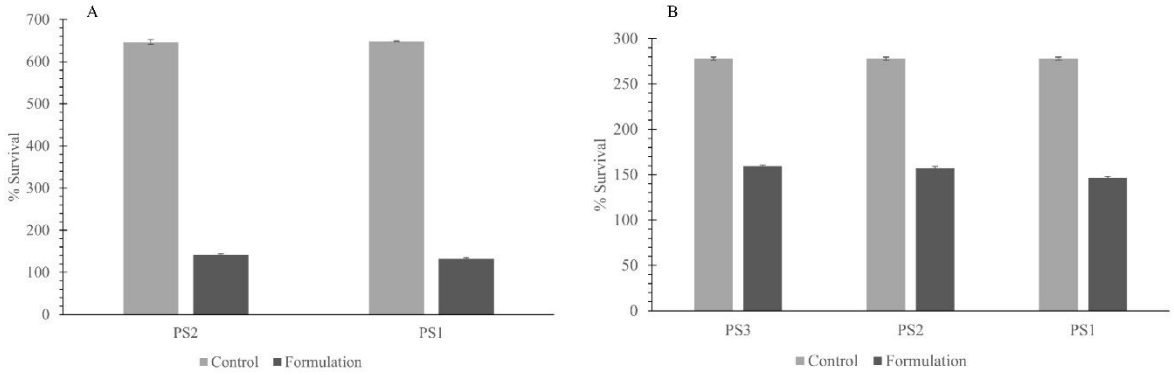

Figure 4. Antibacterial activity effects of palm-based biosurfactants on the growth of (A) *Enterococcus faecalis* ATCC 29212 and (B) *Streptococcus mutans* ATCC 25175. Note: The survivability of other tested bacteria is not shown because the formulated palm oil-based surfactants did not show antibacterial properties on them.

Palm oil and palm kernel oils have previously been shown to have antibacterial activities, particularly against the Gram-positive bacteria. They are more frequent in reducing the viable *S. aureus* compared to other tested bacteria as reported by Ling *et al.* and Jumina *et al.* [27, 28]. Figure 4 shows the antibacterial activity effects of palm-based biosurfactants on the growth of bacteria.

The biosurfactant formulations of PS1 and PS2 containing palm-based fatty acids and PK3 containing palm-based fatty acids ranging from C8 to C20, have slowed down the growth of bacteria upon 20 hours incubation period as shown in Figure 4. However, it was found that PS4 (a biosurfactant containing MCFAs) did not exhibit significant antibacterial effects on the 10 tested bacteria, including Gram-positive and Gram-negative bacteria. There was a study showed that PKOs did not have any antibacterial effects on *S. aureus* [29]. The difference of effects might be caused by different experiment designs and PKO extraction methods from the seeds of the palm. In contrast, the presence of palm-based fatty alcohols in PS1 and PS2 formulations was found to slow down the growing of the tested bacteria, particularly the growth of *E. faecalis* ATCC 29212 when compared to the growth control setup (Figure 4). This finding suggests that palm-based fatty alcohols in biosurfactants might react in halting the bacterial cell division. The growth of *S. mutans* ATCC 25175 (but not for *E. faecalis*) has also been retarded with not more than 200% survival relative to its initiate viability in the biosurfactant formulation of distilled palm kernel fatty acid in PS3, including palm-based fatty alcohols PS1 and PS2 as well. However, *E. faecalis* seems resistant to PS3 containing distilled palm-based fatty acids. It was also noted that neither of these biosurfactants (PS1, PS2 and PS3) showed any effects of bactericidal, bacteriostatic, or slowing the growth in other Gram-positive bacteria, such as *S. aureus* ATCC 29213, *S. aureus* ATCC 43300, and *S. epidermidis* ATCC 12228.

CONCLUSION

Four anionic biosurfactants containing different palm-based oleochemicals were tested for their cytotoxicity and antimicrobial properties. Though these surfactants demonstrated slightly cytotoxicity at higher dosage, they were found safer than SDS and could serve as potential alternatives to SDS. The antimicrobial test indicated that the palm-based biosurfactants generally exhibited antibacterial activity effects on the growth of Gram-positive *E. faecalis* ATCC 29212 and *S. mutans* ATCC 25175. However, neither of these biosurfactants showed any effects of bactericidal, bacteriostatic, or slowing the growth in other Gram-positive bacteria such as *S. aureus* ATCC 29213, *S. aureus* ATCC 43300, and *S. epidermidis* ATCC 12228.

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