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Antibacterial Activity of Self-Nanoemulsifying Drug Delivery System (SNEDDS) Loaded with Mangosteen (*Garcinia mangostana* L.,) Peels against *Bacillus subtilis, Bacillus cereus,* and *Staphylococcus aureus isolated* from Diabetic Foot Ulcer Patients

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ABSTRACT

Diabetic Foot Ulcer is a complication of diabetes mellitus characterized by open sores on the surface of the skin or mucous membranes as well as extensive dead tissue followed by bacterial invasion. Patients with Diabetic Foot Ulcer are resistant to some antibiotics so that alternative therapies such as potential traditional medicines involving mangosteen peels are being developed. Mangosteen peels contain compounds of phenolic, tannin, flavonoid, and xanton. To improve the stability of α-mangostin and improve bioavailability, it requires the preparation of Self-Nanoemulsifying Drug Delivery Systems (SNEDDS). This study aimed to analyze the antibacterial effect of optimal SNEDDS of ethyl acetate fraction from mangosteen peels against *Bacillus subtilis*, *Bacillus cereus,* and *Staphylococcus aureus* and to analyze the comparison of inhibition zone diameter between the optimal SNEDDS formula of the ethyl acetate fraction from mangosteen peels and ethyl acetate fraction without SNEDDS against *the bacteria*. This study began with making 70% ethanol extract from mangosteen peels which was then fractionated to obtain the ethyl acetate fraction. Furthermore, the ethyl acetate fraction was made into optimal SNEDDS formula resulted of *Design Expert software* with Tween 80, PEG 400, and VCO. The optimal SNEDDS formula of the ethyl acetate fraction from the mangosteen peels was tested for its antibacterial effectiveness against *Bacillus subtilis*, *Bacillus cereus,* and *Staphylococcus aureus*. The results revealed that SNEDDS loaded of ethyl acetate fraction from mangosteen peels had antibacterial activity against both types of Gram-positive bacteria, *Bacillus subtilis,* and *Staphylococcus aureus*, which cause diabetes ulcers, and that it did not have antibacterial activity against *Bacillus cereus*. Moreover, the tested SNEDDS loaded ethyl acetate fraction and ethyl acetate fraction without SNEDDS had strong inhibitory activity against *Bacillus subtilis.* Meanwhile, they provided such a smaller inhibition zone as tested against *Staphylococcus aureus* that they had moderate antibacterial activity.

Keywords: Diabetic Foot Ulcer; mangosteen peels; SNEDD;, ethyl acetate fraction

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease known as a silent killer because it often causes complications that lead to death without showing significant symptoms (Ministry of Health Republic of Indonesia, 2014). Diabetes mellitus was one of the most widespread epidemics the world has ever faced, both in developed and developing countries (Zimmert *et al.,* 2016). The latest global estimation of the International Diabetes Federation stated that in 2019 463 million people suffered from diabetes mellitus and the number will have been 700 million by 2045 (IDF, 2019). WHO stated that the number was 422 million in 2014 (WHO, 2016). It is concerned that the poor progression of DM sufferers will lead to complications, both microvascular (retinopathy and neuropathy usually followed by an ulcer, necrosis, and nephropathy) and macrovascular (coronary heart disease, stroke, and peripheral vascular disease). One of the DM complications is Diabetic Foot Ulcer (DFU), which is an infection, ulceration, or destruction of deep tissue associated with neurological abnormalities and various degrees of peripheral vascular disease in the lower extremities. Treatment of DFU required intensive empirical and definitive antibiotic therapy (Susanti *et al.,* 2016). Foot infection was one of the main diabetes mellitus complications and was a significant risk factor for amputation of the lower limb (Hefni *et al.,* 2013). An infection could be caused by pathogenic bacteria originating from the outside environment as well as by bacteria that form physiological microflora of the skin, like *Staphylococcus epidermidis, Staphylococcus aureus*, and *Propionibacterium acnes* (Citron *et al.,* 2007). *Bacteria Proteus*, *Pseudomonas, Staphylococci, Klebsiella, Bacillus,* and *Eschericia coli* were some of the bacteria that often infect diabetes wounds

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(Mohammad *et al.,* 2014).

Given the dangerous state of bacterial resistance to antibiotics which is concerned to cause frequent complications that are difficult to cure but extremely easily disseminated, this study aimed to test the SNEDDS activity of mangosteen peels against *Bacillus subtilis, Bacillus cereus,* and *Staphylococcus aureus* which are resistant to gentamicin and ciprofloxacin. According to Decroli et al. (2008), studying the profile of DFU and the effectiveness of antibiotics in patients with DFU hospitalized in SMF Pathology of Internal Diseases (Agistia *et al.,* 2017), some bacteria have been resistant to antibiotics such as cefotaxime, ceftriaxone, and ciprofloxacin used empirically. The inappropriate use of antibiotics results in unachieved therapy goals and antibiotic resistance. Therefore, to study types of compounds derived from natural materials such as mangosteen peels that can be used to protect against bacteria resistant to antibiotics is necessary.

Garcinia mangostana Linn is a tropical plant commonly known as mangosteen, originating from Southeast Asia. This plant was rich in xanthones and was known to have a variety of natural polysaccharides (Bennett and Lee, 1989). Xanthone compounds had anti-inflammatory, antioxidant, anti glycemic, antiproliferative, and antibacterial activity (Phuong *et al.,* 2017; Sypniewski *et al.,* 2017; Chang *et al.,* 2015). Xanthone has a solubility problem that requires a drug delivery system, one of which is SNEDDS. Self-Nanoemulsifying Drug Delivery Systems could improve the solubility and stability of essential oil-based formulations (Pedro *et al.,* 2013; Ujilestari *et al.,* 2018).

This study aimed to analyze the antibacterial effect of optimal SNEDDS of ethyl acetate fraction (EAF) from mangosteen peels against *Bacillus subtilis, Bacillus cereus*, and *Staphylococcus aureus* and to analyze the comparison of inhibition zone diameter between the optimal SNEDDS formula loaded ethyl acetate fraction from mangosteen peels and EAF without SNEDDS against the bacteria. This study is necessary to overcome antibiotic resistance in diabetic ulcer patients using a drug delivery system.

METHODOLOGY

Materials

The dark purple peels selected for the study were freshly from Central Java, Indonesia. The other materials were 70% ethanol (Dwicentra), n-hexane (Merck, German), ethyl acetate (Merck, German), methanol (Merck, German), aquades (Dwicentra), VCO (Bagoes), PEG 400 (Bratachem), Tween 80 (Bratachem), nutrient agar (NA) (Merck, German), Mueller-Hinton agar (MHA) (Merck, German), standard of α-mangostin (Sigma Aldrich, US) in the HPLC with purity 98%, chloroform (Merck, German), n-hexane (Bratachem), silica gel F254 (Merck, German), and 0.9% sterile NaCl..

Methods

Preparation of Plant Extracts

One kilogram of powdered mangosteen peels was taken in five different extraction thimbles and extracted via maceration for 72 hours using 70% ethanol. Extracted samples were evaporated using water bath until they become thick extracts (Pratiwi *et al.,* 2017a).

Preparation of Plant Fractions

Some condensed ethanol extracts were partitioned with n-hexane to obtain n-hexane soluble fractions and residues. Then, the residues were added with ethyl acetate to obtain ethyl acetate fractions and residues. Furthermore, the extracted soluble fractions of n-hexane, ethyl acetate fractions, and residues were collected and concentrated by a rotary evaporator and a water bath at 40±0.5 °C to obtain a thick fraction (Pratiwi *et al.,* 2017b).

Determination of the Ethyl Acetate Fraction Content of Mangosteen Peels on SNEDDS

Based on the test results for the ethyl acetate fraction content, SNEDDS ethyl acetate fraction from the mangosteen peels in a concentration of 150 mg / 5 mL was used because the concentration of 175 mg / 5 mL on SNEDDS showed that the system was unable to dissolve the fraction. This was indicated by the presence of deposits on three days of observation.

Preparation of SNEDDS Ethyl Acetate Fraction

Surfactants, co-surfactants and oils selected subsequently obtained 14 runs from *Design Expert software* in various mixture compositions for the three components to be optimized, namely Tween 80, PEG 400, and VCO with a ratio of 3.67: 1.67: 1.67; 3: 1: 3; 3: 3: 1; 1: 1: 5; 5: 1: 1; 1: 5: 1; 1.67: 1.67: 3.67; 2.33: 2.33: 2.33; 3: 3: 1; 1: 1: 5; 1: 5: 1; 1: 3: 3; 5: 1: 1; and 1.67: 3.67: 1.67. The fraction of ethyl acetate from mangosteen peels used was based on the results of the drug loading test. Optimum SNEDDS was obtained through assigning values and weights to responses, namely transmittance, pH, and emulsification time to obtain optimal desirability and contour plot formula values. Furthermore, the verification between the optimal SNEDDS prediction software and the optimum SNEDDS was carried out

(Pratiwi, 2021). The optimum SNEDDS was formulated with a combination of surfactant, co-surfactant, and oil, namely Tween 80, PEG 400, and VCO with a ratio of 4.98: 1.02: 1. Furthermore, SNEDDS ethyl acetate fraction (150 mg / 5 mL) was added. The mixture was conditioned in a water bath at 40° C for 10 minutes. The process of dissolving the fraction in the carrier was maximized with a sonicator for 15 minutes (Pratiwi, 2019).

Preparation TLC plate

The TLC plate was activated by placing it in an oven at the temperature of 110 ºC for 20 minutes. The plate was spotted with test and standard preparation, the mounting distance of 8 mm from the edge of a TLC plate. It was developed up to 75 mm in the twin trough chamber using mobile phase, dried in an oven, and subjected for TLC scanning 200-400 nm. Chromatographic analysis (TLC) was performed on silica gel 60 F254 (E.Merck, Germany, 20.0 x 10.0 cm) (Hareesh et al, 2010). The 5µL of standard solutions and samples were spotted on a TLC plate using Linomat 5 (Camag). TLC plate was placed into the vessel that has been saturated with a mixture of chloroform: ethyl acetate (85: 15). The vessel was closed and allowed to mix with the mobile phase until reaching the upper limit. The TLC plate was stabilized by putting them to room temperature for 30 minutes and then were scanned using CAMAG TLC scanner with winCATS software.

Determination of the Active Compound Using TLC-Densitometry Method

A stock solution of samples and standard α-mangosteen were prepared. Standard α-mangostin was in a concentration of 1 mg/10 mL. Prepared standard solutions with a volume of 1 µL, 2 µL, 3 µL, 4 µL, and 5 µL and samples were spotted on a TLC plate using Linomat 5 (Camag). Determination of the active compound was done using the densitometry method. The mobile phase consisted of chloroform: methanol (85:15 v/v) was used. The plate was heated at 110 °C for 10 minutes. The TLC plate was stabilized at room temperature for 30 minutes and then was observed using a CAMAG TLC scanner at 200-400 nm (Misra *et al.,* 2009).

Bacterial Samples from Patients

Bacteria were from the third and fourth Wagner degree of DFU patients in Pontianak's Kitamura clinic. The results of the identification were then tested for antibiotic sensitivity. The bacteria were grown on blood agar and Mac-Conkey. Planting bacteria was performed directly on solid agar media and incubated for 24 hours in an incubator at a temperature of 32-40 °C (Aulia, 2008).

Antibacterial Effectiveness Test

Sterilization of tools and materials was by covering the tools with aluminum foil and cotton. They were put in an autoclave, and it was set at 121°C with a pressure of 15 psi (per square inch) (Radji, 2010).

Nutrient Agar (NA) Media

As much as 23 grams of nutrient was dissolved in 1000 mL of sterile aquadest and was then heated until all dissolved. In the heat state, the solution was then put into Erlenmeyer, followed by checking the pH of the media ranging from 6.8 ± 0.2. The media were then sterilized at 121 °C autoclaves for 15 minutes (Difco, 1977).

Mueller-Hinton Agar (MHA) Media

MHA media were made by dissolving media (38 grams) in 1 L of distilled water while being heated. It was then sterilized by autoclaving at 121 °C for 15 minutes (Difco, 1977).

Bacterial Rejuvenation

The pure culture of the test bacteria from the NA media was etched aseptically with ose needle on the sloping NA media. Scratching was done by zigzagging the surface of the media. Then it was incubated at 37 °C for 24 hours.

Suspension of *Bacillus subtilis, Bacillus cereus dan Staphylococcus aureus*

Cultivating each bacterial suspension inoculated in a 24-hour rejuvenation medium was taken using an ose needle and was suspended into a tube containing 5 ml of 0.9% sterile NaCl solution. The turbidity obtained was then compared to the standard Mc. Farland no. 0.5 which was equivalent to the amount of growth of 1x108 bacterial cells / mL; after equivalent, this suspension was used as a test bacterium (Radji, 2010).

Test Solution Concentration

Mangosteen peels had a MIC value of 200 µg /mL as an antimicrobial (Priya *et al.,* 2010). Based on this, the test solution was made from SNEDDS of ethyl acetate fraction from mangosteen peels in a concentration of 200 µg/mL.

Testing of Antibacterial Activity

The antibacterial activity test used was the Kirby-Bauer disk diffusion test. Suspension of test bacteria as much as 20 µL was put into the media in Petri then rubbed with a sterile cotton cloth on the test media (Difco, 1977). Sterile cotton wool

Tabel I. Plant Determination

Kingdom	*Plantae*
Super Division	*Spermatophyta*
Division	*Magnoliophyta*
Class	*Magnoliopsida* (dicots)
Order	*Malpighiales*
Family	*Clasiaceae*
Genus	*Garcinia*
Species	*Garcinia mangostana L.*
Local Name	Mangosteen

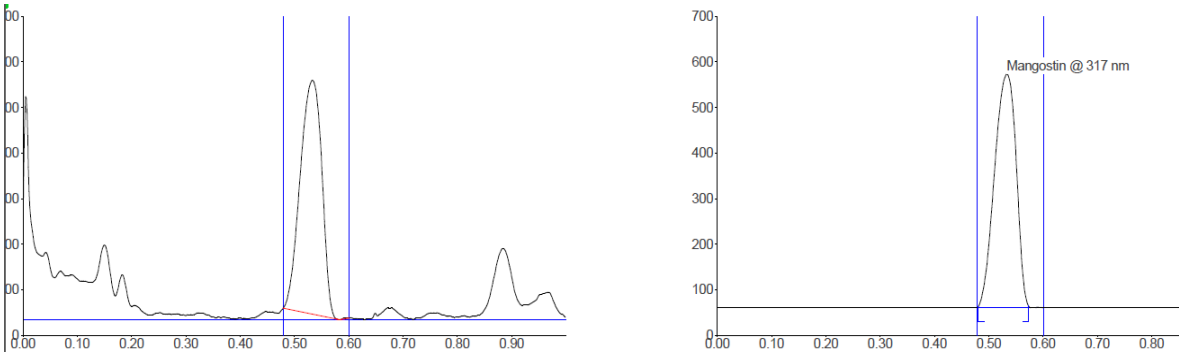


Figure 1. Densitogram profile derived from α-mangostin in ethyl acetate fraction with TLC Scanner

was rotated several times. This procedure was repeated twice. Paper discs used were of 6 mm diameter. The optimal SNEDDS formula was in a concentration of 200 mg/mL. Then the discs were placed on the surface of the media following the desired position. The media were then incubated at 37ºC for 24 hours, then the diameters of the inhibition zone were measured with the calipers expressed in millimeters (Sari *et al.,* 2015).

Data Analysis of SNEDDS Loaded with Ethyl Acetate Fraction

The data were analyzed with an independent sample t-test to compare the significant values of SNEDDS loaded EAF of mangosteen peels and EAF without SNEDDS.

RESULT AND DISCUSSION

Ethyl Acetate Fraction Preparation

The plants used in this study were identified in the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak. Table I shows the results of the determination.

Determination results showed that the plant samples used in this study were mangosteen, *Garcinia mangostana L*. The selection of extraction methods in this study was based on the sensitivity of antioxidant compounds to high temperatures. The extraction method was carried out without heating and at room temperature. The principle of extraction by maceration method was the process of diffusion of solvent in plant cells that contain active compounds. The diffusion made the osmotic pressure inside the cell different from the condition outside the cell so that compounds with the same polarity with the solvent were then pushed out due to different osmotic pressure inside the cell and outside the cell (Dean, 2009). The separation principle of the fractionation process was based on differences in polarity levels and a specific gravity between the two fractions. EAF was used as a sample in this study because of its semi-polar nature, making it easier to enter into the oil phase of SNEDDS. The easier the active compound enters into the oil phase, the better the SNEDDS preparation obtained. Fractionation is a separation procedure that aims to separate the main groups. Polar compounds would go into polar solvents, and non-polar compounds would go into non-polar solvents (Harborne, 1973).

Analysis of α-mangostin Content in EAF with Thin Layer Chromatography (TLC)

The α-mangostin content in the fraction can be obtained by the TLC test. Based on the TLC test, it was found that EAF from mangosteen peels contained 38.07 ± 1.026% α-mangostin. Another study using dichloromethane fraction from mangosteen peels showed α-mangostin content of

Table II. The α-mangostin content in test samples with TLC

Sample	Area	Concentration (mg/mL)	Average α-mangostin content (%) ± SD
Etyl acetate fraction of mangosteen peels	18982.10	37.11	38.07 ± 1.026
	20027.10	39.15	
	19420.70	37.97	

Note:

$\% = \frac{AU}{AP} x \frac{CP}{CU} x\ fx100$

AU : Test sample absorbance; AP : Standard absorbance (using standard AUC values that are closest to the AUC of the test sample); CU : Test sample concentration; CP : Standard Concentration; F : Dilution factor

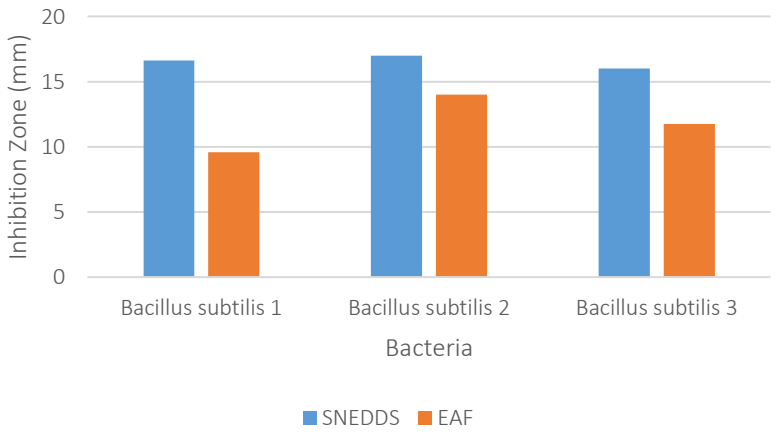


Figure 2. Inhibition zone SNEDDS EAF and EAF without SNEDDS to *Bacillus subtilis*

49.06 ± 0.80% (Widyanati *et al.,* 2014). TLC was a method that is used qualitatively and quantitatively to find out the number of active compound components in various samples (Agrawal *et al.,* 2012).

The effectiveness of SNEDDS loaded EAF against *Bacillus subtilis, Bacillus cereus, and Staphylococcus aureus*

The results showed that the SNEDDS loaded EAF had an inhibition zone of 16.54 ± 0.50 mm in *Bacillus subtilis*. The inhibition zone owned by the EAF loaded SNEDDS was greater than the inhibition zone in the EAF without SNEDDS, which was 11.78 ± 2.20 mm (Figure 2).

The resulted inhibition zone in the SNEDDS loaded EAF test for *Staphylococcus aureus* was of 9.45 mm ± 4.86. EAF without SNEDDS, nevertheless, the obtained inhibition zone was of 6.44 mm ± 0.39 (Figure 3).

Inhibition zones in SNEDDS loaded EAF were greater than EAF without SNEDDS. Based on the data analysis, the p-value was 0.003 <0.05 showing that there is a significant difference between SNEDDS loaded EAF and EAF without SNEDDS.

SNEDDS was able to increase the amount of α-mangostin which was transported through the stratum corneum over a certain time interval that could be revealed by diffusion test. The amount of α-mangostin transported on SNEDDS for the ethyl acetate fraction for 8 hours was greater than that of the ethyl acetate fraction of nanoemulsion. The ethyl acetate fraction without formulation had the smallest amount. This shows that the ethyl acetate fraction of mangosteen peel formulated with SNEDDS and nanoemulsion can increase the penetration of α-mangostin through the stratum corneum (Pratiwi *et al.,* 2017). The SNEDDS formula could improve absorption and dissolution because nano-sized droplets can increase the release of insoluble drugs. SNEDDS of Gem showed a significant increase in release rate compared to conventional tablets under the same conditions, in which 90% drug was released within 15 minutes in comparison to 30% from conventional tablets (Villar *et al.,* 2012). Formulations using polymers could increase the transport of hydrophobic

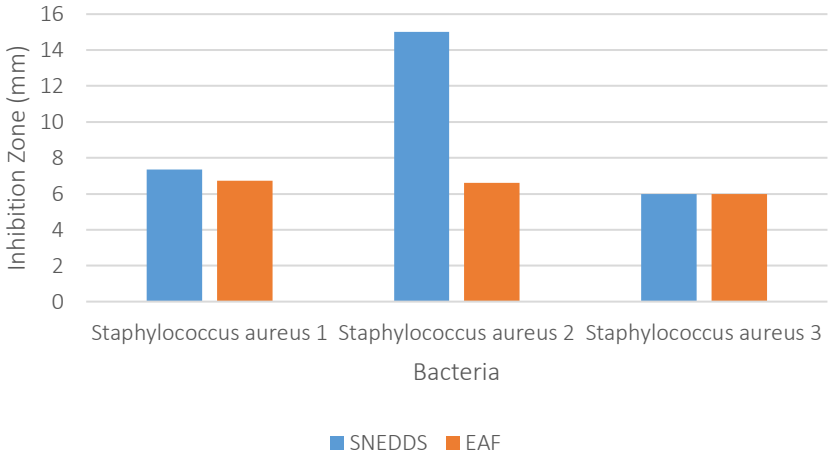
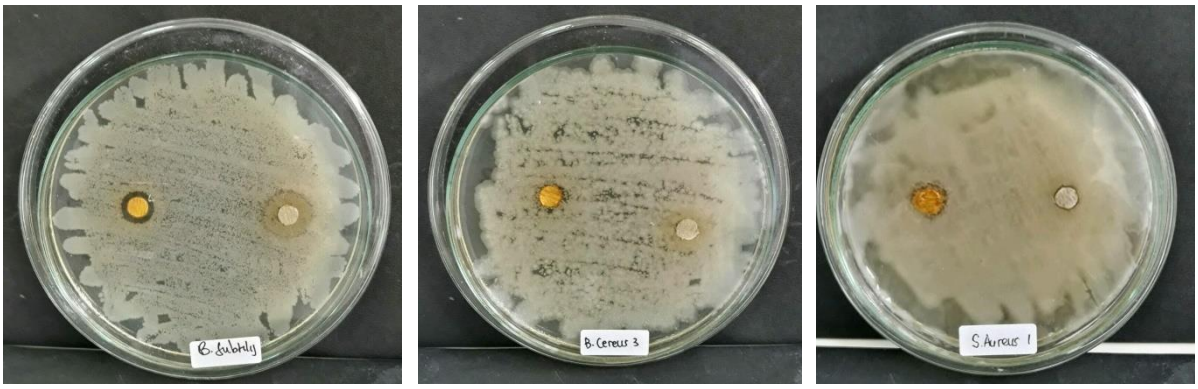


Figure 3. Inhibition zone SNEDDS EAF and EAF without SNEDDS to *Staphylococcus aureus*



(1) (2) (3)

Note: a. Ethyl acetate fraction without SNEDDS; b. SNEDDS ethyl acetate fraction

Figure 4. Effectivity SNEDDS loaded EAF and EAF without SNEDDS against (1) *Bacillus subtilis*; (2) *Bacillus cereus*; (3) *Staphylococcus aureus*

compounds through the stratum corneum into deeper layers of the skin and increase the availability of skin (Šmejkalová *et al.,* 2017). SNEDDS had relatively high lipid solubility to increase drug partitioning of the lipid membrane (Alvi *et al.,* 2011). The main challenge in the formulation was the transport of barriers through the skin (Prasanthi and Lakshmi, 2012). Tween 80 as a surfactant could increase drug flux in the skin by entering into lipid bilayers and causing more permeable regulation and increasing membrane fluidity (Šmejkalová *et al.,* 2017). Tween 80 was able to enhance absorption by inducing lipid fluidization in the stratum corneum, thereby increasing drug penetration (Potts, 1997). Tween 80 was used in a variety of pharmaceutical formulations as enhancers to phospholipid membrane permeability, which causes the breakdown of compounds with low molecular mass. The interaction between Tween 80 and the membrane destroys the epidermal membrane. Specifically, Tween 80 increased the permeability of the sarcoplasmic reticulum (Cserhati, 1995). Two mechanisms determine the rate of drug penetration using non-ionic surfactants: (1) the surfactant penetrated the intracellular area of the stratum corneum, increasing its fluidity then dissolving the lipid component; (2) the surfactant penetration in the intercellular matrix was followed by interactions and bonds in the keratin filament, resulting in interference with the corneocytes (Breuer, 1979). Carriers could affect the increased penetration (Sasivimolphan *et al.,* 2012). In another SNEDDS research, CoQ10 active substance rose to around 33% in an hour and increased to 97% within six hours (Nepal *et al.,* 2010). SNEDDS in ex vivo permeation studies had the potential to increase drug permeability in GIT

(Mustafa *et al.,* 2019). Ex vivo permeation results even experienced a 3.2-fold increase in permeation with LCT14 in rat intestines compared to other formulations (Gupta *et al.,* 2011).

SNEDDS loaded EAF (nano-sized) showed the ability to inhibit bacteria slightly higher than EAF without SNEDDS, which has a larger size. With a smaller size, the contact area became larger than more components contained in the samples that were extracted and synergized to inhibit bacterial activity. In addition, with a smaller size, the components of active compounds would more easily penetrate bacterial cell walls so that it was easier to influence bacterial activity (Sitti *et al.,* 2018). Nano preparations provided many benefits such as increasing the absorption of herbal medicines and solubility compared to particles with larger sizes (Prusty and Sahu, 2009). Gram-positive bacterial cell walls had a single layer containing 1-4% of lipids, while gram-negative bacterial cell walls had three layers consisting of lipoproteins, outer membrane phospholipids, and lipopolysaccharides (Poeloengan and Praptiwi, 2010).

The results of testing on *Bacillus cereus* revealed that no inhibitory zones were seen in both SNEDDS loaded with EAF and EAF without SNEDDS; therefore, both SNEDDS loaded EAF and EAF without SNEDDS did not have activity against *Bacillus cereus*. SNEDDS consisted of nano-sized bioactive droplets containing surfactants and co-surfactants which are amphiphyl compounds, making the ability to penetrate bacterial cells higher (Sugumar *et al.,* 2016; Dai *et al.,* 2015).

EAF without SNEDDS also had activity to inhibit the growth of *Bacillus subtilis* and *Staphylococcus aureus*. This is due to the presence of secondary metabolites contained in EAF. Xanthone was a form of flavonoids contained in mangosteen peels that can denature proteins that cause cell metabolic activity to stop and that is led by the reaction of carbonyl groups in xanthone with amino acid residues in cell membrane proteins, extracellular enzymes, and cell wall proteins, which result in the lost function of proteins (Putera, 2010). The most abundant xanthone derivative and that had the best biological activity on mangosteen peels was α-mangostin (Parveen *et al.,* 1988). Based on the TLC test on EAF, it can be seen that EAF had α-mangostin content of 38.07 ± 1.026. Wijayanti (2018) showed that mangosteen peels extract and fraction contained flavonoids and polyphenols. α-Mangostin was identified as the most active compound against Gram-positive pathogens, and it showed a fast in vitro bactericidal activity; moreover, there was no resistance to α-mangostin in the strain. α-mangostinwas can quickly disrupt the integrity of the cytoplasmic membrane which causes loss of intracellular components. It could also disrupt the bacterial membrane quickly by the bactericidal mechanism (Koh *et al.,* 2013). Gram-positive bacterial cell walls had a single layer containing 1-4% of lipids while Gram-negative bacterial cell walls had three layers consisting of lipoproteins, outer membrane phospholipids, and lipopolysaccharides (Poeloengan and Praptiwi, 2010). Besides xanthone, EAF also contained saponins, tannins, and flavonoids which have antibacterial activity (Poeloengan and Praptiwi, 2010). Tannin compound had a mechanism of protein denaturation and coagulation. Tannins bind to proteins to form H + ions, causing the pH to become acidic so that the protein is denatured. Mechanism of bacterial inhibition by phenol compounds is thought to interfere with the constituent components of bacterial cell peptidoglycan, resulting in incomplete cell layer formation. Instability in the cell wall caused damaged selective permeability functions, active transport functions, and control of the protein composition of the bacterial cell, and this led to the lost shape and lysis of bacterial cells (Robinson, 1995).

According to Davis and Stout (1971), antibacterial activity was considered weak when the inhibition zone is less than 5 mm; it was moderate when the inhibition zone is 5-10 mm, strong when the inhibition zone ranges from 10-20 mm, and very strong when the inhibition zone is more from 20 mm. The results showed that the tested SNEDDS loaded EAF and EAF without SNEDDS had strong inhibitory activity against *Bacillus Subtilis*. Meanwhile, they provided such a smaller inhibition zone as tested against *Staphylococcus aureus* that they had moderate antibacterial activity. Further research is developing SNEDDS preparations to Solid SNEDDS (S-SNEDDS) and testing for antibacterial activity against other types of bacteria, as well as testing other activities to expand the use of mangosteen peels that can benefit society and health.

CONCLUSION

SNEDDS loaded EAF has antibacterial activity against *Bacillus subtilis* with diameter of inhibition zone of 16.54 mm ± 0.39. EAF without SNEEDS against *Bacillus subtilis* activity with a diameter of inhibition zone of 11.78 mm ± 2.2. Both SNEDDS loaded EAF and EAF without SNEDDS do not have inhibitory activity against *Bacillus cereus*. SNEDDS loaded EAF has antibacterial activity against *Staphylococcus aureus* with a diameter of inhibition zone of 9.45 mm ± 4.86. EAF without

SNEEDS has antibacterial activity with diameter of inhibition zone of 6.44 mm ± 0.39. The results showed that the tested SNEDDS loaded EAF and EAF without SNEDDS had strong inhibitory activity against *Bacillus Subtilis*. Meanwhile, they provided such a smaller inhibition zone as tested against *Staphylococcus aureus* that they had moderate antibacterial activity.

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