RESEARCH ARTICLE | MAY 25 2021

Synthesis, characterization, and antibacterial activity of sunflower (*Helianthus annus* Linn) seed oil derivatives FREE

Sutrisno Sutrisno ✉; Rensa Dwi Assyfah; Siti Marfu'ah

AIP Conf. Proc. 2353, 030071 (2021)
https://doi.org/10.1063/5.0052633

Synthesis, Characterization, and Antibacterial Activity of Sunflower (*Helianthus annus* Linn) Seed Oil Derivatives

Sutrisno Sutrisno[a)], Rensa Dwi Assyfah[b)], and Siti Marfu'ah[c)]

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, Jl. Semarang 5, Malang 65145, Indonesia

[a)]Corresponding author: sutrisno.kimia@um.ac.id;
[b)]rensadwiassyfah@gmail.com; [c)]siti.marfuah.fmipa@um.ac.id

Abstract. Sunflower seeds are one of the fourth most widely used sources of edible oil production in the world. The content of unsaturated fatty acids in these oils reaches almost 85%. A previous report showed that a fatty acid can inhibit the growth of bacteria that cause infections which are mostly caused by the nature of bacterial resistance. This study focused on the antibacterial activity of sunflower seed oil derivatives; namely K-salt (K-soap), free fatty acids (FFAs), and fatty acid methyl esters (FAMEs). The fatty acids in sunflower oil is linoleic (49.57%), 11-octadecenoic acid (39.09%), palmitic (9.06%), and stearic (2.28%) acids. The as-synthesized K-soap is solid, white, melting point 167–184 ºC, soluble in water and methanol. FFAs are yellow, liquid, boiling point 201 ºC, density 0.94 g.cm^{-3}, refractive index 1.46, viscosity 57.30 cSt, soluble in methanol, chloroform, and n-hexane, acid value 171.35, saponification value 176.73, and ester value 5.38. The FAMEs are yellow, liquid, boiling point 185 ºC, density 0.89 g.cm^{-3}, refractive index 1.45, viscosity 4.34 cSt, soluble in chloroform and n-hexane. The acid, saponification, and ester values of K-soap, FFAs, and FAMEs are 0.98, 210.96, and 202.98 respectively. They are potential as antibacterial against *Escherichia coli* and *Staphylococcus aureus*.

INTRODUCTION

Chemical exploration originating from renewable resources continues to be developed in line with the increasingly limited supply of unrenewable resources, such as petroleum, natural gas, and coal. In addition, renewable resources are richer with the variety of chemicals they contain, either directly utilized or through derivatization. Biomass is the most widely used renewable resource as a natural feedstock. Carbohydrates, protein, and lipids are the main components present in biomass [1]. Plant lipids whose supplies in nature are quite abundant and have the potential to be empowered through their derivation are triglyceride oils. Structurally, triglycerides are glycerol esters with fatty acids. Therefore, hydrolyzed triglycerides can produce glycerol and fatty acids. The fatty acids that make up triglycerides vary widely, both saturated and unsaturated, and these substances determine the properties of triglycerides. Triglyceride oil is mostly found in plant seeds.

Sunflower seed (*Helianthus annus* Linn) is an industrial plant that can be produce triglyceride oil. According to the Plantation Fund Management Agency (*Indonesia: Badan Pengelola Dana Perkebunan, BPDB*) in 2018, sunflower is the fourth most widely used and traded vegetable oil in the world after soybean oil, crude palm oil, and canola oil [2]. Sunflower seed oil has a saturated fatty acid (SFA) content of 15% and an unsaturated fatty acid (UFA) of 85% [3]. The SFA content in sunflower oil is palmitic and stearic acids, and UFA is oleic and linoleic acids [4]. Long-chain unsaturated fatty acids such as linoleic and oleic acids are reported to have antibacterial activity [5]. This suggests that sunflower seed oil can be developed as antibacterial agent. Infectious diseases are growing rapidly and can threaten health. Many infectious diseases are caused by bacteria [6]. In 2010 the number of deaths caused by pathogenic bacteria reached 15 million people [7]. In addition, infectious diseases have become a serious problem due to widespread bacterial resistance. Bacterial resistance can occur due to the use of antibacterial drugs that are not appropriate or even exceed the dose [6]. This has led to the importance of developing new antibacterial drugs.

International Conference on Life Sciences and Technology (ICoLiST 2020)
AIP Conf. Proc. 2353, 030071-1–030071-7; https://doi.org/10.1063/5.0052633
Published by AIP Publishing. 978-0-7354-4096-8/$30.00

Triglycerides oil does not have antibacterial activity due to their structure relatively large and long [8]. However, fatty acids as one of the triglyceride derivatives have antibacterial activity. The hydroxyl groups of fatty acids are very important for antibacterial activity. Medium and long-chain unsaturated fatty acids are more active against gram-positive than gram-negative bacteria [9]. This suggests that the molecular structure affects antibacterial activity. Other triglyceride derivatives namely potassium soap and fatty acid methyl ester are reported to have antibacterial activity. Potassium soap was able to inhibit the growth of *Bacillus anthrax* in an agar medium with a concentration of 0.1% [10]. The fatty acid methyl ester of *Scenedesmus bijugatus* has antibacterial against *E. coli, S. aureus,* and *C. albicans*. These fatty acid methyl esters include methyl linoleate, methyl stearate, methyl linoleate, and methyl oleate [11]. The objective of the research to synthesize sunflower oil derivatives, namely K-soap, FFAs, and FAMEs including their antibacterial activity study.

EXPERIEMENTAL DETAILS

Synthesis, Characterization, and Identification of K-soap

Sunflower (20 g) and potassium hydroxide solution (60 mL, 15%) were put into a three-neck round bottom flask which has been setting with reflux equipment using an oil bath and magnetic stirrer. Furthermore, the mixture was refluxed until temperature 80 ^{o}C for 3 h [12,13]. The process of reaction (indicated by the appearance of foam) was monitored every 30 min during this time. The mixture of reaction result was cooled at ambient temperature, was filtered. The filtrate was tested using litmus paper (red and blue) and the residue was tested organoleptically. The residue from the filtration is washed and purified by salting-out using a saturated solution of sodium chloride, and dried at 60 ^{o}C until 8 h, obtained powder potassium salt was denoted as K-soap. The physicochemical properties of K-soap powder were characterized its physical properties (phase, color, and melting point), solubility in various solvents (water, methanol, ethanol, chloroform, and n-hexane), and identified using FT-IR spectrophotometer.

Synthesis, Characterization, and Identification of FFAs

Potassium soap (the as-synthesized product) is put into a beaker-glass (250 mL), and distilled water is added gradually until everything forms an emulsion. Furthermore, a solution of 1 M hydrochloric acid is added gradually while stirring using a magnetic stirrer until the emulsion was coagulated. Then filtered, the residue obtained is left to stand, to form 2 layers. The residue from this filtering is the free fatty acids (solid-state) and the filtrate is centrifuged for 30 min, separated by decantation. The upper layer (as the liquid is free fatty acids) obtained was characterized for its physicochemical using a similar procedure of K-soap.

Synthesis, Characterization, and Identification of FAMEs

Sunflower (20 g) and 3% KOH/methanolic solution (15 mL) (mol ratio ≈ 1 : 6) were put into a three-neck round bottom flask which has been setting with reflux equipment using an oil bath and magnetic stirrer. Furthermore, the mixture was refluxed until temperature 60 ^{o}C for 4 h [13,14]. The reaction process (indicated by the change of color) was monitored every 30 min during this time. The mixture of reaction result was cooled at ambient temperature, formed 2 layers, was separated by a separating funnel. The lower and upper layer was tested by red and blue litmus papers, solubility, and flame, respectively. Furthermore, the upper layer (as fatty acids methyl ester, FAMEs) is washed using warm water until the washing water is neutral [14], and dried by magnesium sulfate anhydrate. A similar characterization and identification procedure of K-soap and FFAs was done either for FAMEs.

Antibacterial Activity Test

The media used in this study were liquid (*Nutrient Broth*) and solid media (*Nutrient Agar*). In the first step, making nutrient broth are 0.06 g of beef extract and 0.5 g of bacto peptone are put into Erlenmeyer. Then, add 20 mL of distilled water and heat it to a boil. Also, 10 mL of the mixture was taken and transferred into two test tubes. The test tube is then closed with a cotton plug. The second step, making nutrient agar, which is 10 g of instant nutrient agar, is included in Erlenmeyer, and then 500 mL of distilled water is added and heated until the mixture becomes clear. The total amount of 10 mL each from the mixture and transferred to a petri dish (bubble formation avoided) [15].

The antibacterial activity assay used methanol and n-hexane as negative controls. The samples tested for antibacterial were Sunflower oil, K-soap, FFAs, and FAMEs with concentrations 1% and 2%, respectively. Sunflower oil and FAMEs were dissolved in n-hexane, while K-soap and FFAs were dissolved in methanol. The process of the assay for antibacterial activity is carried out in Laminar Air Flow which has been sterilized with alcohol 70%. Inoculated the *Staphylococcus aureu*s bacteria culture from liquid nutrients into solid media (NA) by rubbing it on the surface of the medium using sterile cotton buds. The same is done for the *Escherichia coli* bacteria. The medium is perforated using a special drill with a diameter of 5 mm to form a hole or well. A sample of 20 µL was inserted into the hole using a micropipette and incubated at 37 ^{o}C for 24 h. The growth of bacterial was observed around the hole and the clear zone formed was measured using a caliper [16].

RESULTS AND DISCUSSIONS

Characterization of Sunflower Seed Oil and Its Derivatives

The visible changes in state or phase and the results of its characterization, it can be explained that there has been a transformation of sunflower seed oil into its derivative. As shown in Figure 1, sunflower seed oil is liquid while K-soap is solid. Dissolving K-soap in water forms foam and slippery like soap in general. It indicated that K-soap was successfully synthesized [17]. The acidification of the potassium salt (solid state) change to a liquid state indicating the formation of FFAs. Likewise, it is formed of two layers of the *trans*-esterification reaction of this oil with KOH-methanolic solution, which indicates that it has been formed of the FAMEs. Physically, the phase of fatty acid methyl ester (clear liquid) is relatively the same as the oil, but the characterization shows a significant difference (Table 1). This is because sunflower seed oil mainly contains unsaturated fatty acids i.e., oleic acid and linoleic acid [18]. A summary of the physicochemical properties of sunflower seed oil and its derivatives is listed in Table 1.

(a) **(b)** **(c)** **(d)**

FIGURE 1. (a) Sunflower Oil (*minyak biji b.matahari*), (b) K-soap (*sabun kalium minyak biji b.matahari*), (c) FFAs (*asam lemak minyak biji b.matahari*), and (d) FAMEs (*metil ester asam lemak minyak biji b.matahari*)

Measurement of boiling point, viscosity, and density (Table 1) showed that fatty acids have the highest value compared to oils and their fatty acid methyl esters. It is thought that presenting the strongest hydrogen bonding between fatty acid molecules affected to the highest density and boiling point. The solubility of a compound follows the "*like dissolves like rule"* [19]. As we expected that sunflower seed oil was soluble in chloroform and *n*-hexane as non-polar solvent. However, potassium soap is soluble in a polar solvent such as water, methanol, and ethanol. Fatty acids are insoluble only in water. Solubility trend of methyl ester is slightly similar to a fatty acid which insoluble methanol as well. This experimental evidence shows that polarity of potassium soap > fatty acid > methyl ester > triglyceride oil, as has been studied in previous studies [20].

The acid value represents the amount of free fatty acid in the oil which shows the number of potassium hydroxide (in milligrams) used to neutralize 1-gram free fatty acids of oil or fat. As shown in Table 1, the higher acid value of fatty acid than sunflower seed oil proved that sunflower seed oil has been transformed into its fatty acids. Meanwhile, fatty acid methyl ester has a lower acid value than sunflower seed oil, indicating that the free fatty acid has been transformed into its fatty acid methyl ester. The free fatty acids in sunflower seed oil have formed its methyl ester fatty acids through *trans*-esterification reaction with alkaline-catalyzed methanol so that the amount of free fatty acids is reduced [13,14].

TABLE 1. Physicochemical Properties of Sunflower Seed Oil and Its Derivatives

No.	Properties	Sunflower Seed Oil	Potassium Soap	Fatty Acid	Methyl Ester
1.	Phase	Liquid	Solid	Liquid	Liquid
2.	Color	Yellow	White	Faded yellow	Yellowish white
3.	Boiling point (ºC)	193	-	201	185
4.	Melting point (ºC)	-	167 - 184	-	-
5.	Refractive index (25 ºC)	1.47	-	1.46	1.45
6.	Viscosity (cSt)	37.72	-	57.30	4.34
7.	Density ($g.cm^{-3}$)	0.93	-	0.94	0.89
8.	Solubility in:				
	H_2O	Insoluble	Soluble	Insoluble	Insoluble
	CH_3OH	Insoluble	Soluble	Soluble	Insoluble
	CH_3CH_2OH	Insoluble	Soluble	Soluble	Soluble
	$CHCl_3$	Soluble	Insoluble	Soluble	Soluble
	n-C_6H_{14}	soluble	Insoluble	Soluble	Soluble
9.	Acid value (mg KOH/g sample)	0.77	-	171.35	0.98
10.	Saponification value (mg KOH/g sample)	189.06	-	176.73	210.96
11.	Ester value (mg KOH/g sample)	188.29	-	5.38	209.98

The saponification value represents the number of potassium hydroxide (in milligrams) needed to saponificate 1-gram triglycerides. This value can be used to describe the molecular weight of triglycerides, which means to describe the size or length of the acyl groups in its fatty acid. As shown in Table 1, the fatty acid methyl ester gave the highest saponification value regarding a lower molecular weight than sunflower seed oil. The ester value which represents the number of potassium hydroxide (in milligram) to saponificate the remaining triglycerides in 1 gram of oil or fat indicated the number of esters in the oil or fat. Based on our result as shown in Table 1, methyl ester gave the highest ester value. It means the fatty acid methyl ester of sunflower seed oil contains high esters.

Identification of Sunflower Seed Oil and Its Derivatives

The fatty acids contained in sunflower seed oil were analyzed by GC-MS. The analysis was carried out on the fatty acid methyl ester obtained through the *trans*-esterification reaction of oil with methanol with BF_3 catalyst in-situ and *trans*-esterification with methanol with KOH catalyst. The results of the GC-MS analysis are listed in Table 2. It showed sunflower seed oil and methyl ester are composed of 4 fatty acids, namely palmitic, linoleic, vaccenic, and stearic acids. Linoleic acid and vaccenic acid are the main components of sunflower seed oil.

The transformation of sunflower oil into its derivatives involves changing the functional groups of the constituent compounds. To support the success of this functional group transformation, it was analyzed by FT-IR spectrophotometry. This FT-IR analysis produces spectra which are characterized by changes in the vibration of its typical groups. The interpretation of the IR spectra of the oil and its derivatives showed that there were typical absorption bands. The typical band on the IR spectrum of sunflower seed oil with a wave number of 1745.58 cm^{-1} (strong and sharp intensities) indicates a stretching vibration of the C=O ester bond. In potassium soap this band appears at 1556.55 cm^{-1} and 1417.68 cm^{-1} (strong and sharp) characterizing the C=O stretching vibration as a carboxylate salt [21]. Typical bands on fatty acids appear at 3008.95 cm^{-1} (weak and widened intensity) indicating O–H stretching vibration and at 1743.65 cm^{-1} (strong and sharp intensity) indicating C=O stretching vibration of carboxylic acid. The typical C=O ester stretch band for the methyl ester appears at the wave number 1743.65 cm^{-1} (strong and sharp). Based on the results of this IR spectra interpretation, it strengthens the evidence that sunflower seed oil has been transformed into its derivatives [22]. In summary, the typical band data of sunflower seed oil IR spectra and their derivatives are shown in Table 3.

TABLE 2. Fatty Acid Content in Sunflower Seed Oil and Fatty Acid Methyl Ester

Fatty Acid Content	Sunflower Seed Oil (%) (*trans*-esterification by CH_3OH/ BF_3)	Methyl Ester (%) (*trans*-esterification, CH_3OH/ KOH)
Palmitic or hexadecenoic acid (16:0)	9.06	6.77
Linoleic or 9,12-octadecadienoic acid (18:2)	49.57	54.60
Vaccenic or 11-octadecenoic acid (18:1)	39.09	35.41
Stearic or octadecanoic acid (18:0)	2.28	2.84

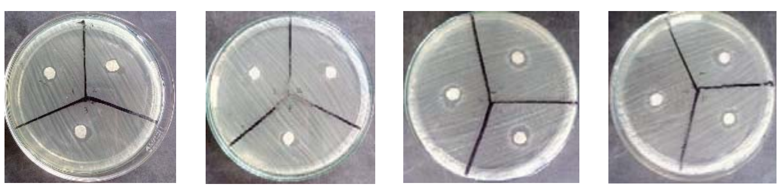
TABLE 3. Sunflower Seed Oil and Its Derivatives Wave Numbers Data

Stretching Vibration	Wave Numbers (cm^{-1})			
	Sunflower Oil	K-soap	FFAs	FAMEs
–OH group	-	-	3008.95	-
C=O group	1745.58	1556.55 1417.68	1743.65	1743.65

Antibacterial Activity of Sunflower Seed Oil Derivatives

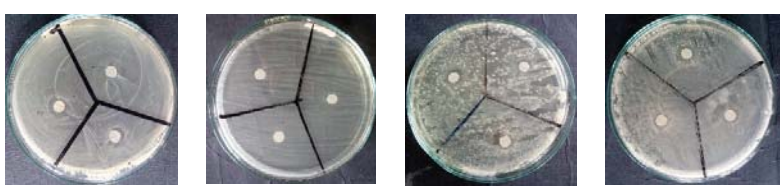
The antibacterial activity of sunflower seed oil derivatives (K-soap, FFAs, and FAMEs) against *Staphylococcus aureus* and *Escherichia coli* in 96% ethanol are shown in Figure 2 - 4 and listed in Table 4. Both bacteria represent gram-positive and gram-negative bacteria. Antibacterial activity (based on inhibition of growth) is indicated by the formation of a clear zone around the well. The larger the clear zone, the stronger the antibacterial activity will be.

The antibacterial activity properties of sunflower seed oil derivatives as shown in Table 4 are as follows: K-soap > free fatty acids > fatty acid methyl esters. Previous report suggested that linoleic acid have antibacterial activity which contains long-chain unsaturated fatty acids [23]. In addition, lower carbon chain fatty acids are more active for gram-positive than gram-negative bacteria [5]. The results showed that the fatty acids from sunflower seed oil had higher antibacterial activity against gram-negative bacteria than gram-positive ones. It is suspected that the fatty acids from sunflower seed oil are more specific to gram-negative bacteria.



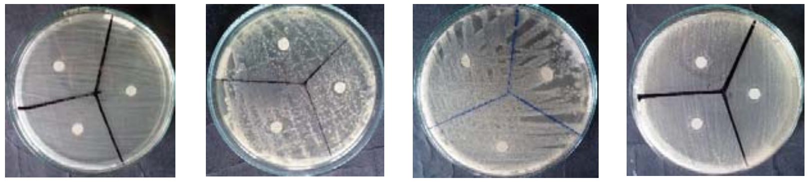
(a) (b) (c) (d)

FIGURE 2. Antibacterial activity of K-soap against 2 types of tested bacteria (a) and (b) K-soap 2% and 1% against *E. coli* , (c) and (d) K-soap 2% and 1% against *S. aureus*.



(a) (b) (c) (d)

FIGURE 3. Antibacterial activity of K-soap against 2 types of tested bacteria (a) and (b) FFAs 2% and 1% against *E. coli* , (c) and (d) FFAs 2% and 1% against *S. aureus*.



(a) (b) (c) (d)

FIGURE 4. Antibacterial activity of FAMEs against 2 types of tested bacteria (a) and (b) FAMes 2% and 1% against *E. coli,* (c) and (d) FAMEs 2% and 1% against *S. aureus*.

TABLE 4. Diameter of the Inhibitory Zone of Sunflower Seed Oil Derivatives

Bacteria	Experiment number	Clear Zone Diameter (mm)						
		Negative Control	K-soap		Fatty Acid		Methyl Ester	
			1 %	2 %	1 %	2 %	1 %	2 %
Escherichia coli	1	6.25	8.25	16.50	8.75	9.98	7.50	7.75
	2	7.05	8.65	8.35	9.65	11.40	6.15	8.75
	3	6.40	9.35	10.00	8.95	8.90	7.00	8.35
	Average	**6.57**	**8.75**	**11.62**	**9.12**	**10.09**	**6.88**	**8.28**
Staphylococcus aureus	1	6.05	8.45	8.80	9.40	10.15	6.15	7.15
	2	6.50	8.45	13.00	7.00	8.05	7.20	6.50
	3	6.20	8.50	9.85	9.00	9.20	6.05	8.80
	Average	**6.25**	**8.75**	**10.55**	**8.47**	**9.13**	**6.47**	**7.48**

Fatty acids have the potential to inhibit various enzymes and in particular unsaturated fatty acids, outperform the enzyme inhibition ability more than saturated fatty acids. The antibacterial activity of fatty acids is due to enzyme inhibition in the membrane or cytosol which is highly influential for the bacteria survival. Unsaturated fatty acids can inhibit the bacterial biosynthesis process *in-vivo* which reorganizes the cell membrane composition of bacteria. It leads to a change in the fluidity and permeability resulting in lysis of cell membrane [23].

Based-on the evidence of the research results, potassium salts and fatty acids from sunflower oil are potential as antibacterial of both tested bacteria. A mixture of potassium salts and fatty acid is recommended as an antibacterial soap from sunflower oil. However, in this study, the antibacterial activity test did not use a positive control (standard antibacterial test), so it was not possible to know its effectiveness and benefits.

In the previous study, there was research on antibacterial activity assays of *Virgin Coconut Oil* (VCO) hydrolysis results using lipase. The result showed that hydrolysis fatty acids act as antibacterial against *Staphylococcus aureus* bacteria with diameter of 8.33 ± 0.58 mm. In addition, hydrolyzed fatty acids have antibacterial activity against *Escherichia coli* with a diameter of 8.67 ± 0.58 mm [24]. In comparison, fatty acids from sunflower seed oil have higher antibacterial activity. On the other hand, other sunflower seed oil derivatives such as potassium soap also have a higher diameter of the inhibition zone but not its methyl esters.

SUMMARY

The derivative compounds of sunflower oil were successfully synthesized. The derivatives are K-soaps (fatty acid potassium salts or potassium salts), free fatty acids (FFAs), and fatty acid methyl esters (FAMEs). K-soap was synthesized by saponification reaction with potassium hydroxide. FFAs were synthesized by acidification reaction with hydrochloric acid. *Trans*-esterification reaction of sunflower oil with methanol which is catalyzed potassium hydroxide was obtained fatty acid methyl esters. The K-soap, FFAs, and FAMEs were active as an antibacterial against *Staphylococcus aureus* and *Escherichia coli*, while the oil does not act as the antibacterial. Antibacterial activities are K-soap > FFAs > FAMEs. Potassium salts and free fatty acids are highly active antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, as a result, both compounds have potential as antibacterial. Based-on the evidence potassium salts and fatty acids from sunflower oil are potential antibacterial. A mixture of the potassium salts and fatty acid of sunflower oil is recommended as antibacterial soap.

ACKNOWLEDGMENTS

This work was supported by the Ministry of RISTEK/BRIN, Indonesia, through the 2020 Thesis Magister Scheme with contract number: 10.3.57/UN32.14.1/LT/2020 No. SPPK No. 053/SP2H/LT/DRPM/2020, March 10, 2020.

REFERENCES

1. R.A. Voloshin, M. V. Rodionova, S.K. Zharmukhamedov, T. Nejat Veziroglu, and S.I. Allakhverdiev, Int. J. Hydrogen Energy **41**, 17257 (2016).
2. Badan Pengelola Dana Perkebunan (2018).
3. M.R. Akkaya, J. Food Sci. Technol. **55**, 2318 (2018).
4. D.B. Konuskan, M. Arslan, and A. Oksuz, Saudi J. Biol. Sci. 1 (2018).
5. A.P. Desbois and V.J. Smith, Appl. Microbiol. Biotechnol. **85**, 1629 (2010).
6. A.W. Adisasmito and S.R.S. Hadinegoro, Sari Pediatr. **6**, 32 (2004).
7. C. Dye, Philos. Trans. R. Soc. B Biol. Sci. **369**, (2014).
8. Murhadi, J. Teknol. Ind. Dan Has. Pertan. **14**, 97 (2009).
9. C.J. Zheng, J.-S. Yoo, T.-G. Lee, H.-Y. Cho, Y.-H. Kim, and W.-G. Kim, FEBS Lett. **579**, 5157 (2005).
10. H. Thormar, H. Hilmarsson, and G. Bergsson, Microb. Pathog. Strateg. Combat. Them Sci. Technol. Educ. 1474 (2013).
11. D. MubarakAli, R. Praveenkumar, T. Shenbagavalli, T. Mari Nivetha, A. Parveez Ahamed, N.A. Al-Dhabi, and N. Thajuddin, RSC Adv. **2**, 11552 (2012).
12. J. Salimon, B.M. Abdullah, and N. Salih, Int. J. Chem. Eng. **2012**, 1 (2012).
13. D.Y.C. Leung, X. Wu, and M.K.H. Leung, Appl. Energy **87**, 1083 (2010).
14. R. Naureen, M. Tariq, I. Yusoff, A.J.K. Chowdhury, and M.A. Ashraf, Saudi J. Biol. Sci. **22**, 332 (2015).
15. M. Mulyadi, W. Wuryanti, and P.R. Sarjono, J. Kim. Sains Dan Apl. **20**, 130 (2017).
16. H. Jahangirian, M.J. Haron, M.H. Shah Ismail, R. Rafiee-Moghaddam, L. Afsah-Hejri, Y. Abdollahi, M. Rezayi, and N. Vafaei, Dig. J. Nanomater. Biostructures **8**, 1263 (2013).
17. Sutrisno, R.D. Assyfah, R. Retnosari, I.B. Rachman, and H.W. Wijaya, in *AIP Conf. Proc. 2231* (2020), p. 040081.
18. A.H. Khine and A.A. Tun, 33 (2013).
19. M.N. Al-Ash'ary, F.M. Supriyanti, and Zackiyah, J. Sains Dan Teknol. Kim. **1**, 150 (2010).
20. M.I. Fitranda, Sutrisno, and S. Marfu'ah, IOP Conf. Ser. Mater. Sci. Eng. **833**, (2020).
21. T. Gocen, S. Haman Bayarı, and M. Haluk Guven, J. Mol. Struct. **1150**, 68 (2017).
22. T.P. Mena, Sutrisno, and S. Marfu'ah, IOP Conf. Ser. Mater. Sci. Eng. **833**, (2020).
23. C.J. Zheng, J.S. Yoo, T.G. Lee, H.Y. Cho, Y.H. Kim, and W.G. Kim, FEBS Lett. **579**, 5157 (2005).
24. T.A. Nguyen, T.D. Le, H.N. Phan, and L.B. Tran, J. Lipids 1 (2017).