

Producing Polyurethane as Wound Plaster using Glycerol Transesterified of Waste Cooking Oil with Moringa Leaf Extract (*Moringa Oleifera Lam.*) as an Antimicrobial

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Abstract

Wound plaster polyurethane can be made using glycerol from waste cooking oil (WCO). Wound plasters should contain antibacterial ingredients in addition to covering the sore. Traditionally various plants have been used to treat sores and contain sundry antibacterial chemical compounds among *Moringa oleifera Lam.* Therefore, it is very potential to make polyurethane wound plasters from glycerol produced from WCO combined with Moringa leaf extract obtained from wound plasters from natural ingredients. Maceration with 96 % ethanol yielded moringa leaf extract. Then, a transesterification reaction was carried out by using KOH-methanol at 60 °C to get glycerol and tested by FTIR spectrophotometry. Polyurethane wound plasters were made with a mixture of methylene 4,4 diphenyl diisocyanate, polyethylene glycol (PEG) and various formulas with variations of glycerol 0, 3, 6, 9, 12, 15 and 18 drops. A characteristic test was enforced with swelling test to find the best formula and functional group analysis using FTIR spectrophotometry. Into the best wound plaster, improved Moringa leaf ethanol extract at an intensity of 1 - 5 %. An antibacterial activity test was carried out on the Moringa leaf ethanol extract and plasters obtained by the agar diffusion method. The FTIR spectrophotometer test showed the formation of glycerol from WCO. The results of the characteristics test for polyurethane wound plaster preparations obtained the best formula using 3 drops of glycerol. The antibacterial activity test results at a concentration of 500 mg/mL Moringa leaf extract produced a muscular inhibition zone of 19.2 mm against *Staphylococcus aureus* and Antiseptic solution of 17.3 mm; *Pseudomonas aeruginosa* of 14.3 mm and Antiseptic solvent of 16.2 mm. Antibacterial activity of polyurethane wound plaster against *Staphylococcus aureus* contains 5 % ethanol extract of 10.3 mm, Plaster Merck X of 11.2 mm, *Pseudomonas aeruginosa*, only gave resistance at 5 % concentration of 8.3 mm and Plaster Merck X of 9.2 mm.

Keywords: Glycerol of WCO (waste cooking oil), Moringa leaves, Polyurethane, Wound plaster, FT-IR, *Pseudomonas aeruginosa*, *Staphylococcus aureus*

Introduction

WCO comes from 2 or more leftovers from frying food ingredients [1]. Chemically, cooking oil (fatty oil) is triglycerides are composed of glycerol and fatty acid compounds, produced from coconut plants, usually from oil palm plants. (*Elaeis guineensis*) family *palmae* [2]. In Medan City there are many fried food traders and substantial industries that use cooking oil, for example, the chips industry. Thus, the percentage level of WCO is very high. WCO still considers waste by the community because it can only consume about 2 - 3 times for frying [3]. If it is excessively used, it can hurt public health. Therefore, many explorations enforce to find alternative ways to make WCO worthwhile. One of them is for use as a raw material in the manufacture of biodiesel [4].

Making biodiesel from WCO produces a by-product in the shape of low-purity glycerol referred to as crude glycerol [5]. There are several ways to obtain crude glycerol, including transesterification, saponification and oil hydrolysis reactions. Transesterification is used to produce glycerol by reacting WCO and methanol with a KOH catalyst [6,7]. The transesterification process uses a base as a catalyst to assist the transesterification reaction enforced at 60 °C. The glycerol produced through this process is then purified, and the results can be used for various products, one of which is the manufacture of Polyurethane (PU).

In this decade PU is one of the most popular materials in the world. PU is a type of heteropolymer or copolymer polymer composed of different monomers, consisting of an organic chain connected by a urethane bond (-NHCOO-) in the main chain. PU is widely used to make foam, elastomeric materials (rubber/plastic), glue, coatings, etc. [8]. Along with technological developments, polyurethane can also be developed as a plaster/wound dressing material with a combination of glycerol from WCO [9].

Plaster material/wound dressing is a dressing product that can control the humidity around the wound according to the type of wound and the accompanying exudate. Wound plasters currently being developed are wound plasters in the form of foam (foam) which can be made from PU, and combined with antimicrobial substances [10]. Various research and development to provide antimicrobial properties to polyurethane enforced by several researchers with various synthetic antimicrobial materials and natural ingredients [11]. Some plants that have potential as antimicrobials or antibacterials include Jengkol leaves, Buas-Buas (*Premna cordifolia L.*), basil leaves and others [12,13]. Other plants that have antibacterial or antimicrobial properties such as Katuk leaves, Ketepeng Cina leaves, potato tubers, moringa leaves and others.

Moringa (*Moringa oleifera Lam.*) is a Moringaceae plant species that is widely used in traditional medicine and as a vegetable in Indonesia. The existence of moringa plants is facile to find in all regions of Indonesia. All Moringa plant parts are edible effective starting from the roots, stems, leaves, seeds, fruits and flowers [14]. Several studies conducted that Simplicia and ethanol a Moringa leaf extract contain flavonoids-related compounds, tannins, alkaloids, steroids and saponins class [15]. Moringa leaves are used as an ingredient in traditional medicine, as antioxidants, and for skin medicine due to fungal infections by rubbing. These properties are closely related to phytochemical compounds such as flavonoids, saponins and tannins which act as antioxidants, antibacterial and antifungal [16,17].

Based on the description above, this study aimed to produce glycerol by transesterification reaction from WCO using a base as a catalyst to obtain glycerol. Resulting in glycerol used to manufacture polyurethane wound plaster preparations as an antibacterial in wound plasters using Moringa leaf extract with various concentration variations.

Materials and methods

The experimental method was employed in this study. Sample identification, glycerol preparation from WCO, and ethanol extraction of *Moringa oleifera Lam.* leaves were all part of this study, functional group analysis using an FTIR spectrophotometer, manufacture of wound plaster preparations in the form of polyurethane foam, testing of wound plasters in the form of foam polyurethane, Moringa leaf ethanol extract antibacterial testing and antibacterial testing of polyurethane wound plasters.

Making moringa leaf simplicia

Fresh samples take from trees, then the leaves are separated from the branches by hand, wet sorting, washed, after being cut into small pieces, it was dried in a drying cupboard at around 60 °C, and crushed using a blender to obtain Simplicia powder [18,19].

The simplicial powder extracts by the maceration method. Weigh the simplicial powder of as much as 500 g put it in a vessel, add 75 parts of the solvent, cover, leave for 5 days protected from light while stirring frequently, sprinkle, squeeze and wash the dregs with 25 parts of the solvent until 100 parts obtained. The mixture was then transferred to a closed vessel, kept cool and light-protected for 2 days and poured or filtered with filter paper. The filtrate is then concentrated using a rotary evaporator.

Examination of simplicia characterization

The water content, water-soluble extract content, ethanol-soluble extract content, total ash content and acid-insoluble ash content are all determined during the Simplicia characterization process [20].

Manufacture of crude glycerol

After filtering the WCO, 500 g is heated to 110 °C until the oil boils. The 5 g KOH in 250 mL methanol. WCO is heated to 60 °C, added to a methanol KOH mixture and stirred for 1 h before standing for approximately 8 h to allow the biodiesel and crude glycerol to completely separate and the crude glycerol to separate from the biodiesel [3].

Crude glycerol purification

The total amount of crude glycerol obtained was 112.22 g, then added sulfuric acid (H₂SO₄) was until pH 6, the glycerol layer separated from the other layers after 3 layers formed, and the glycerol content was

analyzed. Crude glycerol has been purified and added with water 2:3 and 4 % activated carbon washed beforehand. After stirring for 30 min and leaving for 24 h, the mixture was filtered and evaporated with a rotary evaporator at 60 °C to remove residual methanol. The glycerol obtained analyzed by FT-IR [1].

Preparation of polyurethane wound plasters containing Moringa leaf extract

At this stage, the polymerization reaction enforces using PEG and methylene 4,4 diphenyl diisocyanate (MDI) in a ratio of 1:1. Variations in the concentration of polyol (glycerol), namely 0, 3, 6, 9, 12, 15 and 18 drops (PU0, PU1, PU2, PU3, PU4, PU5 and PU6). Polyol (glycerol), PEG and methylene 4,4 diphenyl diisocyanate were put into a closed container, the mixture was then stirred for 3 min (until homogeneous) while nitrogen gas was flowing to produce a viscous liquid. Furthermore, the dense liquid obtained is put into the mold and allowed to harden. Before being characterized, the polyurethane samples were placed in a vacuum oven for 2 h at 40 - 50 °C [21].

After the polyurethane hardens, the best sample is selected based on the characterization results. Polyurethane characterization is enforced through swelling degree tests to determine polyurethane absorption capacity and polyurethane functional group analysis tests using FTIR spectra. Then from the characterization results seen that the most effective polyurethane preparation is PU1 (3 drops).

Then, the addition of Moringa leaf extract in a concentrated form of 1, 2, 3, 4 and 5 % as an antibacterial substance. Then the wound plaster preparation of polyurethane foam was tested and characterized. Polyurethane characterization is accomplished through swelling degree tests to determine polyurethane absorption capacity and polyurethane functional group analysis tests using FT-IR spectra.

Evaluation of wound plaster preparations

Organoleptic test

Organoleptic tests enforce by observing the preparations from the shape, smell and color of the preparations observed visually [22].

Test the degree of inflation (swelling degree)

The Swelling test was carried out by immersing 2 g of the preparation in a glass beaker containing distilled water for 24 h weighing it every 1 h, and recording it. Before the preparation soaked with distilled water, then the percentage degree of inflation is calculated [23].

Antibacterial activity of moringa leaf ethanol extract and polyurethane wound plasters was investigated

Pipette in a sterile petri dish, place 0.1 mL of bacterial suspension containing 10⁶ CFU/mL. Following that, 20 mL of liquid MHA media (45 - 50 °C) was poured, then the cup was shaken over the table's top's surface, so that the media and bacterial suspension were mixed evenly, then set aside to solidify. After that, it punched with a punch hole. Next, 1 mL of ethanol extract from Moringa leaves added with various concentrations. The procedure enforces in the same way for plaster preparations of polyurethane foam with sundry concentrations. Pre-incubation for 15 min, then 18 - 24 h at 36 - 37 °C. A caliper was then used to measure the diameter of the inhibition area (clear zone) surrounding the hole [24]. Likewise, the antibacterial activity test enforces using the bacterium *Pseudomonas aeruginosa*.

Results and discussion

Phytochemical screening

Characterization and phytochemical screening of Moringa leaf ethanol extract

In this study, the maceration method is used for extraction. This method was chosen because it is a simple process with simple equipment that does not harm the compounds in the test sample. A *Simplicia* is of good quality if it meets the requirements stated in the *simplicial monograph* held in the Indonesian Herbal Pharmacopoeia. **Table 1** shows the results of the phytochemical screening.

The findings of the investigation into the simple characterization of Moringa leaves. The extracted content enforces to see the number of compounds that could dissolve in polar and non-polar solvents. The ethanol-soluble extract content was 12.9 %, and the water-soluble extract content was 17.8 %. Examination of the amount of ash used to see the mineral content of *Simplicia*. The ash content was 8.5 %, and the acid-insoluble ash content was 0.5 %. The quality parameters of *Simplicia* are known from the water content contained therein. The information about the quality of the *simplicia* of Moringa leaves can provide by examine the moisture content of the *simplicial*. Based on MMI edition VI, it determined the water content in a *simplicial* is no more than 10 %. The water content did see the amount of water contained in the

simplicial. Based on the results of the water content determination, Moringa leaf *Simplicia* powder, it was found to meet the requirements, namely 4.6 %. The results of determining the simplicial characterization show the results meet the requirements and are guaranteed quality based on the Indonesian *Materia Medika* (MMI).

Table 1 Results of examination of characterization of Moringa leaf *Simplicia*.

No.	Class of chemical compounds	Moringa leaf powder	Moringa leaf ethanol extract
1	Flavonoids	+	+
2	Alkaloids	+	+
3	Saponins	+	+
4	Tannins	+	+
5	Steroids	+	+

Note: (+) = contains the substance under investigation and (-) = does not contain the substance being examined.

Based on the results, Moringa leaf extract contains flavonoids, alkaloids, saponins, tannins and steroids metabolites. Phytochemical screening is required to obtain information on the classes of secondary metabolites found in Moringa leaf ethanol extract.

FTIR functional group analysis of glycerol

The end result of producing crude glycerol were 194.9 g of crude glycerol from 500 mL of WCO. The resulting purification following evaporation from the rotary evaporator was 30.4 g (33 mL), and glycerol obtains using the FTIR (Fourier Transform Infrared Spectroscopy) test. The analysis of pure glycerol and glycerol derived from the transesterification of WCO using FTIR are in **Figure 1**.

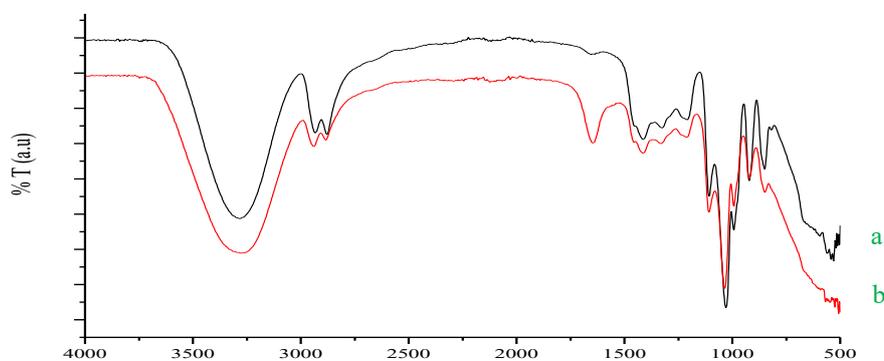


Figure 1 FTIR test results for (a) pure glycerol and (b) glycerol from WCO.

Figure 1 shows converting used cooking oil to glycerol. An extensive band at a wavelength of $3,276\text{ cm}^{-1}$ was observed in glycerol samples from WCO, indicating the presence of OH (hydroxyl) groups. The presence of aliphatic (alkyl) CH groups is indicated by the absorption bands at $2,939$ and $2,883\text{ cm}^{-1}$. The FTIR spectrum revealed that the compound under investigation contains aliphatic OH hydroxyl groups, CH, indicating the presence of glycerol.

The FTIR analysis of glycerol (standard) revealed an extensive band at a wavelength of $3,282\text{ cm}^{-1}$, indicating the presence of OH (hydroxyl) groups. The presence of aliphatic (alkyl) CH groups is indicated by the absorption bands at $2,933$ and $2,878\text{ cm}^{-1}$. The FTIR spectrum of WCO glycerol and pure glycerol results in the glycerol compound obtained from WCO. There is an aliphatic OH and CH group indicate that glycerol is indeed present.

Determination of glycerol density

The density of sample glycerol (WCO) is 1.24 g/mL , and the density of standard glycerol (glycerin) is 1.30 g/mL , both of which the obtained density was close to the density of glycerol (pure/theoretical) this

proves that it is true that there are compounds obtained from the transesterification reaction process on used WCO samples.

Polyurethane evaluation

Polyurethane functional group analysis using FTIR

FTIR analysis of polyurethane functional groups to determine the functional groups and spectral intensities produced by each material used. The results of this test will confirm that the polyurethane was successfully formed. **Figure 2** depicts the FTIR spectra of polyurethane formation.

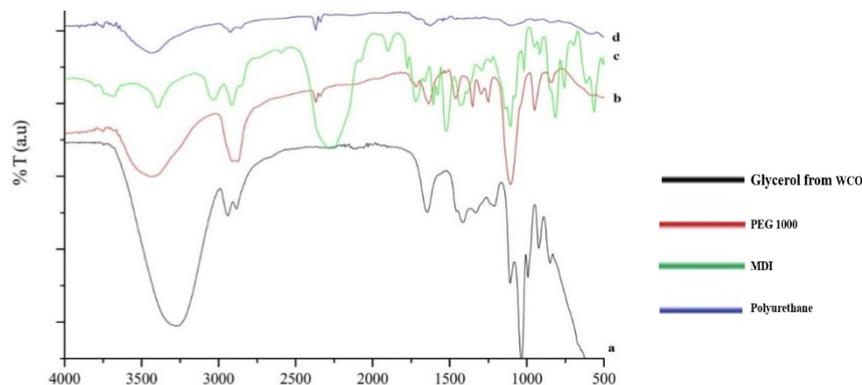


Figure 2 FTIR spectra results for the formation of polyurethane.

The FT-IR spectra of glycerol are shown in **Figure 2**, namely the absorption of hydroxyl groups, C-O and C-H, respectively, at wave numbers 3,276, 1,107 and 2,883 cm^{-1} . The FT-IR spectra of PEG 1000 are shown in **Figure 2**. This figure confirms that there are absorptions of hydroxyl groups, C-O and C-H, respectively, at wave numbers are 3,425, 1,103 and 2,877 cm^{-1} . **Figure 2** shows the FT-IR spectra of methylene 4,4 diphenyl diisocyanate (MDI), confirming the presence of C=C, =C-H, C=O, -NCO and C-H groups, respectively, at absorption wave numbers 1,658, 3,032, 1,720, 2,276 and 2,916 cm^{-1} . **Figure 2** shows the spectra of the synthesized polyurethane with 3 drops of glycerol (PU1), which is a glycerol-based polyurethane mixture, methylene 4,4-diphenyl diisocyanate (MDI) and PEG 1000. This image depicts the absorption of amine groups (N-H), C=O, C-N and C-O at wave numbers 3,425, 1,620, 1,311 and 1,103 cm^{-1} , respectively, which is aided by the reduced intensity of the isocyanate group at wave number 2,276 cm^{-1} originating from MDI, indicating the formation of urethane groups (-NHCO). These criteria represent the formation of polyurethane.

Test the degree of swelling (swelling degree) of polyurethane

The ability of polyurethane wound plaster preparations to absorb water solvents and the presence of cross-links formed in the hard segment area are determined by this test. **Table 2** shows the results of the degree of swelling of the polyurethane wound plaster preparations.

Table 2 Degrees of polyurethane swelling.

No.	Polyurethane	Degree of inflation (%)
1	PU0 (0 drops)	35
2	PU1 (3 drops)	140
3	PU2 (6 drops)	50
4	PU3 (9 drops)	-60
5	PU4 (12 drops)	-15
6	PU5 (15 drops)	75
7	PU6 (18 drops)	35

Table 2 shows the water absorption capacity of 3 drops of glycerol (PU1) polyurethane is greater than the other samples, namely 140 %. It means that PU1 has more hydrophilic properties than other samples, allowing it to absorb liquids more quickly and effectively. Therefore, the polyurethane that has optimum and effective quality is a polyurethane preparation containing glycerol with a concentration of 3 drops of glycerol.

Moringa leaf ethanol extract activity test

The agar diffusion method was used to conduct an antibacterial activity test on Gram-positive Bacteria *Staphylococcus aureus* and Gram-negative Bacteria *Pseudomonas aeruginosa*. **Table 3** shows the results of measuring the inhibition area of Moringa leaf extract.

Table 3 Moringa leaf ethanol extract antibacterial activity test.

Concentration (mg/mL)	The size of the inhibition zone (mm)*	
	<i>Staphylococcus aureus</i> (mm)	<i>Pseudomonas aeruginosa</i> (mm)
100	10.2	9.3
200	13.2	11.2
300	15.3	12.3
400	17.3	13.3
500	19.2	14.3
Antiseptic Solution	17.3	16.2
Ethanol 96 %	-	-

Note: (*) = average result of 3 measurements, (-) = no obstacles and (-) = blank = 96 % ethanol.

Based on the study, the results obtained are the diameter of the inhibition zone around the wellbore treated with Moringa leaf ethanol extract. Showing the effect of antibacterial activity from the Moringa leaf ethanol extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria.

According to the Indonesian Pharmacopoeia V edition, if the inhibition area produces an inhibition zone boundary with a diameter of about 14 mm, it is effective. Inhibition zone diameters of 5 mm or less are considered weak, zones of 5 - 10 mm are considered medium, zones of 10 - 20 mm are considered strong and zones of 20 mm or more are considered very strong. According to the results, treating Moringa leaf ethanol extract with *Staphylococcus aureus* bacteria at a concentration of 500 mg/mL results in an inhibition zone diameter of 19.2 mm in the strong category, while treating *Pseudomonas aeruginosa* bacteria at a concentration of 500 mg/mL results in an inhibition zone diameter of 14.3 mm in the strong category.

Evaluation of polyurethane wound plasters

The active ingredient used in this band-aid is an extract from Moringa leaves which can hydrogen-bond with water and prevent the evaporation of the water. Glycerol is often added to cosmetic preparations to keep the skin moist, and used in antibacterial wound plaster preparations. Moringa leaf extract functions as an antibacterial. Glycerol from the isolation of WCO is also used as a polyol. The most effective and best formula is a polyurethane formula containing 3 drops of glycerol. The 2nd formula enforces by adding Moringa leaf extract with a concentration of 1, 2, 3, 4 and 5 % as antibacterial substances. The combination of Moringa leaf extract will improve the quality of the resulting purification following evaporation and have strong absorption of wound exudate.

Organoleptic testing of wound plaster preparations

The results of organoleptic testing of wound plaster preparations in this study which enforces for ± 2 months (**Table 4**).

Table 4 Organoleptic observations of wound plaster (at room temperature).

Inspection	PU	Observation result
Smell	Polyurethane (PUA)	Typical of Moringa leaves
	Polyurethane (PUB)	Typical of Moringa leaves
	Polyurethane (PUC)	Typical of Moringa leaves
	Polyurethane (PUD)	Typical of Moringa leaves
	Polyurethane (PUE)	Typical of Moringa leaves
Shape (consistency)	Polyurethane (PUA)	Semi solid
	Polyurethane (PUB)	
	Polyurethane (PUC)	
	Polyurethane (PUD)	
	Polyurethane (PUE)	
Texture	Polyurethane (PUA)	Gentle
	Polyurethane (PUB)	Gentle
	Polyurethane (PUC)	Gentle
	Polyurethane (PUD)	Gentle
	Polyurethane (PUE)	Gentle
Color	Polyurethane (PUA)	Yellow
	Polyurethane (PUB)	Yellow brown
	Polyurethane (PUC)	Yellow brown
	Polyurethane (PUD)	Yellowish-brown
	Polyurethane (PUE)	Yellowish-brown

Note: PUA: Glycerol, MDI, PEG and extract 1 % (3:1:1:10). PUB: Glycerol, MDI, PEG and extract 2 % (3:1:1:20). PUC: Glycerol, MDI, PEG and extract 3 % (3:1:1:30). PUD: Glycerol, MDI, PEG and extract 4 % (3:1:1:40). PUE: Glycerol, MDI, PEG and extract 5 % (3:1:1:50).

The organoleptic evaluation of polyurethane wound plasters made from moringa leaf extract and glycerol resulting from the transesterification reaction of WCO is shown in **Table 4**. No change, odor, shape, or color of the preparation was obtained during the test (± 2 months). The addition of Moringa leaf extract with various concentrations did not affect the shape and texture of each PU. The color obtained from each PU there is a slight difference, as, in PUA is yellow, this is because the amount of Moringa leaf extract used is only a small amount (10 mg). However, PUD and PUE have a yellowish-brown color.

Swelling degree test of Moringa leaf extract polyurethane wound plaster

This test determines the ability of polyurethane wound plaster preparations to absorb water solvents and identifies the presence of cross-links formed in the hard segment area. **Table 5** shows the results of calculating the degree of swelling of polyurethane wound plaster preparations.

Table 5 Degree of swelling of polyurethane wound plaster preparations.

No.	Polyurethane	%
1	PUA (1 %)	110
2	PUB (2 %)	100
3	PUC (3 %)	90
4	PUD (4 %)	115
5	PUE (5 %)	140

Based on **Table 5** shows that all samples have a positive value indicating that the sample has optimum and effective absorption. It shows that polyurethane wound plaster preparations have high hydrophilic properties that can absorb exudate or fluid contained in the wound. For PUA, PUB, PUC, PUD and PUE, it is the best and most effective preparation combined with Moringa leaf extract.

Polyurethane wound plaster antibacterial test

Antibacterial test of wound plaster in the form of polyurethane foam containing ethanol extract of Moringa leaves enforces on 5 concentrations: PUA, PUB, PUC, PUD and PUE (1 - 5 %) against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria using the agar diffusion method. **Table 6** shows the results.

Table 6 Antibacterial activity test of polyurethane wound plasters.

Polyurethane wound plaster combination of moringa leaf extract	Diameter of inhibition area (mm)*	
	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
PUA (1 %)	-	-
PUB (2 %)	-	-
PUC (3 %)	9.3 mm	-
PUD (4 %)	10.3 mm	-
PUE (5 %)	10.2 mm	8.3 mm
Plaster Merck X	11.3 mm	9.2 mm
PU base	-	-

Note: PUA-PUE: The wound plaster is in the form of polyurethane foam containing Moringa leaf ethanol extract in various concentrations.

The antibacterial activity of polyurethane wound plaster preparations containing Moringa leaf extract was tested, and the wound plaster formula containing 5 % Moringa leaf extract was found to be the most effective (PUE) produced the largest diameter of inhibition against *Staphylococcus aureus* bacteria, namely 10.2 mm in the strong category, whereas against *Pseudomonas aeruginosa* bacteria produced inhibition of 8.3 mm in the medium category. The polyurethane wound plaster formula without extracts did not show clear zones or inhibition zones, so it concluded all ingredients except extracts did not produce antibacterial activity. It also shows that the inhibition zone produced from the polyurethane wound plaster preparation comes from Moringa leaf ethanol extract has antibacterial activity.

Conclusions

Based on the study, it concluded glycerol produced from the transesterification reaction of WCO, crude glycerol obtained is 194.9 g from 500 mL of WCO. The outcome of purification is 30.4 g (33 mL), and the test results FTIR proves that there are aliphatic CH groups and OH groups in the glycerol produced from this process. Glycerol resulting from the transesterification reaction of WCO made into polyurethane wound dressings by forming urethane groups (-NHCO) from polyurethane FTIR results of the best formula using 3 drops of glycerol. The extracts from Moringa leaves (*Moringa oleifera* Lam.) into the polyurethane wound dressing formula have antibacterial activity. The level of inhibition is strong, namely around 10.2 mm for *Staphylococcus aureus* bacteria and 8.3 mm for *Pseudomonas aeruginosa* bacteria in the moderate category.

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