ENHANCEMENT OF ANTIMICROORGANISM ACTIVITY AND DEGRADABILITY OF PICKLE SKIN BY MODIFICATION WITH NANOPARTICLE PRODUCED FROM RED ALGAE (*Gracilaria sp*.)

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ABSTRACT

The objectives of this research were to characterize nanoparticles prepared with three methods, i.e. extraction, microwave, and ultrasound, and also to study the effect of nanoparticles in modification of pickle skin on hydrophobicity properties, mechanical properties, antimicrobial activity, and biodegradation of skin. A silver nanoparticle was prepared by using an extract of red algae to change silver ion to nano. Extract solution of red algae was produced by using water solvent and then the mixture of extract and nitrate silver solution was shaken for 24 hours in extraction method. The mixture was treated in microwave for 4 min at a power of 300 W to complete the formation of nano. In the ultrasound method, the mixture was ultrasound treatment for 30 min. Characterization was performed using a UV-VIS spectrophotometer and a particle size analyzer to analyze silver nanoparticles, and also an Atomic Absorption Spectrophotometer to determine content of silver in laundry waste of pickle goat skin. Characterization of modified pickle skin was conducted by determining hydrophobicity,

mechanical properties, antibacterial and antifungal activity against Escherichia coli, Staphylococcus epidermidis, and Candida albicans, and also biodegradability of a pickle. Based on the results of UV-VIS and PSA analysis, silver nanoparticles were identified at a wavelength of 416.5 nm with a particle size of 77.2 nm for the microwave method, a wavelength of 421.0 nm with a particle size of 90.4 nm for the extraction method, and a wavelength of 436.5 nm with a particle size of 73.0 nm for the ultrasound method. There were significant differences in the hydrophobicity, mechanical properties, activities of anti-bacteria and anti-fungi, and also degradability of pickle skin.

Keywords: *Candida albicans, Escherichia coli, nanoparticle, pickle skin, red algae, and Staphylococcus epidermidis.*

INTRODUCTION

The goat husbandry industry is one of the industries that develop and produce goat skins as a by-product of industries that have high economic value [1]. Goat skin can be used in various industries, one of them is the textile industry to be used as products such as bags, jackets, wallets, and so on. Most of the goat skin used is in the form of raw skin which is easily damaged due to microorganism activities such as fungi and bacteria [2, 3]. Therefore, it is necessary to have the process of preserving raw goat skin through the pickle process by adding a hard acid. The addition of acid to goat skin is toxic to humans because of the acidic compounds used. Therefore, a new idea emerged to modify the pickle goat skin so that the goat skin is safer and more durable from the attack of microorganisms. One of the innovations is by using silver nanoparticles to modify the pickle goat skin.

Silver nanoparticles have the ability to interfere with microbial metabolic activity and can kill microbes related to microbial cell protein molecules. Silver nanoparticles were chosen because they are not harmful to humans [4-6] are environmentally friendly, antimicrobials that can kill all pathogenic microorganisms [7, 8] and have not reported any microbes resistant to silver nanoparticle [9]. Nanoparticles can be produced through the green chemistry method using plant extracts which functions as a bio-reductor [10]. The plant extracts used in the previous studies were red algae (*Gracilaria sp*.). Red algae is used because it comes from biological natural resources and contains secondary metabolite compounds in the form of alkaloids, flavonoids, tannins, triterpenoids, steroids, and saponins [9, 10] which have functional groups so they can reduce Ag ions to Ag^0. Therefore, red algae can act as a bio-reductor so that the resulting silver nanoparticles are nano-sized. Biosynthesis of silver nanoparticles can be carried out by several methods, namely hydrothermal, sonication, and microwave.

For the manufacture of silver nanoparticles, a stabilizer needs to be added to ensure that the silver nanoparticles produced are stable and minimize the occurrence of agglomeration. The secondary metabolites found in plant extracts act as internal stabilizers. External stabilizers can also be used such as poly vinyl alcohol [9, 10] long-chain fatty acids such as stearic acid, soluble starch (starch), and gelatin [10-12].

Analysis of the silver nanoparticles was performed using UV-VIS spectroscopy and particle size analyzer to determine that silver nanoparticles have formed in the colloid. Pickle goat skin modification is carried out by depositing silver nanoparticles prepared using red algae on the pickle goat skin. The modified goat skin will be characterized to determine significant differences of the skin with and without modification on the test of contact angle, tensile strength, antibacterial and antifungal activity, and also biodegradability.

METHODOLOGY

Materials

The UV-VIS spectrophotometer (Shimadzu series UV-3600), particle size analyzer (Microtrac FLEX 11.1.0.6), atomic absorption spectroscopy (Shimadzu series AA-7000), tensile machine series RTF-2350, laminar air flow bio clean bench (Shimadzu SCB-1000A), autoclaves, shaker, incubator, magnetic stirrer, camera, analytical balance, microwave, digital ultrasonic cleaner, hot plate, and refrigerator have been used in this research.

The pickle goat skin was purchased from the fabric store in Yogyakarta and the red algae plants were collected at Sundak Beach Yogyakarta, Indonesia. Silver nitrate, soluble starch, and alcohol 96% were purchased as commercial products. Nutrient agar and nutrient broth were purchased from Oxoid. Potato dextrose agar, *Escherichia coli*, *Staphylococcus epidermidis*, and *Candida albicans* were obtained from a collection of Department of Biology, Universitas Negeri Yogyakarta.

Preparation of silver nanoparticle using red algae extract

Fresh red algae plant (*Gracilaria sp*.) was collected, then washed and dried under sunlight for 24 hours to reduce the water. The extract was obtained by boiling 25 g of dried plant for 10 min. The next process is cooling until room temperature and filtration of the solution with filter paper Whatmann 42. Mixtures red algae extract with silver nitrate ($AgNO_3$) solution 10^{-3} M in a volume ratio 1:9. For the microwave method, the mixture was heated in a microwave for 4 min at a power of 300 W to complete the bio-reduction [8]. In the ultrasound method, the mixture was ultrasound treatment using a digital ultrasonic cleaner for 30 min [12]. The mixture of 10 mL of red algae extract with 65 mL silver nitrate solution 10^{-3} M was then left to stand for 2 hours. After 2 hours, the starch solution was added. The mixture was shaken for 24 hours [13].

Characterization of silver nanoparticles

The silver nanoparticles that have been prepared through the biosynthesis process were characterized using a UV-VIS spectrophotometer and Particle Size Analyzer (PSA). UV-VIS spectroscopy was used to determine the formation of colloid silver nanoparticles already formed by observing absorption peaks [4, 5, 9, 11] [14, 15], while PSA was used to determine the size distribution of the silver nanoparticle from three biosynthesis methods . Atomic Absorption Spectroscopy (AAS) was used to determine the silver metal content in laundry waste of the pickle goat skin.

Application of silver nanoparticles on pickle skin

The pickle goat skin was cut to the size of 15 x 15 cm^2, then immersed in colloidal of silver nanoparticles from the biosynthesis process with three methods: microwave, ultrasound, and extraction. The final process was the shaker process, the pickle goat skin was treated with a shaker for 24 hours at 150 rpm and then dried. The type of samples which produced in this study is shown in Table 1.

Table 1.

The Type of Samples

Pickle Goat Skin	Code
Pickle Goat Skin	P
Pickle Goat Skin-NanoAg (Microwave)	PN-M
Pickle Goat Skin-NanoAg (Ultrasound)	PN-U
Pickle Goat Skin-NanoAg (Extraction)	PN-E

Characterization of pickle skin

Characterization of modified and unmodified pickle goat skin to determine the comparison of the treatment with hydrophobicity test, mechanical test, measurement of the clear zone to determine antibacterial and antifungal activity against *Escherichia coli*, *Staphylococcus epidermidis*, and *Candida albicans*, and also biodegradation test.

Characterization of pickle goat skin with hydrophobicity test was determined by measuring water contact angle (WCA) using a camera to take a liquid dropped on the surface of pickle goat skin. The images analysis using CorelDraw software to determine contact angle. The WCA of the pickle skin samples without and with modification was analyzed by observing the formed angle (θ/theta) between the water and the pickle skin sample surface using the sessile drop method. Other characterization to determine tensile strength, elongation, and modulus Young using a tensile tester.

In this research, antimicrobial activity of the sample modified and unmodified pickle goat skin to *Escherichia coli* as a gram-negative-bacteria, *Staphylococcus epidermidis* as a gram-positive-bacteria, and *Candida albicans* is a fungus for antifungal activity. Antimicrobial activity by measuring the clear zone is formed around the sample, chloramphenicol, and aqua as a positive and negative controls. All of the media included the bacterial and fungal growth media such as Nutrient Agar (NA), Nutrient Broth (NB), and Potato Dextrose Agar (PDA) and tools were sterilized in an autoclave for 2 hours. Antimicrobial activity using a disk diffusion method. The next step after the sterilization, the bacterial growth media NA poured into each petri and waited about 24 hours, then coated NB which had been overgrown with bacteria. Each sample was inserted into the petri dishes and in the incubator for 24 hours, then observed a clear zone is formed around the sample and controls every three hours for 48 hours. The same way for antifungal activity by using a different fungal growth media which is PDA and NB, then observed a clear zone every 6 hours in 24 hours and one time for 24 hours. The effects of sample type, incubation time, and interaction between samples and incubation time on inhibition activity were analyzed by the ANOVA test [2, 4].

The last characterization was the biodegradation test. Biodegradation of a polymer was characterized by determining the rate of mass loss and degradability of the pickle skin material as the simplest quantitative method [15]. The effect of variation in biodegradation time on biodegradability of pickle skin can be obtained from determining the mass loss rate. The biodegradation test was carried out by the simulation method. The simulation method can be carried out using certain microorganisms or a mixture of known types. The test was carried out for 15 days using activated sludge with the addition of nutrient broth media. The test was carried

out by weighing the mass of the modified and unmodified skin samples before and after the test to determine the mass-loss rate. Weighing the mass of the sample was carried out every 5 days for 15 days as well as replacing the media in the biodegradation test. Determination of the mass loss and mass-loss rate aimed to analyze the biodegradability of variations of biodegradation time and type of pickle skin.

RESULTS AND DISCUSSION

Properties of silver nanoparticles

Biosynthesis was carried out by three methods and resulted in colloidal silver nanoparticles by using a red algae (*Gracilaria sp*.) as a bio-reductor. Figure 1 describes the mechanism for the formation of silver nanoparticles from one of the secondary metabolites contained in red algae, namely flavonoids. Flavonoid compounds belong to the phenolic group which has a hydroxyl group (OH) and a carbonyl group (CO). The two groups can bind the metal by donating electrons to the Ag^+ to produce Ag^0. Ag^+ will be bound to the OH group so that the H in the OH bond will be released by O. There is a reduction in the flavonoid compound so that Ag will be released from O and O to form double bonds and form Ag^0. Preparation of silver nanoparticles is added with a stabilizing agent to prevent agglomeration. The stabilizing agent used is soluble starch as an external stabilizer [16] and secondary metabolites contained in red algae as an internal stabilizer [16]. Figure 2 shows the red algae which used in this biosynthesis. Figure 3 shows results of biosynthesis of nanoparticles by microwave method, (b) extraction method, and (c) ultrasound method.

The biosynthesis product and silver nitrate solution were then characterized using UV-VIS spectroscopy and PSA. Figure 4 shows the UV-VIS spectrum of colloidal silver nanoparticles biosynthesized through (a) microwave method with a wavelength of 416.5 nm, (b) extraction method at a wavelength 421.00 nm, and (c) ultrasound method at a wavelength 436.50 nm. The UV-VIS spectrum results show that the nano wavelength is in the range 400-500 nm [17]. This indicates that there has been reduction of Ag^+ to Ag^0 so that silver nanoparticles have been formed. Characterization of colloidal silver nanoparticles using PSA to aims determine the size of a particle. Figure 5 shows the PSA spectra of colloidal silver nanoparticles (a) microwave method with the size 77.2 nm, (b) extraction method with the size 90.4 nm, and (c) ultrasound method with 73.0 nm. The results of all silver nanoparticle sizes showed that in the size range of silver nanoparticles 1-100 nm [17].

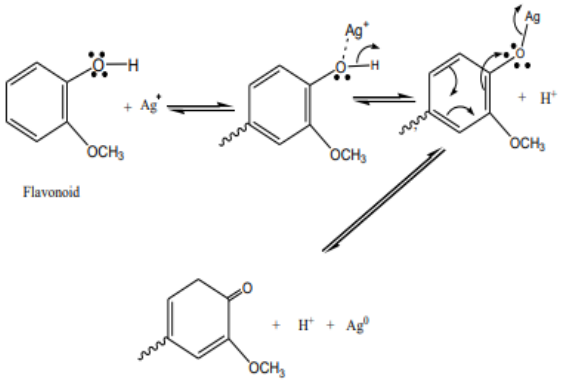
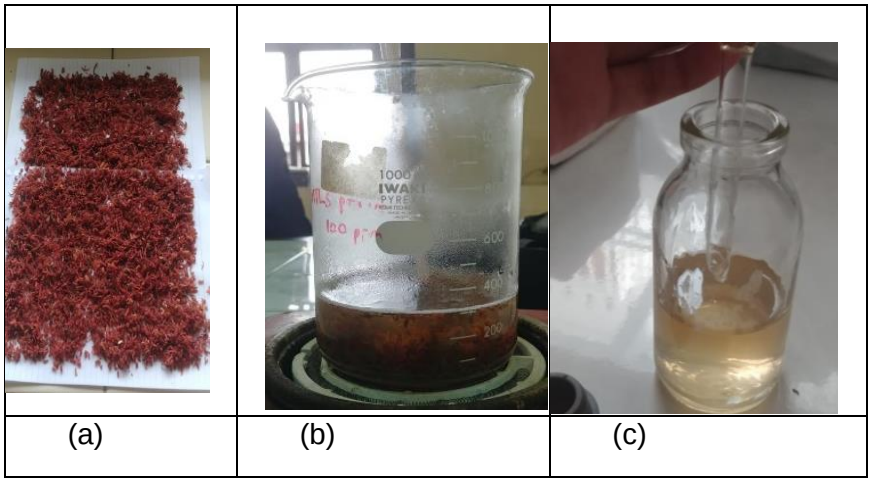
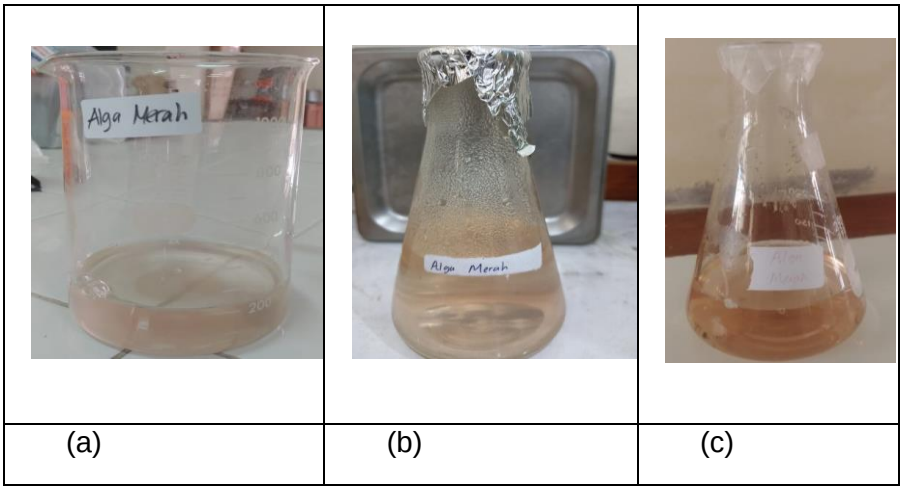


Figure 1. The Mechanism of Formation of Silver Nanoparticles by Flavonoid Compounds



(a) (b) (c)

Figure 2. (a) Red Algae, (b) Heating of Extract of Red Algae, (c) Extract of Red Algae



(a) (b) (c)

Figure 3. **S**ilver Nanoparticle Produced by the Method of (a) Microwave, (b) Extraction, and (c) Ultrasound Method

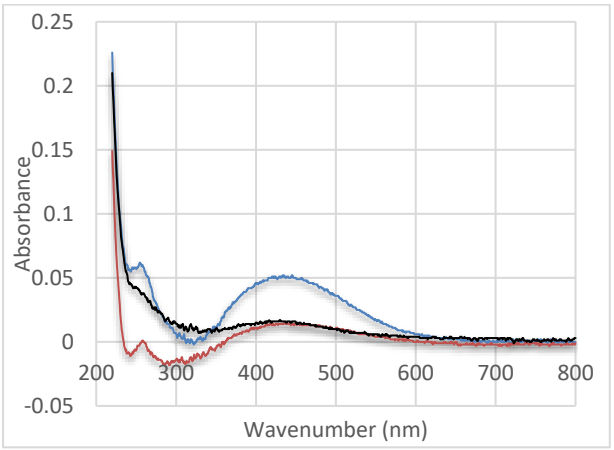


Figure 4. UV-VIS Spectra of Colloidal Silver Nanoparticle Produced by (a) Microwave Method (--), (b) Extraction Method (--) and (c) Ultrasound Method (--).

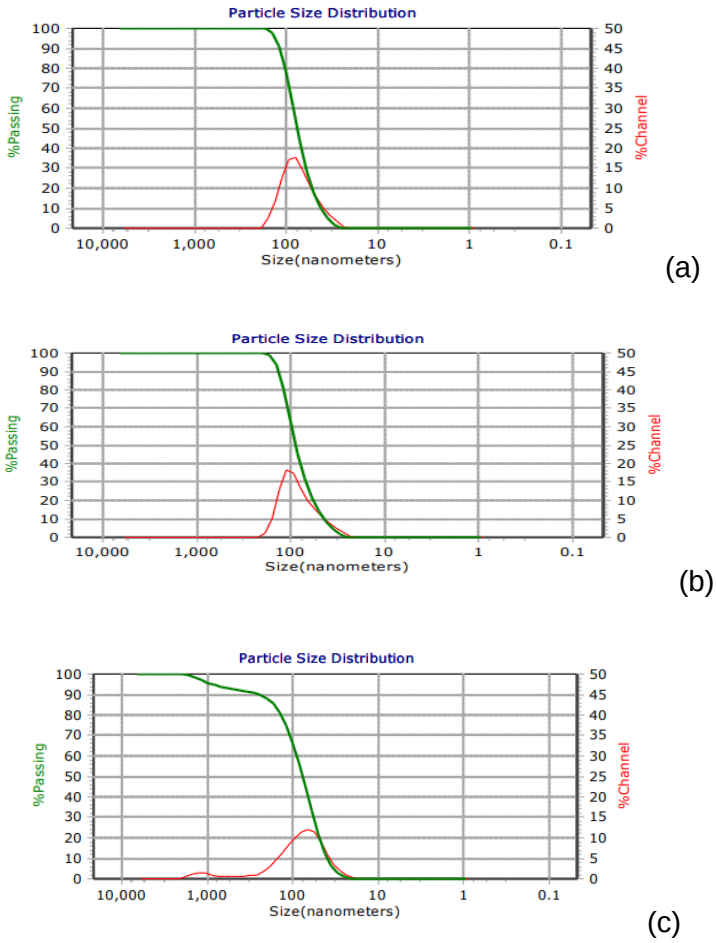


Figure 5. Particle Size Distribution of Colloidal Silver Nanoparticle Produced by the Method of (a) Microwave, (b) Extraction, and (c) Ultrasound.

The water contact angle of pickle skin

Hydrophobicity properties of modified and unmodified pickle goat skin were observed by measuring the contact angle. The result of the contact angle is shown in Table 2.

Table 2

Contact Angle of Pickle Goat Skin

Pickle Goat Skin	Contact Angle (0)
PN-M	61.35
PN-U	63.23
PN-E	53.25
P	46.60

Based on result of the contact angle, can be known that sample of PN-U has the highest contact angle. However, all of the contact angles from modified and unmodified pickle goat skin show hydrophilic surface because the contact angles <90° [18]. The value of the contact angle $<90^0$ can be caused by materials that are easily wet so that water can flow easily, besides that the goat skin used has not been tanned so that water is easy to enter [19, 20]. Modified pickle goat skin showed an effect on the contact angle because modified pickle goat skin has a contact angle greater than the skin without modification [21].

In the results of the contact angle test, a statistical test is needed to determine whether or not there is an effect of modification on the tested goat skin sample. The statistical test using a SPSS program with Two Way ANOVA showed a significance value of 0.017. This significant value is less than 0.05 ($p<0.05$) so that the skin modification treatment effects on the contact angle of pickle skin.

After the ANOVA statistical test is carried out, it is necessary to carry out further tests, the Duncan test to determine the effect of the best skin modification on the contact angle. Result of Duncan test showed that the greatest values was obtained in the sample of PN-U, then followed by the second largest value of PN-M, PN-E, and P.

Mechanical properties of modified and unmodified pickle goat skin

The tensile strength test to determine the quality and strength of the goat skin test sample [22]. Table 4 is the result of mechanical testing, tensile strength of modified and unmodified pickle goat skin. The sample of PN-E showed the highest tensile strength and elongation values [22]. The second highest to lowest tensile strength values were PN-M, P, and PN-U. Meanwhile, the values for the elongation values were PN-M, PN-U, and P. Result of statistical test with Two Way ANOVA showed a significance value of 0.423. This value is greater than 0.05, which means that the modified and unmodified does not have a significant effect on the tensile strength.

Table 3.

Tensile Strength and Elongation of Goat Skin

Various Pickle Goat Skin	Tensile Strength (MPa)	Elongation (%)
NP-M	15.6323	77.0000
NP-U	11.7965	70.9146
NP-E	145.5373	77.4220
P	11.2646	71.1016

Antibacterial Activities of Pickle Skin

Antibacterial activity was carried out by measuring the clear zone diameter that appeared in the sample. The clear zone indicated an inhibition of bacterial growth. Graph of the results of clear zone measurements on *Staphylococcus epidermidis* and *Escherichia coli* are shown in Figure 6 and 7. The diameter of the clear zone of antibacterial activity against *Escherichia coli* on microwave, ultrasound, and unmodified goat skin showed the effective bacterial inhibition rate in the first 9 hours, 10.8 mm, 9.3 mm, and 10 mm. In modified skin with the extraction method resulted in an effective bacterial inhibition in the first 6 hours, which was 9.1 mm, then a decrease occurred at the 9th hour. Based on Figure 6, the graph of antibacterial activity against *Staphylococcus epidermidis* bacteria, it shows that the modified skin of the three methods produced a maximum clear zone diameter at the 9th time of 12.9 mm for PN-E, 11.5 mm for PN-M, and 11.1 mm for the PN-U. Unmodified skin showed a maximum bacterial inhibition rate at 9 hours and for the following hours resulted in smaller clear zone diameter compared to the three types of modified skin. This indicates that the modified treatment of goat skin produces a better effect on the antibacterial activity.

The result of the statistical test with Two Way ANOVA showed the significance value of the two bacteria of 0.000. This value indicated a value less than 0.05 ($p<0.05$). This explains that there were significant differences between the two bacteria with three variations, skin variation, time, and the interaction between skin variation and

time show in antibacterial activities [1, 14, 22]. The variety of preparation of nanoparticles have been carried out by three methods, microwave, ultrasound, and extraction has an effect on antibacterial activity of pickle skin by the appearance of a clear zone. Incubation time also has an effect on antibacterial activity. The results of the Duncan of antibacterial activity of pickle skin against *Escherichia coli* to determine the best skin variation showed that the modified skin by adding nanoparticle prepared with the microwave method has the highest antibacterial activity against *Escherichia coli* bacteria. The results of the Duncan test on the skin modified by the ultrasound method (NP-U) and the extraction method (NP-E) showed the effect of various treatments but with lower values than NP-M and P.

The result of the Duncan of antibacterial activity of skin against *Staphylococcus epidermidis* showed that modified skin with the addition of nanoparticles prepared by using microwave method (NP-M) has the highest antibacterial activity against *Staphylococcus epidermidis*. The largest value was followed by skin modified by ultrasound and extraction methods. This indicated that skin modification by the three methods show a good effect on antibacterial activity.

The results of clear zone measurements and statistical tests showed that the skin modified with antibacterial activity more easily inhibited the growth of *Staphylococcus epidermidis* bacteria as gram-positive bacteria compared to *Escherichia coli* bacteria. *Staphylococcus epidermidis* has a thick peptidoglycan layer so that the structure of the bacteria is more rigid. Besides, the cell wall also contains teichoic acid which consists of -OH and phosphate groups. The functional groups contained in teichoic acid have a role in the interaction relationship between silver nanoparticles and bacteria [10, 18, 22]. The presence of these functional groups in the bacterial cell wall can interact with silver nanoparticles in greater numbers so that the antibacterial activity is greater.

The size of silver nanoparticles is one of the factors that influence antibacterial activity testing. The smaller size of silver nanoparticles with a narrow distribution, the better or increased antibacterial activity will be [19]. This is in accordance with the results of the antibacterial test that the modified skin by adding nanoparticle prepared by the microwave method has the best antibacterial activity compared to other skin types because according to the PSA test results, the modified skin with the microwave method has the smallest size with a narrow distribution.

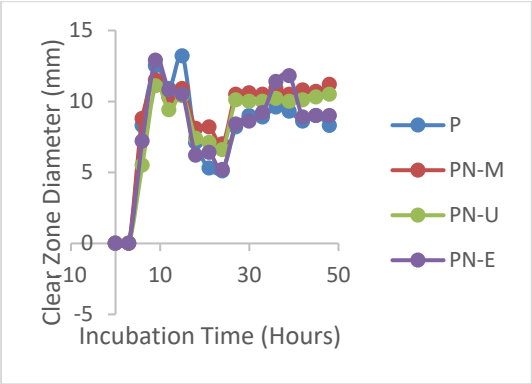


Figure 6. Antibacterial Activity of Pickle Skin against *S. epidermidis*

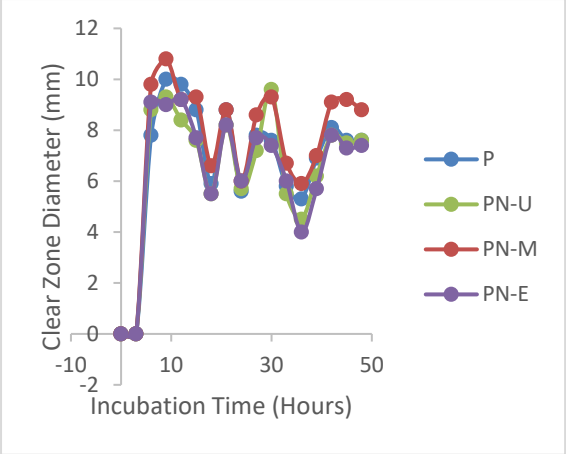


Figure 7. Antibacterial Activity of Pickle Skin against *E. coli*

Antifungal activities of pickle skin

The antifungal activity test was carried out by measuring the zone of inhibition against the fungus *Candida albicans*. Figure 8 shows a graph of the relationship between clear zone diameter and incubation time. Figure 8 shows a graph of an increase in the first 12 hours then decreased at the 18 hours and an increase in the 24 hours. At 24 hours, the PN-M experienced a significant increase with the largest value, as much as 9.4 mm. Meanwhile, the other two modified skins experienced an increase at 24 to 48 hours and the largest increase at 48 hours, as much as 8.7 mm for PN-U and the PN-E of 8.4 mm. Unmodified skin showed smaller values than skin modified by addition nanoparticles via microwave and ultrasound methods.

ANOVA test results on the antifungal test against the fungus *Candida albicans* showed the significance value was 0.000 on the interaction of skin variations, incubation time, and skin variations with incubation time. The significance value of 0.000 is less than 0.05, so that skin variations and incubation time have an effect on the antifungal activity of pickle skin against the fungus *Candida albicans*. The results of the Duncan of antifungal activity of pickle skin against *Candida albicans* to determine the best antifungal activity of skin variation [19] . The modified skin with the microwave method show the greatest value compared to the other two, with and without modified skins. This shows that the microwave method in the preparation of nanoparticle to modify pickle skin has the best antifungal activity.

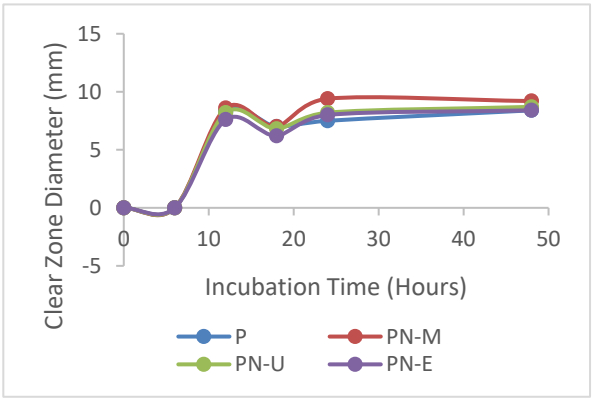


Figure 8. Antifungal Activities of Pickle Skin against *Candida albicans*

Degradability of pickle skin

The result of mass-loss rate of pickle skin is shown in Table 4. The mass loss increased with increasing incubation time in the biodegradation testing. The results of the overall data showed that the PN-M had the largest mass-loss average of 40.60%, the PN-E was 40.11%, the P was 34.91%, and the PN-U was 32.75%.

Table 4.

Type of Pickle Skin	Mass Loss (%)		
	5 days	10 days	15 days
PN-M	19.01	33.09	69.71
PN-U	8.5	42.04	47.72
PN-E	26.54	45.13	48.67
P	14.28	19.04	71.42
Type of Pickle Skin	Mass Loss Rate (mg/day)		
	5 days	10 days	15 days
PN-M	3.802	3.309	4.647
PN-U	1.700	4.040	3.181
PN-E	5.308	4.513	3.245
P	2.856	1.904	4.761

Table 5.

Silver Metal Content in Laundry Waste of Pickle Goat Skin

Type of Goat Skin	Silver Metal Content (ppm)
PN-M	1.83
PN-U	1.68
PN-E	2.25

Based on the results of biodegradation testing, modifications made to goat skin showed a good effect. It is hoped that if the modified goat skin is used in the future and then it is not used again, it will be easily biodegraded so that it does not pollute the environment due to the waste.

Conclusion

Based on the results of the characterization of silver nanoparticles which prepared using red algae (*Gracilaria sp*.) extract showed that the wavelength and particle size of the silver nanoparticle range was 416.5 nm with a size of 77.2 nm for the microwave method, for extraction of 421.0 nm with a size of 90.4 nm, and the ultrasound method of 436.50 nm with a size of 73.0 nm, this indicated that silver nanoparticles have formed. The results of characterization of pickle goat skin showed that there were significant differences between modified and unmodified skin on the contact angle, tensile strength, antibacterial activity, and antifungal activity. The results of biodegradation showed that there were differences in the biodegradability between modified goat skin and goat skin without modification. The mass-loss average of goat skin modified by using nanoparticles via microwave, ultrasound, extraction, and unmodified goat skin was 40.60%, 32.75%, 40.11%, and 34.91%, respectively.

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DECLARATION ON CONFLICT OF INTEREST

There are no conflict of interests to declare.

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