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Formulation of Peel-Off Gel Mask From Manilkara Zapota (L) P. Royen Leaves Extract and Antibacterial Activity Against

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Abstract: Sawo leaves contain alkaloids, flavonoids, tannins, saponins and steroids which are antibacterial. This study aims to obtain the best concentration of Sawo leaf ethanol extract (*Manilkara zapota (L) P. Royen*) formulated as a *peel-off* gel mask preparation based on antibacterial activity test against *Staphylococcus epidermidis* bacteria. This study used laboratory experimental method. Sawo leaf extract peel-off gel mask preparation formula was made with concentration variations of 0.1%; 0.3%; 0.6%; 2.5%; 5% and 10%. Sawo leaf plant extract was obtained by maceration using 96% ethanol solvent. Identification of compounds was carried out using phytochemical screening, spray TLC and Bioautography TLC. Antibacterial testing of extracts was carried out using solid dilution and liquid dilution methods. The antibacterial test of the preparation using the wells diffusion method had the largest diameter of the inhibition zone at a concentration of 8% with an inhibition zone diameter of 14.1 mm and the inhibition ability was categorized as strong. It can be concluded that the concentration of 2%; 4%; 6%; 8% is good to be formulated as a *peel-off* gel mask preparation and is categorized as strong inhibition ability.

Keywords: Antibacterial, TLC Bioautography, Peel Off Gel Mask, Sawo (*Manilkara zapota (L) P. Royen*), *Staphylococcus epidermidis*

INTRODUCTION

Facial skin beauty for a woman both teenagers and adults is very important, a clean and healthy face is the dream of all women. All means will be done for the health and beauty of the face, one of which is with facial care to avoid any disturbances that cause the face to be damaged. Skin is an important organ that is sensitive and protective against external influences. On the skin can occur problems such as acne. Diseases such as acne are characterized by involving hyperactive secretion of oil glands and the colonizing effect of bacteria (WahdanWahdaningsih *et al*., 2018).

Acne (*Acne vulgaris*) is a skin disease that almost everyone has experienced, often considered a physiologically arising skin disorder and a disease that generally occurs in the skin area of the face, neck, to the chest to the back (Sawarkar *et al*., 2010). Normally acne often occurs in adolescents to adults and does not recognize the existence of gender (Lestari *et al*., 2020). According to the Global Burden of Disease (GBD) study, 85% of adults aged 12-25 years suffer from acne vulgaris. Research in Germany found 64% of 20-29 year olds and 43% of 30-39 year olds suffer from acne vulgaris. In addition, research in India explains that the disease most commonly affects >80% of the world's population during some period of life and 85% of adolescents in developed countries. The prevalence of acne vulgaris in the Southeast Asian region is 40-80% of cases while according to records from Indonesian cosmetic dermatology there continues to be an increase, namely 60% of patients with acne vulgaris in 2006, 80% in 2007 and reached 90% in 2009 (Afriyanti, 2015). The results of a research report by Indonesian Cosmetic Dermatology showed that the percentage of acne sufferers increased by 10% each year, namely 60% in 2006, 80% in 2007, and 90% in 2009 (Sibero *et al*., 2019).

Acne can arise due to several factors, namely genetics, hormones, food, skin conditions, psychology, weather, bacterial infections, work, cosmetics and other chemicals (Noventi & Carolia, 2016). The presence of excess oil glands and dirt from outside the money clogs the pores of the skin, then accumulates in dead skin cells will be a good growth medium for bacteria which can eventually cause acne (Meilina & Hasanah, 2016). Acne can be caused by excessive oil gland activity and exacerbated by bacterial infection. The bacteria that cause acne consist of *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus* epidermidis (Meilina & Hasanah, 2013). Previous research in Korea found that patients with Acne Vulgaris found bacteria in the form of *Staphylococcus epidermidis* and *Propionibacterium* acnes (Moon et al., 2012). These bacteria are not pathogenic under normal conditions, but when there is a change in skin conditions, the bacteria become invasive. Secretions of sweat glands and sebaceous glands that produce fatty acids, amino acids, urea, water and salt are sources of nutrients for bacterial growth. The mechanism of acne is that bacteria damage the stratum corneum and stratum germativum by secreting chemicals that can destroy the pore wall. The condition can also cause inflammation. So that fatty acids and oils in the skin are clogged and harden into acne bumps. If the pimple is touched with dirty hands or nails, the inflammation extends so that the hardened fatty acid and skin oil solids will be enlarged (Miratunnisa *et al*., 2015).

Research Primadiamanti *et al.* (2018) reported that the mustard plant is known to have antibacterial activity against *Staphylococcus aureus. Staphylococcus aureus* and *Staphylococcus epidermidis* are both gram-positive bacteria, so it can be assumed that the *Staphylococcus epidermidis* bacteria may also be known to have antibacterial activity against mustard plants. The content of secondary metabolites in mustard leaves and bark are flavonoids, phenolics and saponins which are known to be able to inhibit bacterial growth (Octaviani & Syafrina, 2015; 2018) *Staphylococcus aureus* and *Staphylococcus epidermidis* are gram-positive bacteria that can cause skin infections, one of which is acne (Brooks *et al.*, 2008).

Until now, there have been many types of treatments to treat skin problems such as acne, either physically (laser therapy) which requires a lot of money or is classified as expensive. Chemically (use of antibiotics) such as tetracycline, erythromycin, and clindamycin. However, these drugs also have side effects such as antibiotic resistance and skin irritatio (Muharram *et al*., 2022). Therefore, other alternative treatments are needed with relatively low prices and minimal side effects, namely by utilizing natural ingredients as an alternative treatment (Fatmawaty *et al*., 2022). Although synthetic drugs have been widely used, traditional medicines are still widely used by the community because people believe that natural ingredients are able to treat diseases with minimal side effects compared to drugs made from synthetic materials (Fatmawaty *et al*., 2016).

Sawo *(Manilkara zapota (L.) P. Royen)* belongs to the Sapotaceae family and is one of the plants that can be used in traditional medicine. Traditionally, people use young sawo fruit to treat diarrhea and can be used as an alternative treatment for skin-related diseases such as acne (Basuki & Wiyono, 2019). Sawo leaves *(Manilkara zapota (L.) P. Royen)* contain active metabolite compounds contained in sawo, namely alkaloids, flavonoids, saponins, tannins and terpenoids which have been known to have antibacterial activity (Islam *et al*., 2013). Research Distiani (2021) reported that sawo leaves (*Manilkara zapota (L.) P. Royen)* are plants that have potential as antibacterials, because they contain flavonoid compounds. Other studies have also shown that water and methanol extracts of sawo leaves have antibacterial activity (Nair & Chanda, 2008). In research Sari *et al*. (2015) reported that the minimum inhibitory concentration (MIC) value of the ethyl acetate fraction against *Staphylococcus epidermidis* bacteria was at a concentration of 125 mg/mL. The results showed that the ethyl acetate fraction of butoh keling extract has antibacterial activity against *Staphylococcus epidermidis* so that it can be used as an antibacterial alternative. In research Suryana *et al*. (2017) obtained the minimum inhibitory concentration (MIC) value of *Leucaena leucocephala* ethanol extract against *Staphylococcus epidermidis* is 62.5 µg/ml. The Minimum Kill Concentration (MBC) value of Camellia sinensis and Psidium guajava L ethanol extracts is 125 µg/ml.

Masks are cosmetics that are currently in great demand among the public, especially teenagers, to overcome facial skin problems. The use of masks is generally quite complicated, so mask products that are easier and more practical to use are needed, for example, *peel-off* gel masks. A *peel-off* mask is a type of dosage form that is gently applied to the surface of the facial skin and will peel off after a few minutes of application. This mask preparation is used as a deterrent to bacteria such as *Staphylococcus epidermidis* to treat facial skin-related problems such as wrinkles, premature aging, acne and is mainly used to open pores that are closed due to dust deposition (Merwanta *et al*., 2019).

But until now there has been no research on the antibacterial activity of mustard leaf extract against *Staphylococcus epidermidis*. So in this study, an assessment of the peel-off gel mask preparation formulation of ethanol extract of mustard leaves and an antibacterial activity test against *Staphylococcus epidermidis* as one of the bacteria that cause acne were carried out. The peel-off gel mask preparation formulation of ethanol extract of mustard leaves was chosen because this mask preparation formulation is practical in use so it does not require other ingredients such as water to rinse off (Rahmawanty *et al*., 2015). Some of the advantages of peel-off face masks include improving and treating facial skin from wrinkles, aging, acne and can also be used to shrink pores (Grace *et al*., 2015).

METHODS

Tools and Materials

The materials used in this study were sawo leaf simplisia (*Manilkara zapota (L.) P. Royen*), Polyvinyl alcohol (PVA), Hydroxypropyl methylcellulose (HPMC), TEA, glycerin, propylenglycol, methyl paraben, ethanol 96%, distilled water, clindamycin, DMSO 10%, magnesium powder, *Staphylococcus epidermidis* bacterial culture, Nutrient Agar (NA), Nutrient Broth (NB), 1% H2S04 solution, Aluminum chloride reagent ($AlCl_3$), chloroform, ethyl acetate ($C\ H\ O_{482}$), NaCl 0.9%, Dragendorff, Mayer, Wagner, Libermann Burchard, Hydrochloric Acid (HCl), $FeCl_3$ (1%).

The tools used in this study are beakers, measuring cups, petri dishes, maceration vessels, test tubes, erlemeyers, stirring rods, analytical scales, spatulas, LAF, spiritus lamps, ose needles, cotton swabs, sterile cotton, pH meters, incubators, drip pipettes, funnels, knives, weighing paper, waterbaths, autoclaves, calipers, rulers, vacuum rotary evaporators, GF 254 TLC plates, capillary tubes, uv 254 & 366 lamps, TLC chambers, filter paper, preparation glass,

Brookfield Viscometer, aluminum foil, labels, tissues, preparation containers, micro pipettes, horn spoons, spreaders, incubators, waterbaths, autoclaves, calipers, vacuum rotary evaporators, TLC plates GF 254, capillary pipes, uv lamps 254 & 366, TLC chamber, filter paper, preparation glass, Brookfield Viscometer, aluminum foil, label, tissue, preparation container, micro pipette, horn spoon, spreader, incubator.

Extraction

Sampling of sawo leaves (*Manilkara zapota* (L.) *P. Royen)* was taken from Wolo Village, Penawangan, Grobogan Regency, Central Java as much as 1 kilogram. The part used was sawo leaves (*Manilkara zapota* (L.) *P. Royen). The* picked sawo leaves (*Manilkara zapota* (L.) *P. Royen*) were cleaned of dirt, washed with water until clean and drained, then cut into thin pieces into 3 parts. Furthermore, the sawo leaves (*Manilkara zapota* (L.) *P. Royen)* are dried directly using sunlight for about 2 weeks until the leaves dry completely. The purpose is to prevent damage to certain secondary metabolite compounds, especially flavonoids and facilitate the pollination process. The process is continued by pollinating the sawo leaves using a grinder (pollinator machine) (Lailiyah *et al*., 2021). Then the sample was sieved using a flour sieve mesh no. 60 to remove impurities that remained during the drying process.

A total of 500 grams of sawo leaf powder was extracted using the maceration method, the solvent used was 5000 mL of 96% ethanol. The first simplisia powder is put into a maserator or glass jar then enter 96% ethanol solvent, soak for 3x24 hours while occasionally stirring. Maceration is carried out within 1 day then separated between filtrate and residue by filtering using filter paper. Then the extraction process is continued by remacerating for approximately 2-3 days until the extract is colorless, the residue is added with 96% ethanol solvent. The obtained maceration filtrate is then evaporated with a rotary evaporator at 40°C until a thick extract is obtained and weighed (Indriani *et al*., 2019). Calculated the yield of 96% ethanol extract by weighing the weight of the total thick extract with the weight of the total simplisia. The formula is as follows:

$$\text{Yield (\%)} = \frac{\text{weight of total thick extract } (gram)}{\text{initial weight of total simplicia } (gram)} \text{x } 100\%$$

TLC and Bioautography TLC

The stationary phase used is GF254 silica plate with a size of 10 x 2 cm as many as 5 plates. The silica plate was given a lower and upper edge line of 1 cm as the limit of the initial position of bottling and the upper edge as a sign of the limit of the elution process. The mobile phase used was chlorophyllom:ethyl acetate (7:3). The mobile phase is mixed and then inserted in the chamber and closed tightly then saturation of the mobile phase using filter paper for 10 minutes. The purpose of saturation of this mobile phase is to equalize the vapor pressure in the entire vessel. (Paputungan *et al*., 2019).. The extract is bottled on each plate using a capillary pipe, then the TLC plate that has been given a totolan extract is inserted into the chamber and wait for the eluent to rise to the limit mark and then dried. After that, the TLC plate is sprayed with the appropriate solvent, observed with UV 254 nm and UV 366 nm lights and calculate the Rf value with the formula:

$$\text{Rf: } \frac{\text{distance traveled by the spot } (cm)}{\text{distance traveled by the eluent (cm)}}$$

Extracts that have been eluted with chlorophom:ethyl acetate (7:3) eluent were then identified by spraying Dragendorf, AlCl3, FeCl3 and Liberman Burchard (LB) spray reagents. Compound identification was carried out on 5 plates of extract bottling results using spray reagents. The identification carried out is on alkaloid compounds using Dragendorff reagent, flavonoid identification using Mg powder reagent and HCl solution, tannins using FeCl reagent$_3$ 1%, saponins using Libermann Burchard reagent and steroids using Libermann Burchard reagent (Rijayanti, 2014).

The bioautography test was carried out using agar diffusion. NA media was poured into a sterile Petri dish as much as 20 ml. After solidifying the medium, the *Staphylococcus epidermidis* test bacteria were inoculated. using the smear method (catton swab) evenly on the media. Then the previously eluted TLC plate was placed on the media for 30 minutes then the plate was removed and the cup was incubated for 24 hours at 37°C. The zone of inhibition of bacterial growth was observed, characterized by the presence of a clear area at the position of the stain and compared with the chromatogram of the TLC results. The TLC plate was observed under UV light or lamp with wavelengths of 254 and 365 nm and the Rf value of the clear zone was calculated (Abidin *et al*., 2022).

Table 1. Compound Groups, Reagents and Spray TLC Testcolors

Compound	Reagents	Color (+)
Alkaloids	Dragendorff	Orange
Flavonoids	AlCl3	Yellow
Tannins	$FeCl_3$ 1%	Blue-black and green-black
Saponins	Libermann Burchard	Green, yellow, red, blue
Steroids	Libermann Burchard	Blue, green

Dosage Formulation

Table 2. Formulation of Peel-Off Gel Mask Preparation of Ethanol Extract of Sawo Leaf *(Manilkara Zapota (L.) P. Royen).*

	Formula % (b/v)					Usability
	Fo	FI	FII	FIII	FIV	
Ethanol extract of sawo leaf	-	2%	4%	8%	10%	Active substance
Polyvinyl alcohol	10%	10%	10%	10%	10%	Film forming
HPMC	1%	1%	1%	1%	1%	Gel base
Glycerin	12%	12%	12%	12%	12%	Humectants
Triethanolamine (TEA)	0,15%	0,15%	0,15%	0,15%	0,15%	Surfactants
Nipagin (Methyl Paraben)	0,2%	0,2%	0,2%	0,2%	0,2%	Preservatives
Aquadest	Ad 100 ml	Ad 100 ml	Ad 100 ml	Ad 100 ml	Ad 100 ml	Solvent

HPMC was developed first in cold water by sprinkling it little by little and allowed to stand for ± 24 hours until it expands perfectly. PVA was developed in warm distilled water until fully expanded and homogeneous. Then, glycerin, which had been dissolved in hot aquadestilata, HPMC, and TEA were added successively to the PVA mass, stirring until homogeneous. Then sterilized in an autoclave at 121˚C with 1 atmosphere pressure for 15 minutes. After that, mustard leaf extract was added which had been diluted in distilled water and then stirred until homogeneous. (Puluh *et al*., 2019).

Antibacterial Testing

Tool Sterilization

The tools that will be used in this antibacterial activity research are washed thoroughly using soap, then dried after which they are sterilized first using an autoclave at 121 °C pressure 1 atm for 20 minutes.

Nutrient Agar (NA)

The nutrient agar medium was weighed as much as 5 grams of NA and then put into an Erlenmeyer flask and then added 50 mL of distilled water. Media that has been homogeneous is sterilized in an autoclave for 15 minutes at a temperature of 121° C. After that, the media was waited until it cooled slightly around 40-45° C. NA media that has been cooled then poured into Petri dishes as much as 25 mL. NA media that has been poured into Petri dishes is allowed to solidify (Indarto *et al*., 2019).

Nutrient Broth

The nutrient broth media was weighed as much as 0.8 grams dissolved in 100 ml of distilled water, poured into test tubes of 10 ml each homogenized and sterilized by autoclaving at 121° C for 1 hour (Indarto *et al*., 2019).

Preparation of Positive Control and Negative Control

Positive Control

The positive control used was clindamycin 1.2% (Salsabilla *et al*., 2023). Clindamycin is used as a positive control because *Staphylococcus epidermidis* bacteria are known to have a high sensitivity to the antibiotic clindamycin (Kamala & Permana, 2020). The positive control test solution was made by dissolving 120 milligrams of clindamycin in 10 ml of sterile distilled water (Gunarti *et al*., 2021).

Negative Control

The negative control used is 10% DMSO because DMSO has no activity to inhibit bacteria (Amanda *et al*., 2021). In addition, DMSO is a solvent that can dissolve almost all compounds, both polar and non-polar (Rahmi & Putri,

2020). The negative control used is 10% DMSO. DMSO 10% is made by putting 10 mL of DMSO into a measuring cup, then adding 100 mL of distilled water.

Bacterial Rejuvenation

The bacterial rejuvenation process is carried out by taking a pure culture of the test bacteria as much as one ose. The needle is then scratched zig zag on NA media in a Petri dish and incubated at 37ºC for 24 hours. Rejuvenation or regeneration of bacteria aims to restart metabolism in stored test bacteria (Wijayati *et al*., 2014).

Preparation of Bacterial and *McFarland* Test Suspensions

Preparation of Bacterial Suspension

Test bacteria on agar media were taken 1 ose using a sterile ose wire and then suspended into a tube containing 10 mL of 0.9% NaCl solution in a test tube then shaken until evenly distributed and then equalized with Mc Farland. (Retnaningsih *et al*., 2019).

Preparation of 0.5 Mc Farland Solution

Preparation of *McFarland* 0.5 standard solution is commonly used as a comparison of turbidity of bacterial culture in liquid medium with a density between 1 x 107 cells/ml - 1 x 108 cells/ml. The work sequence for making *McFarland* 0.5 solution is as follows. A total of 0.05 ml of 1% Barium Clorida (BaCl) in distilled water was added to 9.95 ml of 1% Sulfuric Acid ($H_2 SO_4$). Then stored in a place that is protected from direct sunlight (Aviany & Pujiyanto, 2020).

Antibacterial Activity Test of Extracts by Liquid Dilution and Solid Dilution Methods

In the liquid dilution method, a multistage dilution method was used. A total of 8 sterile test tubes were prepared. Each tube included mustard leaf extract, 10% DMSO 10 ml and 1 mL of *Staphylococcus epidermidis* bacteria equivalent to 0.5 Mc Farland standard. Each test tube was labeled 1-6, tube 5 was labeled K (+) and tube 6 was labeled K (-) which is the negative control, which is a tube containing 10 ml DMSO. Tube 1 included 4 ml NB and 1 gram of sawo leaf extract, then dissolved in 20 ml of 10% DMSO which had been diluted with 100 ml of distilled water and inserted 4 ml into the first tube then given 1000 µl of *Staphylococcus epidermidis* bacterial suspension. Tube 2 was given 4 ml NB and added 4 ml from tube 1 then given 1000 µl of *Staphylococcus epidermidis* bacterial suspension. Tube 3 was given 4 ml NB and added 4 ml from tube 2 then given 1000 µl of *Staphylococcus epidermidis* bacterial suspension. Tube 4 was given 4 ml NB and added 4 ml from tube 3 then given 1000 µl of *Staphylococcus epidermidis* bacterial suspension. Tube 5 was given 4 ml NB and added 4 ml from tube 4 then given 1000 µl of *Staphylococcus epidermidis* bacterial suspension. Tube 6 was given 4 ml NB and added 4 ml from tube 5 then given 1000 µl of *Staphylococcus epidermidis bacterial* suspension. Then tube 7 was given the contents of clindamycin antibiotic and 1000 µl of bacterial suspension. Tube 8 is a negative control, which is a tube containing 10 ml of DMSO and 1000 ml of bacterial suspension. Furthermore, the treatment tube media was incubated for 1x24 hours, all tubes were visually examined for turbidity. If the turbidity of each tube is still equal to or more turbid than the K(-) tube containing DMSO according to the McFarland 0.5 turbidity standard, it means that the bacteria can still grow, but if the solution in the tube looks clearer than the K(-) tube, it means that bacterial growth is starting to be inhibited. This is what indicates the minimum inhibitory concentration (MIC). The minimum inhibitory concentration is determined by the smallest concentration of extract in the treatment tube that has begun to inhibit bacterial growth (Munira & Nasir, 2023).

In the solid dilution method, the determination of the MBC value refers to research (Rollando, 2019) Testing in determining the MBC value is carried out by taking 100 µL of mixed samples (sawo leaf extract and bacterial suspension) and then poured on NA media that has been prepared in a Petri dish and then leveled using a sterile spreader bar. The solution used is a MIC determination test solution that is clear or has no signs of bacterial growth, then incubated for a period of 24 hours. The MBC value is determined by observing the presence or absence of bacterial growth on the Petri dish.

Antibacterial Activity Test of Peel-Off Gel Mask with Well Diffusion Method

The last test is testing the antibacterial activity of peel-off gel mask preparations of ethanol extract of sawo leaves (*Manilkara zapota* L.) carried out against *Staphylococcus epidermidis* bacteria using the well diffusion method. The positive control used, namely clindamycin gel, aims to be used as a comparison to determine the effectiveness of the peel-off gel mask preparation extract of ethanol extract of sawo leaves (*Manilkara zapota* L.). While the negative control used is a peel-off gel preparation without extract to ensure that the inhibition zone of the peel-off gel mask preparation of sawo leaf ethanol extract (*Manilkara zapota* L.) produced is not the influence of the solvent and base used. Antibacterial strength has several provisions, namely if the inhibition area of 20 mm or more means very strong, the inhibition area of 10-20 mm means strong, 5-10 mm means moderate and the inhibition of 5 mm or less means weak.

NA media in a Petri dish was waited until it solidified then four holes were made for concentration, two holes for positive control and negative control. NA media is planted with bacterial suspensions using the *swab* method, this technique aims to move microbes that are on the surface of samples that have a large surface and are generally difficult to move using a cotton swab / cotton bud. Then each sample was pipetted as much as 100 µl and then dripped into the wells, clindamycin was inserted as a positive control and *peel-off* gel mask preparation base as a negative control into each well. Then incubated at 37°C for 24 hours and then observed the inhibition zone formed around the wells using a caliper (Wulandari & Ariyani, 2020).
Can be calculated using the formula (Tambunan & Sulaiman, 2018):

$$\frac{(DV - DS) + (DH - DS)}{2}$$

Description:
DV: Vertical Diameter
DH: Horizontal Diameter
DS: Well Diameter

Physical Evaluation of Peel-Off Gel Mask Preparations
Organoleptical Test

Includes sensory testing by observing changes in terms of color, odor and also the shape of the preparation. (Setiyadi & Qonitah, 2020).

Homogeneity Test

Apply 1 g of gel mask preparation to the preparation, then observe whether there are parts that are not mixed. Homogeneous gel mask preparations are characterized by the absence of visible coarse grains (Shanti Septiani et al., 2011).

pH test

The pH check is carried out using a pH meter tool, by weighing the mask preparation as much as 1 g and then dissolving it in 10 mL of distilled water and then stirring it until it dissolves, after which a pH meter that has been standardized is dipped into the solution and the results are recorded, the pH of the preparation must show a pH that matches the pH of the skin, which is around 4.5 - 6.5 (Rachmalia *et al*., 2008).

Spreadability Test

Spreadability testing is done to ensure even distribution of the gel when applied to the skin by looking at the diameter of the spread. Gel as much as 1 gram is placed in the center of the glass, above the gel is placed another glass and allowed to stand for 1 minute, measure the diameter of the spread using a ruler and then record the measurement results, then place the weight and allowed to stand again for 1 minute, measure the diameter of the spread using a ruler and record the measurement results again.

Adhesion Test

A total of 0.25 g of peel-off gel mask was placed between 2 glass objects and then pressed with a load of 1 kg for 5 minutes. Then the glass object was mounted on the adhesion test device and given a load weighing 80 g. The peel-off mask release time was recorded using a stopwatch (Pratiwi & Wahdaningsih, 2018). Good adhesion for topical preparations is more than 4 seconds.

Drying Time Test

The gel mask preparation is applied to the back of the hand, then the drying time of the preparation is calculated until it forms a film layer using a stopwatch. The drying time of a good peel-off gel mask preparation is between 15-30 minutes (Saputra *et al*., 2019).

RESULT AND DISCUSSION

In this study, the preparation of Sawo leaf ethanol extract peel-off gel mask using PVA (polyvinyl alcohol) base as a plasticizer, Hydroxypropyl Methylcellulose (HPMC) as a viscosity enhancer, glycerin as a humectant, TEA as an emulsifying agent, Nipagin (Methyl Paraben) as a preservative and distilled water used as a solvent. Sawo leaf wet samples were obtained as much as 2 kg and 1kg of dry samples were obtained. This drying aims to prevent damage to certain secondary metabolite compounds to facilitate the pollination process. Drying also prevents the growth of molds and fungi so that samples can be stored for a long time. Then produce a simplisia powder of 500 gr. Making

this simplisia aims to increase the surface area of the sample which will interact with the solvent so that the compounds in the sample can dissolve more.

Extraction is carried out using the maceration method, which is one of the most common extraction methods carried out by putting plant powder and the appropriate solvent into an inert container that is tightly closed at room temperature. However, there are also major disadvantages of this method, including that it can take a lot of time, the solvent used is quite large, and it is likely that some compounds can be lost. In addition, some compounds may be difficult to extract at room temperature. On the other hand, the maceration method can also avoid the risk of damage to compounds in thermolabile plants (Mukhtarini, 2014). The choice of solvent in extraction is one of the important factors because it can affect the success of extraction. The solvent used in this extraction is 96% ethanol because it is a solvent that is universal and selective in dissolving the desired chemical compounds and is more efficient in degrading cell walls that are non-polar so that polyphenols will be extracted more. The thick extract obtained during the maceration process is 82 grams.

TLC & TLC Bioautography TLC test was conducted at the Analytical Chemistry Laboratory, Faculty of Medicine, State University of Semarang. The mobile phase used was chlorophyllom:ethyl acetate (7:3). Performed by preparing a 10x2 cm TLC plate as many as 5 plates, then prepare the eluent and saturate first using filter paper. Performed as much as 2x totolan on the TLC plate and inserted into the chamber containing eluent wait until the limit mark and dried. Then spraying reagents until the results are obtained in accordance with the parameters and observed using UV 254 and 366 lamps. The results show that sawo leaves (*Manilkara zapota (*L*) P.Royen*) contain secondary metabolite compounds in the form of alkaloids, flavonoids, tannins, saponins and steroids. The results of the Rf TLC values are presented in the following table:

Table 3. Rf Value of TLC

No.	Compound Name	Rf Value	
		254	366
1.	Alkaloids	0,25; 0,43	0,25; 0,43
2.	Flavonoids	0,31; 0,81; 0,87	0,12; 0,81
3.	Tannins	0,12; 0,93	0,18; 0,93
4.	Saponins	0,25; 0,75; 1	0,25; 0,75; 1
5.	Steroids	0,12; 1	0,12; 0,75

The results of the TLC analysis of Sawo leaf (*Manilkara zapota* (L.) *P. Royen)* are presented in table 6 (Appendix 7).

Table 4. Monitoring Of TLC Results

Parameters	Reagents	Color	Results
Alkaloid Test	Dragendorff	Orange	(+)
Flavonoid Test	AlCl3	Yellow	(+)
Tannin Test	$FeCl_3$ 1%	Blackish green	(+)
Saponin Test	Libermann Burchard	Red, yellow	(+)
Steroid Test	Libermann Burchard	Green	(+)

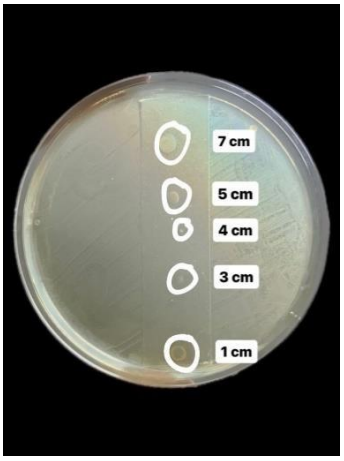

Figure 1. Bioautography TLC Results

The TLC Bioautography test was conducted at the Analytical Chemistry Laboratory of the Faculty of Medicine, State University of Semarang for the preparation of eluents and TLC plate printing, followed by pasting TLC plates onto media that had been planted with bacteria and incubated for 24 hours at the Diponegoro University Microbiology Laboratory. Bioautography TLC results were obtained by observing the presence or absence of a clear zone on the media that had been incubated for 24 hours.

TLC-Bioautography analysis obtained 5 active stains with Rf 0.12; 0.37; 0.5; 0.62; 0.87 in inhibiting the growth of *Staphylococcus epidermidis* bacteria. From these results it can be seen that Sawo manila leaves can inhibit the growth of *Staphylococcus epidermidis bacteria* due to the presence of alkaloid, flavonoid, tannin, saponin and steroid content. In testing anti-bacterial compounds using the TLC-bioautography method, the contact bioautography method was chosen in order to obtain the process of transferring active compounds into the agar medium so as to produce a larger inhibition zone with reduced sensitivity and the ability to distinguish between active compounds with the same Rf value.

The results of the antibacterial test of mustard leaf extract using liquid dilution MIC of mustard leaf ethanol extract are at a concentration of 0.3%. And the results of the MBC of sawo leaf ethanol extract obtained a clear zone at a concentration of 5%. The antibacterial test of the extract using the solid dilution method obtained the results presented in table 3.

Table 5. Results of Solid Dilution Test

Concentration	Bacterial Growth
10%	-
5%	-
2,5%	-
0,6%	-
0,3%	+
0,1%	+
K (+)	-
K (-)	+

Description:
K (-) : Control (-) i.e. 10% DMSO + bacterial isolate + growth medium
K (+) : Control (+) i.e. clindamycin + bacterial isolate + growth medium
(-) : No bacterial growth occurs
(+) : Bacterial growth occurs

The results of the antibacterial test of mustard leaf extract using the well diffusion method obtained clear zones at concentrations of 2%, 4%, 6% and 8%. Presented in table 6.

Table 6. Results of Antibacterial Activity Test of *Peel-Off* Gel Mask Preparations

Group	Vertical	Horizontal	Wells	Inhibitory Power
F1	15,2	18,1	6	10,65
F2	18,1	17,7	6	11,9
F3	18,1	18,1	6	12,1
F4	20,1	20,1	6	14,1
K (+)	16,02	16,02	6	10,02
K (-)	-	-	6	-

The physical evaluation test of the preparation in the organoleptic test aims to observe the color, texture and odor of the mustard leaf *peel-off* gel mask preparation that has been made. The results of observations on the preparation of peel-off gel masks with variations in PVA and HPMC bases obtained results with a semi-solid texture. Based on observations made on all formulas, it is found that all formulas have the same texture and aroma. The texture produced is thick like gel preparations in general. The smell produced is the typical aroma of sawo leaf simplisia and the resulting color is blackish green.

Table 7. Organoleptical Test

Formula	Organoleptics		
	Color	Smell	Shape
Formula 0	Clear	-	Gel
Formula 1	Blackish Green	Typical Simplisia	Gel
Formula 2	Blackish Green	Typical Simplisia	Gel
Formula 3	Blackish Green	Typical Simplisia	Gel
Formula 4	Blackish Green	Typical Simplisia	Gel

The preparation will be said to be homogeneous if no clumps or particles are found in the preparation. (Sholikhah & Apriyanti, 2020). The homogeneity test aims to determine the presence or absence of lumps or inhomogeneous mask parts that are physically visible. Based on the homogeneity test that has been carried out on the preparation of sawo leaf ethanol extract masks, homogeneous results are obtained on the base and in the mask formulas F1, F2, F3 and F4.

In the pH test, this measurement is carried out to determine whether the pH of a preparation is included or not in the skin pH range which ranges from 4.5-6.5. The pH value of the tea leaf extract peel-off gel mask is 6 and 5, stated to be in accordance with the requirements. When the pH value obtained is too acidic, it will cause irritation and if the pH value obtained is also too basic, it will cause dryness on the skin (Andaryekti *et al*., 2015). The following is a table of pH testing on the 4 formulas.

Table 8. pH Test

Formula	pH
Formula 1	7
Formula 2	7
Formula 3	7
Formula 4	6

The spreadability test was conducted to determine the ability of the peel-off gel mask to spread when applied to the skin. The easier it is to spread, the greater the contact surface area of the mask with the skin, so that the absorption process of substances on the skin will be optimized. Based on the spreadability test conducted, it was found that the increasing use of extracts in the formula, the spreadability will decrease and the diameter of the gel will spread when there is a load. The decrease in spreadability occurs, due to an increase in the size of the molecular unit that has absorbed the solvent, so that the liquid is retained and increases the resistance to flow and spread. (Andaryekti *et al*., 2015). The following is Table 10 Spreadability test on 3 formulas.

Table 9. Spreadability Test

Formula	Load	Replication	Time (seconds)	Average (seconds)
Base	Glass		8,5; 8,5; 8,9; 9	10 cm
	50 grams	1	10; 10; 10; 10	
Formula 1	Glass		6,3; 6,3; 6,3; 6,3	9.45 cm
	50 grams	1	8,2; 8,2; 8,1; 8,6	
	100 grams	2	8,8; 8,7; 8,3; 9,1	
	150 grams	3	9,5; 9,5; 9,1; 9,1	
	200 grams	4	9,5; 9,5; 9,3; 9,1	
Formula 2	Glass		7; 7,4; 7,2; 7,4	9 cm
	50 grams	1	8; 8,2; 8; 8,1	
	100 grams	2	8,5; 9; 8,5; 8,5	
	150 grams	3	9; 9; 9; 9	
Formula 3	Glass		6,5; 6,1; 6,8; 6,8	9.675 cm
	50 grams	1	7,3; 7,4; 7,7; 7,8	
	100 grams	2	8; 8,2; 8,1; 8,5	
	150 grams	3	9; 9; 9; 9	
	200 grams	4	9; 9,4; 9,7; 9	
	250 grams	5	9; 9,7; 9,7; 10	
Formula 4	Glass		7,5; 8; 7,4; 8,4	9.875 cm
	50 grams	1	8,5; 8,5; 8,3; 8,8	
	100 grams	2	9; 9; 8,8; 9,5	
	150 grams	3	9,5; 9,5; 9,5; 9,5	
	200 grams	4	9,7; 9,8; 10; 10	

The adhesion test aims to determine the ability of the peel-off mask to adhere to the skin. The adhesion of the preparation is said to be good if it is not more than 4 seconds. The difference in base concentration variations can affect how long the peel-off gel mask preparation will stick, the more base concentration in the peel-off gel mask preparation, the longer the adhesion obtained (Pradiningsih and Mahida, 2019). The time of the adhesion test in formula 1 averaged 5.18 seconds, formula 2 averaged 33.5 seconds, formula 3 averaged 3.60 seconds and formula 4 averaged 2.5 seconds. From the results obtained, it is concluded that the mask has met the peel-off mask adhesion test standard, which is less than 7 minutes 14 seconds which presented in table 12.

Table 10. Adhesion Test

Formula & Base	Time (seconds)
Base	2,76 S
Formula 1	5,18 S
Formula 2	33,5 S
Formula 3	3,60 S
Formula 4	2,5 S

Testing the drying time of the preparation is carried out to determine how long the *peel-off* gel mask preparation can dry and form a film layer using a stopwatch. Drying time is the ideal time for applying masks in general, the range of drying times for a good peel-off gel mask preparation is between 15-30 minutes. (Saputra *et al*., 2019). The results of observing the drying time of the preparation in the four dosage formulations showed a decrease in drying time in each dosage formulation. One of the factors that can cause the mask to dry for a long time is related to the homogeneity of the preparation.

Table 11. Drying time test

Formula & Base	Time (minutes)
Base	8
Formula 1	15
Formula 2	15
Formula 3	13
Formula 4	14

CONCLUSION

Sawo Manila leaves (*Manilkara zapota* (L) *P. Royen*) are known to contain compounds such as alkaloids, flavonoids, tannins saponins and steroids which are proven to inhibit *Staphylococcus epidermidis* bacteria. The largest concentration of Sawo Manila leaf ethanol extract (*Manilkara zapota* (L) *P. Royen*) formulated as a *peel-off* gel mask preparation based on antibacterial tests against *Staphylococcus epidermidis* bacteria The results of the antibacterial test of Sawo leaf extract using liquid dilution KHM of Sawo leaf ethanol extract are at a concentration of 0.3%. The results of the MBC of ethanol extract of mustard leaves obtained a clear zone at a concentration of 5%. The antibacterial test of *peel-off* gel mask preparations of ethanol extract of manila sawo leaves was carried out using the well diffusion method, which uses nutrient agar as a medium by perforating or making wells. Then put the sample into the media and incubated for 18-24 hours and observed the clear zone. The highest clear zone was found at a concentration of 8% which amounted to 14.1 mm.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest

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