Journal of Science and Technology Research for Pharmacy
Volume 4 Issue 2 Year 2024 Pages 1-17

E-ISSN 2776-0685
https://journal.unnes.ac.id/journals/JSTRP/index

Antibacterial Activity Test of Guava Leaf Ethanol Extract against *Staphylococcus epidermidis* and its Formulation as Anti-Acne Serum

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Abstract: Skin is a layer of tissue that spreads over the entire surface of the body. On the surface of the skin, sweat glands secrete waste products through the skin pores in the form of sweat. Acne (Acne vulgaris) is a condition where the pores are clogged and cause pockets of pus to become inflamed. *Guava* leaves *(Psidium guajava L.)* contain alkaloid, flavonoid, tannin, saponin and steroid compounds that are believed to have antibacterial activity. The purpose of this study was to determine the antibacterial activity of *guava leaves (Psidium guajava L.)* against *Staphylococcus epidermidis* made in serum dosage form. In this study, guava leaf extract was tested to determine the value of MIC (Minimum Inhibitory Level) using the liquid dilution method and MBC (Minimum Bactericidal Level) using the solid dilution method by measuring the diameter of the inhibition zone. The results showed that the MIC of guava leaf extract was obtained at a concentration of 0.05 while the MBC results of guava leaf extract were obtained at a concentration of 5%. Serum preparation is a low viscosity preparation that delivers active substances through the skin surface which has more active ingredients and less solvent content. Serum preparation formulations were made in several concentrations, namely F0 0%, F1 2%, F2 4%, and F3 6%. Physical evaluation of the preparation included organoleptic, homogeneity, adhesion, spreadability, pH, and viscosity. The antibacterial activity test of serum preparations was carried out by the pitting diffusion method. The results showed that F3 had the largest inhibition zone of 13 mm and was included in the strong category.

Keywords: acne, guava leaf, serum, *staphylococcus epidermidis*

INTRODUCTION

Skin health is one of the most important parts that needs to be taken seriously by everyone, both teenagers and adults. The skin is an organ that protects the body from various dangers. The skin contains nerve cells that can detect and communicate changes in the environment, maintain water and electrolyte balance and thermoregulation. However, the skin can experience disorders such as *acne* or what is commonly referred to as acne. *Acne* or commonly called *Acne Vulgaris* is a skin disease due to chronic inflammation with complex pathogenesis, involving sebaceous glands, follicular hyperkeratinization, excessive bacterial colonization, immune reactions, and inflammation. (Madelina & Sulistiyaningsih, 2018). Acne is a common skin disease that affects 85% of the world's population aged 11-30 years. (Okoro, 2016). Generally, acne appears during puberty (age 8-9 years) where the production of androgen hormones increases dramatically and results in an increase in keratin sebum secretion (Winarno, 2014). (Winarno, 2014). The triggers of acne include genetics, hormonal activity in the menstrual cycle, stress, hyperactive sebaceous gland activity, hygiene, food, and the use of cosmetics. Acne is caused by blockage of skin pores so that oil secretion becomes inhibited and then enlarges and dries into pimples. Increased estrogen and progesterone hormones in adolescent girls, as well as testosterone hormones in adolescent boys lead to increased production of oil and sweat glands. In addition to the hormonal factors above, acne is often exacerbated by bacterial activity that infects inflamed skin tissue (Karim *et al.,* 2018). The most common acne-causing bacteria besides *Propionibacterium acnes* is *Staphylococcus epidermidis. Staphylococcus aureus* is a bacterium that causes blockages in the skin, causing inflammation and acne. *Staphylococcus epidermidis* is a normal flora that can cause various infections in body tissues such as skin infections, namely acne. (Sarlina *et al*., 2017).

Many anti-acne drugs on the market still contain synthetic antibiotics and many of them have side effects that can cause skin irritation in long-term use. Therefore, there is a need for an alternative to overcome acne problems, such as antibacterial agents derived from natural ingredients. Indonesian native medicinal plants have been known since long before the existence of health services that use modern medicines. One of the native Indonesian medicinal plants that has been used by the community since long ago as an antibacterial is *guava* leaf (*Psidium guajava L*.). (Afifi, 2018). Guava leaves are scientifically proven to have anti-acne activity. Guava leaves contain secondary metabolites consisting of tannins, flavonoids, terpenoids, monoterpenoids, cystulterpenes, alkaloids, quinones and saponins. The content of these compounds such as flavonoids, tannins and saponins, and terpenoids is proven to have antibacterial activity.

In research conducted previously, guava leaves are known to have potential as antibacterial against *Propionibacterium acnes and Staphylococus aureus bacteria* with inhibition of *Propionibacterium acnes bacteria* at a concentration of 60% of 6.987 mm while at a concentration of 90% of 9.777 mm, and for *Staphylococus aureus* bacteria results at a concentration of 60% of 7.533 mm while at a concentration of 90% of 9.373 mm. The inhibition results are included in the moderate activity category (5-10 mm). The inhibition diameter of guava leaf extract at 90% concentration against *Propionibacterium acnes* was greater than that against Staphylococus aureus, namely 9.77 mm and 9.37 mm, respectively. (Amina, 2022). In a previous study, an antibacterial guava leaf activity test was also conducted against *Escherichia coli* growth. The diameter of the largest inhibition zone was found at 100% concentration of 13.63mm and the diameter of the smallest inhibition zone was at 25% concentration of 9.23mm. 12.5%, 6.25%, 3.125% and 1.56% concentrations have no potential inhibition against Escherichia coli bacteria. (Girsang *et al*., 2019).. Gunarti (2018) has also researched the preparation of facial wash gel from guava leaf extract, where from formula F1 which has the best antibacterial activity of 2.688 mm which shows a strong inhibitory response to *Propionibacterium acnes bacteria.* (Gunarti, 2018). Previous research has also tested antibacterial activity using guava leaf fractions against *Propionibacterium acnes* bacteria using the pitting method. The results obtained are the average inhibition zone in each sample with a concentration of 10,000 µg/mL and 100,000 µg/mL 96% ethanol extract of 12.20 mm and 19.25 mm while in the ethyl acetate fraction obtained at 12.49 mm and 13.22 mm and in the methanol: water fraction obtained at 13.46 mm and 17.95 mm and positive control 38.33 mm. In this test, the sample with a dose of 1,000 µg/mL and the n-hexane fraction sample had no inhibition zone. While the sample that has the largest inhibition zone is 96% ethanol extract 100,000 µg/mL. So it can be concluded that the results of the inhibition zone have antibacterial activity and are able to inhibit the growth of *Propionibacterium acnes* bacteria with strong inhibitory strength (Putri, 2022).

Research on the antibacterial activity of guava leaves against *Staphylococcus epidermidis* has been done before. However, so far it is not known what compounds are responsible for the antibacterial effect of *Staphylococcus epidermidis*. Therefore, this study will investigate the antibacterial activity of guava leaf extract against *Staphylococcus epidermidis* bacteria and its formulation as a serum preparation. Serum is a preparation with low viscosity, because of its low viscosity serum is categorized as an emulsion preparation. Serum has the advantage of having a high concentration of active ingredients so that the effect is absorbed faster by the skin, can provide a more comfortable effect and is easier to spread on the skin surface because the viscosity is not too high. (Kurniawati & Wijayanti, 2018). The use of serum on the skin can make the skin firmer, smoother texture, shrink pores and increase skin moisture. The serum preparation will be tested again for antibacterial activity against *Staphylococcus aureus* and a preparation evaluation test will be carried out. Furthermore, a bioautographic TLC test will also be carried out, which is a detection method to find an antimicrobial compound that has not been identified by localizing the antimicrobial activity on a chromatogram. This method utilizes the notion of thin layer chromatography (Paputungan *et al*., 2019).Based on the description above, the authors are interested in knowing the antibacterial activity contained in guava leaves and formulating anti-acne serum preparations from guava leaf extract.

METHODS

The materials used in the study were *guava* leaf powder *(Psidium guajava L.)* 96% ethanol, Hydroxyethyl cellulose, Tetrasodium EDTA, Glycerin, Triethanolamine (TEA), DMDM Hydantion, distilled water (aquadestilata), concentrated sulfuric acid (H2SO4), Dimethylsulfoxide (Dmso) 100%, concentrated hydrochloric acid (HCl), magnesium powder (Mg), chloroform, ferric chloride (FeCl3 1%), Staphylococcusepidermidis bacterial culture, Clindamycin, NaCl 0.9%, Mannitol Salt Agar (MSA), Nutrient Broth (NB), Nutrient Agar (NA).

The tools used in the research are oven, maceration vessel, measuring cup, mortar and stamper, stirring rod, waterbath, sudip, analytical balance, refrigerator, filter paper, funnel, glass object, pH stick, viscotester tool, adhesive power test tool, gloves, ose, Petri dish, test tube, test tube rack, bunsen, lighter, hot plate, tweezers, micropipette, autoclave, sterilizer, incubation device, volume pipette, dropper pipette, rotary evaporator, erlenmeyer, beaker glass, cotton swab, crock drill, sterile cottond swab, vernier, and ruler.

Extract Preparation

Guava leaf simplisia powder *(Psidium guajava L.)* was weighed as much as 500g. Extract preparation was carried out by maceration method using 96% ethanol solvent with the ratio of material and solvent 1:5 (Ratna *et al.,* 2015). Putting guava leaf powder in a maceration container, then adding 96% ethanol solvent as much as 5 liters until submerged and stirring until homogeneous, then allowed to stand for 3 days and then stored in a room protected from sunlight. During soaking, stir occasionally for 15 minutes. After soaking, filtering was done using filter paper to

get the filtrate. Next, evaporate the macerate with a rotary evaporator at a temperature of 50° C until a thick extract is obtained.

Phytochemical Screening

1. Flavonoids
   A sample of 0.5 mL is added with 0.5 g of Mg powder plus 5 mL of concentrated HCI (drop by drop) the color change to red/yellow there is foam is a characteristic that the extract contains flavonoids. (Hidayah *et al*., 2020).
2. Alkaloids
   Alkaloids are tested by means of 0.5 mL of sample added 5 drops of chloroform and 5 drops of mayer reagent (1 g KI dissolved in 20 mL of distilled water and added 0.271 g $HgCl_2$ to dissolve), if the color of the solution turns brownish yellow and a yellow precipitate appears, it indicates the presence of alkaloid compounds. (Hidayah *et al*., 2020).
3. Tannins
   A sample of 2 g was added with ethanol until the sample was submerged. Then as much as 1 mL of sample solution was transferred into a test tube and added 2-3 drops of 1% $FeCl_3$ solution. Positive results are indicated by the formation of a bluish or greenish black color. (Hidayah *et al*., 2020).
4. Saponins
   Samples were taken as much as 0.5 g added with 10 mL of hot aquadestilata, cooled and then shaken for 10 minutes. The formation of a stable foam (lasts a long time) indicates positive saponins. (Hidayah *et al*., 2020).
5. Steroids
   A sample of 0.5 mL was then given 0.5 mL of glacial acetic acid, and 0.5 mL of H_2SO_4, and the color changed to a brick red color because the extract was positive for terpenoids. (Hidayah *et al*., 2020).

Thin Layer Chromatography (TLC)

The sample used was ethanol extract of *guava* leaves (*Psidium guajava L.*). A total of 10 mg of extract was dissolved in 1 ml with the appropriate solvent, then bottled on the stationary phase and then eluted. The TLC method uses a stationary phase on silica gel GF254 with a size of 2x10 cm with a distance of 1 cm.

1. **Alkaloid Identification**
   The TLC plates that have been photographed with extracts and fractions are eluted with the mobile phase Chloroform: Ethyl acetate (7:3). Test alkaloid compounds using Dragendorff spray reagent (stain spotter). Positive results of alkaloids are indicated by the formation of orange spots in visible light and red fluorescence spots in UV 366.
2. **Flavonoid Identification**
   The TLC plates that have been photographed with extracts and fractions are eluted with the mobile phase Chloroform: Ethyl acetate (7:3). Flavonoid compound test using a spray reagent (stain spotter) $AlCl_3$ 10%. Positive results of flavonoids are indicated by the formation of brown, yellow, green or blue color spots.
3. **Identification of Tannins**
   The TLC plates that have been photographed with extracts and fractions are eluted with the mobile phase Chloroform: Ethyl acetate (7:3). Test tannin compounds using a 5% $FeCl_3$ stain reagent. Positive results are indicated by the formation of a blue-black spot on the light and on condensed tannins indicated by the formation of a brownish green spot.
4. **Identification of Saponins**
   The TLC plates that have been photographed with extracts and fractions are eluted with the mobile phase Chloroform: Ethyl acetate (7:3). Test for saponin compounds using Liberman Bouchardat spray reagent (stain spotter). Positive results for saponins are indicated by the appearance of a yellowish green or bluish green color.
5. **Steroid Identification**
   The TLC plates that have been photographed with extracts and fractions are eluted with the mobile phase Chloroform: Ethyl acetate (7:3). Test steroid and terpenoid compounds using 10% H_2SO_4 (sulfuric acid) spray reagent. Positive steroid results are indicated by the presence of purple, red, green or blue color spots in UV light (Maulana, 2019).

Media Creation

NA media was made by weighing 10 grams of NA dissolved in 500 ml of distilled water. The homogeneous media was sterilized in an autoclave for 15 minutes at 121°C. After that, the media was waited until it cooled slightly around 40-45°C. NA media that has cooled down will then be poured into Petri dishes as much as 20 mL. NA media

that has been poured into Petri dishes is left to solidify (Narulita, 2017). Making NB media is done by weighing as much as 0.8 grams of NB dissolved in 100 ml of distilled water. The homogeneous media was sterilized in an autoclave for 15 minutes at 121°C (Narulita, 2017).

Bacterial Inoculation

Colonies of Staphylococcus epidermidis bacteria were taken using a sterile ose needle and then implanted on agar media and incubated at 37°C for 24 hours.

Preparation of Bacterial Suspension

H2SO4 solution of 9.95 ml was mixed with 1% BaCl solution of 0.5 ml in an erlenmeyer. Then shaken until a turbid solution is formed. This turbidity is used as a standard turbidity of the test bacterial suspension. After making the standard solution, one ose of bacterial culture that has been inoculated on NA media is suspended into a test tube containing 10 ml of NB media and incubated for 24 hours at 37°C. The bacterial suspension was diluted using sterile 0.9% NaCl until the turbidity was equivalent to a standard solution of 0.5 Mc. Farland I standard solution (liquid culture whose turbidity is equivalent to 0.5 Mc).

Preparation of Variation of Extract Concentration

The concentrations of guava leaf extract used were 5%, 2.5%, 1.25%, 0.625%, 0.3%, 0.1%, 0.05% and 0.025% with multilevel dilutions using 10% DMSO solvent.

Preparation of Positive and Negative Controls

The positive control used was clindamycin 1.2%. Clindamycin was used as a positive control because it has strong antibacterial activity against S. epidermidis. The positive control test solution was prepared by weighing 1 gram of clindamycin in 10 ml of sterile distilled water. The negative control used is DMSO 10% because it is one of the solvents that is not bactericidal or has no activity to inhibit bacteria (Natheer et al, 2012). DMSO 10% was made by putting 10 ml of DMSO into a measuring cup and then adding 100 ml of distilled water.

Antibacterial Activity Test

Testing the antibacterial activity of extracts using liquid dilution and solid dilution methods. Prepare sterilized tools and materials. Prepare test tubes and label each extract concentration on the tube. Then put 4 ml of NB media into each tube. Put 1 ml of extract concentration solution into each tube except positive and negative control tubes. After that, incubate for 24 hours at 37°C and observe the turbidity. Determine the MIC value by visually observing the turbidity and clarity of each test medium and then comparing it with the control solution. The lowest concentration shows inhibition of bacterial growth characterized by clarity in the test media. Next, take the NA media that has been made then take a sample from each tube using a *cotton swab* and then apply it to the NA media that has been labeled with the concentration of the extract. After that, incubate for 24 hours at 37°C. The MBC value can be determined by looking at the growth of bacteria which is characterized by the presence of a clear zone in the agar medium after incubation.

Serum Formulation

The standard formula used in this study was modified from the formula of (Hikmah *et al*., 2023).

Table 1. Modified Formula of Guava Leaf Extract Serum (Psidium guajava L.)

Material	Formula Composition (%b/v)				Function
	F0	F1	F2	F3	
Guava leaf ethanol extract	-	2	4	6	Active Substance/ anti-acne
Glycerin	30	30	30	30	Humectants
Hydroxyethyl cellulose	2	2	2	2	*Gelling agent*
Triatholamine	1	1	1	1	Surfactants
Tetrasodium EDTA	0,18	0,18	0,18	0,18	*Chelating agent*
DMDM Hydantion	0,1	0,1	0,1	0,1	Preservatives
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100	Solvent

*Description:

F0 (-) : Serum Formulation without Extract

FI : Serum Formulation with 2% Extract Concentration

FII : Serum Formulation with 4% Extract Concentration

FIII : Serum Formulation with 6% Extract Concentration

Serum Preparation

Weigh the HEC gelling agent according to the formulation weighing and then dissolve it in 15 ml of distilled water at 50°C on a hot plate. After the gelling agent dissolves, add TEA until homogeneous. Add EDTA little by little until homogeneous. Add DMDM Hydantion little by little and then grind until homogeneous. Next, grind the glycerin until homogeneous. The serum base that has been formed, then add the active substance, namely the ethanol extract of guava leaves then add 100 ml of distilled water and stir until homogeneous. The last step is to put the serum preparation into the container and then carry out the serum preparation evaluation test.

Serum Preparation Evaluation

1. **Organoleptical Test**
   Serum testing includes color, aroma, and sensation on the skin by observing the visual appearance and sensation on the skin. (Hasrawati *et al.*, 2020).
2. **Homogeneity Test s**
   This homogeneity test was conducted by researchers to see whether the serum gel preparation was homegen or not. Samples were placed evenly on two glass slides and evaluated. An excellent preparation is homogeneous and free of lumps (Mardhini *et al.,* 2018).
3. **pH test**
   A total of 0.5 g of serum was diluted with 5 mL of distilled water, then the pH stick was dipped for 1 minute. The color change that occurs on the pH stick indicates the pH value of the preparation.
4. **Adhesion Test**
   The adhesion test is carried out to determine whether the preparation can adhere or stick to the skin surface. Adhesion is one of the characteristics responsible for the effectiveness of the preparation in providing pharmacological effects. The longer the adhesion of a preparation at the application site, the greater the pharmacological effect produced. A sample of 0.25 grams is placed between 2 glass objects in the adhesion test device, then a 1 kg load is pressed for 5 minutes, the load is lifted and given an 80 gram load on the device and the serum release time is recorded (Tilarso *et al*., 2022).
5. **Spreadability Test**
   A 0.5 g sample was placed on the glass and waited for 1 minute. The spread diameter of the sample was measured. Next, 150 g of load was added and allowed to stand for 1 minute and then a constant diameter was measured (Warnida *et al*., 2016).
6. **Viscosity Test**
   The serum gel preparation is inserted into the viscotester using rotor number 2 with a readable viscosity range of 100-4000 dPas (Hasrawati *et al*., 2020).

Antibacterial Activity Test of Serum Preparation

Testing the antibacterial activity of guava leaf extract serum against *Staphylococcus epidermidis* was carried out using the well diffusion method. A bacterial suspension of 50 µl was prepared in a test tube. Take the NA media that has been made then dip a sterile cotton swab in the bacterial suspension then swab the NA media. Next, a well hole with a diameter of 6 mm was made using a *cork borer.* Each formula, positive control (clindamycin 1%) and negative control (preparation base) were inserted into the wells that had been made. After that, incubate for 24 hours at 37°C. Observe the clear zone produced around the wells containing the serum preparation and measure the diameter of the inhibition using a caliper.

TLC-Bioautography

The results of the TLC identification with the mobile phase chloroform: ethyl acetate (7:3) were continued with the TLC-bioautography test by weighing 0.56 g of Nutrient Agar and adding it with distilled water to 20 mL and dissolving it until homogeneous. The media that has been homogenized is then sterilized using an autoclave at 121 °C for 15 minutes, and left until the media cools. Next, the liquid nutrient agar media was poured into each Petri dish as much as 20 mL and allowed to solidify. The chromatogram of the results of the separation of compounds by TLC was placed on the solidified medium. Let stand for 60 minutes in the refrigerator, the chromatogram plate was lifted and removed from the medium. Then incubated for 24 hours at 37°C (Paputungan *et al*, 2019).

RESULT AND DISCUSSION

The yield of guava leaf extract is 13.4%, so it meets the requirements for extract yield in the Indonesian Herbal Pharmacopoeia, which is not less than 13.1% extract yield.

Table 2: Yield results of guava leaf extracts

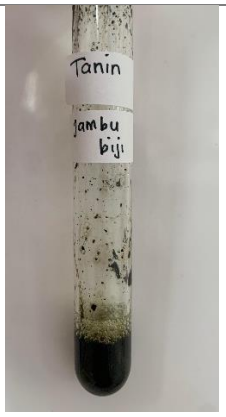
Simplified Powder Weight (gr)	Extract Weight (gr)	Yield (%)	FHI Requirements
500	67	13,4	>13,1%

Phytochemical Screening

Phytochemical screening is carried out with the aim of knowing the presence or absence of bioactive components contained in bijoi guava leaves (Riris, *et al*,. 2020). Phytochemical screening is carried out using the reagent test method by looking at the color reaction formed. The tests carried out at the phytochemical screening stage with this reagent test method include alkaloid tests, flavonoid tests, tannin tests, saponin tests, and triterpenoid / steroid tests.

Table 2 Phytochemical Screening Results of Extracts by Reaction Test Method

Phytochemical Test	Treatment	Terms	Observation Results	Conclusion
Alkaloids	Tube I → 0.5 g extract + 0.5 ml HCl 2N + 3 drops mayer reagent	Forms a yellowish-white precipitate	 Forms a yellowish-white precipitate	(+)
	Tube II → 0.5 g extract + 0.5 ml HCl 2N + 3 drops wagner reagent	Forms a brown precipitate	 Forms a brown precipitate	(+)
	Tube III → 0.5 g extract + 0.5 ml HCl 2N + 3 drops dragendorff reagent	Formed orange color	 Formed orange color	(+)

Flavonoids	0.5 g extract + 0.5 g Mg powder 5 ml + 3 drops HCl	Forms a yellow or red color	 Yellow color formed	(+)
Tannins	0.5 g extract + 3 drops of 1% FeCl3 solution	Forms a blackish-green or bluish color	 Forms a blackish-green color	(+)
Saponins	0.5 g extract + 10 ml hot distilled water then shaken vigorously for 10 minutes	Formation of froth/foam for not less than 10 seconds, 1 - 10 cm high	 Formed a froth/foam with a size of 3 cm that lasts for 10 seconds	(+)
Steroids	0.5 g extract + 0.5 ml glacial acetic acid + 3 drops concentrated H2SO4	Forms a bluish-green color	 Forms a bluish-green color	(+)

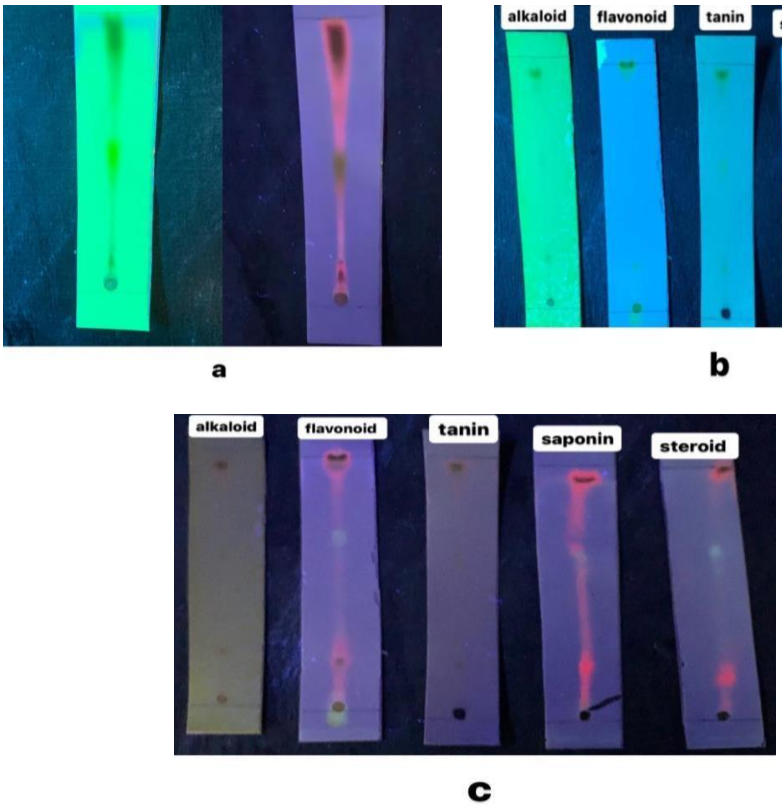
Based on the phytochemical screening data in table 4 positive results were obtained in the alkaloid, flavonoid, tannin, saponin and steroid tests. The alkoloid test is carried out by adding the extract with 2N HCl. The addition of HCl aims to make the atmosphere acidic because alkaloids are basic (Shofa, 2020). Alkaloids were tested with Mayer, Wagner and Dragendorff reagents. The test results showed positive results marked by yellow discoloration and the presence of a white precipitate in the Mayer reagent, Wagner's reagent was marked by a brown precipitate, while the color change in the Dragendrof reagent was orange. Flavonoid test is done by adding the extract with magnesium powder and then adding HCl as much as 3 drops. The purpose of adding magnesium powder and HCl is to reduce the

glycoside bond with flavonoids. In order for flavonoids to be identified, the glycoside bond with flavonoids in plants must be broken by reducing the bond, which is the result obtained positive because a yellow color is formed. Tannin test is done by adding the extract with FeCl3 as much as 3 drops. This color change occurs when the addition of FeCl3 reacts with one of the hydroxyl groups present in the tannin compound. The presence of phenolic groups is indicated by a blackish green or dark blue color after being added with FeCl3 (Ramadhan *et al*., 2020). Saponin test is done by adding the extract with hot distilled water, cooled then shaken vigorously for 10 seconds the results obtained are positive for saponin because it forms a froth as high as 1 cm for no less than 10 minutes. Steroid test is done by adding the extract with glacial acetic acid and H2SO4 as much as 3 drops. The purpose of adding glacial acetic acid is to break the steroid-terpenoid relationship with other groups. In addition, the purpose of adding this acetic acid is so that the sugar bond in the compound is broken, so that the terpenoid steroid sugar group is released. The results were positive with the formation of a bluish green color.

Thin Layer Chromatography (TLC)

The separation and purification process was firstly done using thin layer chromatography (TLC) technique. TLC aims to find the best type of eluent that will be used in column chromatography. Determination of the best mobile phase is done by trying different mobile phases to elute the extract. The next step is saturation for the mobile phase. This stage aims to maximize the spot movement process by distributing the mobile phase evenly. This process is done by inserting the mobile phase into the TLC chamber that has been cleaned and has been given filter paper. If the mobile phase has reached the top end of the indicator paper, then the mobile phase is saturated. After saturation, the GF 254 TLC plate that has been prepared is bottled with extract using a capillary pipe. The plate is inserted into a chamber or vessel containing a saturated mobile phase and observed until the mobile phase moves up to the mark then dried and observed under UV light 254 and 366 nm. Next, spray some reagents that match the compounds to be tested and observe again under UV light 254 and 366 nm as in Figure 4.4 below.
TLC results of guava leaf extract with chloroform: ethyl acetate (7:3) mobile phase.

Figure 4.4 Thin Layer Chromatography (TLC)



Description:
(a) visible light
(b) UV light 254 nm
(c) UV light 366 nm

Table 3. TLC results of guava leaf extract with chloroform: ethyl acetate (7:3) mobile phase.

Compound	Color				Rf	Ket
	Without Reagent		Add Reagent			
	254	366	254	366		
Alkaloids	Yellow, Green	Red, Yellow, Brown	Hjau	Red	0,4, 0,6, 0,9	+
Flavonoids	Yellow, Green	Red, Yellow, Brown	Green	Red, Green, Brown	0,4, 0,7, 1	+
Tannins	Yellow, Green	Red, Yellow, Brown	Green, Yellow	Brown, Yellow, Red	0,4, 0,6, 0,9	+
Saponins	Yellow, Green	Red, Yellow, Brown	Green	Red	0,4, 0,6, 1	+
Steroids	Yellow, Green	Red, Yellow, Brown	Green	Red	0,4, 0,6, 0,9	+

The data obtained is in the form of Rf values and stain colors on the chromatogram as a result of TLC plate elution which will provide information about the compounds that are thought to be contained in guava leaf ethanol extract. The Rf values obtained indicate differences in the nature of the compounds and can be used to identify compounds. Compounds that have a larger Rf mean they have low polarity, and vice versa. This is because the stationary phase is polar. More polar compounds will be strongly retained on the stationary phase, resulting in a low Rf value. Separation on TLC occurs due to competition between the stationary phase and mobile phase to bind the components contained in the mixture to be separated. The competition is caused by the polarity of the stationary phase and liquid components. Components that have the same polarity as the stationary phase will interact more strongly and as a result the component will be absorbed by the stationary phase.

Spot detection is done under UV lamp with short wavelength (254 nm) and long wavelength (366 nm). Observations at UV lamps are based on the principle that at a short wave of 254 nm, the plate gives a flouresence while the sample is dark in color, the stains that appear arise due to the interaction between UV light and fluorescence indicators contained in the TLC plate. The long wave 366 nm gives the opposite situation where the stain gives flouresence and the plate is dark, the stain that appears to arise due to the interaction between UV light and the chromophore group bound by the auxochrome present on the stain. The results obtained that guava leaf extract can be separated well using the mobile phase chloroform: ethylacetate (7:3). Chloroform is a solvent that is more non-polar so that it can attract many compounds. The results of the observation of the TLC profile show that the ethanol extract of jeruju leaves contains a variety of compounds which can be seen from the colored stains on the slab obtained from the pengelusian results with various Rf values. These stains indicate that there are active compounds, where one stain can contain many active compounds while one compound is contained in one stain.

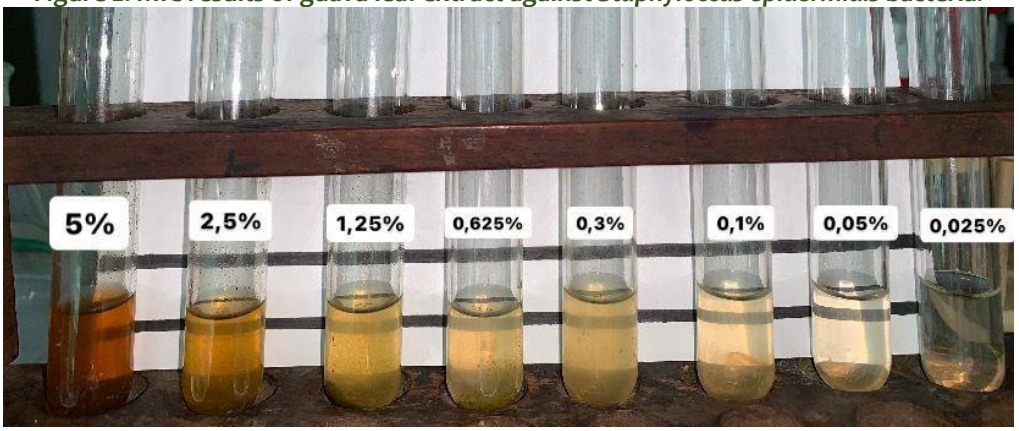
Antibacterial Activity Test

The antibacterial activity test of the extracts in this study used liquid dilution and solid dilution methods. The liquid dilution method was used to measure the MIC (Minimum Inhibitory Concentration) while the solid dilution method was used to determine the MBC (Minimum Bactericidal Concentration). The method performed in the liquid dilution method is by making a series of dilutions of antimicrobial agents in liquid media added with test microbes. The solid dilution method is carried out by inoculating test microbes on agar media containing antimicrobial agents (Etikasari *et al*., 2017).

The variations of extract concentrations used were 5%, 2.5%, 1.25%, 0.625%, 0.3%, 0.1%, 0.05%, 0.025%. In addition to making concentration variations, this study also used positive and negative controls. The control aims as a comparison. The negative control uses DMSO because DMSO has no antibacterial function and is generally only used as a solvent. While the positive control uses clindamycin antibiotics dissolved in distilled water. (Rachmawati *et al*., 2016).

Determination of the MIC test results was carried out after each extract concentration was incubated for 24 hours at 37 °C, by observing the changes that occurred in each extract concentration that had been given a suspension of test bacteria. The concentration of guava leaf extract that looks clear indicates that the concentration provides activity to inhibit *Staphylococcus epidermidis* bacteria, while the concentration of extract that looks cloudy indicates that the guava leaf extract solution does not provide inhibitory activity against *Staphylococcus epidermidis* bacteria. The test results of guava leaf extract can be seen in table 4.3 below.

Figure 2: MIC results of guava leaf extract against *Staphyloccus epidermidis* bacteria.



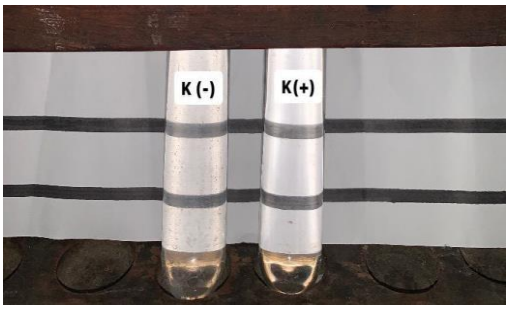


Table 4. MIC results of guava leaf extract against *Staphylococcus epidermidis* bacteria

No.	Tube	Description
1.	5%	Cloudy
2.	2,5%	Cloudy
3.	1,25%	Cloudy
4.	0,625%	Cloudy
5.	0,3%	Cloudy
6.	0,1%	Clear
7.	0,05%	Clear → MIC
8.	0,025%	Clear
9.	Positive Control (Clindamycin)	Clear
10.	Negative Control (DMSO)	Cloudy

Based on the results in the table above, it can be seen that guava leaf extract has a minimum inhibitory concentration against Staphylococcus epidermidis bacteria, the average clarity is obtained at a concentration of 0.05%. According to (Sariadi, et al., 2019), in determining a MIC value, it is selected from the lowest concentration that still shows clarity (Noval, *et al*. 2021). In table 4, it can be seen that the negative control has turbidity, meaning that bacterial growth occurs because DMSO is known to have no antibacterial activity. The positive control tube looks clear, meaning that no bacterial growth occurs, this is in accordance with the function of clindamycin which is an antibiotic that is sensitive to *Staphylococcus epidermidis* bacteria. The tube containing guava leaf extract shows clarity at a concentration of 0.05% so it can be concluded that the MIC value is at a concentration of 0.05%.

Determination of MBC can be done by looking at the clear zone on the media. The results of testing the inhibition zone for each concentration of guava leaf extract against the growth of *Staphylococcus epidermidis* bacteria are obtained as shown in Figure 3 and Table 5 below.

Figure 3. Guava leaf extract against *Staphylococcus epidermidis* bacteria.

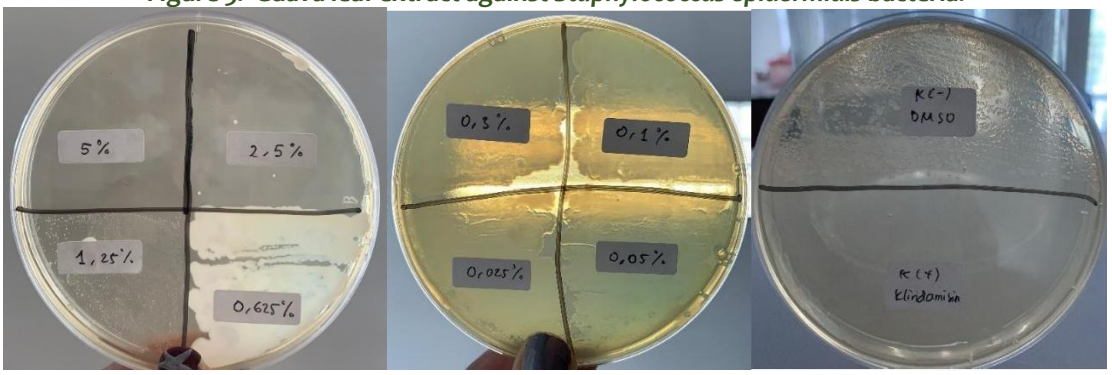


Table 5. MBC results of guava leaf extract against *Staphylococcus epidermidis* bacteria

Extract Concentration	Results
5%	-
2,5%	+
1,25%	+
0,625%	+
0,3%	+
0,1%	+
0,05%	+
0,025%	+
Positive Control (Clindamycin)	-
Negative Control (DMSO)	+

Description:
Negative sign (-) : no bacterial growth
Positive sign (+): bacterial growth

The extract tube which is the MIC value is continued with testing to see the MBC (Minimum Bactericidal Concentration) value. The MBC value can be seen in table 4.6, namely the presence of a clear zone at a concentration of 5%, which means that the MBC value is at a concentration of 5% due to the antibacterial activity of guava leaf ethanol extract against *Staphylococcus* epidermidis bacteria in accordance with the function of metabolite compounds contained in the extract, including flavonoids, alkaloids, tannins, saponins, and steroids. The positive control shows a clear zone, this is certain because clindamycin is proven to have antibacterial activity as an antibiotic.

Antibacterial Activity Test of Preparations

Testing the antibacterial activity of guava leaf extract serum preparations against *Staphylococcus epidermidis* was carried out using the well diffusion method by perforating the media using a drill with a size of 6 mm. After the media was perforated, a bacterial suspension was taken using a sterile cotton swab and then swabbed on the media. Furthermore, serum preparations with variations in concentration that have been made, namely 2%, 4%, and 6%, are inserted into the holes that have been made in the media and incubate for 1x24 hours. In addition to the extract, the base of the serum preparation was also used as a negative control and clindamycin gel as a positive control. After incubation, the results can be measured and then calculated the inhibition zone as shown in Figure 4 and Table 6 below.

Figure 4. Diameter of inhibition zone of guava leaf extract serum preparation against *Staphylococcus epidermidis* bacteria

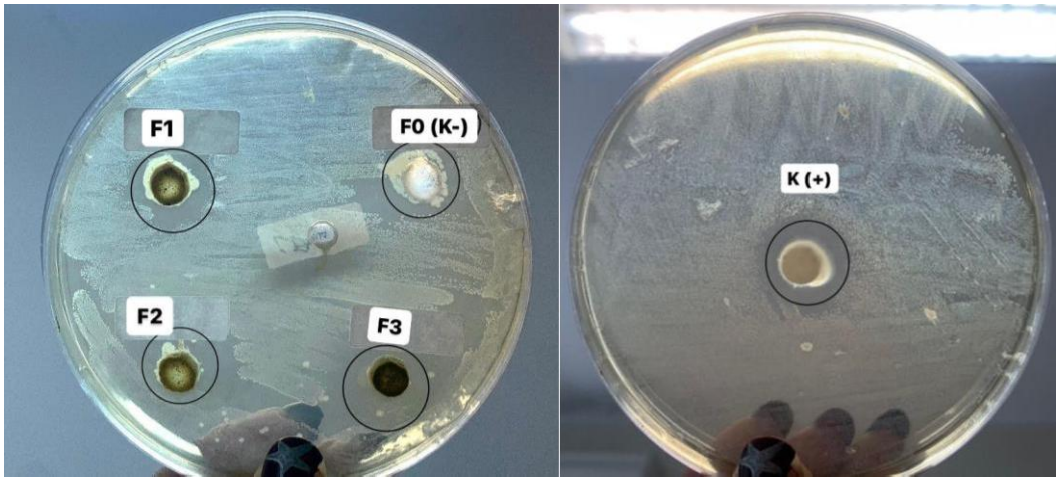


Table 6. Results of antibacterial activity test of guava leaf extract serum preparation against *Staphylococcus epidermidis* bacteria.

	Vertical	Horizontal	Well Diameter (mm)	Inhibition (mm)	Category
F1	17,5	18	6	11,75	Strong
F2	18,5	19	6	12,75	Strong
F3	19	19	6	13	Strong
Control (+)	16	16	6	10	Strong
Control (-)	-	-	6	-	None

Based on the category of bacterial inhibition according to Davis & Stout (1971) and Rahayu *et al*., (2019) if the diameter of the inhibition zone formed is greater than 20 mm, the antibacterial power category is very strong, if the diameter of the inhibition zone formed is 11-20 mm, the antibacterial power category is strong, while if the diameter of the inhibition zone formed is around 5-10 mm, the antibacterial power category is moderate, and if the diameter of the inhibition zone is smaller than 5 mm, the antibacterial category is weak. The observation results showed that the serum preparation of ethanol extract of guava leaves was able to inhibit the growth of *Staphylococcus epidermidis* bacteria. F1, F2, and F3 showed a zone with an inhibition zone diameter of 11.75 mm; 12.75 mm; 13 mm with a strong inhibition response. The smallest inhibition zone is F1 and the largest inhibition zone is F3. While in the positive control of clindamycin gel with an inhibition zone of 10 mm, which means that the antibiotic is strong in inhibiting the growth of *Staphylococcus epidermidis* bacteria. In contrast to the negative control against *Staphylococcus epidermidis* bacteria, the results did not show the ability to inhibit bacterial growth because no clear zone was formed around the wells. So it can be concluded that the higher the concentration of guava leaf ethanol extract, the greater the content of active compounds, thus the larger the inhibition zone formed.

Physical Evaluation of Preparations

1. **Organoleptics**

Table 7. Serum Organoleptical Test Results

Formulation	Organoleptics		
	Color	Smell	Shape
F0	Clear	-	Semi Solid
F1	Green	Typical	Slightly Thick
F2	Green	Typical	Semi Solid
F3	Green	Typical	Semi Liquid

Description:
F0 : Serum formulation without extract
F1 : Serum formulation with 2% extract concentration

F2 : Serum formulation with 4% extract concentration
F3 : Serum formulation with 6% extract concentration

Organoleptic tests are carried out to determine the physical form of the finished preparation where observations are made directly including the shape, color, and smell of the serum gel preparation using the five senses (Widia *et al*., 2012). The results of the organoleptic test of the serum formulation made are green preparations with a distinctive smell of simplisia and a slightly thick, semi-solid and semi-liquid form.

2. Homogeneity

Table 8. Homogeneity Test Results

Formulation	Homogeneity
F0	Homogeneous
F1	Not Homogeneous
F2	Not Homogeneous
F3	Not Homogeneous

Description:
F0 : Serum formulation without extract
F1 : Serum formulation with 2% extract concentration
F2 : Serum formulation with 4% extract concentration
F3 : Serum formulation with 6% extract concentration

The homogeneity test is carried out by applying a serum sample to a piece of glass or other suitable transparent material, the preparation must show a homogeneous arrangement and no coarse grains are visible (Hasrawati *et al*., 2020). The results of the homogeneity test of the serum formula made without extract are homogeneous. While the results of the homogeneity test of the serum formula preparation with extracts are not homogeneous, this is because there are some coarse particles in the serum preparation made, causing the preparation to be inhomogeneous.

3. pH

Table 9. pH Test Results

Formulation	pH Test Results
F0	6
F1	6
F2	6
F3	6

Description:
F0 : Fromulation without extract
F1 : Serum formulation with 2% extract concentration
F2 : Serum formulation with 4% extract concentration
F3 : Serum formulation with 6% extract concentration

In testing the pH of the serum preparation of ethanol extract of guava leaves is carried out using a pH meter. The pH test aims to see the safety of a preparation when used on the skin and also to determine the acid and base levels of a preparation, especially in topical preparations. The results of the pH test of the guava leaf ethanol extract serum preparation have an average pH of 6. The results of the guava ethanol extract gel pH test are safe to use on the skin because they are in accordance with the skin pH range of 4.5-6.5.

4. Stickiness

Table 10: Adhesion Test Results

Formulation	Adhesion Test Results
F0	2.3 seconds
F1	1.32 seconds
F2	0.35 seconds < 1 second
F3	0.26 seconds < 1 second

Description:
F0 : Formulation without extract
F1 : Serum formulation with 2% extract concentration
F2 : Serum formulation with 4% extract concentration
F3 : Serum formulation with 6% extract concentration

Adhesion testing aims to measure how much the gel preparation adheres to the skin when used so that drug delivery can function optimally. From the results obtained, F0, namely the base without extract, has a longer adhesion, this is because the base has a large viscosity so that it affects the longer adhesion while F3 has a fast adhesion, this is because the serum with a concentration of 6% in the formula has more water content. The results of the guava leaf ethanol extract serum adhesion test are in accordance with the requirements for the adhesion of semisolid preparations, which is more than 1 second.

5. Spreadability

Table 11: Results of the Spreadability Test

Formulation	Results of Spreadability Test
F0	9 cm
F1	8 cm
F2	5 cm
F3	7.5 cm

Description:
F0 : Serum formulation without extract
F1 : Serum formulation with 2% extract concentration
F2 : Serum formulation with 4% extract concentration
F3 : Serum formulation with 6% extract concentration

Scatterability testing aims to determine the ability of serum to spread evenly when used on the surface of the skin, because it can affect the speed of release of active substances and also affect drug absorption (Ardana *et al*., 2015). The results of the serum spreadability test had an average spreadability of 5.1 cm. The results obtained are in accordance with the requirements for the spreadability of semisolid preparations, which is 5-7 cm.

6. Viscosity

Table 12: Viscosity Test Results

Formula	RPM Viscosity (cps)					
	4	5	10	20	50	100
F0	262,44	-	-	-	-	-
F1	-	-	1560	937,3	512,3	-
F2	-	-	2039	1165	-	-
F3	-	-	69,0	73,5	43,6	39,3

The viscosity test is carried out to determine the amount of viscosity of a preparation, where the viscosity or viscosity value states that the amount of resistance of a liquid to flow. The viscosity of serum preparations is usually proportional to the amount and molecular weight of the thickener added. The highest viscosity was obtained by F2 with a concentration of 4% and the lowest viscosity was obtained by F3 with a concentration of 6%. These results are still within the required limits, namely the standard viscosity of serum preparations is 5000-6000.

TLC-Bioautography

TLC-Bioautography is an advanced test that serves to determine what chemical components provide antibacterial activity from extra ethanol guava leaves. The eluent used is chloroform and ethyl acetate in a ratio of (7:3). The method used in TLCBioautography is the contact method, namely by attaching the TLC plate above the agar medium that has been inoculated with test bacteria.

Figure 5. TLC-Bioautography results

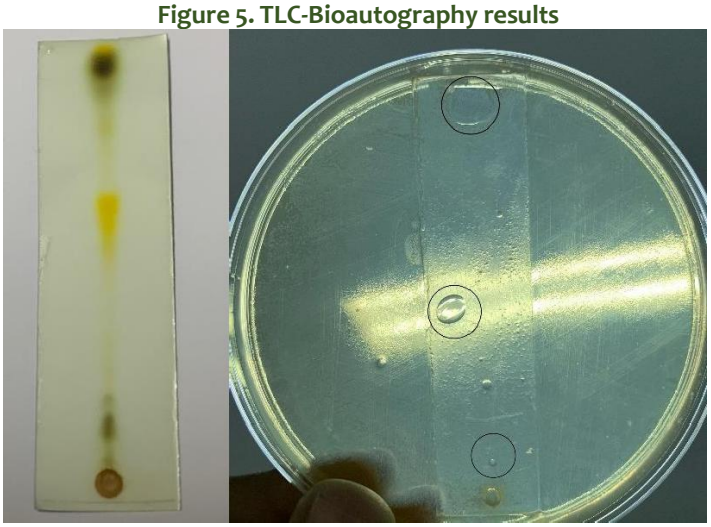


Table 13: TLC-Bioautography Test Results of Guava Leaf Extracts

Eluent	Stains	Rf	Results
Chloroform (7): ethyl acetate (3)	1	0,15	There is a clear zone
	2	0,25	
	3	0,5	

Bioautography TLC results of guava leaf extracts give positive results where a clear zone is formed on the chromatogram plate, which means that guava leaf extract is proven to have antibacterial activity so that it can inhibit *Staphylococcus epidermidis* (Aslah *et al*., 2019).

CONCLUSION

Based on the results of research on "Antibacterial Activity Test of Ethanol Extract of *Guava* Leaf (*Psidium guajava L.*) Against *Staphylococcus epidermidis* and its Formulation as Serum" it can be concluded that:

1. Ethanol extract of *guava* leaves (*Psidium guajava L.*) can inhibit the growth of *Staphylococcus epidermidis* bacteria. The extract that provides the strongest antibacterial activity is at a concentration of 5%.
2. The phytochemical contents identified in the ethanol extract of *guava* leaves (*Psidium guajava L.*) are alkaloids, flavonoids, tannins, saponins and steroids.
3. Serum preparation of ethanol extract of guava leaves (Psidium guajava L.) can provide antibacterial activity against *Staphylococcus epidermidis* bacteria. *The* formula that provides the strongest antibacterial activity is F3 with a concentration of 6% (13 mm).

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

We declare that we have no conflict of interest

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