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Antibacterial Activity of Ethanol Extract of Kersen Bark (*Muntingia Calabura* L) & It's Formulation as an Anti-Acne Cream

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Abstract: **Background**: Kersen (*Muntinga calabura*) bark is one of the medicinal plants that can be used to treat acne. The ethanol extract of kersen bark contains alkaloid, flavonoid, tannin, saponin, and terpenoid compounds that have potential as antibacterials. **Objective**: This study aims to make a cream preparation from ethanol extract of kersen bark and to determine the antibacterial activity against acne-causing bacteria *Staphylococcus epidermidis*. **Methods**: Extraction was done by remaceration method and 96% ethanol solvent was used to obtain the extract of kersen stem bark. Phytochemical screening was carried out to determine the secondary metabolites in the extract. The antibacterial activity test of the extract was carried out using the solid liquid dilution method to determine the KHM and KBM values. The extracts were then formulated into M/A (oil in water) cream preparations, then physically evaluated and tested for antibacterial activity using the pitting diffusion method. **Results**: Kersen bark contains alkaloids, flavonoids, tannins, saponins, and terpenoids that show antibacterial activity against *Staphylococcus epidermidis* with KHM and KBM values at a concentration of 3.125%. The physical properties of the creams showed that all creams met the requirements with 5% concentration as the minimum limit for the preparation to have antibacterial activity. **Conclusion**: Kersen bark extract cream formulation with the greatest antibacterial activity was obtained at a concentration of 10%, where the higher the concentration of extract used, the higher the antibacterial activity.

Keywords: Kersen bark, antibacterial, *Staphylococcus epidermidis*, cream preparation

INTRODUCTION

Acne is defined as a skin disease caused by oil buildup due to clogged pores on the face, triggering bacterial activity and skin inflammation (Nurjanah *et al*., 2018). Acne is often made worse by bacterial activity that infects the skin when inflamed. The bacteria that most commonly infect the skin and form pus include *Staphylococcus epidermidis* (Marliana *et al*., 2018)[2].

Staphylococcus epidermidis is a Gram-positive bacteria that belongs to the normal flora of the skin, but is often involved in the pathogenesis of acne. The mechanism of acne is that bacteria will damage the stratum corneum and stratum germivum by secreting chemicals that can destroy the pore wall and cause inflammation. This condition will make fatty acids and oils in the skin clogged and harden into acne bumps (Imasari & Emasari, 2022).

The current selection of therapies for acne treatment includes antibiotics, laser treatments, and cosmetics containing chemicals such as sulfur, resorcinol, salicylic acid, and benzoyl peroxide. But unfortunately, these types of treatments are quite expensive and prone to irritation (Adhi, 2020). The utilization of natural ingredients as new antibacterial agents and therapeutic options in the treatment of acne is increasingly attracting public interest. The use of natural medicine is considered to have a lower risk of side effects compared to synthetic drugs and has a more affordable price (Mufida et al., 2018).

The kersen plant (*Muntingia calabura* L) is a plant that grows in Indonesia and several Southeast Asian countries. In previous studies, kersen bark samples were shown to contain secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids (Siara *et al*., 2017) which were used for antioxidant, antidiabetic, anticancer, and antibacterial testing on Gram-negative bacteria. However, there has been no further research related to the activity of kersen bark extract against *Staphylococcus epidermidis* bacteria which is a Gram-positive bacteria.

Considering the potential of kersen stem bark as an antibacterial, in this study, the formulation of anti-acne cream from kersen stem bark and antibacterial activity test against acne-causing bacteria, namely *Staphylococcus epidermidis*, were carried out. One of the advantages of cream in cosmetic products is its ability to spread evenly when used, so that it can prevent inflammation and provide protection to its users (Suwandi *et al*., 2023). Therefore, this study was conducted with the aim of evaluating the potential of kersen stem bark ethanol extract cream as a natural antibacterial agent to treat acne caused by *Staphylococcus epidermidis* bacteria. By knowing the potential of kersen stem bark ethanol extract as a natural antibacterial agent, this study is expected to provide a more natural and safe alternative to acne treatment, and help reduce dependence on synthetic drugs which are increasing.

METHODS

Extraction

Kersen stems were obtained from Demak Regency, Central Java. Kersen bark was collected manually, washed and wet sorted. Kersen stem bark was kneaded, dried, sorted on the dried stem bark simplisia, and weighed again. The dried stem bark simplisia was pulverized into fine powder using a blender. 350 grams of kersen bark powder was put into a container, then 3.5 liters of 96% ethanol (1:10 ratio) was added. Soaking was carried out for 3x24 hours and solvent replacement was carried out after 24 hours. The results of maceration were combined and the solvent was evaporated with a rotary evaporator at 40˚C until a thick extract of kersen bark was obtained.

Phytochemical Screening

Phytochemical testing was conducted to determine the content of secondary metabolites contained in kersen stem bark extract.

a. Alkaloid test
   2 ml of extract was put into a test tube and 2-3 drops of mayer reagent were added to the tube. The formation of a yellowish white precipitate after the addition of mayer's reagent indicates the presence of alkaloids (Suleman *et al*., 2022).
b. Flavonoid test
   Put 2 ml of kersen bark extract into a test tube, then add 3 drops of 10% AlCl3. The test results are said to be positive for flavonoids if a yellow color is formed in the solution (Ni'ma *et al*., 2022).
c. Tanin test
   Put 2 ml of kersen bark extract into a test tube, then add 2-3 drops of 1% $FeCl_3$ solution. If the result is positive, there will be a change in color to bluish black or green (Ngajow *et al*., 2013).
d. Saponin test
   Pour 2 ml of kersen bark extract into a test tube, then add distilled water. Shake the mixture vigorously until foam forms. If the foam remains for 5 minutes, the result will be positive (Ngajow *et al*., 2013).
e. Terpenoid test
   To test for the presence of terpenoids, the sample mixture is added with anhydrous acetate and concentrated sulfuric acid. If the test results show a positive result, this indicates the presence of terpenoid compounds with the formation of a purplish red color.

Preparation of test solution concentration

The 50% concentration was made by weighing 2 grams of thick extract of kersen stem bark and added to a volumetric flask with 4 ml of DMSO solution. Multilevel dilution was carried out by taking 1 ml of 50% concentration extract added with 1ml of NB media. A concentration of 25% was obtained and multilevel dilution was carried out as in the previous treatment. Concentrations of 50%; 25%; 12.5%; 6.25%; and 3.125% were obtained.

Antibacterial activity test of kersen bark extract

Antibacterial testing was carried out using liquid and solid dilution methods to determine the KHM and KBM values of the extract. Liquid dilution testing was carried out by making 1 ml of sterile NB (*Nutrient broth*) media in 12 tubes added with various concentrations of extracts tested with 2 repetitions, DMSO (negative control), and clindamycin (positive control) in each tube. Furthermore, each tube was added with 1 mL of S. epidermidis bacteria suspension. All test tubes were incubated at 37˚C for 18-24 hours and observed for turbidity. In the solid dilution method, testing was carried out using Mannitol Salt Agar (MSA) media. Sterilized Mannitol Salt Agar (MSA) was put in a Petri dish as much as 15 ml and let stand until solid. Each test concentration and control from the liquid dilution was applied to the MSA media, then incubated at 37˚C for 18-24 hours, then observe the growth of the bacteria (Fitriyanti, 2023).

Preparation of kersen bark extract cream

Kersen bark extract cream was formulated as M/A type cream (vanishing cream). The components of the oil phase (stearic acid, propyl paraben, and cetyl alcohol) and the water phase (TEA, glycerin, methyl paraben, and water) were separated first. The oil phase and water phase were heated at the same temperature, 70°C. After the oil phase had melted completely, the oil phase was put into a preheated mortar container. Next, the water phase was slowly added to the mortar containing the oil phase while stirring continuously. Kersen bark extract was added and all ingredients were crushed to produce a homogeneous cream mass (Husnani & Rizki, 2019).

Table 1. Cream Formulation of Kersen Stem Bark Extract

Ingredients	Function	Fo (%)	F1 (%)	F2 (%)	F3 (%)
Kersen stem bark extract	Active substance	-	3	5	10
TEA	Emulgator	1,5	1,5	1,5	1,5
Stearic acid	Emulgator	6	6	6	6
Cetyl alcohol	Surfactant	2	2	2	2
Gliycerin	Humectant	3	3	3	3
Methyl Paraben	Preservative	0,18	0,18	0,18	0,18
Propyl Paraben	Preservative	0,02	0,02	0,02	0,02
Aquadest	Base	Ad 100	Ad 100	Ad 100	Ad 100

Testing the physical properties of the cream

a. Organoleptical test was conducted by visual observation, including changes in shape, color, phase separation, texture, and changes in odor (Hakim *et al*., 2020).
b. pH measurement using a pH meter and pH stick. The measuring electrode is immersed so that the entire tip of the electrode is submerged, and the pH value obtained is recorded (Hakim *et al*., 2020).
c. Homogeneity test was carried out by weighing 0.5 grams of cream placed on a glass plate and distributed evenly (Hakim *et al*., 2020).
d. Spreadability is done by weighing 0.5 gram of cream and placed on a glass plate. Above the cream is given another glass and leave it for 1 minute, then measure the diameter of the spread of the cream. Then add a load weighing 50 grams, and let the load stand still for 1 minute. After that, measure the actual spread diameter of the cream. Repeated tests were carried out with the addition of loads of 100 grams to 150 grams with the same time interval (Hakim *et al*., 2020).
e. Adhesion test was modified based on researc (Yuni *et al*., 2023). A total of 0.5 gram of cream was weighed and placed on a glass plate. Another glass plate was placed on top of the cream and a load of 0.5 kg was applied for 5 minutes. Remove the load and record the time until both glass plates are released.
f. The Emulsion Type Test was modified based on study (Yuni *et al*., 2023). The test was conducted using the dilution method. The cream is added to the preparation with a certain amount of distilled water and then stirred. If the preparation remains homogeneous and does not experience phase separation, the preparation belongs to the M/A type (Megantara *et al*., 2017).

Antibacterial test of kersen bark extract cream

The pitting method was used in the antibacterial power test in this study. The steps began with preparing *Nutrient agar* (NA) medium as much as 10 grams dissolved in 500 ml of distilled water. The medium was sterilized by autoclaving it at 121°C for 15 minutes. Then, the sterilized *nutrient agar* medium was poured as much as 15 ml into 20 sterile Petri dishes and allowed to solidify. To make wells, tips with a diameter of ± 6 mm were used to make holes in the *nutrient agar* medium. Next, a suspension of *Staphylococcus epidermidis* bacteria was prepared, then the suspension was streaked on the solid agar medium using a sterile cotton bud. After that, cream samples with various concentrations that have been made as well as positive controls (clindamycin cream) and negative controls (cream base), are inserted into the wells. Petri dish was then incubated for 24 hours at 37°C. Measurements were made on the clear zone formed around the wells, indicating the presence of a zone of inhibition of bacterial growth (Suleman *et al*., 2022).

RESULT AND DISCUSSION

Extraction

The extraction method carried out in this study is using the remaseration method. In the remaceration method, the solvent is divided for the soaking process, so that the solvent is not directly poured for the extraction process. The remaceration method was chosen because it does not involve a heating process that can damage the active substances in the simplisia, the time used for taking active substances is shorter, and the resulting yield is more (Indarto *et al*., 2019).

Kersen bark powder of 350 grams was dissolved using 3.5 L of 96% ethanol in a ratio of 1: 10. The solvent used in this study is 96% ethanol which is a universal solvent and can dissolve both polar and nonpolar substances. The reason for using ethanol solvent is because ethanol has semi-polar properties that can extract compounds with various polarities. It is expected that the resulting yield will be more and able to extract all secondary metabolites in the sample (Indarto *et al*., 2019).

The extract obtained was evaporated using a rotary evaporator at 40˚C. This was done with the aim of separating the solvent from the active compounds contained in the kersen bark (Handoyo, 2020). The evaporation process produced a thick dark brown extract and smelled typical of kersen stem bark simplisia with a weight of 28.2 grams and a yield of 8.06%. The higher the yield obtained indicates that the extract produced is greater.

Phytocemical screening

Phytochemical screening is carried out to determine the content of secondary metabolites contained in kersen stem bark using certain solvents so that compounds that have potential as antibacterials can be identified.

Tabel 2. Phytochemical Screening Test Results of Kersen Stem Bark

Compound group	Reagent	Observation	Result
Alkaloid	Reagensia mayer	Yellowish white precipitate	Positive (+)
Flavonoid	$AlCl_3$ solution 10%	Formed yellow color	Positive (+)
Tanin	$FeCl_3$ solution	Changes to bluish black or green	Positive (+)
Saponin	Aquadest	There is a foam	Positive (+)
Terpenoid	Reagensia liberman	Formed purplish red color	Positive (+)

The alkaloid test, positive results are indicated by the presence of a yellowish-white precipitate after the addition of the mayer reagent. The formation of the precipitate is due to the alternation of lignans. In the process of making reagents mayer will form a red precipitate HgI_2 as a result of the reaction of $HgCl_2$ and KI. If excess KI is added, it will produce the $K_2[HgI_4]$ compound. So when the mayer reagent is reacted with the sample, alkaloid compounds that have nitrogen atoms will react with metal K+ from $K_2[HgI_4]$ and will form a potassium-alkaloid complex precipitate (Kopon *et al*., 2024).

The flavonoid content in this study was determined based on the color change that occurred after the addition of $AlCl_3$. The principle of flavonoid determination using $AlCl_3$ is the formation of complexes between $AlCl_3$ with keto groups at C-4 atoms and hydroxy groups at C-3 or C-5 atoms that are neighbors of flavones and flavonols (Estikawati & Lindawati, 2019) . The tannin test was determined by changing the color to bluish black or green after the addition of FeCl3 1%. Tannins tend to be polar because they have hydroxy groups. Changes in the sample to blue-black or green after the addition of 1% $FeCl_3$ indicate that the tannin compound is hydrolyzed. The reaction that occurs can be expressed as follows (Kopon *et al*., 2024):

$FeCl_3$ (aq) + 6ArOH (s) → 6H+ + 3Cl- + $[Fe(OAr)6]_3$- (aq)

Identification of saponin compounds was carried out by adding distilled water to the extract and shaking. Positive results are indicated by the formation of foam in the solution. The formation of foam indicates the presence of glycosides that are able to form foam in water which is hydrolyzed into glucose and other compounds (Kopon *et al*., 2024). Terpenoid testing is determined by a change in color to purplish red after the addition of liberman reagent. The test is based on the ability of terpenoid compounds to form concentrated H_2SO_4 color in acid anhydride solvent. The principle of this reaction is the release of H_2O and coupling with carboxyation (Kopon *et al*., 2024).

Antibacterial test results of extracts

Testing the antibacterial activity of extracts was carried out using the liquid dilution method and continued with solid dilution. The use of the dilution method is intended to determine the KHM (Minimum Inhibitory Concentration) and KBM (Minimum Kill Concentration) values of the test microbes using liquid and solid medium. Tests were carried out with 2 repetitions using five variations of extract concentrations, namely 50%; 25%; 12.5%; 6.25%; and 3.125%.

In the liquid dilution method, positive control (Clindamycin), negative control (DMSO 10%), and extracts that have been diluted to concentrations of 50%; 25%; 12.5%; 6.25%; and 3.125% are added with test microbes and bacterial growth media. While the solid dilution method was carried out by inoculating positive controls, negative controls, and extracts that had been added to test microbes on agar growth media. DMSO was chosen as a negative control with the aim of comparing that the solvent used as a diluent does not affect the antibacterial test results of the tested samples (Najia *et al*., 2022). Meanwhile, clindamycin was chosen as a positive control because clindamycin contains very strong antibacterial against gram-positive bacteria, such as *Staphylococcus epidermidis* (Indarto *et al*., 2019).

The bacterial growth medium used in liquid dilution is NB (*Nutrient broth*). The use of NB as a liquid medium has several advantages including turbidity in the test solution can be observed visually. In addition, NB is a complex

medium that provides complete nutritional needs for bacterial growth (Wahyuni *et al*., 2018). The Minimum Inhibitory Concentration (MWC) of the extract was determined by the lowest concentration of extract in the tube that produced a clear medium without visible microbial growth at a concentration of 3.125%.

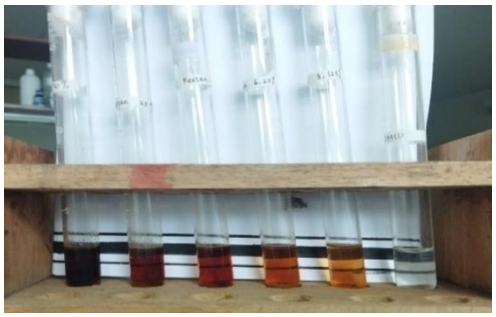

Figure 1. Dilution results of experiment 1

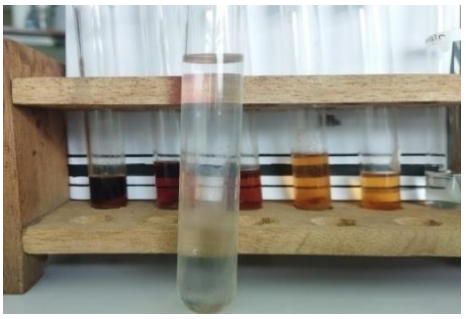

Figure 2. Dilution results of experiment 2

Tabel 3. Results of Solid Dilution Testing

Concentration % (b/v)	Bacterial Growth	
	First replication	Second replicatior
3,125%	-	-
6,25 %	-	-
12,5%	-	++
25%	-	-
50%	-	+
K (+)	-	-
K (-)	+++	+++

In the solid dilution method antibacterial testing, the media used is MSA (Mannitol Salt Agar). MSA media was chosen in this study because MSA is a selective medium used to isolate or culture Staphylococcus bacteria[22]. In experiment 1, the concentration of the extract tested had no bacterial growth which was characterized by 0% colony count. This shows that the kersen bark extract contains antibacterial substances that can inhibit and kill bacteria. The Minimum Kill Concentration (KHM) was determined by scratching each extract suspension from the liquid dilution results on Mannitol Salt Agar (MSA) media. The minimum kill concentration of the extract was obtained at a concentration of 3.125%, the agar media that had been scratched with the suspension showed no bacterial growth.

In the second replication at concentrations of 12.5% and 50% there was bacterial growth of ±30% and ±10%. This can occur due to the liquid dilution medium that has been added to the bacterial suspension is only vortexed not so long so that the bacterial suspension does not mix with the extract. As a result, the antibacterial ability of the extract is not maximized. In addition, the presence of bacteria can also be caused by the lack of an aseptic process when scraping bacteria from liquid dilution media into agar (Kulla & Herrani, 2022).

Cream preparation test

Organoleptical

Tabel 4. Organoleptical Testing Results of The Cream

Formula	Observation		
	Form	Color	Odor
F0 (Base)	Half solid	White	Typical of cream
F1	Half solid thicker than base	Chocolate	Typical aroma of kersen bark extract
F2	Half solid thicker than base	Chocolate	Typical aroma of kersen bark extract
F3	Half solid thicker than base	Slightly dark chocolate	Typical aroma of kersen bark extract

Based on the table, it can be seen that the results of organoleptical testing show that the extracts added to the formula affect the results of the cream dosage form made. The F0 dosage form is semi-solid with high water content because F0 is a cream base without the addition of kersen bark extract. F1, F2, and F3 tend to

have almost the same dosage form/texture but have a thicker texture and a more intense color than the base due to the addition of kersen bark extract (Sulastri *et al*., 2023).

Homogeneity

Tabel 5. Cream Homogeneity Test Results

Formula	Homogeneity
F0 (base)	Homogeneous
F1 (3%)	Homogeneous
F2 (5%)	Homogeneous
F3 (10%)	Homogeneous

Based on the homogeneity test, it shows that all formulations do not show any coarse grains when the cream is applied on a transparent glass plate. Homogeneous cream preparations indicate that the mixing of the base and kersen bark extract is good, so there are no lumps or coarse grains in the preparation that can cause irritation and unevenness of the cream when distributed (Sulastri *et al*., 2023).

pH

Measurement of the pH of the cream preparation aims to adjust the pH of the cream to the pH of normal skin so that it does not irritate the skin. The epidermis is the outermost layer of skin that has a pH ranging from 4.5-6.5 and functions as a protector against bacteria, chemical irritants, and allergies (Azkiya *et al*., 2017).

Tabel 6. Cream pH test results

Cream Formula	Replication 1	Replication 2	Replication 3	Mean ± SD
F0 (Base)	6,44	6,40	6,83	6,56 ± 0,19
F1 (3%)	6,45	6,47	6,54	6,49 ± 0,04
F2 (5%)	5,54	5,49	5,48	5,5 ± 0,03
F3 (10%)	5,18	5,19	5,17	5,18 ± 0,01

Based on observations of the pH of kersen bark extract cream preparations F1, F2, and F3 have normal skin pH, which ranges in the range of 4.5-6.5. In the cream base, the pH value is slightly over the range limit, which is 6.56. This is because the pH value of the preparation is influenced by the pH value of the constituent components. Stearic acid tends to cause a decrease in the pH of the preparation due to the presence of acidic groups so that it is necessary to have triethanolamine which is alkaline to balance the pH in the preparation. However, giving high triethanolamine can increase the pH value of the preparation because triethanolamine is an alkaline material (Arifin *et al.*, 2022).

Spreadability test

Scatterability testing is done to determine the ability of the cream to spread when applied to the skin.

Tabel 7. Results of Cream Spreadability Test

Cream Formula	Weight addition	Replikasi 1	Replikasi 2	Replikasi 3	Mean ± SD
F0 (Base)	Weightless	7,0 cm	6,6 cm	7,4 cm	7,0 ± 0,33
	50 gram	8,6 cm	7,6 cm	8,0 cm	8,1 ± 0,41
	100 gram	9,4 cm	8,8 cm	8,2 cm	8,8 ± 0,49
	150 gram	10 cm	9,6 cm	8,6 cm	9,4 ± 0,59
F1 (3%)	Weightless	5,1 cm	5,1 cm	5,0 cm	5,1 ± 0,05
	50 gram	5,7 cm	5,7 cm	5,7 cm	5,7 ± 0
	100 gram	6,1 cm	6,4 cm	6,4 cm	6,3 ± 0,14
	150 gram	6,7 cm	6,8 cm	6,8 cm	6,8 ± 0,05
F2 (5%)	Weightless	5,0 cm	4,9 cm	5,1 cm	5,0 ± 0,08
	50 gram	6,0 cm	6,5 cm	5,9 cm	6,1 ± 0,26
	100 gram	6,3 cm	6,0 cm	6,1 cm	6,1 ± 0,12
	150 gram	6,6 cm	6,2 cm	6,2 cm	6,3 ± 0,19

Cream Formula	Weight addition	Replikasi 1	Replikasi 2	Replikasi 3	Mean ± SD
F3 (10%)	Weightless	4,8 cm	4,7 cm	5,1 cm	4,9 ± 0,17
	50 gram	5,3 cm	5,1 cm	5,4 cm	5,3 ± 0,12
	100 gram	5,5 cm	5,3 cm	5,7 cm	5,5 ± 0,16
	150 gram	5,7 cm	5,6 cm	5,9 cm	5,7 ± 0,12

The data from the spreadability test results showed that the preparation can spread in a diameter range of 4.9 - 9.4 cm. This shows that the cream made meets the requirements of spreadability, with the criteria that the cream is very easy to spread because it has a diameter of more than 5 cm 27 (Mintoro *et al*., 2022).

Adhesion test

Tabel 8. Cream Adhesion Test Results

Cream Formula	Replication 1	Replication 2	Replication 3	Mean ± SD
F0 (Base)	1,26 s	1,30 s	1,20 s	1,25 ± 0,04
F1 (3%)	2,33 s	2,26 s	2,31 s	2,30 ± 0,03
F2 (5%)	2,21 s	2,62 s	2,64 s	2,49 ± 0,20
F3 (10%)	2,64 s	2,87 s	2,50 s	2,67 ± 0,15

Based on the testing of ethanol extract cream of kersen stem bark has an average value of adhesion of 2.30 - 2.67 seconds. These results meet the standard of cream adhesion which is more than 1 second, so it is expected that the active substances contained can be absorbed well. The value of cream adhesion is related to the spreadability of the cream, where the smaller the spreadability of the cream, the longer the time the cream adheres to the skin surface. Conversely, if the spreadability of the resulting cream is large, the faster the time for the cream to adhere. This is because the consistency of the cream affects the spreadability and adhesiveness tests[28].

Emulsion type test

Emulsion type testing is carried out to determine whether the preparation made is in accordance with the expected type of preparation. Preparations that remain homogeneous after the addition of distilled water are type M/A, while preparations that experience phase separation and are not homogeneous after the addition of distilled water indicate that the preparation is type A/M.

Tabel 9. Cream Emulsion Type Test Results

Formula	Homogeneity
F0 (base)	Homogeneous
F1 (3%)	Homogeneous
F2 (5%)	Homogeneous
F3 (10%)	Homogeneous

The test results on F0, F1, F2, and F3 creams show that all of the cream formulas have an oil-in-water (M/A) cream type. The advantage of using the M/A cream type is that it is easily washed off with water and if used on the skin there will be evaporation and an increase in the concentration of water-soluble drugs so as to encourage absorption into the skin tissue. The M/A type of cream also increases the permeability of the skin due to the hydration effect so that drug penetration increases and reduces the risk of inflammation (Yuni *et al*., 2023).

Cream antibacterial activity test

The antibacterial activity test of the cream preparation aims to determine the ability of the kersen stem bark extract cream preparation to inhibit the bacteria tested. The bacteria used in this test are *Staphylococcus epidermidis* bacteria which are normal skin flora and are bacteria that play a role in the formation of acne. Antibacterial activity testing was carried out using the agar well diffusion method.

Tabel 10. Antibacterial Activity Test Results of Cream

Cream formula	Replikasi 1	Replikasi 2	Replikasi 3	mean ± SD
K (+)	16,6 mm	15,9 mm	16,35 mm	16,28 ± 0,29

F0 (base)	-	-	-	-
F1 (3%)	-	-	-	-
F2 (5%)	8,3 mm	10 mm	8,5 mm	8,93 ± 0,76
F3 (10%)	11,4 mm	12,2 mm	11,6 mm	11,73 ± 0,34

Based on antibacterial testing, it shows that kersen bark extract cream can inhibit *Staphylococcus epidermidis* bacteria at a minimum concentration of 5%. This is characterized by the formation of clear zones around the wells. The classification of antibacterial inhibition zones starts from the weak, medium, to strong category. If the diameter of the inhibition zone produced is <5 mm calculated from the diameter of the entire clear zone area, it is said to be weak. An inhibition zone diameter of 5-10 mm indicates that the antibacterial ability is in the moderate category. An inhibition zone of 10-20 mm indicates that the bacterial growth inhibition response is categorized as strong and an inhibition zone of >20 mm is categorized as very strong (Geofani *et al*., 2022).

The positive control (clindamycin) showed a clear zone with an average inhibition zone diameter of 16.28 mm which is included in the strong inhibition category. The use of positive control aims to compare the diameter of the inhibitory power of the anti-acne cream preparation with the resulting kersen stem bark extract cream preparation. In the negative control, there was no clear zone around the well, indicating that the base or additives used in the formula had no effect on the growth of *Staphylococcus epidermidis* (Waznah *et al*., 2021).

The results of the antibacterial activity test in the study showed that at a concentration of 3% kersen stem bark extract in the cream preparation was not able to kill the test bacteria. This can be seen from the absence of a clear zone around the wells containing cream formula 1 (3%). Factors that can cause this activity are the presence of active substances trapped in the cream preparation so that it has difficulty passively diffusing out of the preparation to the target location, namely the bacterial growth medium (Irianto *et al*., 2020).

In testing creams with 5% and 10% extract concentrations showed that each extract concentration had different antibacterial activity. At a concentration of 5%, it was found that the cream had moderate antibacterial activity because the inhibition zone formed was around 8.93. While the cream with an extract concentration of 10% has strong antibacterial activity, because the clear zone value obtained is 11.73 mm.

Antibacterial activity in the cream is due to the presence of secondary metabolite compounds contained in kersen bark extract. Based on the results of phytochemical tests, secondary metabolite compounds contained in the extract include alkaloids, flavonoids, tannins, saponins, and terpenoids. The mechanism of inhibiting bacterial growth in each phytochemical compound contained has different activities. With the presence of secondary metabolites contained in the extract, the inhibition of bacterial growth can be observed.

CONCLUSION

Based on the results of the antibacterial activity test of ethanol extract of kersen (*Muntingia calabura* L.) stem bark and its formulation as an anti-acne cream, it can be concluded that kersen (*Muntingia calabura* L.) stem bark extract contains secondary metabolites in the form of alkaloids, flavonoids, tannins, saponins, and terpenoids which have potential as antibacterial against *Staphylococcus epidermidis* with KHM and KBM values of 3.125%. All of the cream formulations with kersen bark extract as the base ingredient have good physical properties. The cream formulation of kersen bark extract with the greatest antibacterial activity was obtained at a concentration of 10%, where the higher the concentration of extract used, the higher the antibacterial activity.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST

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