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Antibacterial Activity of Extract Combination Gel of Cherry Leaf (*Muntingia calabura* L.) and Beluntas Leaf (*Pluchea indica* L.) againts *Staphylococcus aureus*

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ABSTRACT

Staphylococcus aureus infection is a crucial problem because it has been found that there are S. aureus that are resistant to antibiotics. An alternative treatment for infection is with herbal plants such as cherry (*Muntingia calabura* L.) and *beluntas* (*Pluchea indica* L.) leaves since they contain antibacterial compounds. Cherry and *beluntas* leaves are formulated in gel form because they are easy to use. This study aims to determine the physical properties, microbial contamination, and antibacterial activity of the gel preparation. A combination of cherry leaf extract and *beluntas* leaf extract with a concentration ratio of 2.5:0.5 was formulated in gel preparations with concentrations of 3%, 6%, and 12%. The gel was later tested for physical properties, including an organoleptic test, a pH test, a spreadity diameter test, an adhesity test, and a viscosity test. The gel was also tested for the number of yeasts and antibacterial activity by the agar-well diffusion method. The test results were analyzed with SPSS 20.0.0 One-Way Annova. The results of the physical properties test for the gel preparation for viscosity were (5666.18; 6369.23; and 8540.11) cps, the pH value was (7.58; 7.11; and 6.45), the scattering diameter was (6.16; 5.88; and 5.00) cm, and the value of adhesity was (74.66; 80.99; and 98.18) seconds. The results of the yeast mold number test were obtained at 3x102 CFU/mL. The results of the inhibition zone diameters were 6.00, 7.90, and 10.00 mm. Based on the research, the difference in extract concentration affects the physical properties of the gel. The gel formula with a 12% concentration has physical properties reaching the requirements, meets the requirements for the value of yeast mold numbers, and provides the most optimal antibacterial activity.

INTRODUCTION

In tropical-climate areas like Indonesia, various diseases often occur. Infection is one of the examples. Infections are usually caused by bacteria, such as *Staphylococcus aureus* (Fikri and Siregar, 2020). *S. aureus* causes diseases such as impetigo, boils, acne, and wound infections. Treatment for patients with *S. aureus* infection is antibiotics (Wikananda et al., 2019).

In the case of *S. aureus* infection, S. aureus bacteria were found resistant to antibiotics such as Methicillin-Resistant *Staphylococcus aureus* (MRSA), with an estimated MRSA number of 28% in Indonesia and Hong Kong and 70% in Korea (Nismawati et al., 2018). The number of bacterial strains resistant to antibiotic drugs continues to increase, prompting the importance of efforts to find and use alternative medicines made from natural ingredients to prevent infectious diseases (Widiastuti and Pramestuti, 2018).

Indonesia has a variety of plants that most people believe are medicines, including cherry (*Muntingia calabura* L.) and *beluntas* (*Pluchea indica* L.) plants. Cherry leaves can be used as an antibacterial because they contain

compounds including flavonoids, tannins, and saponins (Nau'e et al., 2020). Another plant that can be used as an antibacterial is *beluntas* leaves. *Beluntas* leaves contain various compounds, including alkaloids, flavonoids, tannins, essential oils, sodium, potassium, aluminum, calcium, magnesium, and phosphorus (Suru et al., 2019).

In Saputra's (2020) research, the combination of cherry and *beluntas* leaf extract tested by the liquid dilution method using a checkerboard microplate flat-bottom polystyrene 96 wells had a synergistic effect in inhibiting the growth of S. aureus. support the level of its antibacterial activity, therefore the development will be reasonable to do. Gel preparations have numerous advantages, such as being easy to apply. When it is applied without any pressure, it gives a cool sensation to the skin, leaving no marks on the skin and being easy to use (Anggraeni et al., 2012). Gel preparations are created because *S. aureus* infections mostly attack the skin.

The aim of this study is to determine the physical properties, the value of yeast mold numbers, and the optimum concentration of inhibition in the combination gel preparation of cherry (*M. calabura* L.) and *beluntas* leaves (*P. indica* L.).

METHODS

Materials

The materials in this study are *beluntas* leaves, cherry leaves, 96% ethanol (PT. Brataco), *Staphylococcus aureus* bacteria obtained from Balai Laboratorium Kesehatan Daerah Istimewa Yogyakarta (Yogyakarta Special Region Health Laboratory), 0.9% NaCl solution (Widatra), Sabouraud Dextrose Agar (Oxoid) and Muller Hinton Agar (Oxoid), Brain Heart Infusion (Oxoid), chloramphenicol and clindamycin gel, and gel ingredients: Carbopol® 940, HPMC (PT. Brataco), propylene glycol (PT. Brataco), methyl paraben (pharmaceutical grade), propyl paraben (pharmaceutical grade), distilled water (pharmaceutical grade).

Extraction

The extraction process uses the maceration method, with the solvent used being 96% ethanol in a ratio of 1:10 (Widhiana Putra et al., 2020). The dry powder of cherry and *beluntas* leaves is measured at 150 grams. The dry powder is put into a glass container and added to 96% ethanol solvent in a ratio of 1:10 or until completely submerged, which is later homogenized with a stirrer for 4 hours and allowed to stand for 24 hours at room temperature. The maceration results are filtered using Whatman filter paper with the help of a separating funnel (Koirewoa et al., 2012). The extract is concentrated using a rotary evaporator at a temperature of 45°C. The concentrated extract was later measured, and the yield value was calculated (Rahmawati, 2014). The extract obtained was then removed for non-polar compounds by washing with *n*-hexane.

Plant determination

Plant determination is to obtain the determination of the test plant sample and to avoid the error of material for research (Devi and Mulyani, 2017). The determination of cherry and *beluntas* was conducted by Heri Setiyawan, a botanist from the Biology Laboratory, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, Yogyakarta.

Extract ethanol free test

One mililiter of concentrated acetic acid (CH_3COOH) and sulfuric acid (H_2SO_4) were added to a number of test solutions. Then it is homogenized, and after the mixture is homogeneous, it is heated with Bunsen fire. A positive result of an ethanol-free extract occurs if the test results do not smell the ester odor as a characteristic odor of alcohol (Ballo et al., 2021).

Extract moisture test

Each extract measured 1.5 g and was put on an aluminum plate. The plate is later inserted into the halogen moisturizer analyzer to determine the water content of the extract (Nurani et al., 2017).

Extract phytochemical screening test

A phytochemical screening test of the extracts was conducted using a tube test.

Flavonoid

It is measured as much as 0.5 g of extract, then put in a test tube and added to 5 mL of ethanol. The mixture is then heated for 5 minutes. Then, 10 drops of concentrated HCl and 0.2 g of Mg powder were added. The extract contains flavonoid compounds if a red-brown precipitate is formed (Tarukbua et al., 2018).

Saponin

As much as 0.5 g of extract was put into a test tube, along with 10 mL of distilled water. The test tube was shaken for 1 minute and

allowed to stand for 10 minutes while observing the foam that formed. The extract contains saponins if the foam persists for 10 minutes (Tarukbua et al., 2018).

Phenol

It is measured as much as 0.5 g of extract, then added to 10 mL of distilled water and dropped with $FeCl_3$. The blackish-green color formed indicates the presence of phenolic compounds (Susanti et al., 2017).

Gel formulation of cherry and *beluntas* extracts

The materials used are measured according to the amount in the formula (Table 1). Carbopol® 940 was developed by sprinkling it in hot water until it rose. Later, it was stirred, and triethanolamine (mass 1) was added. HPMC was developed using hot water and then stirred until a homogeneous mass (mass 2) was formed. Methyl paraben and propyl paraben were dissolved in propylene glycol (mixture 3). Mass 1 was then added to mass 2, mass 3, and a combination of cherry leaf extract and *beluntas* leaf extract. The mixture was stirred homogeneously using a stemmer until a gel mass was formed. Then the gel was stored in a gel pot and labeled (Yunita and Anwarudin, 2020).

Evaluation of gel preparation

Organoleptic test

The gel organoleptic test was conducted by observing the gel based on color, consistency, and odor (Sikawin et al., 2018).

Viscosity test

A viscosity test was conducted using a Rheosys Merlin VR Viscometer and a cone and plate with 2.0/30 mm. 500 mg of gel was placed on a plate and squeezed by a cone. The test parameters were made similar for all formulas so that all formulas achieved the same treatment. After that, it was operated using a computer with the Rheosys Micra application.

Table 1. Formulation of gel

Materials	F1 (%)	F2 (%)	F3 (%)
Extract combination	3	6	12
Carbopol® 940	0.5	0.5	0.5
HPMC	7	7	7
Propylene Glycol	15	15	15
Methyl Paraben	0.075	0.075	0.075
Propyl Paraben	0.025	0.025	0.025
Triethanolamine	2	2	2
Aquadest ad	100	100	100

pH test

The pH test of the gel used a pH meter. The gel pH test was conducted by dipping the pH meter electrode into a gel preparation that had previously been dipped in distilled water. The pH meter tool will show the pH of the gel preparation (Sikawin et al., 2018).

Spreadibility diameter test

The gel was measured at 1 g and then placed in the middle of the glass, and another glass was placed on it for 1 minute. On the glass tool, 125 grams were added for 1 minute. The diameter of the scatter was measured using a ruler (Garg et al., 2002).

Adhesity test

The gel, which measured as much as 0.25 g, was placed between two glass objects on the test equipment. The glass object was later placed on top of a load weighing 1 kg for 5 minutes. Remove the 80-gram load from the appliance to determine the gel's adhesive. Gel adhesive was determined as the time for two glasses to separate from each other (Sikawin et al., 2018).

Gel's mold/yest number

The media used is SDA (Sabouraud Dextrose Agar). Each petri dish was poured with as much as 12–15 mL of SDA media at a temperature of no more than 45 °C, homogenized, and allowed to solidify. From each dilution of the gel suspension, 0.1 mL is put into a petri dish. It is spread and leveled using bent rods. For each dilution, two dilutions were carried out (Kemenkes RI, 2020). The media used was also tested for sterility by pouring SDA media on a petri dish and letting it sit until solid without being filled with samples at various dilutions. Incubate Sabouraud Dextrose Agar plates at a temperature of 20°–25° for 5-7 days. For the calculation of the number of yeasts, the dish was selected from one level of dilution with the highest number of colonies, which was less than 50 colonies. The average number of colonies in the culture medium was calculated and expressed as the number of colonies per g or per mL of preparation (Kemenkes RI, 2020). The limit of yeast mold numbers for gel preparations according to BPOM Regulation No. 32 of 2019 is ≤ 104 colonies/g (BPOM RI, 2019).

Antibacteria activity testing

The media used was BHI (Brain Heart Infusion), and the solid media used was MHA

(Mueller Hinton Agar) media. The pure cultured *S. aureus* bacteria were streaked on the MHA medium aseptically using a sterile ose needle. After being scratched, the media was incubated at 37°C for 18–24 hours (Fauziah and Darmawan, 2017). *S. aureus* bacteria as much as 1 ose taken with sterile ose, then put into 1 mL of liquid BHI in a test tube, incubating the media for 4-6 hours at 37°C. From the incubation medium, take 100 µL of the suspension and dilute it with 0.9% NaCl solution until the turbidity is equivalent to the standard McFarland 108 CFU/mL (Fauziah and Darmawan, 2017). Then 100 µL of the bacterial suspension was diluted again using 10 mL of 0.9% NaCl media (1:100) until the turbidity of the suspension was 106 CFU/ml (Pamuladiman and Widiyastuti, 2021).

The suspension of *S. aureus* bacteria was taken in 100 µL, poured on MHA media, and leveled with the help of a spreader. The suspension was allowed to stand until it was absorbed into the MHA medium. After that, 5 wells with a diameter of 6 mm were made on MHA media (Balouiri et al., 2016). The distance between the wells in the media is made equal. Each combination extract gel of cherry leaves and *beluntas* leaves, a positive control (clindamycin gel), and a negative control (gel base) were taken and put in their respective wells and incubated for 24 hours at 37°C (Sikawin et al., 2018). Clindamycin gel was used as a positive control. The clear area formed around the well indicates the inhibition area formed. The diameter of the inhibition zone is calculated by subtracting the total diameter of the clear area from the diameter of the well with a caliper or ruler (Pamuladiman and Widiyastuti, 2021).

RESULTS AND DISCUSSION

Plant determination

The research begins with the process of determining the plants to be used as samples. Plant determination aims to determine if the plant sample used is the expected plant and to prevent sample errors for the research (Devi and Mulyani, 2017).

Extraction

The extraction process of cherry and *beluntas* leaves uses the maceration method with 96% ethanol as a solvent. According to the results of the research, cherry leaf extract had a yield of 9.30%. From the obtained yield of cherry leaf extract, it showed smaller results than previous studies; in Saputra's (2020) research, the yield was 13.27%. The yield of *beluntas* extract obtained was 11.50%. The yield obtained has met the requirements of FHI because the extract obtained is not less than 8.30% (Kemenkes, 2017).

From the extract obtained, the non-polar compound was removed by washing it with n-hexane. The solvent n-hexane is used in the defatting process. Defatting is a process conducted with the aim of removing non-polar content such as fat and chlorophyll possessed by the extract (Giri, 2020). After being washed with n-hexane, the extract was concentrated again with a water bath to remove the remaining n-hexane and measured, and the weight and yield of the n-hexane-washed samples were calculated. The yield of cherry extract obtained after washing with *n*-hexane was 9.09%, and the yield of *beluntas* extract obtained after washing with n-hexane was 11.33%. The yield of washing with n-hexane is smaller than the yield obtained before washing with n-hexane.

Extract ethanol free test

The ethanol-free test was carried out to ensure that the extract obtained was pure without the presence of ethanol as a contamination, which was the solvent of the maceration process that had been carried out. Ethanol also has antibacterial and antifungal properties, which can cause false positives in the tests carried out on samples, so it must be ensured that the extract does not contain ethanol (Kurniawati, 2015).

Non-containing ethanol extract can be ascertained by the absence of a distinctive ethanol odor after the test is carried out. From the ethanol-free test, the result obtained was that cherry and *beluntas* extracts were free of alcohol compounds. It was characterized by the absence of a characteristic alcohol ester odor.

Extract moisture test

The extract obtained was tested for water compounds using a halogen moisture analyzer. From the test results, cherry extract has 8.6% water content, and *beluntas* extract has 4.55%. The standard water compound of the extract is <10% in cherry extract and <9.6% in *beluntas* extract (Kemenkes, 2017). The results of the test show that the extract complies with the quality requirements. The water content of the extract is related to the purity of the extract. If the extract has a lower water content, then the extract may have been contaminated, such as by

the growth of fungi and microbes, which will be smaller (Gangga et al., 2017).

Table 2. The result of the extract phytochemical test

Extract	Test	Result	Note
Cherry	Flavonoid	+	Red precipitate
	Phenol	+	The test results are blackish green.
	Saponin	+	There is constant foam.
Beluntas	Flavonoid	+	Red precipitate
	Phenol	+	The test results are blackish green.
	Saponin	+	There is constant foam.

Table 3. The results of the organoleptic test of the gel preparation

Criteria	Base	F1	F2	F3
Smell	Carbopol-HPMC	Thypical extract	Thypical extract	Thypical ekstract
Color	White	Light brown	Light brown	Brown
Consistency*	+	+	++	+++

*Consistency = + (Less thick), ++ (Thick), +++ (Very thick)

Extract phytochemical test

A phytochemical test of the extract compound was performed using a tube test in order to determine the presence of flavonoid compounds, saponins, and phenols contained in cherry and *beluntas* extract as compounds that have antibacterial activity. The results of the phytochemical test of the extract are listed in Table 2.

The experimental results show that cherry and *beluntas* extracts contain flavonoids, saponins, and phenols. This is in accordance with the results of prior research, which show cherry extract contains flavonoid compounds, tannins, and saponins that have antimicrobial properties (Nau'e et al., 2020), and *beluntas* extract contains alkaloids, phenols, flavonoids, sterols, and quinones that are useful as antimicrobials (Yuliani et al., 2017).

Gel formulation

The components used in the preparation of the gel are cherry leaf extract and *beluntas* leaf extract with different concentrations as active substances, carbopol and HPMC as gelling agents, propylene glycol as humectants, triethanolamine as alkalizing agents, methyl parabens and propyl parabens as preservatives, and distilled water.

In this study, the formulation of cherry leaf extract and *beluntas* leaf extract was made into a gel dosage form based on HPMC and carbopol. HPMC is able to form a gel that is neutral, clear in color, has a stable pH with a pH range of 3 to 11, and is durable because it remains stable when stored for a long time and has good resistance to possible microbial contamination (Rowe et al., 2009). Carbopol is a material that can be used as a gel base that does not cause hypersensitivity to the skin when used topically, so Carbopol is a safe ingredient. Carbopol can also be strong and adhere well. The use of a combination of HPMC and carbopol as a base is better when compared to using it alone because it is able to form a gel with a better physical shape, a high viscosity value, good drug release and dissolution, and good bioavailability (Tambunan and Sulaiman, 2018). Propylene glycol is used as an ingredient to improve the properties of carbopol if it binds to the drug too strongly by increasing the solubility of the drug substance. The drug will be easily separated from the base if it has a high solubility, so if it is easily released, the drug will be much more effective to use. Triethanolamine is used as an alkalizing agent to increase the pH of carbopol, which will form a clear and thick gel (Tsabitah et al., 2019). Methyl paraben and propyl paraben are used as preservatives and growth inhibitors for fungi and molds in gels. The antimicrobial activity of the combination of methyl and propyl increases when used in combination because of the additive effect (Rowe et al., 2009).

Evaluation of gel preparation

Organoleptic test

The organoleptic test of the gel preparation aims to see the appearance of the gel that has been made. The gel that has been made is observed in the form of consistency, color, and smell. Organoleptic testing is related to the convenience of using gel as a drug preparation (Afianti and Murrukmihadi, 2015). The results of the organoleptic test of the gel preparation are presented in Table 3.

The combination gel of cherry and beluntas extract showed that the concentration of the added extract increased the smell, color, and consistency of the gel. The increase in the concentration of the extract resulted in a stronger smell, a darker color, and a thicker viscosity. All gel formulations made have a thick consistency; the color matches the active substance used, so the aesthetics of the gel made are less attractive for topical drug preparations.

Gel viscosity test

Viscosity shows the resistance of a preparation to flow. The purpose of this test is to determine the viscosity of the gel preparation. The viscosity value is related to the viscosity of a preparation; a large viscosity value indicates the thickness of the preparation (Afianti and Murrukmihadi, 2015). The value of gel viscosity is opposite to the value of the diameter of the spread gel; the higher the diameter of the spread gel, the smaller the value of the viscosity of the gel. A good gel has a viscosity value of 2000–50,000 cps (Sinko, 2011; Sulistyani et al., 2021). The gel viscosity value was tested with a Rheosys Merlin VR Viscometer using 5 rpm points. The viscosity value of the gel preparation was taken from one rpm point, namely at 25 rpm. Table 4 shows that all gel formulas fall within the range of viscosity value requirements. The higher the concentration of the extract used in the gel, the greater the viscosity value of the gel. Gel Formula 3 has the highest viscosity value because it has the thickest gel viscosity.

From the results of the gel viscosity, the type of gel flow can be determined. The results of the examination showed a non-Newton system of plastic flow and thixotropic The flow properties obtained from the rheogram, which has thixotropic flow properties with an ascending curve on the right and a descending curve on the left, indicate that the preparation has a lower viscosity value at each shear velocity value of the descending curve than the ascending curve. The plastic flow curve does not pass through the point (0.0) but intersects the shear stress axis. Thixotropic flow properties are defined as a change in the structure of the preparation that will not return to its original state immediately after the loss of applied pressure. This thixotropic flow property is good for topical preparations because it has a high consistency in the container, so when used by consumers, gel, cream, and gel-cream preparations are easy to spread, making it easier to apply (Chandra and Fitria, 2019).

The test results show that there is a correlation between the viscosity value and the spread diameter value. In gel preparations that have a high viscosity value, the value of the diameter of the spread is small because the more viscous the resulting gel preparation, the greater the value of the viscosity and the smaller the value of the diameter of the spread. The viscosity value is also related to the adhesive value; the higher the viscosity value, the higher the gel adhesive value.

pH test

The pH value is a value that indicates the level of acidity or alkalinity of a preparation being tested. The pH value in this study was measured with a pH meter. The pH test of the gel preparation is related to the safety of the gel. A good topical preparation has a pH value equivalent to the pH of human skin, so it does not cause irritation to the skin (Afianti and Murrukmihadi, 2015). The pH value of the preparation that is in accordance with the pH of the skin and does not cause irritation is 4.5–6.5 (Irianto et al., 2020). The results of the gel pH test (Table 4) show that the higher the concentration of the extract, the lower the pH of the gel. This decrease in pH can be caused by the presence of acidic flavonoids and phenols, so the pH of the gel will decrease with the addition of the extract (Widiyanto, 2007; Tarigan, 2020). The results showed that only Formula 3 gels were included in the safe range of pH for topical preparations of 4.5–6.5, while for bases and gels, Formula 1 and Formula 2 were not included in the standard pH range because they had a pH value of more than the range of 4.5–6.5.

Spreadibility diameter test

The spread diameter indicates the ability of the gel preparation to spread on the skin surface when used. The diameter of the spread of the gel describes the level of comfort in using the gel. The spread diameter test of the gel preparation aims to determine the ability to spread the gel on the skin surface; the easier the gel to spread, the faster it can provide a therapeutic effect (Purgiyanti, 2019). The results of the gel spread diameter test are presented in Table 4.

According to Garg et al. (2002), the diameter of a good gel preparation spread is 5-7 cm. Table 1 shows that the bases and gels of Formula 1, Formula 2, and Formula 3 are in the

range of good dispersion; the higher the concentration of extract, the smaller the diameter of the resulting gel spread will be. Formula 3 gel has the smallest spreading diameter because it contains the most extracts. In addition, the value of gel viscosity also affects the value of the diameter of the spread, where the higher the value of viscosity, the smaller the value of the diameter of the spread. The Formula 3 gel has the largest viscosity value, so it has the smallest spread diameter value.

Adhesive power test

The adhesive test of the gel preparation aims to test the adhesive ability of the gel when used on the skin for a certain period of time. Gels with good adhesion can maximize drug delivery effects (Afianti and Murrukmihadi, 2015). The results of the adhesive test are presented in Table 4.

Gel adhesive has a value opposite to the value of the diameter of the gel spread. A gel that has a small scattering diameter means that the gel has a high adhesive value, and vice versa. Adhesiveness will affect drug absorption; the longer a drug is attached to human skin, the better the absorption of the drug will be, which causes the goal of drug therapy to be achieved and maximized. A good gel has a stickiness of more than 1 second (Yusuf et al., 2017). Table 4 shows that all gel formulas have adhesive for more than 1 second, and the higher the concentration of extract used, the greater the adhesive value produced. Gel Formula 3 has the greatest adhesive value because it has the highest concentration of extract combinations and has the smallest dispersion diameter value.

Mold/ yeast number of the gel

One of the important standardization stages in processing traditional medicinal preparations is the microbial contamination test, which is a requirement from the Indonesian Herbal Pharmacopoeia (FHI) to have a proper amount of contamination in accordance with Regulation No. 32 of the Head of BPOM of the Republic of Indonesia concerning Quality Requirements for Traditional Medicines. The gel preparation that has been made is tested for yeast mold numbers because the gel contains water, which can cause mold and yeast growth (Prasetyo and Inoriah, 2013), and the gel contains carbopol, which, when made in a solution form, can cause mold and yeast growth (Rowe et al., 2009).

The number of yeast molds is calculated using:

$$\text{AKK} = \text{colony number} \times \frac{dilution\ factor}{ml\ sample}$$

The results of the yeast mold number test for gel preparations presented in Table 5 show that the number of colonies growing below 50 for gel and gel bases for each concentration has the highest number of colonies in Formula 1, with an average colony of 3 colonies. In the control media, no yeast colonies were found. The value of the gel yeast mold number was 3x102 CFU/mL. This result is safe and does not exceed the maximum limit set by BPOM Regulation No. 32 of 2019, which is ≤ 104 colony/g.

Antibacterial Activity

The antibacterial inhibition test in this study was carried out by the well method. Each MHA medium consisted of a combination gel of cherry and beluntas extract with different concentrations of 3%, 6%, and 12%, a positive control containing clindamycin gel, and a negative control containing a gel base. After all, the wells were filled and then incubated for 24 hours at 37°C. Then observations were carried out, and the inhibition zone formed was measured with a caliper instrument. The inhibition was seen in the formation of a clear-colored area around the well. The larger the diameter of the clear zone formed, the greater the inhibition. The description and analysis stated that the combination gel of cherry extract and beluntas extract could inhibit the growth of S. aureus colonies. The results of the inhibition of the Staphylococcus aureus antibacterial test are presented in Figure 1.

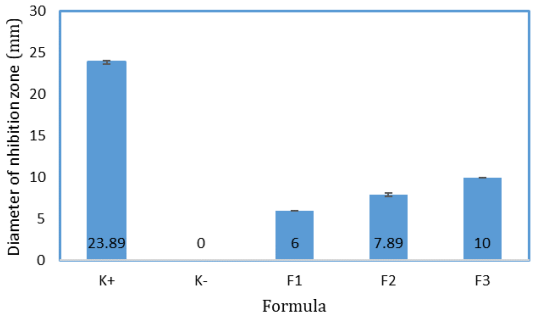

Figure 1. The relationship between the formula and the diameter of the inhibition zone. Note : K + : control +, K- : control +, F1 : formula 1, F2: formula 2, F3: formula 3

Tabel 4. Viscosity, pH, spreadibility, and adhesive power of each gel formula

Formula	Viscosity(cps)	pH	Spreadibility diameter (cm)	Adhesive power (s)
Base	3900.06 ± 130.25	7.88± 0.01	6.16 ± 0.06	69.24 ± 0.44
F1	5666.18 ± 251.68	7.58± 0.01	5.88 ± 0.04	74.66 ± 0.40
F2	6369.23 ± 185.18	7.11 ± 0.01	5.44 ± 0.02	80.99 ± 1.04
F3	8540.11 ± 532.11	6.45 ± 0.01	5.00 ± 0.00	98.18 ± 0.34

Table 5. Yeast mold number (AKK) in gel preparations

Sample	Preparation	Colony number		$\underline{x}$	AKK
		R1	R2		
Base	10^{-1}	0	1	0.5	$5x10^1$
	10^{-2}	0	0	0	0
	10^{-3}	0	0	0	0
F1	10^{-1}	3	3	3	$3x10^2$
	10^{-2}	0	0	0	0
	10^{-3}	0	0	0	0
F2	10^{-1}	1	1	1	$1x10^2$
	10^{-2}	0	0	0	0
	10^{-3}	0	0	0	0
F3	10^{-1}	1	2	1.5	$1.5x10^2$
	10^{-2}	1	2	1.5	$1.5x10^3$
	10^{-3}	0	0	0	0
Media control		0	0	0	0

The results of the normality test show that the data are normally distributed because the significant value is 0.196 and shows > 0.05. The data from the activity test results were normally distributed and then continued with the homogeneity test to determine whether the data obtained were homogeneous. The test results obtained a significant value of 0.001 (<0.05). The test results were then followed by a nonparametric test, namely the Kruskal-Wallis test. The results obtained a significant value of 0.008 (<0.05), which showed that each gel formula had a significant difference. Furthermore, the Mann-Whitney test was carried out, which aims to determine the difference between the two groups. Each group was compared with positive control and negative control to find out whether each group had a significant difference or not. The results obtained are comparisons between groups showing significantly different inhibitions because the significance value is <0.05.

The inhibition zone formed was influenced by the content of compounds in cherry leaf extract, and beluntas leaf extract had antibacterial activity. The contents contained in cherry extract and beluntas extract are flavonoids, phenols, and saponins. There has been no previous report on the use of a combination of cherry and beluntas extracts for gel preparations. However, research on the antibacterial activity of 15% beluntas leaf extract gel was reported to produce antibacterial activity with an inhibition zone diameter of 14.3 to 17.6 mm at various concentrations of the carbopol-hpmc combination as a gel base (Alvionida et al., 2021). As for the 12% combination gel in this study, which contained only 2% beluntas leaf extract, it produced an inhibition zone diameter of 10 mm. Kersen leaf extract gel 15% was also reported to produce antibacterial activity with an inhibition zone diameter of 12 mm (Manarisip et al., 2019). However, it is quite difficult to compare the results of the three studies due to differences in the composition of the base gel and the composition of the extracts in the gels.

CONCLUSIONS

The physical properties of the gel combination of cherry leaf extract and beluntas leaf extract were influenced by the concentration of the extract. The higher the concentration of the extract, the higher the adhesion and viscosity values, and the lower the pH and dispersion values. The gel concentration of 12% met all the requirements for the physical properties of the gel. The value of the yeast mold number in the gel preparation is 3x102 CFU/mL, so it does not exceed the safety limits according to the requirements of BPOM Number 32 of 2019 so that the gel is safe to use. The results of the antibacterial test data for gel preparations were (6.00) mm, (7.90) mm, and (10.00) mm. The higher the concentration of cherry extract and beluntas extract, the greater

the inhibition. The most optimal antibacterial concentration is 12%.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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