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Antibacterial cream activity test of Banyuru extract combination (*Pterospermum celebicum* Miq) DAN Bee pollen

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ABSTRACT

A study has been conducted on the formulation and antibacterial effects of banyuru cream extract (*Pterospermum celebicum* Miq) and bee pollen. The aim of this research is to know the antibacterial effect of cream preparation from banyuru extract (*Pterospermum celebicum* Miq) and bee pollen on *Staphylococcus aureus* and *Pseudomonas aeroginosa* isolates by agar diffusion method. The banyuru extract (*Pterospermum celebicum* Miq) and bee pollen were formulated in cream dosage form with the concentration ratio of banyuru extract (*Pterospermum celebicum* Miq) and beepollen F1 (3: 3) %, F2 (3: 2)%, and F3 (3: 1)%. Evaluation of the preparation includes organoleptic examination such as odor, color and homogeneity, pH, viscosity and antibacterial. The results showed that inhibition zone of F1, F2 and F3 on *Staphylococcus aureus* was 19.60 mm, 18.44 mm and 17.1 mm while in *Pseudomonas aeroginosa* that was 18.90 mm, 17.91 mm and 16.15 mm. The results showed homogeneous preparations, pH according to skin pH and viscosity increased. The results of antibacterial cream evaluation showed that formula 1 gave the best effectiveness with inhibit zone of 19.60 mm and 18.90 mm against *Staphylococcus aureus* and *Pseudomonas aeroginosa* isolates.

***Key words**: Antibacterial, Cream, Banyuru, Beepollen, S. aureus, P. aeroginosa*

Introduction

South Sulawesi has various resources of biodiversity, one of which is the Banyuru plant (*Pterospermum celebicum* Miq) is a type of plant that is classified as family of *Sterculiaceae* (Sosef *et al.*, 1988). The wooden part of the plant's trunk is used as material for the manufacture of plywood, furniture, shipping, bridges, and paper which can withstand termite attacks. Also, this plant has been widely used as traditional medicine by the community for its efficacy as a medicine for dysentery, wounds on the gums, as a medicine for itching and anti-infection drugs (Marzuki *et al.*, 2014).

According to research conducted by Marzuki (2015), it shows that the ethanol extract of the Banyuru stem bark (*Pterospermum celebicum* Miq) potential as an anti-bacterial infection, *Pseudomonas*

Aeruginosa, *Streptococcus Mutant*, *Staphylococcus aureus* and *Eschericia coli* with the inhibition zone of 16,12: 15,98: 15,78 dan 15,00 mm (Marzuki *et al.*, 2015).

The results of current studies reveal that Bee pollen is an important source of healthy, bioactive natural products (ie, polyphenols, flavonoids, and phenolic acids). Due to its diversity in its chemical position Beepollen can be useful in the prevention of diseases in which free radicals are involved. It also noted that this product is a potential source of antibacterial agents that act on gram-positive and gram-negative bacteria (Abdel A.A. Mohdaly, 2015).

Bee pollen exhibits antibacterial activity against gram-positive bacteria viz *Staphylococcus aureus* and *Listeria monocytogene* with each MIC value of 0.78 mg/ml dan 0.30 mg/ml while the MBC value is 0.85 mg/ml and 0.35 mg/ml. Whereas in gram negative bacteria, namely *Escherichia coli* and *Salmonella enterica* with each MIC value of 1,25 mg/ml dan 1,35 mg/ml while the MBC value is 1,35 mg/mL and 1,45 mg/ml (Mohdaly, 2015).

Research also conducted by Abouda, (2010), reported that bee pollen has antimicrobial activity against gram-positive and gram-negative bacteria, namely *Staphylococcus aureus* inhibition at concentrate 50, 60, 70 and 80% and *Pseudomonas Aeruginosa* inhibited at concentration 80 dan 90% (Abouda *et al.*, 2011).

Bee pollen has various compounds including flavonoids, fatty acids, and phenolics which are known to have antimicrobial activity. Based on these data, it can be concluded that the bee pollen combination has the potential to increase the antibacterial activity of the Banyuru stem bark (*Pterospermum celebicum* Miq). So that a cream formulation can be made with a combination of the Banyuru bark extract (*Pterospermum celebicum* Miq) and the beepollen.

The cream is a topical dosage form with a semisolid form, in the form of an emulsion containing one or more medicinal substances that have been dissolved or dispersed in a suitable base material and contain not less than 60% water. Cream bases are preferred for daily use because they have the advantage of having a cool effect on the skin, are not oily, and have good dispersibility (Voight, 1995).

Research conducted by Syukur (2017) regarding the Banyuru bark extract cream formulation showed good physical characteristics and stability at a 3% Banyuru extract concentration and an emulator concentration of 5% with an optimum inhibition zone diameter against Propionibacterium acne of 15,81 mm (Syukur, 2017).

This study aims to determine the antimicrobial activity of the combination cream of Banyuru (*Pterospermum celebicum* Miq.) And Beepollen extracts against the test bacteria *Staphylococcus aureus* and *Pseudomonas aeroginosa*.

Materials and Methods

The materials used in this study were distilled water, pure culture of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, NA medium, MHA medium, sterile aquadest, 0.9% NaCl solution, gentamicin cream, stearic acid, α-tocopherol, Phytocream® emulator, methylene blue, methylparaben, propylene glycol, propylparaben, cetyl alcohol, Banyuru extract (*Pterospermum celebicum* Miq.) and beepollen.

Method of Work

Sample Preparation

Banyuru stem bark samples (*Pterospermum celebicum* Miq) were taken from the Bantaeng district. The Banyuru stem bark sample is first separated between the bark and the wood then washed the Banyuru bark with running water until it is clean. Then it is chopped into small sizes and dried in an oven at 50 °C until dry. After drying the Banyuru bark is powdered using a grinding machine to obtain dry Simplicia powder with a sieve number 18. Meanwhile, bee pollen is taken from the stock that has been provided.

Banyuru Extract Made

The sample that has been weighed as much as 500 g, is extracted by maceration using 70% ethanol as much as 4 liters for 5 days and stirred occasionally, then filtered and the obtained filtrate is collected. The residue is macerated again with the same solvent and volume for 3 days, this is done three times. The collected filtrate is compressed using a rotary evaporator, until the extract becomes thick then lyophilized using a freeze-dryer and the extract is obtained in powder form.

Cream Formula Design

A total of 3 antibacterial cream formulas were made with various concentrations of Banyuru bark extract and beepollen extract.

Formula creation

The ingredients are weighed according to the design formula in Table 1 by separating the ingredients into two parts, namely part 1 consisting of stearic acid, cetyl alcohol, propylparaben, α-tocopherol, and phytocream® (oil phase) and part 2 consisting of propylene glycol, methylparaben, and aquadest. The ingredients for the oil and water phases are each heated and stirred until they reach a temperature of 75 °C until they are homogeneous. Once homogeneous, the mixture from both phases was stirred under continuous stirring for 15-20 minutes and then homogenized at 4000 rpm until a homogeneous emulsion was obtained. When the temperature starts to drop to 45 °C, the extract is added to the previous mixture while continuing to be homogenized at 2000 rpm for 15-20 minutes.

Table 1. Antibacterial Cream Formula with various concentrations of the ethanol extract of the bark of Banyuru (*Pterospermum celebicum* Miq) and beepollen extract

Material Name	Cream Formula (%b/b)		
	F1	F2	F3
Banyuru bark extract	3	3	3
Bee pollen	3	2	1
Setil alkohol	2.5	2.5	2.5
Asam stearat	2	2	2
Propilen glikol	15	15	15
Metil paraben	0.2	0.2	0.2
Propil paraben	0.02	0.02	0.02
Phytocream®	5	5	5
α-tokoferol	0.05	0.05	0.05
Aquadest	69.23	70.23	71.23

Evaluation of the Physical Stability of the Formula

Evaluation of the physical stability of the formula includes organoleptic, pH, viscosity, and physical stability of the cream with the Freeze-thaw test method, determined by storing the preparation at 5°C and 35 °C alternating for 12 hours for 10 cycles.

Organoleptic Examination

Organoleptic examination was carried out visually, the observations made included changes in shape, color, smell, and texture resulting from the preparation formula that had been made.

pH measurement

pH measurements were carried out on cream preparations that had been made before and after storage conditions in the climatic chamber for 10 cycles. Measurements were made using a pH meter. The pH measurement is done by immersing the electrode in the cream preparation.

Viscosity Measurement

Viscosity measurements were carried out on cream preparations that had been made before and after storage conditions in the climatic chamber for 10 cycles. Viscosity measurements were carried out using a Brookfield viscometer.

Cream Type Examination

Dilution Method

The cream that has been made is put into a vial, then diluted with water. If the emulsion can be diluted then the emulsion type is m/a.

Antibacterial Testing

Sterilization Tool

The tools used in this antibacterial activity study are washed and immersed in a detergent solution for 15 minutes to 3 minutes. Then rinse with clean water. The tools are dried upside down in the open air. After drying, then wrapped in parchment paper. Non-scale glass utensils are sterilized by using an oven at 180 °C for 2 hours. Meanwhile, the sterilized glass tools were autoclaved at 121 °C at 2 atm pressure for 15 minutes.

Making Medium

All ingredients are weighed according to need then put into Erlenmeyer, then dissolved in distilled water to a volume of 100 ml, then check the pH of 7.0 then add the volume to 1000 ml distilled water, then sterilize in autoclave at 121 °C for 15 minutes.

Preparation of Test Bacteria

Microbial Rejuvenation Test

The test microbes used were divided into *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria. Each of the test microbes was taken one loop, then inoculated on a sloping Nutrient Agar (NA) medium, then incubated at room temperature 37 °C for 24 hours.

Preparation of Test Microbial Suspension

The rejuvenated test microbes were taken with a

sterile loop wire and then suspended into a tube containing 3 mL of 0.9% NaCl solution to obtain the same turbidity as the standard.

Antimicrobial Activity Test Procedure

Tools and materials are prepared. MHA medium (Mullen Hilton Agar) is sterile at a temperature of about 40 ºC – 50 ºC poured into 15 ml of Petri dishes aseptically and allowed to solidify, this is called the base layer. After that, 10 ml of MHA (Mullen Hilton Agar) medium was mixed with 1 ml of the bacterial suspension *Pseudomonas aeruginosa* in a dilution bottle then poured over the base layer and allowed to solidify (seed layer). Prepare 5 pieces of tamper for each concentration, then make a well on the media so that it has been compacted using a tip hole tool or a buffer. After that, the samples were inserted into the wells hole at each concentration, 3 anchoring pieces were dripped with each sample of 20 µL, 1 anchoring fruit containing gentamicin, and 1 other containing a cream base. Incubated at 37 ºC for 24 hours. Observed and measured the zone of inhibition. Do the same with the test microbe *Staphylococcus aureus* and other samples.

Results and Discussion

Extraction Process

A total of 500 g of dried Banyuru bark were extracted by maceration using 4 L of 70% ethanol extractor to obtain a dry extract of 46 g with a percentage of Andaman of 9.2% which is brown and has a distinctive smell of Banyuru's bark. The results of the observations can be seen in the following Table:

Cream Making

This research was conducted by formulating the Banyuru and beepollen extracts in a cream based on oil in water (M / A) with a phytocream emulator. The process of making the cream chosen is the melting method. The smelting method is a method of making preparations by melting all or a few of the components of the material, then cooling it with constant stirring (Ansel, 1989). The oil phases used are stearic acid and cetyl alcohol. The water-insoluble phase is melted in a water bath until melted. Water-soluble material, namely propylene glycol, is dissolved in warm distilled water.

Table 2. Results of Observation of Banyuru Stem Bark Ethanol Extract

Parameter	Results
Color	Brown
Smell	typical

Banyuru and beepollen extracts which have antibacterial properties are made in cream formulations based on several things, including having a good dispersion ability, easily wetted, not greasy, and gives a cool effect on the skin. The combination cream formulation of the Banyuru and beepollen extract uses a phytocream emulator which aims to reduce the interface tension of water and oil. The concentration used in the formulation is 5%, which is known to have the best level of stability. Phytocream is a natural surfactant, does not contain ethylene oxide which is safe, easy to formulate, and easy to handle and is used specifically for cosmetic ingredients.

Other additives used are methylparaben and propylparaben as a preservative, propylene glycol as a humectant, alpha-tocopherol as an antioxidant, and cetyl alcohol as an emollient. The use of preservatives is needed in cream preparations because water and oil content is high enough to be a good medium for the growth of microorganisms (Directorate General of Drug and Food Control, 1985).
Evaluation of the Physical Stability of the Formula

Organoleptic Testing

The purpose of the organoleptic test was to determine the physical appearance of the cream preparation including shape, color, odor, and homogeneity. Based on the organoleptic test results of the three formulas, the dosage form was semi-solid, brown by in accordance with the color of the extract and the resulting odor was a distinctive odor. The homogeneity test aims to see and determine the mixture of cream preparation ingredients. The results obtained were the absence of lumps which indicated that the cream preparation was homogeneous. This is because the Banyuru and beepollen extracts easily mix with the M / A base so that there is no clumping or phase separation.

From the organoleptic test series, there were no changes before and after accelerated storage in the three cream preparation formulas. This is presumably because the cream base is inert and the ingredients used are ingredients that dissolve in each of the water and oil phases and the manufacturing method is homogeneous so that it shows the same

organoleptic bo[7] before and after the storage is accelerated. The results of the observations can be seen in the following Table:

pH testing

pH testing is important to do to find out whether the cream formulated is suitable for use on the skin. The pH requirement for good preparation is that it must have a pH value that falls within the pH range of the skin, which is around 4.5-6.5. Based on the [25]erage measurement of cream preparations, the pH of the cream is good for the pH of the skin. There was no specific pH value because the researcher was constrained by the tool. The results of pH testing on the F1, F2, and F3 formulations were each obtained with a pH of 5 and there was no change before storage and after storage in each cream formula, so that the three formulas can be said to be safe for the skin. The results of pH testing can be seen in the following table:

Viscosity Testing

The viscosity test aims to determine the thickness of a cream preparation. The viscosity measurement of the cream formula was carried out before and after storage was accelerated using a visc[21]eter with spindle number 7 at a speed of 50 rpm. Based on the results of the viscosity test conducted, it showed that there was an increase in viscosity before and after storage was accelerated in the three combination creams of Banyuru and beepollen extracts. This occurs because changes in temperature alternately during the accelerated storage process can cause water evaporation from the preparation so that the viscosity of the cream increases. In general, creams become thinner at high temperatures and become thick when allowed to reach room temperature (Voight, 1994). From the test results, it is also seen that the concentration of the active substance affects the v[1]cosity value of the cream preparations. So that the higher the concentration of active substances added to the cream, the higher the viscosity value of the cream. So it can be concluded that the results of the viscosity measurement show that the three cream formulas with phytocream® emulgators that have the most stable viscosity change are the F1 formula. The results of the viscosity measurement before and after accelerated storage are as follows:

Emulsion Type Testing

Emulsion type testing aims to determine the type of emulsion on the preparation. The results of the emulsion type test with the dilution test before and after accelerated storage show that the cream formula can be diluted in aquadest. This is because the volume of the water phase (dispersing phase) is greater than the oil phase (dispersed phase), so that the oil droplets can be dispersed into the water and the water phase is more dominant which makes the cream diluted, therefore this process is said to be an emulsion of oil in w[1]ter (M/A). The results of the emulsion type test before and after the accelerated storage conditions of the three formulas showed that F1, F2, and F3 had the M/A emulsion type. The results of emulsion testi[1]g before and after accelerated storage conditions can be seen in the following table:

Table 4. Results of pH Formula Testing

Formula	pH Before Accelerated Storage	pH After Accelerated Storage
F1	5	5
F2	5	5
F3	5	5

Antimicrobial Testing

This test aims to determine the effect or efficacy of the test formulas (F1, F2, and F3) on test microbes which can inhibit and kill bacterial activity. The bacteria used in this study were *Staphylococcus aureus* and *Pseudomonas aeroginosa*. The reason these bac[26]teria are used is that these bacteria represent gram-positive and gram-negative bacteria and are normal

Table 3. Organoleptic Observation Results of Cream Formulas

Formula	Before accelerated storage			After accelerated storage		
	Color	Smell	Homogeneity	Color	Smell	Homogeneity
F1	Brown	Specific	Homogeneous	Brown	Specific	Homogeneous
F2	Brown	Specific	Homogeneous	Brown	Specific	Homogeneous
F3	Brown	Specific	Homogeneous	Brown	Specific	Homogeneous

Table 5. Cream viscosity test results

Formula	Viscosity (CPS)	
	Before Expedited Storage	After Expedited Storage
F1	19.200	21.866,66
F2	18.666,66	21.066,66
F3	17.733,33	19.400

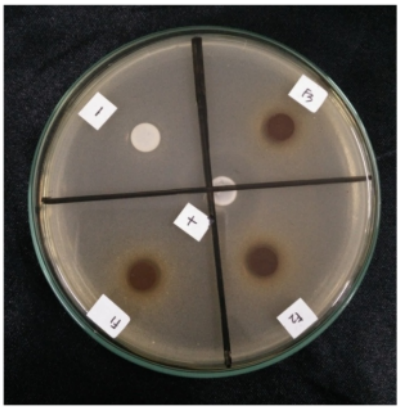
skin flora and in an imbalance of numbers, it can cause mild to severe infections in humans.

In this study, positive control was used in the form of gentamicin antibiotic cream and the average diameter of inhibition against *Staphylococcus aureus* was 22.73 mm and *Pseudomonas aeroginosa* was 19.52 mm. The negative control used was a cream base without extract, based on the test results obtained data that the negative control tested against *Pseudomonas aeroginosa* did not provide an inhibitory power on the test against *Staphylococcus aureus*, it was seen that there was an inhibitory power of 7 mm.

Based on the data from the test results of antibacterial activity from the combination cream formula of banyuru and beepollen extracts, it shows that there is an inhibitory power against *Staphylococcus aureus* and *Pseudomonas aeroginosa* bacteria with the largest average diameter, namely formula F1, and the lowest is formula F3. This is in line with research conducted by Marzuki, A (2015) where it is known that the ethanol extract of Banyuru stems has antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeroginosa* with an inhibitory diameter of 15.78 mm and 16.12 mm, respectively. After testing the combination cream of banyuru and beepollen extracts and compared with the literature, it can be concluded that the combination of banyuru and beepollen extracts has greater antibacterial activity than the banyuru extract without the combination.

Table 6. Observation Results of Emulsion Type Test

Formula	Before Expedited Storage	After Expedited Storage
F1	M/A	M/A
F2	M/A	M/A
F3	M/A	M/A



Gambar 1. Hasil uji daya hambat sediaan krim ekstrak banyuru dan beepollen terhadap *Pseudomonas aeroginosa*

Conclusion

From the results of the study, it can be concluded that the combination cream of Banyuru extract (*Pterospermum celebicum* Miq) and beepollen with various concentrations of F1 (3%: 3%), F2 (3%: 2%) and F3 (3%: 1%) were formulated using phytocream. 5% stable based on the organoleptic test, emulsion type, viscosity, and pH by Freeze-thaw method. The results showed that the zone of inhibition at F1, F2, and F3 against *Staphylococcus aureus* was 19.60 mm, 18.44 mm, and 17.1 mm, while those in *Pseudomonas aeroginosa* were 18.90 mm, 17.91 mm and 16.15. mm. The combination cream of banyuru and beepollen extracts have anti-

Table 7. The results of the inhibition test for the combination of banyuru and beepollen extracts

Formula	Treatment	Average Inhibition Zone Diameter(mm)	
		S.aureus	*P.aeroginosa*
F1	Banyuru and beepollen extract (3:3) %	19,60	18,90
F2	Banyuru and beepollen extract (3:2) %	18,44	17,91
F3	Banyuru and beepollen extract (3:1)%	17,1	16,15
Control (+)	Creamgentamisin	22,73	19,52
Control (-)	Base without extract	7,3	-

microbial activity with optimum inhibition against *Staphylococcus aureus* and *Pseudomonas aeroginosa* is F1 (3%: 3%) with inhibition zone diameters of 19.60 mm and 18.90 mm, respectively.

It is necessary to research on clinical trials and irritation tests for the combination of Banyuru extract (*Pterospermum celebicum* Miq) and beepollen.

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