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The influence concentration gel agent propolis combination of mulberry leaf on the growth of the bacteria *Propionibacterium acnes*

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Abstract: This research was carried out with the aim of determining the inhibitory power of the combination gel of propolis extract and mulberry leaf extract against *Propionibacterium acnes* bacteria and determining the concentration of propolis extract that provided the greatest inhibitory power against the test bacteria. The research method includes the extraction of mulberry leaves by maceration using 70% ethanol as a solvent and the propolis extract used is a ready-to-use extract. The formulations were designed with various concentrations of propolis (0.5%, 1%, 1.5%) and 0.5% mulberry leaf concentration. Testing the antimicrobial activity of propolis extract in combination with mulberry extract in gel preparations against the growth of *Propionibacterium acnes* bacteria was carried out using the agar diffusion method. The results showed that the average diameter of the inhibition area at 0.5% concentration was 36.87 mm, at 1% concentration was 44.557 mm and at 1.5% concentration was 45.97 mm, positive control was 49.21 mm. Based on the data obtained, it is concluded that the gel formula with a propolis concentration of 1.5% is the preparation with the largest zone of inhibition.

Keywords: Inhibition test, gel, propolis extract, mulberry leaf extract.

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Introduction

Acne is an inflammation of the skin characterized by the presence of closed comedones (white heads), open comedones (black heads), nodules (papules or nodules) or purulent nodules (pustules or cysts) on the surface of the skin that are reddish and fatty, called seborrhea. In general, acne problems are experienced by more than 80% of the population aged 12-44 years (Florentinus, 2014).

According to other studies that have been conducted, propolis extract with a concentration of 1% is effective in inhibiting the growth of Propionibacterium acne as one of the causes of acne (Fauziah, 2013).

Based on Thomas' research, acne can interfere with quality of life because it can cause disturbances in self-image and cause anxiety. Acne prone skin usually has scars or final residue in the form of black spots that can interfere with appearance, so a compound is needed to overcome this (Florentinus, 2014).

Mulberry (Morus alba folium) is one of the plants that can be used to reduce spots (acne scars), which has been proven by research by Hamzah and Sang Hee Lee, namely the antioxidant effect and inhibition of tyrosinase activity which converts dopa into dopachrome in the process of melanin biosynthesis in mulberry leaves. (Morus alba folium) with a concentration of 0.5% (Hamzah, 2012 and Lee, 2002).

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So that it can be formulated Propolis (*Trigona sp.*) gel extract combined with mulberry leaf extract in gel dosage form as a pharmaceutical preparation that can inhibit bacterial growth and then as an antioxidant and melanin biosynthesis that can inhibit spots on acne scars.

This is the reason why it is necessary to conduct research on the inhibition of the preparation of the combination of propolis gel extract (*Trigona sp.*) and mulberry leaf extract against Propionibacterium acnes bacteria.

The formulation of the problem this study are:

1. Is there any effect of gel preparation of a combination of propolis extract and mulberry leaf extract on the inhibition of *Propionibacterium acnes* bacteria?
2. Does variation in propolis concentration have an inhibitory effect on Propionibacterium acnes bacteria?

The purpose of this research is to make a combination gel of propolis extract and mulberry leaf extract with various concentrations of propolis extract

The aims of this research are:

1. Determine the effect of the combination gel of propolis extract and mulberry leaf extract on Propionibacterium acnes bacteria.
2. Determine the effect of the concentration of propolis extract in the gel preparation that provides the greatest inhibitory power to the test bacteria.

The benefits of this research are:

As a source of reference and scientific data for further research, other researchers and students about the most effective variation of the concentration of propolis extract from propolis extract gel preparations combined with mulberry leaf extract to inhibit the growth of Propionibacterium acnes bacteria.

Materials and Methods

a. Tools and materials used

The tools used are, petri dish, porcelain dish, beaker, measuring cup, incubator, caliper, electric stove, Erlenmeyer flask, Laminar Air Flow (LAF), spirit lamp, measuring flask, autoclave, oven, water bath, pH meter, scale pipette, dropper, test tube, thermometer, analytical balance. The ingredients used are 70% ethanol, distilled water, mulberry leaf, propolis, Hydroxyethyl cellulose (HEC), carbopol, nipagin, propylene glycol, triethanolamine, Propionibacterium acnes, disc blank.

b. Equipment sterilization

Tools made of glass were sterilized using an oven at 180^0C for 2 hours. Plastic utensils that are not resistant to high heating and scaled glass utensils are sterilized in an autoclave at 121^0C for 10-15 minutes. Tools in the form of ose, tweezers are sterilized by incandescent over a direct fire just before use.

c. Sampling and Processing

1. Sampling

The propolis sample used was a ready-to-use propolis extract obtained from the beekeeping at Hasanuddin University, South Sulawesi, the extract was obtained from the extraction using the maceration method with 70% ethanol as a solvent.

Mulberry leaf samples were taken in Daya Makassar, South Sulawesi. Sampling was carried out at 8 am by picking.

2. Sample processing

The mulberry leaves are washed with water and then drained, cut into small pieces, dried by aerating in a place that is not exposed to direct sunlight for one day. After that the sample was extracted by maceration method.

3. Mulberry Leaf Extract

A total of 500 grams of mulberry leaf simplicia was put into a maceration vessel with 2000 ml of 70% ethanol added, the sample was stirred. After 2 days the filter fluid was replaced with 2000 ml of new 70% ethanol. Replacement of the filter fluid was carried out 3 times. The 70% ethanol extract obtained was then collected and the liquid was evaporated using a rotary evaporator and put into a desiccator to obtain a thick extract.

Formulation gel

Table 1. Gel formula design

Ingredients	Function	Concentration of ingredients in formula (%)				
		FI	FII	FIII	K(-)	K(+)
Propolis extract	Active substance	0,5	1	1,5	-	Gel Agent Acnes
Mulberry extract	Active substance	0,5	0,5	0,5	-	
HEC	Gel base	0,5	0,5	0,5	0,5	
Methyl paraben	Preservative	0,2	0,2	0,2	0,2	
Propylene glycol	Cosolvent	10	10	10	10	
Glycerin	Humectants	10	10	10	10	
Aquadest ad	Solvent	100	100	100	100	

d. Gel making procedure

The method of making HEC (hydroxyethyl cellulose) base gel is as follows:

The gel was made by dissolving methyl paraben with distilled water while heated to a temperature of 700C,

then added (hydroxyethyl cellulose) HEC was stirred until it swelled to form a gel (mixer method). The ethanolic extract of propolis and ethanolic extract of mulberry leaves dispersed with propylene glycol and glycerin in a mortar was then added to the gel base that had been formed, stirring until homogeneous.

e. Medium Setup

The FTM medium was weighed 3 grams, then dissolved with 100 mL distilled water heated until pink, added so that 1.5 grams was then sterilized in an autoclave at 1210C, pressure 1 atm for 30 minutes.

f. Preparation of Test Bacteria

The test bacteria used were Propionibacterium acne bacteria obtained from the stock of the Pharmacy Microbiology Laboratory of the Indonesian Muslim University Makassar.

1. Pure Culture Rejuvenation of Propionibacterium acne

Pure culture of Propionibacterium acne, taken 1 ose and inoculated aseptically by streaking on agar slanted from FTM medium, then incubated anaerobically at 37°C for 24 hours.

2. Preparation of Pure Suspension of Propionibacterium acne

The rejuvenating test bacteria were suspended in a physiological solution (0.9% Nacl) until a transmittance of 25%T was obtained on the spectrophotometer.

g. Test Bacteria Prosedure

Suspension of Propionibacterium acnes, 20µL pipetted and put into a sterile petri dish, shaken, added 15 ml of FTM agar medium, allowed to stand until half solid.

h. Resistance Test

Testing the antimicrobial activity of proplis extract in combination with mulberry leaf extract in gel preparations against the growth of Propionibacterium acnes bacteria was carried out using the agar diffusion method. The three gels that have been formulated, negative control (gel base), and positive control (wardah gel preparation), are put into the well, allowed to stand for about 15 minutes, then the distance between the wells from the edge of the petri dish is about 2-3 cm.

i. Observation of Antimicrobial activity

Observations were made after an incubation period of 24 hours. The diameter of the barrier in the form of a clear zone was measured using a caliper.

j. Data Processing

The data obtained from the resistance measurements were tabulated and then averaged and processed using the ANOVA statistical method.

Result and Discussion

Research has been carried out on the inhibition of propolis extract in gel preparations against the inhibition of the growth of Propionibacterium acnes bacteria after an incubation period of 24 hours, the results obtained (can be seen below this table)

Table 2. Result of Inhibition Diameter of propolis gel combination of mulberry leaves

Replications	Diameter of Barriers with Concentration of Propolis Extract (mm)				
	F1	F2	F3	K(+)	K(-)
I	35,1	43,35	35,1	0	0
II	34,91	42,97	52,74	67,67	0
III	40,6	47,35	50,07	79,96	0
Tot	110,61	133,67	137,91	253,21	0
Average	36,87	44,557	45,97	84,403	0

Discussion

Propionibacterium acnes is a bacterium that usually lives on normal skin. These bacteria participate in the pathogenesis of acne by producing lipase, which breaks free fatty acids from skin lipids. These fatty acids can cause tissue inflammation and contribute to acne. (Jawetz Ernest, 1996)

Propolis is known from the 7th to the 20th centuries as an anti-bacterial, anti-fungal, anti-viral and anti-inflammatory. Several studies have stated that the pharmacologically active molecules in propolis are galangin compounds, caffeic acid, pinocembrin, and ferulic acid which have antibacterial properties. (Susilo B., 2009)

Research conducted by Dicky (2012) proves that propolis is a natural ingredient that is proven to be the most effective against Propionibacterium acnes bacteria, with a concentration of 0.1% it can inhibit 5.53 mm. The purpose of this study was to make a gel combination of propolis extract and mulberry leaf extract with various concentrations of propolis extract.

The aim was to determine the inhibitory power of the combination gel of propolis extract and mulberry leaf extract against Propionibacterium acnes bacteria and determine the concentration of propolis extract in the gel preparation that provided the greatest inhibitory power to the test bacteria.

Preparation of the preparation is done by first extracting the raw material, namely mulberry leaves. This process is carried out with the aim of attracting the components of compounds present in plants. The extraction method used is the maceration method, because seeing the simplicia to be extracted is the leaf part of the plant which has a soft texture and is not resistant to heating. Apart from that, the maceration method is an extraction method that is easy and economical to work on.

The method used in the process of testing this inhibitory power is the agar diffusion method with the aim of knowing the diameter of the inhibition area formed after an incubation period of 1x24 hours, namely the test method where the sample will diffuse from the reservoir to the agar medium. In this study, a buffer was used to facilitate the gel diffusion into the agar medium due to the consistency of the gel being semi-solid.

The results showed that at a concentration of 0.5% propolis extract in gel preparations could inhibit the growth of Propionibacterium acnes bacteria with an average diameter of inhibition area of 36.87 mm, at a concentration of 1% of 44.557 mm and at a concentration of 1.5% of 45. .97mm. Meanwhile, the area of inhibition in the positive control (Wardah gel preparation) was 49.21 mm and the negative control area had no obstacles. Then continued with the method. Statistically using the One Way ANOVA (Analysis of Variance) method obtained significant results at all concentrations.

The results of the ANOVA test can be seen in the significant number, if the significant value is > 0.05 then H0 is accepted while if H0 <0.05 then H0 is rejected. Because the significance value is < 0.05, then H0 is rejected and H1 is accepted. H0 there is no significant difference in the diameter of the inhibitory power due to differences in concentration. While in H1 there was a significant difference in the diameter of the inhibition due to the difference in gel concentration. From the data obtained that H1 was accepted, a further Tukey test was carried out and from the results of Tukey's follow-up for all gel concentrations there was a significant diameter value of resistance by looking at the asterisk in the Mean Difference column. From these data, it can be seen that differences in the concentration of gel preparations can affect the diameter of the resistance. So that the most significant difference in the concentration of gel preparations is the diameter of the inhibitory power, namely the gel preparation concentration of 1.5%.

The size of the inhibition area formed due to differences in the concentration of propolis extract contained in each preparation. This is due to the presence of compounds or active substances contained in propolis gel which are antibacterial.

The increase in concentration is generally followed by an increase in the diameter of the barrier as mentioned by Pelczar and Chan (1998), that the higher the concentration of antimicrobial substances used, the higher their ability to control microorganisms.

Conclusion

Based on the results of the research data, it can be concluded that:

Preparation of Propolis Extract Gel The combination of Mulberry leaves is very influential in inhibiting Propionibacterium acnes bacteria

The effect of variations in the concentration of propolis extract in acne gel preparations which have the largest diameter inhibition inhibiting the growth of Propionibacterium acnes in the anti-acne gel formula propolis extract combination of mulberry leaves is the extract concentration of 1.5%

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