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FORMULATION OF DRAWINGS (DRYMOGLOSSUM PILOSELLOIDES L. PRESL) ETHANOL EXTRACT OF ACNE GEL ON THE GROWTH OF THE BACTERIA PROPIONIBACTERIUM ACNES

Zola Efa Harnis[1], Nina Irmayanti Harahap[2], Rika Puspita Sari[3] , Sarviana Lubis[4]
[1 2 3] Fakultas Farmasi, Institut Kesehatan Delihusada, Delitua, Indonesia

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ABSTRACT

Dragon scales (Drymoglossum piloselloides L. Presl) is a plant that has antibacterial activity against the bacteria Propionibacterium acnes and Staphylococus aureus, Bacillus subtilis, Escherichia coli, and Streptococcus pneumonia. The content of compounds that act as antibacterial are saponins, polyphenols, essential oils, sterols / triterpenes, phenols, flavanoids, sugars and tannins. This study aims to make a formula of dragon scales ethanol extract gel against the growth of propionibacterium acnes bacteria. The method used in this study was experimental laboratory. The formulation of dragon scales ethanol extract gel is made with a concentration variation of 5%, 10%, 15%. Antibacterial activity test is carried out with diffusion agar.

Email :
zolaharnis19@gmail.com
hrpnina19@gmail.com
rikapuspitatambunan@gmail.com

1. INTRODUCTION

Acne is an inflammatory disease that can occur on the skin of the face, neck, chest and back. This disease is caused by overactivity of the oil glands and is exacerbated by infection with the bacteria *Propionibacterium acnes* [1]. Acne or acne vulgaris arises due to inflammation of the pilosebaceous follicles which is characterized by the appearance of comedones, pustules, and nodules on the face, shoulders, chest and upper back, and upper arms [2]. Acne treatment is done by correcting follicular abnormalities, reducing sebum production, reducing the number of Propionibacterium acne colonies or their metabolic products and reducing inflammation in the skin [3].

Terdapat berbagai macam faktor yang bisa menjadi etiologi timbulnya jerawat, diantaranya There are various factors that can be the etiology of acne, including heredity or genes, race, psychological, hormonal, or more commonly a bacterial infection [4]. *Propionibacterium acne* is a bacterium that has an important role in the pathogenesis of acne vulgaris by producing lipases that break down free fatty acids from skin lipids. These fatty acids can cause tissue inflammation when linked to the immune system and promote acne vulgari. Acne vulgaris is an inflammation that leads to the ducts of the skin's oil glands [5], [6].

Dragon scale leaves are widely used, especially as antibacterial and anti-herbal medicine. Dragon scales is an epiphytic plant with round to oblong leaves and is widely found in Indonesia, especially in humid areas. This plant is commonly used by the community to treat mumps, dysentery, rheumatism and vaginal discharge. Dragon scale leaf alcohol extract contains terpenoid compounds, phenols, flavonoids, tannins and sugars. Terpenoid compounds, phenols and tannins contained in dragon scales

are thought to have an antibacterial effect[7]. Several derivatives of flavonoid compounds, sterols/triterpenes and tannins in plants can have antibacterial activity. The presence of antibacterial activity is caused by the -OH group contained in the chemical structure formula of each compound which can cause denaturation of bacterial cell proteins [8].

2. METHOD

Tools and Matrials

The tools used in the research are autoclaves, incubators, refrigerators, pH meters, rotary evaporators, colony counters, blenders, maceration tools, water content determination tools, drying cabinets, ose needles, bunsen, hot plates, dropper pipettes, aluminum foil, parchment paper, tissue, petri dish, sterile cotton, watch glass, caliper, mortar, disc paper, jar, tweezers, vortex, water bath, vial, spirit lamp, filter paper, kiln, stamper, viscometer, analytical balance, sieve mesh no . 200, whatman filter paper no. 42, funnel, stirring rod, measuring cup, erlenmeyer, mixer, degree object, spatel, gel pot, label, tweezers, test tube, test tube rack, stirrer, laminar air flow, centrifugator, micro pipette, metal screw, scale ruler.

The ingredients used in the research were 96% ethanol, the ingredients for the gel formula consisted of dragon scales extract (Drymoglossum piloselloides L. Presl), HPMC (Hydroxypropyl methylcellulose), 96% ethanol, propylene glycol, glycerin, TEA, aquadest, acnes sealing gel, H2SO4 0.36 N, BaCl2.2H2O 1.175%, NaCl 0.9%, Media Nutrient Agar and Propionibacterium acnes bacteria, NaCL 0.9%, the chemicals used are of high quality, as a comparison, namely Clindamicin Gel.

Extract Making

Dragon scales are washed and then drained. Then dried in a drying cabinet at a temperature of 40-50°C. The dry sample was made into powder. As much as 1 kg of simplicia powder was macerated by adding 7.5 liters of 96% ethanol then the container was closed and left for 5 days protected from sunlight and occasionally stirred, then filtered and pressed with flannel, then the pulp was macerated again with 2.5 liters of ethanol 96 % and then left in a cool place and protected from light for 2 days and then filtered. The macerate is heated with the help of a rotary evaporator at a temperature of not more than 70°C and then heated over a water bath until a thick extract is obtained [9].

Identification of Alkaloids

A total of 2 g of simplicia powder was weighed, added 1 ml of 2 N hydrochloric acid and 9 ml of distilled water, heated on a water bath for 2 minutes, cooled and filtered. The filtrate is used for the alkaloid test. Three test tubes were taken, then 0.5 ml of the filtrate was added to each test tube. On the tube:

a. Added 2 drops of Bouchardat reagent
b. Added 2 drops of Dragendorff's reagent
c. Added 2 drops of Meyer's reagent

Alkaloids are said to be positive if a precipitate occurs.

Identification of Saponins

A total of 0.5 g of the sample was put into a test tube and added 10 ml of hot distilled water, cooled and then shaken vigorously for 10 seconds, a steady foam formed no less than 10 minutes as high as 1-10 cm. Added 1 drop of 2N hydrochloric acid solution, if the foam does not disappear, it indicates the presence of saponins [10].

Identification of Flavonoids

A total of 10 g of simplicia powder was then added with 100 ml of hot water, boiled for 5 minutes and filtered in hot conditions, the filtrate obtained was then taken 5 ml and then added 0.1 g of Mg powder and 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol, shaken and allowed to separate. Positive flavonoids if there is a red, yellow, orange color in the amyl alcohol layer [11][12].

Identification of Tannins

A total of 0.5 g of the sample was macerated with 10 ml of distilled water, filtered and the filtrate was diluted with distilled water until it was colorless. Take 2 ml of the solution and add 1 to 2 drops of iron (III) chloride reagent [13].

Steroid Identification

As much as 0.5 g of sample was weighed, added n-hexane, then macerated for 2 hours, filtered, the filtrate was heated in an evaporating dish, in the remainder added acetic anhydride and concentrated sulfuric acid. If a blue green color appears, it indicates the presence of steroids [14].

Gel Preparation Antibacterial Activity Test

Test the activity of the dragon scales ethanol extract gel preparation with the disc diffusion method consisting of 3 concentrations, namely 5%, 10% and 15%. 15 ml of Nutrient media was poured, then the bacteria that had been diluted according to Mc Farland's turbidity was taken 1 ml and spread over a cup petri evenly. Furthermore, the paper backing that had been soaked in formula 0, formula 1, formula 2, formula 3 and positive control (Clindamicin gel) was placed on the solid surface of the media, then incubated in an incubator at 37°C for 18–24 hours, after that The diameter of the inhibition zone (clear zone) growth around the reservoir was measured using a caliper.

Table 1. Dragon scales Ethanol Extract Gel Formulation

Material	F0 (%)	F1 (%)	F2 (%)	F3 (%)	Control Positif
Ethanol extract dragon scales	-	5	10	15	Gel Klindamicin
HPMC (g)	1,5	1,5	1,5	1,5	
Gliserin (ml)	20	20	20	20	
Propilen glikol (g)	12	12	12	12	
TEA (ml)	2	2	2	2	
Aquadest ad (ml)	100	100	100	100	

Evaluation of Gel Preparations

Organoleptic Observations

Organoleptic observations were seen directly on the shape, color, and smell of the dragon scales ethanol extract gel preparation. This test is carried out to improve the quality of a preparation.

pH measurement

Examination of pH using a pH meter on gel preparations must be in accordance with the pH of the skin. The pH of the gel preparation must be in accordance with the pH of the skin, namely 4.5-6.5

Homogeneity Test

The homogeneity test using a gel was weighed as much as 0.1 g and smeared on an object glass, homogeneity was demonstrated in the absence of granules [15].

Viscosity Measurement

Viscosity test is carried out by placing 100 mL of gel into a tubular container and then installing a spindle 64. The spindle must be submerged in the test preparation. The viscometer is turned on and it is ensured that the rotor can rotate at a speed of 60 rpm. Observed the pointer of the viscometer that points to the number on the viscosity scale and then recorded and multiplied by a factor of 100.13

Spreadability Test

A total of 0.5 grams of the gel sample was placed on a round glass, the other glass was placed on it and left for 1 minute. The diameter of the gel spread was measured. After that, 100 grams of additional load were added and allowed to stand for 1 minute and then a constant diameter was measured [16].

Adhesion Test

The ethanol extract gel of dragon scales leaves is placed on a glass object that has a predetermined area, another glass object is placed on top of the gel, a 500 g load is placed for 5 minutes, the glass

object is mounted on an adhesive test instrument, then releases the 80 gram load and records the time required. glass objects to separate from each other [17].

3. RESULTS AND DISCUSSION

Phytochemical Screening

Based on the phytochemical screening that has been carried out, positive results were obtained for flavonoid compounds, saponins, and tannins.

Gel Preparation Formula

Prepare all the materials used. The ingredients are weighed according to the above formulation. Hydoxypropyl methylcellulose (HPMC) as much as 1 gram, was developed in a porcelain dish with a little hot aquadest, after expanding, was stirred continuously so that it was completely dispersed. Next, add 10 ml of propylene glycol and 10 ml of glycerin and stir again until homogeneous using a mixer. Then add 1 ml of TEA and stir again until evenly mixed, after that add the extract with a concentration of 5% then add 10 ml of distilled water and stir again until everything is completely homogeneous. For the manufacture of gels with concentrations of 10% and 15% carried out in the same way. After that, the three gel formulations were stored at room temperature overnight at 100C-150C.

Preparation Evaluation

Organoleptic Observations

The results of organoleptic observations of gel formulas have different characteristics. The results of the organoleptic test can be seen in Table 3

Table 3. Results of Organoleptic Test of Dragon Scales Gel Preparation

Organoleptic examination	F0(Basic)	F1(5%)	F2 (10%)	F3(15%)
Form	semi solid	semi solid	semi solid	semi solid
Flavour	No special smell	Special	Special	Special
Colours	White	Light green	Blackish Green	Blackish Green

pH test

The test results on the pH of the gel preparations obtained indicate that the gel preparations produced are in accordance with the pH of the skin and can be used and do not cause irritation to the skin, because the pH of the gel preparations must be in accordance with the pH of the skin, namely 4.5-6**).**

Table 4. Results of pH measurements

Formulation	Observation timen	
	Week 1	Week 2
Control -	5,30	5,82
Formulation 1	4,97	5,92
Formulation 2	6,13	6,25
Formulation 3	5,09	5,4

Spreadability Test

The results of the evaluation of the spreadability of gel preparations carried out on the four formulas (table 5) met the predetermined requirements, namely, 5-7 cm

Table 5. Spreadability Test Results

Preparation Formula	Spreadabillity (cm)
F0	5

F1	5,3
F2	5,3
F3	5,7

Viscosity Test

Viscosity testing using a Brookfield Viscometer using a spindle 65 at a speed of 2.5 RPM**.**

Table 6. Viscosity value of dragon scales gel preparation

Formulation	Viscosity (cPoise)
F1	3180
F2	3090
F3	1692

Based on the results obtained, the higher the concentration of dragon scales extract, the lower the viscosity value.

Testing the Inhibitory Power Width (LDH) of Gel Preparations

The Inhibitory Width Test (LDH) was carried out to compare with the existing preparations on the market. Testing of antibacterial activity using the disc method. The results of the antibacterial activity test of the gel preparation have antibacterial activity against the bacteria Propionibacterium acne. This can be seen in the results of the Inhibitory Width (LDH) measurement that is formed, namely the formation of a clear zone that can be seen around the paper disc. So dragon scales have antibacterial activity that is not significantly different from gels on the market. The results of the LDH test can be seen in Figure 1.

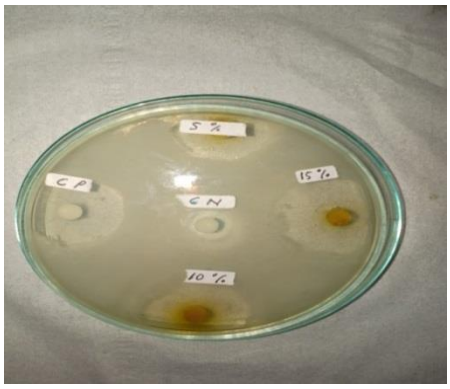

Figure 1. LDH Sediaan *Gel*

F1 = Formulation 1 (5%); F2 = Formulation 2 (10%); F3 = Formulation 3 (15%); CN = Control Negatif (HPMC); CP = Comparison Control (*gel Klindamicin*)

It can be seen that each preparation has antibacterial activity by inhibiting the growth of Propionibacterium acne bacteria. Formulas 1 and 2 have small inhibitory activity because the higher the concentration of longan leaves used, the greater the width of the inhibition zone formed. Meanwhile, the gel base has a narrow zone of inhibition. The formation of an inhibitory zone around the paper disc is thought to be because the dragon scales gel contains flavonoids, tannins, saponins, and steroids.

Tannin compounds can break down cell wall polypeptides causing bacterial damage, saponin compounds can dissolve lipids or are lipophilic, resulting in the destruction of bacteria. Flavonoid compounds can inhibit nucleic acid synthesis, further disrupting the function of the cytoplasmic membrane and energy metabolism of bacteria. steroids work by damaging the lipid membrane, so that liposomes leak. 12

The value of the Inhibitory Width obtained was then analyzed using the ANOVA method if there were differences, the Tukey further test was carried out. Based on the results of the inhibition of Propionibacterium acne gave significantly different results.

Table 7. Results of Inhibitory Zone Diameter

Perlakuan	I	II	III	Rata-rata
5 %	16,75	18,25	20,5	18,5
10 %	22,5	24,4	26,6	24,5
15 %	28,9	30,1	32,57	30,52
Kontrol (+)	28,26	23,95	29,1	27,10
Kontrol (-)	0	0	0	0

Oneway ANOVA results of the diameter of the inhibition zone of the bacteria Propionibacterium acnes (appendix 22), showing a significant value of 0.000 (sig <0.05) so that H0 is rejected and H1 is accepted, it shows that there is a significant difference between variations in the concentration of dragon scales ethanol extract gel preparations against Propionibacterium bacteria. acne.

The results of the Tukeys B test on the inhibition zone produced by Propionibacterium acne (appendix 22) showed that the concentration of 5% was not significantly different from the concentration of 10%. This means that 5% and 10% concentrations of dragon scales ethanol extract gel preparations have the same inhibitory effect on Propionibacterium acne bacteria. Concentration of 5%, and 10% were significantly different from the concentration of 15%. The results of the analysis showed that variations in concentration affected the inhibition of the growth of Propionibacterium acne bacteria, where an increase in concentration indicated an inhibitory activity of the growth of Propionibacterium acne bacteria.

4. CONCLUSION

Ethanol extract of dragon scales can be formulated into a gel preparation. Concentrations that can inhibit the growth of Propionibacterium Acnes are all concentrations, but the concentration of 15% maximum inhibition zone diameter is 30.52 mm (strong category). All concentrations had good physical stability. However, gel preparations of dragon scales ethanol extract with concentrations of 5%, 10%, and 15% gave an anti-acne effect against Propionibacterium Acnes. Dragon scales ethanol extract gel with a concentration of 15% greater inhibitory power than the concentrations of 5% and 10%, where the higher the concentration the greater the inhibitory power produced. The gel preparation in the market has an inhibitory power of 27.10 mm, compared to the gel preparation of dragon scales ethanol extract which has a strong inhibitory power of 30.52 mm.

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