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Antibacterial Activity Test of Ashitaba Leaf Extract Ointment Formulation (*Angelica Keiskei* (miq) *Koidz*) against *Staphylococcus epidermidis* Bacteria

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ABSTRACT

Acne is a condition in which the pores of the skin are blocked, causing inflamed pus pockets. One of the factors causing acne is bacteria. Staphylococcus epidermidis bacteria are bacteria found in acne. The use of medicinal plants is an alternative as a treatment for diseases including diseases caused by bacterial infections. Ashitaba leaves (*Angelica Keiskei* (miq) *Koidz*) positively contain alkaloids, saponins, tannins and flavonoids which play a role as antibacterials. The purpose of this study was to determine the antibacterial activity of the formulation of ashitaba leaf extract ointment preparations against *Staphylococcus epidermidis* bacteria that cause acne, using the well diffusion test method. Observation of the inhibitory power was tested on ashitaba leaf extract and ashitaba leaf extract ointment preparations with extract concentrations of Formula I 10%, Formula II 15%, Formula 20%. The results of this study are that the formulation of ashitaba leaf extract ointment preparations can have inhibitory power against *Staphylococcus epidermidis* bacteria. The highest inhibition zone in Formula III with an extract concentration of 20% with an inhibition zone diameter value of 7.27 mm.

Keywords : Acne, Ashitaba leaves, *Staphylococcus epidermidis* bacteria

INTRODUCTION

The skin is an organ that covers the entire human body and has a function to protect against external influences by substances found in the environment, including microorganisms that grow and live in the environment. Naturally, the skin has tried to protect itself from attacks by microorganisms with a layer of fat on the skin obtained from the sebaceous glands and a few sweat glands from the skin and the presence of an outer skin layer that functions as a skin barrier. However, in certain conditions, these natural protection factors are insufficient and often due to bacteria attached to the skin causing skin infections, wounds, boils and acne inflammation infections accompanied by pain occur in the abscess formation process so that an action is needed to remove the fluid and limit the growth and spread of bacteria (Radji, 2011).

Acne is a condition in which the pores of the skin are blocked, causing inflamed pus pockets. The inflammation that occurs in acne is triggered by the bacteria Propionibacterium acnes, Staphylococcus epidermidis and Staphylococcus aureus. These bacteria are normal flora on the skin, but can be invasive. Acne caused by these bacteria has different effects, in *Staphylococcus epidermidis* bacteria develop in the sebaceous glands and become blocked, will produce substances that will cause irritation to the surrounding area then will swell, burst and then spread inflammation to the skin tissue (Kursia et al., 2016). Therefore, a drug is needed to cure bacterial infections in acne. Acne treatment can use pharmacological therapy with the use of antibiotics such as tetracycline, erythromycin, doxycycline, and clindamycin. This therapy has side effects of irritation, resistance, organ damage and immunohypersensitivity (Putri et al., 2019).

Excessive use of antibiotics and over a long period of time will cause resistance of microorganisms and provide side effects that are harmful to human health. Therefore, an alternative is needed to reduce the use of antibiotics by utilizing natural sources found in plants. The use of plant extracts that have antibacterial activity is very helpful in curing infections caused by bacteria (Sheikh

et al., 2012). One of the plants that has antibacterial properties is the ashitaba plant. One part of the ashitaba plant that can be used as a treatment is the leaves.

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METHODS

Research Tools and Materials

The tools used are analytical scales (SF 400C), scales and weights, grinders, blenders, Erlenmeyer flasks (Pyrex), measuring cups (Pyrex), test tubes (Pyrex), watch glasses, porcelain cups, weighing bottles, mortars and stampers, glass objects, mouster balances, pH paper, evaporators, water bars (Memmert), viscometers (Rion VT-6), stirring rods, spatulas, petri dishes, pipettes, beaker glasses (Pyrex), aluminum foil, tweezers, volume pipettes, Materials used Ashitaba leaf extract (Pharmacy Laboratory of the Strada Indonesia Kediri Health Sciences Institute), Propylene glycol or PEG 400 (Bratacho), PEG 4000 (Bratacho), Nipagin (Bratachem), Nutrient Agar (Oxoid), Staphylococcus epidermidis bacteria, Mg powder, HCl, ethanol 96% (Merck), FeCl3 1%, Mayer's reagent, Dragendorff's reagent, Wagner's reagent, 0.9% NaCl (Sigma), Ashitaba leaf extract ointment preparation, gentamicin ointment (Medipharma).

1. Making Ashitaba Leaf Extract
Weigh 400 g of ashitaba leaf powder. The fine powder of ashitaba leaves is put in a brown bottle then added 3000 ml of 96% ethanol covered with aluminum foil, macerated for 3 days, then filtered using a Boucher funnel, separate the filtrate and residue. 2000 ml of ethanol is added to the residue, left for 2 days then filtered. The filtrate is then combined into one, the filtrate obtained is evaporated using a rotary evaporator at a temperature of 400C, continued using an oven at a temperature of 500C until a thick extract is formed for 5 days (Susanti, 2012). The thick extract obtained is then weighed and the percent yield is calculated.

Table 1. Ashitaba leaf extract ointment formulation table

Material	Formula I	Formula II	Formula III
Ashitaba leaf extract	10%	15%	20%
PEG 400	30%	50%	65%
PEG 4000	60%	35%	15%
Nipagin	0,15%	0,15%	0,15%

2. Making Ashitaba Leaf Extract Ointment
Prepare the tools and materials, weigh the ashitaba leaf extract according to the concentration, PEG 400, PEG 4000 and nipagin 0.3 grams. PEG 4000 is put into a porcelain cup then melted over a water bath. The melted base is stirred until homogeneous in a mortar. PEG 400 is added, then stirred until a thick and homogeneous mass is formed

and nipagin is added then stirred until homogeneous. Ashitaba leaf extract is added little by little, then stirred until homogeneous and forms an ointment mass. Put into an ointment pot and evaluated (Zulfa et al., 2015).

3. Evaluation of Ashitaba Leaf Extract Ointment Preparation

a. Organoleptic test Organoleptic testing is carried out by observing the ointment preparation from the shape, smell, and color of the preparation (Lasut et al., 2019)
b. Homogeneity test Applying ointment to a piece of glass, then visually observing the parts that are not mixed well in the ointment (Isnaeni and Suherman, 2019). The requirements for

a homogeneous ointment are characterized by the absence of lumps in the application results, a flat structure and having a uniform color from the starting point of application to the end point of application (Lasut et al., 2019)

c. pH test Ashitaba leaf extract ointment extract is diluted with 10 ml of distilled water in a test tube then tested on a pH meter for 1 minute. DI the value listed on the tool (Naibaho et al., 2013). A good pH value is 4.5-7.

d. Spreadability test: 0.5 grams of ointment is placed on a round glass with a diameter of 15 cm, another glass is placed on top and left for 1 minute. The diameter of the ointment spread was measured. After that, 100 grams of load was added and left for 1 minute then the diameter was measured (Astuti, 2010)

e. Adhesion test The adhesion test is carried out by weighing 1 gram of ointment placed on one surface of the object glass and then covering it with another object glass. The object glass was pressed with a weight of 200 g for 5 minutes. The object glass that is close together is then installed on the adhesion test tool and at the same time as applying a load to the adhesion test tool, the stopwatch is turned on (Zulfa et al, 2015).

RESULTS

1. Making Ashitaba Leaf Extract

Powder Weight	Weight of Thick Extract	% Yield
450 g	50,6 g	11,24%

2. Organoleptic Test Results

The results of organoleptic testing can be seen in table

Table. 2 Organoleptic test results

Observation	Formula		
	F I	F II	F III
Color	Deep Green	Deep Green	Blackish Green
Shape	Thick	Thick	Thick
Smell	Distinctive aroma of Ashitaba leaf extract	Distinctive aroma of Ashitaba leaf extract	Distinctive aroma of Ashitaba leaf extract

3. Homogeneity Testing

The results of the Homogeneity Test can be seen in Table 3.

Table 3. Homogeneity Test Results

Formula	Observation
F I	Homogeneous
F II	Homogeneous
F III	Homogeneous

4. Results of pH testing of ointment preparations

Table 4. pH Test Results

Replication	Observation of pH		
	FI	FII	FIII
1	6,01	6,33	6,54
2	6,02	6,34	6,55
3	6,00	6,32	6,53
Average	6,01	6,33	6,54

5. Results of the ointment spreadability test

Table 5. Results of ointment spreadability testing

Replication	Observation of spreading power (cm)			
	FI	FII	FIII	K+ Gentamicin
1	7,2	6,5	5,8	5,5
2	7,1	6,6	5,9	5,6
3	7,3	6,4	5,7	5,4
Average	7,2	6,5	5,8	5,5

6. Adhesion test

Table 6 Results of ointment adhesion test

Replication	Observation of adhesive force (cm)			
	FI	FII	FIII	K+ Gentamicin
1	10,1	8,4	5,3	6,4
2	10,2	8,8	5,8	6,7
3	10,0	8,2	5	6,1
Average	10,1	8,2	5,3	6,4

7. Results of the Activity Test of the Antibacterial Ointment Preparation of Ashitaba Leaf Extract 15%

Table 7. Results of the activity test of the antibacterial ointment preparation using ashitaba leaf extract

Formula	Replication Inhibition Zone Diameter (mm)			Average diameter of inhibition zone (mm)
	Replication 1	Replication 2	Replication 3	
I	5,65	5,5	5,7	5,61
II	6,4	6,7	6,5	6,56
III	7,1	7,3	7,4	7,27
Gentamicin	8,3	8,5	8,9	8,57

DISCUSSION

1. Ethanol free test

Ethanol-free test is done to prove that there is no ethanol solvent content in the extract, in addition ethanol acts as an antibacterial and antifungal so that it affects the antibacterial activity test. Testing by ashitaba leaf extract is done by adding 1 ml of acetic acid and concentrated sulfuric acid in a test tube then heated over a bunsen flame. It is said to be ethanol-free if there is no distinctive ether odor (Kurniawan, 2015). The results of this study are that the extract does not smell of ester, only the distinctive odor of ashitaba leaves, so it can be concluded that ashitaba leaf extract is ethanol-free.

2. Identification of Chemical Compound Content of Ethanol Extract of Ashitaba Leaves
Phytochemical testing in this study was qualitative, namely by observing the presence of sediment or color changes formed after the addition of several reagents for testing alkaloids, saponins, flavonoids and tannins. The results obtained showed that the ashitaba leaf extract positively contained flavonoids, alkaloids, saponins and tannins.

3. Evaluation Results of Ashitaba Leaf Extract Ointment Preparation
The thick extract obtained is made into an ointment formulation. Ashitaba leaf extract as the active substance and water-soluble base carrier substance, namely Propylene glycol (PEG). The ointment base was chosen because it does not contain fatty ingredients, so it is good for anti-acne preparations. Fatty ingredients can trigger excess oil production on the face, which can cause acne.

4. Homogenity testing
Homogeneity testing of the Ashitaba leaf extract ointment preparation formula I, formula II, formula III did not show any coarse particles, resulting in a homogeneous ointment preparation. This shows that all additional ingredients and extracts as active substances used in making the ointment preparation are mixed evenly.

5. Results of pH testing of ointment preparations

The pH test of the ointment preparation is carried out to see the acidity level of the ointment preparation produced using a pH meter. A good ointment preparation has a pH between 4.5-7 which is the same as the normal pH of the skin (Swastika et al., 2013). The ointment preparation is expected to have a pH that matches the normal pH of the skin so that it is safe when applied and does not cause irritation. The pH of the ointment preparation that is too low (acidic) can harm the skin and irritate it, while if the pH of the ointment preparation is too low (alkaline) it can dry out the skin (Ambarwati, 2021).

6. Results of the ointment spreadability test
Spreadability testing for each ointment preparation is carried out to see the ability of the preparation to spread on the skin, where an ointment base should have good spreadability to ensure satisfactory administration of medicinal ingredients using several loads within a certain time.

7. Results of the Activity Test of the Antibacterial Ointment Preparation of Ashitaba Leaf Extract 15%
Based on the results in the table and can be seen in the image of the antibacterial activity test of ethanol extract of ashitaba leaves against Staphylococcus epidermidis shows the presence of an inhibition zone formed in the three concentration variations. The diameter of the inhibition zone formed increases with the increase in the test concentration.
The difference in the average inhibition zone of each concentration can be caused by the difference in the concentration levels used. This is in accordance with research conducted by Auliyah in 2016, the higher the concentration of the extract, the higher the number of compounds or active substances in it that work to inhibit bacteria. In addition, an increase in extract concentration will be followed by an increase in the inhibition of bacterial growth.

CONCLUSION

The results of the research on the activity test of the antibacterial ointment preparation of ashitaba leaf extract showed that the highest inhibition zone diameter was in Formula 3 with an average value of the inhibition zone diameter of 7.27 mm.

REFERENCE

Ansel, H.C. (2013) Bentuk Sediaan Farmasetis dan Sistem penghantaran Obat. edisi 9. Jakarta: EGC

Djamil, r., & zaidan, s. (2017). Isolasi Senyawa Flavonoid dari Ekstrak Metanol Daun ashitaba (Sauropus androgynus (L.) Merr), Euphorbiaceae. Jurnal Ilmu Kefarmasian Indonesia, 14(1), 57-61.

Djumaati, F. (2018). Formulasi Sediaan Salep Ekstrak Etanol Daun kelor (Moringa oleifera Lamk.) Dan Uji Aktivitas Antibakterinya Terhadap Bakteri Staphylococcus aureus. Pharmacon, 7(1).

Zukhri, S., Dewi, K. M. S., & Hidayati, N. (2018). Uji Sifat Fisik dan Antibakteri Salep Ekstrak Daun ashitaba (sauropus androgynus (l) merr.). Jurnal Ilmiah Kesehatan, 11(1).

Zulfa, E., Prasetyo, T. B., & Murukmihadi, M. (2015). Formulasi Salep Ekstrak Etanolik Daun Binahong (Anrederacordifolia (Ten.) Steenis) Dengan Variasi Basis Salep. Jurnal Ilmu Farmasi dan Farmasi Klinik, 12(2), 41- 4