



Antibacterial Activity Test of Ashitaba Leaf Water Fraction 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 saponin, tannin and flavonoid compounds which play a role as antibacterial. The purpose of this study was to determine the antibacterial activity of the formulation of the ointment preparation of the water fraction of ashitaba leaves 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 ointment preparations of ashitaba leaf water fraction with a concentration of water fraction Formula I 10%, Formula II 15%, Formula 20%. The results of this study are the formulation of ashitaba leaf extract ointment preparations can have inhibitory power against *Staphylococcus epidermidis* bacteria. The highest inhibition zone in Formula III with a water fraction concentration of 20% with an inhibition zone diameter value of 7.8 mm.

Keywords: acne, ashitaba, fraction, *staphylococcus epidermidis*

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BACKGROUND

Daily activities often cause problems that are classified as mild, moderate or severe. One of the mild risks that can occur is an accident that causes injury to certain parts of the body and can cause infections that can be dangerous if not treated immediately. The emergence of infection cases is usually caused by several microorganisms such as bacteria, parasites, and fungi. Among the bacteria that often cause infections in humans is *Staphylococcus epidermidis*. *Staphylococcus epidermidis* bacteria are one of the normal flora on the surface of human skin. These bacteria often cause mild infections accompanied by abscesses (Rahmawati, 2017).

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 in 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 in the long term will cause resistance of microorganisms and give 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 water fractions that have antibacterial activity is very helpful in healing infections caused by bacteria. One of the dosage forms that is widely used to treat infected wounds is the topical ointment dosage form. An ointment preparation consists of the main active substance and the ointment base. In the selection of the ointment base must be considered carefully. The ointment base must be inert, meaning it will not affect and reduce the therapeutic effect of the main substance. This ointment dosage form is widely chosen because it is effective and easy to apply in various conditions and situations. Based on this, an ointment preparation will be made that is efficacious as an antibacterial against *Staphylococcus epidermidis* bacterial infections from the ashitaba leaf water fraction.

METHODS

Making Ashitaba leaf water fraction

Ten (10) g of extract is added with a certain amount of ethanol to dissolve the extract in a beaker, then add enough distilled water to 50 ml, then put it into a separating funnel. Fractionation with nonpolar solvents first, namely n-hexane as much as 1/3 (15 ml) in the separating funnel. Before shaking, turn the separating funnel upside down and open the tap to release the gas. Shake slowly so that no emulsion is formed, then open the separating funnel tap to release the gas. The process is repeated 2 times. The fractionation results are washed again with distilled water as much as 1/3 (10 ml) of the volume of the n-hexane fraction (ideally), then the water is put back into the water phase and then collected and evaporated again with a rotary evaporator.

Making Ashitaba Leaf Water Fraction Ointment

Prepare the tools and materials, weigh the Ashitaba leaf water fraction 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 water fraction 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).

Evaluation of Ashitaba Leaf Water Fraction Ointment Preparation 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).

Homogeneity test

Apply the ointment to a piece of glass, then visually observe 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).

pH test

Ointment water fraction Ashitaba leaf water fraction 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.



Spreadability test

A total of 0.5 grams of ointment was placed on a round glass with a diameter of 15 cm, another glass was placed on top and left for 1 minute. The diameter of the ointment spread was measured. Afterwards, 100 grams of load was added and left for 1 minute and then the diameter was measured (Astuti, 2010).

Adhesion test

Adhesion test is done by weighing 1 gram of ointment placed on one surface of the object glass and then covered with another object glass. The object glass was pressed with a load of 200 g for 5 minutes. The overlapping object glasses are then installed on the adhesive strength tester and at the same time as the load is applied to the adhesive strength tester, the stopwatch is turned on (Zulfa et al., 2015).

RESULTS

1. Identification of Chemical Compound Content of Ethanol Fraction 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 saponins, flavonoids and tannins. The results obtained showed that the extract of ashitaba leaves positively contained flavonoids, saponins and tannins.

2. Evaluation Results of Ashitaba Leaf Water Fraction Ointment Preparation

The thick extract obtained was formulated into an ointment preparation. Ashitaba leaf extract as the active substance and water-soluble base carrier is 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.

Hasil Pengujian Organoleptis

The results of the organoleptic test can be seen in table 3

Table 3. Results of the organoleptic test

Observation	Formulas		
	F I	F II	F III
Color	Deep Green	Deep Green	Blackish Green
Form	Thick	Thick	Thick
Smell	The distinctive aroma of the water fraction of ashitaba leaves	The distinctive aroma of the water fraction of ashitaba leaves	The distinctive aroma of the water fraction of ashitaba leaves

3. Homogeneity Testing

The results of the Homogeneity Test can be seen in table 4.

Tabel 4. Homogeneity Test Results

Formulas	Observation
F I	Homogen
F II	Homogen
F III	Homogen

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.

4. 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). The results of the pH test of the ointment preparation can be seen in table 5.

Tabel 5. pH Test Results

Replication	pH observation		
	FI	FII	FIII
1	5,6	5,9	6,4
2	5,2	5,8	6,8
3	5,3	6,1	6,9
Average	5,4	5,9	6,7

5. Results of testing the spreadability of the ointment

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. The results of the ointment spreadability test can be seen in table 6.

Tabel 6. Results of ointment spreadability testing

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



Based on the test and data obtained, it can be seen in table 6. that the spreadability of the Ashitaba leaf extract ointment, the ability to spread in formula I has not met the requirements. The ointment preparation is expected to have good spreadability, which is around 5-7 cm.

6. Adhesion Test

The purpose of the adhesion test is to determine the time required for the ointment to adhere to the skin.

The results of the ointment adhesion test can be seen in table 7.

Tabel 7. Results of the ointment adhesion test

Replication	Observation of dispersion power (cm)			
	FI	FII	FIII	K+ Gentamicin
1	9,9	8,4	5,5	6,4
2	9,8	8,6	5,2	6,7
3	10,0	8,1	5,4	6,1
Average	9,9	8,4	5,4	6,4

Based on the table of results of the adhesive power test of the Ashitaba leaf water fraction ointment preparation, formula I, formula II, III and positive control meet the standard requirements for the adhesive power test, namely not less than 4 seconds.1. Activity Test Results of Antibacterial Ointment Preparation of Ashitaba Leaf Water Fraction. The activity test results of the antibacterial ointment preparation of Ashitaba leaf water fraction can be seen in table 8.

Tabel 8. Results of the activity test of the antibacterial ointment preparation of the water fraction of Ashitaba leaves

Formulas	Replication Inhibition Zone Diameter (mm)			Average diameter of inhibition zone (mm)
	Replication 1	Replication 2	Replication 3	
I	5,4	5,2	5,4	5,3
II	6,3	6,2	6,5	6,3
III	7,9	7,7	7,8	7,8
Gentamicin	8,3	8,5	8,9	8,6

Based on the results in the table and can be seen in the image of the antibacterial activity test of the ethanol water fraction of ashitaba leaves against *Staphylococcus epidermidis*, an inhibition zone was formed in all three concentration variations. The diameter of the inhibition zone formed increased with increasing test concentration. The difference in the average inhibition zone of each concentration can be caused by differences 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 the concentration of the fraction 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 the water fraction of ashitaba leaves showed that the highest inhibition zone diameter was in Formula 3 with an average value of the inhibition zone diameter of 7.8 mm.

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