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Effectiveness and physical quality test of Ashitaba leaf extract facial wash in an effort to reduce the growth of *Staphylococcus aureus* Bacteria that cause acne

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Abstract

Background: Acne (acne vulgaris) is a skin problem that is often experienced by women or men and is common during adolescence or puberty. Acne is a common skin disease that attacks 85% of the population in Indonesia aged 11-30 years. Pharmaceutical preparations in the form of facial wash that contain antibacterial content are in accordance with the needs of individuals with acne, in addition this anti-acne facial wash is also practical, effective and efficient in its use. The purpose of this study was to obtain the best formulation of facial wash with ashitaba leaf extract and to determine the best concentration in inhibiting the growth of *Staphylococcus aureus* bacteria that cause acne using the diffusion method.
Method: The research method used was the maceration method extraction, then a formulation was made from facial wash preparations with several concentrations, namely 4%, 6% and 8%, then physical quality testing was carried out followed by antibacterial testing of Staphylococcus aureus bacteria using the diffusion method.
Results: The results of the research on ashitaba leaf extract have flavonoid, tannin, alkaloid and saponin compounds. The results of the physical quality test of the antibacterial facial wash formulation of ashitaba leaf extract which has the best physical quality is formula II because in the pH, viscosity and foam tests it is within the specified standard range, the facial wash preparation formulation which has the highest inhibition zone is formula III with an extract concentration of 6% with an average area of inhibition zone of 18.21 mm which is included in the strong category.
Conclusion: Test results of antibacterial facial wash formulation using ashitaba leaf extract which has antibacterial activity.

Keywords: Ashitaba; Extract; *Facial wash; Staphylococcus aureus.*

INTRODUCTION

Acne (acne vulgaris) is a skin problem that is often experienced by both women and men and is common in adolescence or puberty. Acne is a common skin disease that attacks 85% of the population in Indonesia aged 11-30 years.(1) Factors that cause acne include clean living habits or hygiene, obesity, air pollution, etc. While the pathogenesis factors that cause acne include increased sebum production(2), *Propionibacterium acne* bacteria and *Staphylococcus aureus* bacteria.(3) The use of natural ingredients is currently a trend in the world of skincare and medicine (4), an example of a plant that has high potential is Ashitaba. Ashitaba leaves have antibacterial, anti-inflammatory and antioxidant activities.(5) Ashitaba leaves have a fairly high flavonoid content (6), Xanthoangelol is a chalcone (Flavonoid) which shows antibacterial effects on Gram-Positive bacteria, for example *Staphylococcus aureus* bacteria that cause acne.(7)

In general, acne often grows on the face, so facial cleanliness needs to be considered in order to minimize or reduce bacterial growth, so researchers have developed a pharmaceutical preparation, namely Ashitaba leaf extract Facial wash which functions as a facial cleanser while reducing the occurrence of acne-causing bacteria on the face.

Acne is a skin problem that often appears on the face, especially in adolescence or puberty, where acne can cause hormonal, physical, psychological and social changes.(8) So that the emergence of acne in adolescents can reduce the level of self-confidence when socializing. The emergence of acne is also closely related to the individual's own hygiene patterns, so it is necessary to increase cleanliness, especially in the facial area so that acne does not grow. Pharmaceutical preparations in the form of facial wash that contain antibacterial content are in accordance with the needs of individuals with acne, besides this anti-acne facial wash is also practical, effective and efficient in its use.(9) Previous research conducted namely methanol extract of ashitaba herb showed moderate antioxidant activity(10). Methanol extract of ashitaba herb has antibacterial activity against *Staphylococcus epidermidis* bacteria at a concentration of 10% with an inhibition zone of 14.41%(11). Research conducted by Hamid in 2023 showed that methanol extract of ashitaba leaves has antibacterial activity against *Staphylococcus aureus*, namely at a concentration of 10%, having an inhibition zone in the ethyl acetate fraction of 16.75 mm and the n-hexane fraction of 12.9 mm. Ashitaba stem extract has antibacterial activity against Streptococcus mutans with a concentration of 50% resulting in an inhibition zone diameter of 18 mm, so researchers conducted further research related to Ashitaba leaves by utilizing Ashitaba leaf extract in the form of a Facial wash formulation and by testing the physical quality of the formula. The purpose of this study was to obtain the best formulation of Ashitaba leaf extract facial wash which has the best potential in inhibiting the growth of *Staphylococcus aureus* bacteria that cause acne(12). So that pharmaceutical preparations in the form of facial wash which in addition to functioning to cleanse the face for hygiene purposes can also inhibit the growth of bacteria that cause acne and there has been no research on the facial wash formula from Ashitaba leaf extract as an effort to reduce the growth of *Staphylococcus aureus* bacteria that cause acne.

METHOD

1. Tools

Autoclave, incubator, oven, test tube, Erlenmeyer, analytical balance (AND GF-02), evaporator cup, ose needle, Bunsen, rotary evaporator, Sterling-Bidwell tool, moisture balance, UV 366, Laminar Air Flow.

2. Materials

Ashitaba leaves, *Staphylococcus aureus*, Crystal Violet 1% (Brand), Aquadest, Alcohol, Safranin, Iodine, Nutrient Agar, silica gel GF254, safranin (Brand), Mc.Farland solution 0.5 and DMSO 10%, EDTA-4Na, Glycerin, Citric Acid, SLS (Sodium Lauryl Sulfate), TEA (Triethanolamine), Propylene glycol, Nipagin, Perfume, Carbophol, Aquadest,

Extract Preparation

The preparation of ashitaba leaf extract is by maceration method, weighing 1000 grams of ashitaba powder, soaking using 70% ethanol for 3 days using a dark glass bottle with occasional shaking of the bottle slowly. Continued concentration of the extract using a rotary evaporator until the extract becomes thick, continued calculating the extract yield.(13)

Table 1. Formulation

Material	F1 (%)	F2 (%)	F3 (%)
Extract	4	6	8
EDTA-4Na	0,1	0,1	0,1
Gliserin	2	2	2
Citric Acid	1	1	1
SLS (*Sodium Lauryl Sulfat*)	0,5	0,5	0,5
TEA (*Triethanolamine*)	3	3	3
Propylen glycol	1	1	1
Nipagin	0,2	0,2	0,2
Parfum	0,1	0,1	0,1
Carbophol	1	1	1
Aquadest	Ad 100	Ad 100	Ad 100

Ashitaba extract facial wash in the form of gel preparation is made by homogenizing aquadest, Nipagin, EDTA-4Na, Glycerin and Propylene glycol using a magnetic stirrer then adding SLS, then heated to a temperature of 40oC, added perfume, propylene glycol, citric acid, ashitaba leaf extract mixed until homogeneous, add carbopol gel, carbopol gel is made by dissolving carbopol in aquadest stirred until dissolved, then added TEA stirred until homogeneous and thickened.

Physical Quality Test
- Organoleptic Test
Organoleptic tests include the Shape, Color and Smell of the Ashitaba leaf extract facial wash which are observed visually.
The homogeneity test is tested to observe whether the preparation is homogeneous or not. The homogeneity test method is by applying the facial wash preparation to the top of the glass plate, touching it then when rubbing the facial wash mass must show a homogeneous composition, that is, no solid glass material is felt.(14)

- pH Test
This examination is tested using a pH meter. The tool is calibrated using a standard solution each time a measurement is carried out which has the function of maintaining the accuracy of the measurement, namely pH 4.7 and 10. The electrode is rinsed using distilled water and then dried. The pH measurement of this preparation is carried out by: 1 gram of sample is dissolved in hot distilled water up to 10 milliliters. The electrode is dipped in the container, let the needle move until it reaches a constant position.

- Viscosity Test
Viscosity testing was carried out using a Brookfield DV2T Viscometer using a spindle with no. 4 because the facial wash preparation from the formula was rather thick, and a speed of 200 rpm by pouring the preparation into the viscometer glass and the viscosity value was known by reading the numbers on the appropriate scale. Viscosity is the resistance of a liquid to flow, where the greater the viscosity, the greater the resistance.(15)

- Foam power test
The foam power test on distilled water was carried out by: weighing one gram of the sample, putting it in a test tube, adding distilled water up to 10 ml, shaking it by turning the test tube upside down for 5 seconds, then immediately measuring the height of the foam obtained. Then, the tube was left for 5 minutes, then the height of the foam produced was re-measured after 5 minutes.
Antibacterial Activity Testing
In the antibacterial testing treatment of facial wash formulation of ashitaba leaf extract using the age diffusion method with 4 treatment groups. Negative control of facial wash base, formula 1 concentration 4%, formula 2 concentration 4%, formula 3 concentration 6%. The antibacterial effectiveness of facial wash of ashitaba leaf extract can be observed from the formation of an inhibition zone measured in the clear zone area.

RESULTS

1. Extraction Results

Table 2. Yield of ethanol extract of ashitaba leaves

Powder (g)	Thick extract(g)	Yield(%)
1.000	121,51	12,151

Based on table 2, the crude extract results were 121.51 grams. The calculation of the yield by dividing the weight of the thick extract by the weight of the herbal medicine and obtaining the results of the yield of 12.151%. The extract results obtained were optimal because (>10%) the extract was well extracted.(16) The extraction method chosen in the extraction process can affect the quality of the extract, so the selection of the extraction method needs to consider the nature of the material, the stability of secondary metabolites, costs and time

efficiency. The selection of the extraction method by maceration is because this method includes simple extraction, the processing process is carried out by soaking the herbal medicine powder in a solvent, so that it does not require heating and damage to the compounds contained can be avoided.(17)

2. Physical Quality Test Results

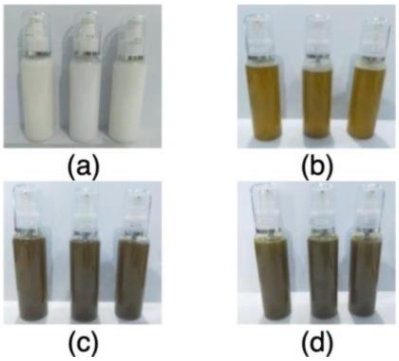

Figure 1. Image of (a) Negative control; (b) Formula 1; (c) Formula 2; (d) Formula 3

Organoleptic Test Results

Table 3. Organoleptic Test Results

Parameter	Formula	Result
Homogenity	Control -	Homogen
	Formula 1	Homogen
	Formula 2	Homogen
	Formula 3	Homogen
Form	Control -	A bit thick
	Formula 1	A bit thick
	Formula 2	Thick
	Formula 3	Thick
Colour	Control -	White
	Formula 1	Light brown
	Formula 2	Brown
	Formula 3	Brown
Smell	Control -	Odorless
	Formula 1	Typical
	Formula 2	Typical
	Formula 3	Typical

Organoleptic examination tested by observing the facial wash preparation is the color, odor and form of the soap that has been made. Organoleptic testing aims to determine the physical appearance of the facial wash preparation with ashitaba leaf extract, by observing the shape, odor and color of the preparation. The test results obtained all homogeneous samples, meaning that all formulas are evenly mixed in the facial wash preparation. In the test of the form of the preparation, the higher the concentration of extract in the facial wash formula, the thicker it is, as well as the color produced, the higher the content of ashitaba leaf extract, the darker the color of the formula, for the smell in formulas I, II and III have a distinctive ashitaba smell.

pH testing Result

Table 4. Results of pH testing

Sample	Replication	Result	Average ± SD
Control -	Replication 1	4,3	4,2 ± 0,06
	Replication 2	4,2	
	Replication 3	4,3	
Formula 1	Replication 1	5,4	5,6 ± 0,15
	Replication 2	5,6	
	Replication 3	5,7	
Formula 2	Replication 1	6,5	6,3 ± 0,15
	Replication 2	6,2	
	Replication 3	6,4	
Formula 3	Replication 1	7,4	7,4 ± 0,2
	Replication 2	7,2	
	Replication 3	7,6	

Generally, the pH of bath soap ranges from 4.5-6.5. If the pH value is very high, it can cause scaly skin, if the pH is acidic, skin irritation can occur. The results of a relatively alkaline soap pH can help the skin to open its pores, then the foam from the soap binds sebum and other dirt that sticks to the skin.(18) Based on the results of the study, the negative control showed a pH result that was too acidic, and in formula III showed a pH with a value of 7.3, which means that the facial wash is included in the alkaline category, which can cause damage to the outermost skin and can cause the skin to feel dry, so formulas I and II are good formulas because they have a range of results 4.5 - 6.5.

Viscosity Test Results

Viscosity testing is carried out to determine the thickness of a preparation using a viscometer and measured at several speeds. In testing this facial wash preparation, spindle number 4 was used, using a speed of 200 rpm.

Table 5. Viscosity Test Results

Sample	Replication	Result	Average
Kontrol -	Replication 1	3010	
	Replication 2	3016	3042,7
	Replication 3	3102	
Formula 1	Replication 1	3502	
	Replication 2	3513	3513,3
	Replication 3	3525	
Formula 2	Replication 1	3978	
	Replication 2	3988	3986
	Replication 3	3992	
Formula 3	Replication 1	4325	
	Replication 2	4352	4346
	Replication 3	4361	

According the good viscosity range of facial wash is 3000-5000.(19) The viscosity of facial wash affects the product's acceptance by consumers, there is a fairly high viscosity of the preparation that can reduce the frequency of collisions between particles so that the preparation becomes more stable. The international unit of viscosity is pascal-second (pa.s) or simply with the poise unit (P).(15) The results of the study showed that all samples were within the good viscosity range. According to Putri 2017 it is influenced by the water content in the soap. The less water content in the soap, the higher the viscosity of the soap, and conversely the more water content in the soap, the lower the viscosity.

Foam Test

Table 6. Foam Test Results

Sample	Replication	Result (mm)	Average± SD
Control -	Replication 1	42	
	Replication 2	33	40,67 ± 7,1
	Replication 3	47	
Formula 1	Replication 1	67	
	Replication 2	66	65,6 ± 1,5
	Replication 3	64	
Formula 2	Replication 1	74	
	Replication 2	75	73,7 ± 1,5
	Replication 3	72	
Formula 3	Replication 1	87	
	Replication 2	86	87,3 ±1,5
	Replication 3	89	

Foam is one of the most important parameters to determine the quality of a cosmetic product, especially facial wash. The purpose of foam testing is to observe the foam power of facial wash. Stable foam over a long period of time is needed because foam can help clean sebum. The Indonesian national standard for foam testing is 13-220 mm, facial wash is identical to the formation of foam during use. The characteristics of facial wash foam are influenced by several factors, namely the presence of SLS (Sodium Lauryl Sulfate) surfactants, foam stabilizers and other ingredients that make up facial wash.(20) In addition, the presence of saponin content in leaf extracts in Formulas I, II and III can increase optimal foam test results (Putri, 2017). So it can be concluded that all formulas fall into the category of requirements of the Indonesian National Standard. The highest foam is found in Formula 3 with an average of 87.3 mm. The function of foam in soap is to prevent redeposition, meaning that dirt particles that have been dissolved in water by soap do not fall or settle again so that the dirt can be removed with the previous water.(18)

Antibacterial activity test

Table 7. Diffusion test results

Concentration	Replication	Inhibition Zone (mm)	Average ± SD	Category
Control -	1	7,21	7,24 ± 0,11	Currently
	2	7,14		
	3	7,36		
Formula 1 (Extract 4%)	1	14,13	14,3 ± 0,21	Strong
	2	14,23		
	3	14,54		
Formula 2 (Extract 6%)	1	16,92	16,67 ±0,24	Strong
	2	16,67		
	3	16,43		
Formula 3 (Extract 8%)	1	18,45	18,21 ± 0,21	Strong
	2	18,87		
	3	18,58		

In this study using the selected disc diffusion method to determine the sensitivity of bacteria, the diffusion method was chosen because it is more practical and does not require special equipment. Each disc is filled with 3 types of extract concentrations (4%, 6% and 8%) and the negative control only contains a facial wash base without the addition of ashitaba leaf extract.

DISCUSSION

The results of the study, in ashitaba leaves contain several compounds, namely

flavonoids, tannins, alkaloids and saponins. Flavonoids have various uses, namely antibacterial, antitumor, anti-inflammatory (anti-inflammatory) etc. Flavonoids function as antibacterials by forming complex compounds using extracellular proteins, dissolved proteins, and disrupting the integrity of the bacterial cell membrane. The working mechanism of tannin antibacterials has antibacterial power using the mechanism of precipitating proteins. The working mechanism of alkaloids as antibacterials is by disrupting the components of peptidoglycan in bacterial cells, so that the cell wall layer does not form completely and causes the cell to die. The working mechanism of saponins as antibacterials is that they can cause leakage of proteins and enzymes from within the cell (21), so that facial wash from ashitaba leaf extract can reduce the growth of *Staphylococcus aureus* bacteria.

The results of the study that have been conducted on negative controls have an average inhibition zone of 7.24 mm, this is because there are several ingredients that have antibacterial potential, such as citric acid so that in negative controls there is an inhibition zone in the moderate category. In formulas I, II and III the largest inhibition zone is in formula III, with an ashitaba leaf extract content of 8% with an average inhibition zone area of 18.21 mm which is included in the strong category, this is because the greater the ashitaba extract content contained in the facial wash, the greater the compound content contained in the facial wash so that if the compound content is greater, the greater the inhibition zone / clear zone produced.

Analysis of Results

The data from the research on the antibacterial activity of facial wash from several concentrations of ashitaba leaf extract were analyzed using SPSS version 24 to see whether ashitaba leaf extract was able to inhibit the growth of *Staphylococcus aureus* bacteria.

ANOVA

Inhibition Zone Results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	222.808	3	74.269	1804.845	.000
Within Groups	.329	8	.041		
Total	223.137	11			

Figure 2. Results of the Anova test table

From the results of the SPSS analysis on the one-way Anova test, it shows that the normality and homogeneity test of the inhibition zone is >0.05. The Anova test shows a p-value of 0.000 which means that there is a significant difference between the negative control, 4% extract, 6% extract, and 8% extract, so it is proven that the data is homogeneous. The results of the analysis can be seen in Figure 2.

CONCLUSIONS

The antibacterial facial wash formulation with ashitaba leaf extract that has the best physical quality is formula II, the facial wash preparation formulation that has the highest inhibition zone is formula III with a concentration of 8% producing an average inhibition zone area of 18.21 mm with a strong category.

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