Open Access Health Scientific Journal
https://oahsj.org/index.php/oahsj Vol. 7 No. 1 February 2026, pp. 9-16
Research article

Effectiveness and Physical Quality Test of Ashitaba Leaf Extract Exfoliating Toner against Staphylococcus epidermidis and Propionibacterium Acnes

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ARTICLE INFO

Keywords:
Acne,
Ashitaba
Staphylococcus epidermidis,
Propionibacterium acnes,
Toner.

ABSTRACT

Background: Acne (acne vulgaris) is a chronic inflammatory skin disease with a complex pathogenesis, involving the sebaceous glands and excessive colonization of acne-causing bacteria. In the modern era, the trend of using natural cosmetics is gaining momentum, not only in Indonesia but also globally. An example of a plant with great potential is Ashitaba. Ashitaba leaves have antibacterial. This study aimed to observe the physical quality test and antibacterial test of ashitaba leaf extract toner against Staphylococcus epidermidis and Propionibacterium acnes bacteria**.**
Methods: The design in this study is an experimental method, physical quality tests include, Organoleptic tests are carried out visually, the components evaluated are the smell, shape, color and texture of the preparation, pH test and viscosity test. analysis of data from the physical quality test of the Ashitaba leaf extract facial toner, the physical characteristics of the preparation, including organoleptic, homogeneity, viscosity, and pH, of all three formulas met the parameters.
Results: The toner tested against Staphylococcus epidermidis and Propionibacterium acnes bacteria had the largest inhibition zone, namely with an extract concentration of 20%.
Conclusion: The toner tested against Staphylococcus epidermidis bacteria with a 20% extract had the largest inhibition zone of 27.6 mm, while Propionibacterium acnes bacteria with a 20% extract had the largest inhibition zone of 27.9 mm.

I. Introduction

Acne vulgaris is a chronic inflammatory skin disease that affects the pilosebaceous unit and is characterized by the presence of non-inflammatory lesions such as comedones as well as inflammatory lesions including papules, pustules, nodules, and cysts. Acne is one of the most prevalent dermatological conditions worldwide and predominantly affects adolescents and young adults. The pathogenesis of acne is complex and multifactorial, involving increased sebum production by sebaceous glands, abnormal follicular keratinization, inflammatory processes, and excessive colonization of acne-associated bacteria such as Staphylococcus epidermidis, Staphylococcus aureus, and Propionibacterium acnes (currently classified as Cutibacterium acnes) (Todd, 2024).

Sebaceous gland hyperactivity is strongly influenced by androgen hormones, particularly during puberty and adolescence. Elevated androgen levels stimulate sebaceous glands to produce excessive sebum, which creates an optimal environment for bacterial growth within the follicle. Excess sebum, combined with impaired desquamation of keratinocytes, leads to follicular obstruction and the formation of microcomedones, which represent the earliest stage of acne development. These microcomedones may progress into visible acne lesions when inflammation and bacterial proliferation occur.

Received 12 October 2025; Accepted 3 December 2025; Published 3 February 2026
 DOI: https://doi.org/10.55700/oahsj.v7i1.134

Bacterial colonization plays a significant role in acne progression. Cutibacterium acnes produces lipase enzymes that break down triglycerides in sebum into free fatty acids, triggering inflammatory responses in the skin. In addition, this bacterium activates the innate immune system by stimulating toll-like receptors, leading to the release of pro-inflammatory cytokines. Although Staphylococcus epidermidis is part of the normal skin microbiota, its excessive growth may contribute to inflammation and biofilm formation, while Staphylococcus aureus can exacerbate acne lesions through secondary infection.

In Indonesia, acne is highly prevalent, with approximately 85% of adolescents and young adults aged 11–30 years affected by this condition (Lestari et al., 2021). This high prevalence indicates that acne is not merely a cosmetic concern but also a public health issue. Factors such as tropical climate, genetic predisposition, dietary habits, and lifestyle changes associated with modernization may contribute to the high incidence of acne in this population.

Although acne is not a life-threatening disease, it has a considerable impact on psychological and emotional well-being, particularly among adolescents. Visible facial acne often leads to decreased self-confidence, excessive embarrassment, social withdrawal, and reduced quality of life. In some cases, persistent acne is associated with anxiety and mild depression. The psychological burden of acne may persist even after physical symptoms improve, emphasizing the importance of comprehensive acne management that addresses both physical and psychosocial aspects.

Lifestyle factors have been increasingly recognized as contributors to acne development and severity. Dietary habits such as frequent consumption of fast food, high-glycemic-index foods, and sugar-sweetened beverages can increase insulin and insulin-like growth factor-1 levels, thereby stimulating sebaceous gland activity and worsening acne. In addition, the widespread use of cosmetic products among adolescents, particularly those with comedogenic ingredients, can contribute to follicular blockage if not properly removed. These behaviors are currently common among adolescents and young adults (Husna et al., 2024).

Along with these lifestyle changes, the demand for effective and safe skincare products continues to increase. In the modern era, there has been a growing global trend toward the use of natural and plant-based cosmetic products due to concerns about the long-term side effects of synthetic chemicals and increased awareness of environmental sustainability. Indonesia, with its rich biodiversity, offers significant potential for the development of natural cosmetic ingredients derived from medicinal plants. One plant with promising potential is Ashitaba (Angelica keiskei), which has been traditionally used for its health-promoting properties. Ashitaba leaves are known to possess antibacterial, anti-inflammatory, and antioxidant activities, primarily due to their high flavonoid content. Xanthoangelol, a major chalcone-type flavonoid found in Ashitaba, has demonstrated strong antibacterial activity against gram-positive bacteria, including Staphylococcus epidermidis and Cutibacterium acnes (Wahyuni et al., 2024). These properties make Ashitaba a promising natural ingredient for acne management.

Acne lesions most commonly occur on the facial area, making facial cleansing a crucial step in acne prevention and treatment. Proper cleansing helps remove excess sebum, dirt, makeup residue, and microorganisms from the skin surface. One commonly recommended method is double cleansing, which involves the use of an oil-based cleanser followed by a water-based cleanser. This method effectively removes both lipid-soluble and water-soluble impurities and may reduce the risk of pore blockage.

Another contributing factor to acne formation is the accumulation of dead skin cells on the skin surface, which can obstruct hair follicles and promote comedone formation. Exfoliation is therefore important in maintaining normal skin turnover. To address this issue, researchers have developed cosmetic and pharmaceutical preparations in the form of exfoliating toners formulated with natural active ingredients. An exfoliating toner containing Ashitaba leaf extract is designed to gently remove dead skin cells while simultaneously providing antibacterial, antioxidant, and anti-inflammatory effects.

Ashitaba leaves also contain other bioactive compounds such as 4-hydroxydericin and xanthoangelol, which work synergistically to reduce bacterial growth, suppress inflammation, and neutralize free radicals. These combined activities may help reduce acne lesions and prevent the formation of new acne in acne-prone skin (Riadi et al., 2024). Therefore, the development of an exfoliating toner containing Ashitaba leaf extract represents a promising approach for acne management that aligns with current trends in natural cosmetic use.

This study aimed to observe the physical quality test and antibacterial test of ashitaba leaf extract toner against Staphylococcus epidermidis and Propionibacterium acnes bacteria.

METHODS

Materials

The materials in this study are ashitaba leaves, 96% ethanol (PT. Brataco), *Staphylococcus epidermidis* bacteria and *Propionibacterium acnes* bacteria, 0.9% NaCl solution (Widatra), Nutrient Agar, Nipagin, Nipasol (pharmaceutical grade), Salicylic acid (pharmaceutical grade), Glycerin (pharmaceutical grade), Propilenglikol (pharmaceutical grade), Fragrance (pharmaceutical grade), Tween 80 (pharmaceutical grade), Aquadest (pharmaceutical grade).

Extraction

The extraction process uses the maceration method, with the solvent used being 96% ethanol in a ratio of 1:10 (Widhiana Putra et al., 2020). The dry powder of ashitaba leaves is measured at 700 grams. The dry powder is put into a glass container and added to 96% ethanol solvent in a ratio of 1:10 or until completely submerged, which is later homogenized with a stirrer for 4 hours and allowed to stand for 24 hours at room temperature. The maceration results are filtered using Whatman filter paper with the help of a separating funnel. The extract is concentrated using a rotary evaporator at a temperature of 45°C. The concentrated extract was later measured, and the yield value was calculated.

Plant determination

Ashitaba determination was conducted at the Batu Malang Herbal Materia Medika Laboratory to determine the plant's authenticity and validity

Extract ethanol free test

One mililiter of concentrated acetic acid (CH_3COOH) and sulfuric acid (H_2SO_4) were added to a number of test solutions. Then it is homogenized, and after the mixture is homogeneous, it is heated with Bunsen fire. A positive result of an ethanol-free extract occurs if the test results do not smell the ester odor as a characteristic odor of alcohol (Ballo et al., 2021).

Extract moisture test

Each extract measured 1.5 g and was put on an aluminum plate. The plate is later inserted into the halogen moisturizer analyzer to determine the water content of the extract.

Extract phytochemical screening test

Flavonoid

It is measured as much as 0.5 g of extract, then put in a test tube and added to 5 mL of ethanol. The mixture is then heated for 5 minutes. Then, 10 drops of concentrated HCl and 0.2 g of Mg powder were added. The extract contains flavonoid compounds if a red-brown precipitate is formed.

Saponin

As much as 0.5 g of extract was put into a test tube, along with 10 mL of distilled water. The test tube was shaken for 1 minute and allowed to stand for 10 minutes while observing the foam that formed. The extract contains saponins if the foam persists for 10 minutes.

Tanin

Phytochemical testing of tannin compounds using FeCl3, where 1 ml of FeCl3 solution is added to the extract sample until a positive result is obtained, indicated by the formation of an inky blue or blackish green color.

Toner formulation of ashitaba extracts

Making an exfoliating toner formulation using Ashitaba leaf extract with the following formula:

Table 1. Formulation of toner

Material	Control	F I	F II	FIII	Utility
Ashitaba Leaf Extract	-	10%	15%	20%	Active Ingredients
Nipagin	0.02%	0.02%	0.02%	0.02%	Preservative

Nipasol	0.02%	0.02%	0.02%	0.02%	Preservative
Salicylic acid	0.5%	0.5%	0.5%	0.5%	Exfoliating
Glycerin	10%	10%	10%	10%	Humektan
Propilenglikol	10%	10%	10%	10%	Humektan
Fragrance	q.s	q.s	q.s	q.s	Fragrance
Tween 80	0.5%	0.5%	0.5%	0.5%	Surfaktan
Aquadest	Ad 100	Ad100	Ad 100	Ad 100	Solvent

All ingredients were mixed in a beaker and stirred using a magnetic stirrer until homogeneous, with positive control, namely toner preparations circulating on the market.

Evaluation of gel preparation

Organoleptic test

The gel organoleptic test was conducted by observing the gel based on color, consistency, and odor (Sikawin et al., 2018).

Viscosity test

A viscosity test was conducted using a Rheosys Merlin VR Viscometer and a cone and plate with 2.0/30 mm. 500 mg of gel was placed on a plate and squeezed by a cone. The test parameters were made similar for all formulas so that all formulas achieved the same treatment. After that, it was operated using a computer with the Rheosys Micra application.

pH test

The pH test of the gel used a pH meter. The gel pH test was conducted by dipping the pH meter electrode into a gel preparation that had previously been dipped in distilled water. The pH meter tool will show the pH of the gel preparation.

Antibacteria activity testing

Bacterial identification was then carried out, followed by testing the exfoliating toner formula against *Staphylococcus epidermidis* and *Propionibacterium acnes* using the well diffusion method with three concentrations of ashitaba leaf extract (10%, 15%, and 20%), a negative control without ashitaba leaf extract, and a positive control exfoliating toner product available on the market.

RESULTS

Plant determination

The research begins with the process of determining the plants to be used as samples. Plant determination aims to determine if the plant sample used is the expected plant and to prevent sample errors for the research specifically Ashitaba leaves.

Extraction

The extraction process of cherry and beluntas leaves uses the maceration method with 96% ethanol as a solvent. According to the results of the research, ashitaba leaf extract had a yield of 9.40%. From the obtained yield of cherry leaf extract, it showed smaller results than previous studies; in Saputra's (2020) research, the yield was 12.17%.

Extract ethanol free test

The ethanol-free test was carried out to ensure that the extract obtained was pure without the presence of ethanol as a contamination, which was the solvent of the maceration process that had been carried out. Ethanol also has antibacterial and antifungal properties, which can cause false positives in the tests carried out on samples, so it must be ensured that the extract does not contain ethanol (Kurniawati, 2015). Non-containing ethanol extract can be ascertained by the absence of a distinctive ethanol odor after the test is carried out. From the ethanol-free test, the result obtained was that ashitaba extracts were free of alcohol compounds. It was characterized by the absence of a characteristic alcohol ester odor.

Extract phytochemical test

A phytochemical test of the extract compound was performed using a tube test in order to determine the presence of flavonoid compounds, saponins and tannin contained in ashitaba extract as compounds that have antibacterial activity.

Table 3. The result of the extract phytochemical test

Test	Result	Note
Flavonoid	+	Red precipitate
Saponin	+	There is constant foam
Tannin	+	blackish green color

All three classes (flavonoids, tannins, saponins) exhibit *multimodal antibacterial activity*, often coupling membrane damage with interference in metabolism or gene expression. Flavonoids tend to have more diverse intracellular targets (DNA, enzymes, efflux pumps) in addition to membrane effects. Tannins are especially strong protein binders and metal chelators, they often act extracellularly or at the cell envelope, interfering with proteins and enzymes. Saponins' amphiphilicity makes them potent at disrupting membranes, but they may need careful dose control to avoid toxicity. Because bacteria differ in cell envelope structure (Gram-positive vs Gram-negative), the relative potency of these compounds often depends on bacterial type.

Evaluation of gel preparation

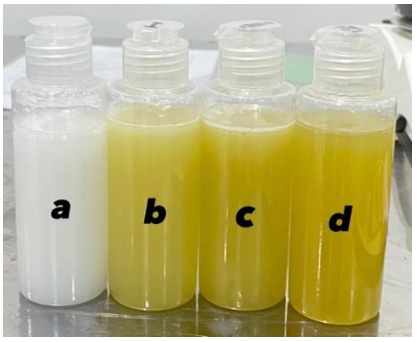

Figure 1. a. Negative control b. 10% Ashitaba leaf extract toner, c. 15% Ashitaba leaf extract toner, d. 20% Ashitaba leaf extract toner

Organoleptic Testing

The organoleptic test of the toner preparation aims to observe the appearance of the prepared toner. The toner is observed for its consistency, color, and odor. The organoleptic test relates to the ease of use of the toner as a medicinal preparation. Ashitaba extract combination toners showed that the concentration of the added extract improved the aroma, color, and consistency of the gel. Increasing the extract concentration resulted in a stronger aroma, darker color, and thicker viscosity. All toner formulations were designed to have a thick consistency; the color corresponded to the active ingredients used.

Toner viscosity test

Viscosity testing is performed to determine the viscosity level of a product. The standard viscosity for facial toner is <5 cPs, measured using a viscometer with spindle number 1 at 60 rpm (Sari et al., 2021). The viscosity evaluation results before stability testing for Formula I yielded an average value of 1.47 cPs, Formula II an average value of 3.82 cPs, and Formula III an average value of 4.7, thus ensuring that the facial toner meets the requirements (<5 cPs).

pH test

The pH value indicates the level of acidity or alkalinity of a substance being tested. The pH value in this study was measured using a pH meter. The standard pH for skin is 4.5-6.5, measured using a pH meter (Aji, 2020). The desired pH for the Ashitaba leaf extract facial toner preparation is 5.5 ± 0.5 for comfortable use. Too low a pH can cause skin irritation, while too high a pH can cause dry skin and an itchy sensation (Sari et al., 2021). The pH evaluation results for formula I showed an average pH of 5.68,

formula II an average pH of 5.74, and formula III an average pH of 5.82. Therefore, the facial toner preparation meets the skin pH requirements (4.5 - 6.5) and fits the researcher's desired pH value of 5.5 ± 0.5.

DISCUSSION

Antibacterial Activity

The antibacterial inhibition test in this study was conducted using the blank disk method. Each NA medium consisted of a combination of toner and ashitaba leaf extract at various concentrations: 10%, 15%, and 20%, a positive control containing a commercially available toner, and a negative control containing a toner base. The disks were then incubated for 24 hours at 37°C. Observations were made, and the zones of inhibition formed were measured using a caliper. Inhibition was demonstrated by the formation of a clear zone around the well. The larger the diameter of the clear zone, the greater the inhibition. The description and analysis showed that the ashitaba extract toner can inhibit the growth of *Staphylococcus epidermidis* and *Propionibacterium acnes* colonies.

Table 4. Results of the antibacterial activity test of *Staphylococcus epidermidis*

Tested	Inhibiton zone (mm)	Average ± SD
Control -	0 0 0	0 ± 0
Control +	29.4 29.9 29	29.4 ± 0.45
Extract 10%	20.3 20.5 20.1	20.3 ± 0.2
Extract 15%	23.5 23.1 23.7	23.4 ± 0.3
Extract 20%	26.3 26.7 26.4	27.6 ± 0.2

The ANOVA results showed an F value of 5355.162 with a significance (Sig.) of 0.000 ($p < 0.05$), meaning that the difference in inhibition zones between groups was statistically significant. In other words, the ashitaba leaf extract toner was significantly able to inhibit the growth of *Staphylococcus epidermidis*, and the effect increased according to the dose orconcentration of the extract.

Table 5. Results of the antibacterial activity test of *Propionibacterium acnes*

Tested	Inhibiton zone (mm)	Average ± SD
Control -	0	0 ± 0
	0	
	0	
Control +	29.4	29.4 ± 0.45
	29.9	
	29	
Extract 10%	22.5	22.5 ± 0.3
	22.7	
	22.3	
Extract 15%	24.6	24.5 ± 0.2
	24.3	
	24.7	
Extract 20%	27.9	27.9 ± 0.1
	27.8	
	28	

The ANOVA results showed an F value of 7260.489 with a significance (Sig.) of 0.000 ($p < 0.05$), which means the difference in inhibition zones between groups was statistically significant. In other words, the ashitaba leaf extract toner was proven to be effective in inhibiting the growth of *Propionibacterium acnes* bacteria, and its effectiveness increased with the concentration of the extract.

CONCLUSION

Based on the evaluation and analysis of data from the physical quality test of the Ashitaba leaf extract facial toner, the physical characteristics of the preparation, including organoleptic, homogeneity, viscosity, and pH, of all three formulas met the parameters. Furthermore, the toner tested against *Staphylococcus epidermidis* bacteria with a 20% extract had the largest inhibition zone of 20.3 mm, while *Propionibacterium acnes* bacteria with a 20% extract had the largest inhibition zone of 27.9 mm.

ACKNOWLEDGMENTS :

Thanks to the Ministry of Education and Science for the financial support so that the researcher can complete this research and all parties who have helped complete this research

CONFLICTS OF INTEREST

No conflict of interest was found during the research

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