ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

Jurnal Kefarmasian Indonesia
Available online at https://jkefarind.com/index.php/jki
Original Research Article
JKI

Formulation and Antibacterial Activity of Garlic Bulbs Ethanol Extract Shampoo Against *Staphylococcus aureus*

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ARTICLE INFO

Article history:
Received 21 January 2026
Revised 26 March 2026
Accepted 31 March 2026
Published online 30 June 2026

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Citation: Whardhani SK, Sharonita EV, Novia J, Rangka FM. Formulation and Antibacterial Activity of Garlic Bulbs Ethanol Extract Shampoo Against Staphylococcus aureus. Jurnal Kefarmasian Indonesia. 2026;16(1):12-24

ABSTRACT

Dandruff is a scalp condition characterized by the excessive shedding of dead skin cells, often exacerbated by *Staphylococcus aureus*. This study explores the potential of garlic bulbs extract as an antibacterial agent in shampoo formulations to address dandruff. This approach offers a safer alternative to Zinc Pyrithione (ZnPT), which can cause side effects such as scalp dermatitis, hair damage, and discoloration. This study aimed to formulate an herbal shampoo containing garlic bulbs ethanol extract and evaluate its physical properties and antibacterial activity against *Staphylococcus aureus*. Three formulations, F1 (6%), F2 (8%), and F3 (10%), were prepared and assessed for organoleptic characteristics, pH, foam height, viscosity, homogeneity, and stability through cycling tests. Antibacterial activity was determined using the well diffusion method. The inhibition zone diameters obtained were 19.80 mm (F1), 23.65 mm (F2), and 25.73 mm (F3), with inhibition categories classified according to CLSI as intermediate for F1 and susceptible for both F2 and F3. Increasing extract concentration improved antibacterial activity; however, F2 was identified as the optimal formulation due to its strong inhibition (23.65 mm), stable physical characteristics, and more efficient extract utilization compared to F3. All formulations fulfilled Indonesian National Standard (SNI) requirements and remained stable after six cycling cycles. These findings demonstrate that garlic bulbs extract shampoo possesses significant antibacterial activity against *Staphylococcus aureus* and exhibits suitable physical stability, supporting its potential as a safer herbal alternative for dandruff management.

***Keywords*:** Antibacterial; Dandruff; Garlic bulbs; Shampoo; *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is one of the primary microorganisms associated with scalp disorders, including dandruff and other pilosebaceous conditions. Indonesia's tropical climate, characterized by high humidity and elevated temperatures, provides an ideal environment for the proliferation of such bacteria. These conditions contribute to the high prevalence of dandruff among Indonesians, where both *Staphylococcus aureus* and *Propionibacteria* have been identified in scalp samples as key factors in its development.[1,2] Dandruff is a common and persistent scalp disorder that affects nearly half of the population at some point in life. It can negatively influence quality of life by causing symptoms such as itching, visible flaking, and social discomfort, with notably higher incidence among young adults. Despite its prevalence, the precise underlying mechanisms of dandruff remain incompletely understood, which continues to make the development of

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

effective long-term treatments challenging.[3,4]

Increasing resistance to commonly used topical antibiotics, such as erythromycin and clindamycin, has further underscored the need to explore herbal-based alternatives for managing infections caused by these microorganisms.[5] According to data from the International Database, US Census Bureau (2004), approximately 18,4% of the Indonesians suffer from dandruff, ranking fourth after China, India, and the US. Dandruff can affect individuals of all ethnicities and genders, including children, typically presenting with mild severity. The severity of dandruff is influenced by age, particularly during puberty and middle age.[6]

Mild dandruff can be managed by washing the hair to reduce excess oil production on the scalp, which serves as a medium for various microorganisms. Shampoo is an ideal cosmetic product for preventing bacteria on the scalp. It is commonly used by various demographics, including adults and children, to cleanse the hair and scalp, resulting in a clean, soft, manageable, and shiny appearance.[7] Gel formulations are preferred in shampoo production due to advantages such as longer contact time, ease of rinsing, and appealing appearance.[8]

One common anti-dandruff shampoo contains active ingredients like Zinc Pyrithione (ZnPT), which functions as an antibacterial and antifungal agent. The use of ZnPT in anti-dandruff shampoos is well recognized, with regulatory limits set at 2% for rinse-off products and 0.1% for leave-on products according to the Regulation of the Head of the Food and Drug Supervisory Agency No. 18 of 2015. However, excessive use of ZnPT can lead to scalp dermatitis and hair damage, including hair loss, discoloration, and breakage.[9] Therefore, there is a need for alternative natural ingredients that can mitigate the side effects of excessive ZnPT use and prevent bacterial growth on the scalp. Herbal shampoos have become increasingly popular as natural substitutes for synthetic hair-care products because they offer a gentler cleansing effect, are environmentally friendly, and provide additional therapeutic benefits.[10]

Previous research by Magryś et al. (2021) indicated that a 70% ethanol extract garlic bulb showed strong antibacterial activity against *Staphylococcus aureus* with a 35 mm inhibition zone at a 100% concentration, compared to gentamicin with a 25 mm diameter and the lowest MIC of 6.25%.[11]

Allicin, one of the principal organosulfur compounds found in garlic bulbs, exhibits broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. Its mechanism involves inhibiting RNA synthesis and disrupting lipid metabolism, thereby interfering with amino acid and protein formation as well as phospholipid construction in the bacterial cell membrane, ultimately suppressing bacterial growth and survival. As a naturally occurring antibacterial constituent, allicin plays a key protective role in garlic by preventing bacterial invasion. [12,13] Garlic bulbs contain secondary metabolites such as alkaloids, flavonoids, saponins, and tannins that exhibit antibacterial properties.[14,15]

Based on research indicating that garlic bulbs have activity against bacterial growth, the author is interested in testing the antibacterial efficacy of a 70% ethanol extract from Garlic bulbs (*Allium sativum* L.) against *Staphylococcus aureus* using the well diffusion method to identify the antibacterial potential of Garlic bulbs that can be utilized in scalp care products. This will be followed by the formulation of a shampoo containing garlic bulbs extract. It is hoped that this formulation will not only provide antibacterial benefits but also enhance scalp health. By leveraging the natural properties of garlic bulbs, the resulting product is expected to be a safe and effective option for preventing bacterial growth.

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ISSN: 2354-8770 (Electronic)

METHODS

Equipment and materials

The equipment used in this study includes herb grinders (Fomac), analytical balances (Ohaus), beakers (Borosil), funnels (Pyrex), rotary evaporators (Heidolph), test tubes (Borosil), Erlenmeyer flasks (Borosil), autoclaves (Biobase), ovens (Memmert & NUVE), Petri dishes (Borosil), magnetic stirrers, hot plate (Heidolph), micropipettes (Socorex), inoculating needles, Bunsen burners, microscopes (Olympus), Eppendorf tubes, vortex mixers (Heidolph), incubators (Memmert), mortars, measuring glasses (Iwaki), spatulas, 913 pH meters Metrohm, and Brookfield Ametek DVPlus viscometers.

The materials used in this study were Garlic bulbs, ethanol (Merck), 70% distilled water, filter paper (Whatman) , sulfuric acid (Merck), acetic acid (Merck), ammonia (Merck), chloroform (Merck), 10% HCl (Merck), Dragendorff's reagent, 1% FeCl3 (Merck), magnesium powder (Merck), concentrated HCl (Merck), amyl alcohol (Merck), 6 N NaOH, ether, and Lieberman-Burchard reagent, nutrient agar (Himedia), Mueller Hinton agar (Himedia), 70% alcohol, crystal violet (Merck), iodine solution (Merck), decolorizer (Merck), safranin (Merck), 10% DMSO (Merck), clindamycin, McFarland standard 0.5 (10^8 CFU/ml) (Merck), sodium lauryl sarcosinate (BASF), cocamide DEA, xanthan gum (Merck), glycerin (Merck), panthenol (Merck), citric acid (Merck), menthol (Merck), phenoxyethanol (Merck).

Work Procedures

Plant Determination

Plant determination is conducted to validate the identity of the plant material used in this research. The determination of garlic bulbs (*Allium sativum* L.) was performed at *Badan Riset dan Inovasi Nasional* (BRIN) Bogor, West Java.

Preparation of Garlic bulbs Simplicia (*Allium sativum* L.)

Approximately 5 kg of garlic bulbs (*Allium sativum* L.) was sourced from a farm in Temanggung, Central Java, and underwent a thorough cleaning process, including peeling, wet sorting, and washing. After cutting the bulbs into smaller pieces for quicker drying, they were air-dried, dry sorted, ground using an herb grinder to enhance extraction efficiency, and stored at room temperature away from direct sunlight.[16]

Preparation of Garlic bulbs Extract (*Allium sativum* L.)

The garlic bulbs extract (*Allium sativum* L.) was prepared using the maceration method. A total of 200 g of simplicia was mixed with 70% ethanol at a 1:5 ratio and soaked for 24 hours with occasional stirring. This procedure was repeated four times to achieve optimal extraction. The resulting macerate was then filtered to obtain the filtrate, which was subsequently concentrated using a rotary evaporator and dried in a water bath to produce a thick Garlic bulbs extract.

Measurement of Extraction Yield

The extract yield was calculated using the following formula:

$$\%\text{Yield} = \frac{\text{Weight of extract (g)}}{\text{Weight of simplicia (g)}} \times 100\%$$

Determination of Water Content

Moisture content was determined using a moisture analyzer set at 105°C for 10 minutes. A total of 1 g of the Garlic bulbs extract was weighed, placed onto a metal plate, and evenly spread before analysis. According to the Indonesian Herbal Pharmacopoeia, the maximum allowable moisture content for garlic bulbs (*Allium sativum* L.) extract is not more than 12%.[17]

Ethanol-Free Test

The ethanol-free test was performed to determine whether any residual ethanol remained in the extract. For this test, 1 mL of the thick extract was transferred into a

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

test tube, followed by the addition of 2 drops of H_2SO_4 and 2 drops of acetic acid. The mixture was then heated. The extract was considered ethanol-free if no characteristic ester odor derived from ethanol was detected.

Phytochemical Screening Identification

Phytochemical screening of garlic bulbs extract (*Allium sativum* L.) using Dragendorff's and Mayer's reagents.The phenol test with 1% $FeCl_3$. Flavonoid test using magnesium powder, concentrated HCl, and amyl. Quinone test with 6N NaOH. Saponin test using 1% HCl. For steroids and triterpenoids, treatment with Liebermann-Burchard reagent. The tannin test, include catechin and gallo tannins using 1% $FeCl_3$, gelatin, and Steasny reagent.

Formulation of Garlic bulbs Extract Shampoo

The formulation of garlic bulb (*Allium sativum* L.) extract shampoo was developed to incorporate the antibacterial properties of the extract into a stable and practical dosage form suitable for scalp applications. Each ingredient in the formulation was selected based on its functional role. The concentrations of garlic bulb extract were varied to evaluate their effects on the physical characteristics and antibacterial activity of the resulting shampoo formulations. The complete composition of each formulation is presented in Table 1.

The Making Process of Garlic bulbs Extract Shampoo (*Allium sativum* L.)

Shampoo preparation began by weighing all ingredients and heating distilled water in a beaker. Xanthan gum was then dispersed in the hot water and stirred using a magnetic stirrer at a speed of 350–400 rpm until homogeneous, followed by the gradual addition of glycerin. Panthenol, phenoxyethanol, menthol, and dissolved citric acid were then added and stirred. After that, the fully dissolved Garlic bulbs extract was added to the solution and stirred until a uniform mixture was achieved without heating (mixture 1). In a separate beaker, distilled water was heated, and sodium lauryl sarcosinate was added gradually while stirring until dissolved.

Table 1. Formulation of Garlic bulbs Extract Shampoo

Name of Material	Function	F1% (w/v)	F2 % (w/v)	F3 % (w/v)
Garlic bulbs Extract	Active ingredient	6	8	10
Peppermint Essence	Corrigen odoris	q.s.	q.s.	q.s.
Sodium Lauroyl Sarcosinate	Anionic surfactants	3	3	3
Cocamide DEA	Non-ionic surfactants	4	4	4
Xanthan Gum	Gelling agents	0.4	0.4	0.4
Citric Acid	Buffer	q.s.	q.s.	q.s.
Menthol	Cooling sensation	0.5	0.5	0.5
Phenoxyethanol	Preservative	1	1	1
Glycerin	Humectant	10	10	10
Panthenol	Moisturizer	0.5	0.5	0.5
Distilled Water	Solvent	ad 100	ad 100	ad 100

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ISSN: 2354-8770 (Electronic)

The temperature was raised to 60°C, and Cocamide DEA was added gradually while stirring until homogeneous (mixture 2). Finally, mixture 2 was slowly added into mixture 1 while stirring continuously until the final shampoo formulation was fully homogeneous.

Physical Evaluation of Garlic bulbs Extract Shampoo (*Allium sativum* L.)

To produce garlic bulbs extract shampoo that complies with national standards, it is essential to perform safety and quality tests for traditional medicines in accordance with BPOM Regulation No. 32 of 2019. These tests include organoleptic evaluation, pH measurement, foam height assessment, viscosity analysis, homogeneity check, and stability testing of the garlic bulbs extract shampoo.

Organoleptic Test

This test involves observing the physical characteristics of the product, such as shape, colour, and aroma. According to SNI 06-2692-1992, liquid shampoo should not exhibit sedimentation.

pH Test

The pH measurement ensures that the shampoo does not cause skin irritation. Testing is performed using a calibrated pH meter by dissolving 0.1 g of shampoo in 10 mL of distilled water. The pH value must comply with SNI 06-2692-1992 requirements (5.0–9.0) and align with the natural pH of hair and scalp (4.5–5.5).[18]

Foam Height Test

This test evaluates the surfactant's ability to produce foam, which aids in cleansing and prevents hair tangling. The procedure involves dissolving 0.1 g of shampoo in 10 mL of water, shaking for 20 seconds, and measuring foam height at the 5th and 15th minutes. The acceptable foam height range is 13–220 mm.[19]

Viscosity Test

Conducted on 100 g of shampoo using a Brookfield Viscometer (spindle 63, 30 rpm), the ideal viscosity specification is 400–4000 cps.[20]

Homogeneity Test

1 g sample is spread thinly on a transparent glass surface. The shampoo should exhibit uniform composition without coarse particles.

Stability Test

Performed using the cycling method, this accelerated test alternates storage at 4°C and 40°C for 24 hours each, repeated for six cycles. After each cycle, organoleptic, pH, foam height, viscosity, and homogeneity tests are conducted to ensure product stability.

Antibacterial Activity of Garlic bulbs Extract Shampoo (*Allium sativum* L.)

The antibacterial activity of the garlic bulbs (*Allium sativum* L.) shampoo formulation against *Staphylococcus aureus* was evaluated using the well diffusion method. Each well was filled with 50 µL of the test preparation. The shampoo formulations contained garlic bulbs extract at concentrations of 6%, 8%, and 10%. Clindamycin (10%) was employed as the positive control, while the shampoo base served as the negative control. The inclusion of the shampoo base as the negative control ensured that any observed antibacterial activity was attributable solely to the added active ingredient. MIC or zone diameter value breakpoints and interpretive categories are established per

Table 2. Interpretive Categories of Antibacterial Effectiveness

Interpretive Category	MIC (µg/mL)	Zone Diameter (mm)
Susceptible	≤ 4	≥ 20
Susceptible-dose dependent	8–16	15–19
Intermediate	8–16	15–19
Resistant	≥ 32	≤ 14
Nonsusceptible	> 1	< 17

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

CLSI document M23 for categories of susceptible, intermediate, and resistant (and susceptible-dose dependent and nonsusceptible, when appropriate), which are summarized below for clarity. [21]

RESULTS AND DISCUSSION

Plant Determination

The plant determination was carried out to ensure the taxonomic accuracy of the plant material used in this study. The garlic bulbs employed as raw material were botanically identified as *Allium sativum* L. through determination conducted at Herbarium Bogoriense, *Badan Riset dan Inovasi Nasional* (BRIN) Bogor, West Java. The identification was verified and documented under certificate number B-634/II.6.2/IR.01.02/2/2025. Accurate botanical confirmation was essential to guarantee the authenticity of the plant material and to support the validity and reliability of the subsequent pharmacological and formulation evaluations conducted in this study.

Yield Extract Measurement

From 5 kg of fresh Garlic bulbs, 644 g of dried simplicia was obtained after oven drying at 40°C. The drying process reduced moisture content, preventing microbial growth and degradation of active compounds. The simplicia exhibited characteristic garlic bulbs odour, white colour, and spicy taste. Grinding increased surface area, enhancing solvent penetration during extraction. Maceration using 70% ethanol produced 118.2 g of thick extract from 400 g of garlic bulbs powder, yielding 29.55%. This value exceeds the minimum standard of 26% set by the Indonesian Herbal Pharmacopoeia, indicating efficient extraction.[22] The extract displayed a characteristic garlic bulbs odour and brown colour, typical of ethanol extracts of garlic bulbs.

Water Content Testing of the Extract

Water content serves as a non-specific characterization parameter aimed at determining the amount of residual water present in the extract. A high moisture content may promote the growth of bacteria and fungi, which can degrade the chemical constituents of the extract and potentially reduce its biological activity during storage. Using a moisture analyzer, the garlic bulb extract demonstrated a moisture content of 6.71%. This value complied with the maximum limit specified in the Indonesian Herbal Pharmacopoeia, which requires that the moisture content of *Allium sativum* L. extract does not exceed 12%.[17,23]

Ethanol-Free Test

The ethanol-free test was conducted to confirm the absence of residual ethanol, which served as the extraction solvent for isolating antibacterial compounds from the garlic bulb extract. This assessment was essential to ensure that no remaining ethanol interfered with or produced false-positive outcomes in the antibacterial activity evaluation of the extract. Based on the procedure, the resulting garlic bulb extract was confirmed to be free of ethanol contamination and its suitability for antibacterial testing.

Phytochemical Screening Identification

The result of phytochemical screening identified secondary metabolites in the extract, are presented in Table 3. The results indicate that the 70% ethanol extract of garlic bulbs contain biologically active compounds, including alkaloids, phenols, flavonoids, saponins, and tannins, known for their antibacterial properties.[14] The presence of these compounds suggests potential for antibacterial activity, as supported by previous research.[24] Alkaloids disrupt bacterial cell wall synthesis, flavonoids inhibit membrane function, saponins damage cell membranes, and tannins inactivate essential bacterial enzymes, all contributing to their antibacterial effects.[25,26]

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ISSN: 2354-8770 (Electronic)

Table 3. Phytochemical Screening Identification

Secondary Metabolite	Reagent	Result	Description
Alkaloid	Dragendorff	+	A brick-red precipitate was formed
	Mayer	+	A white precipitate was formed
Phenol	FeCl3	+	A green color was formed
Flavonoid	Mg + HCl	+	An orange color was formed
Quinone	NaOH 1 N	-	No red color was formed
Saponin	HCl 1 N	+	Stable foam was formed
Steroid/Triterpenoid	Lieberman-Burchard	-	No blue to green or red to purple color was formed
Tannin	FeCl3 1%	+	A green color was formed

Explanation:
(+) = Detected
(–) = Not detected

Organoleptic Test

The organoleptic evaluation and stability test results for all three formulations of garlic bulb extract shampoo are presented in Table 4. Table 4. shows that the garlic bulb shampoo remained stable throughout all stability cycles, maintaining its brown liquid-gel form and peppermint aroma. Peppermint was added as a corrigen odoris to mask the strong sulfur-derived odor of *Allium sativum* L. No sedimentation was observed, in accordance with SNI 06-2692-1992, indicating good physical stability and homogeneous dispersion of the polar garlic extract within the shampoo base.[27]

Table 4. Organoleptic Evaluation of Garlic Bulb Shampoo

Formula	Organoleptic Parameter	Observation results Before Stability Testing	Observation results After Stability Testing (Cycle 1–6)
F1, F2, F3	Form Odour Colour	Liquid gel Peppermint Brown	Liquid gel Peppermint Brown

pH Test

The pH and stability test results for all three formulations of garlic bulb extract shampoo are presented in Table 5. The garlic bulb extract shampoos showed stable pH values throughout six stability cycles, ranging from 5.16 to 5.44. This indicates that the addition of Allium sativum L. extract did not affect shampoo pH during storage. Because the extract itself is slightly acidic (pH ~6.15), less citric acid was required to adjust the formulation. All formulations met both the natural scalp pH range (4.5–5.5) and the SNI requirement (pH 5.0–9.0). An acidic pH helps inhibit bacterial growth, as *Staphylococcus aureus* grows optimally at pH 7.0–7.5. Therefore, maintaining an acidic environment in the shampoo contributes to reducing bacterial proliferation.[28]

Table 5. pH Test Evaluation Results of Garlic Bulbs Extract Shampoo

Formula	Mean ± SD before stability test (n=3)	Mean ± SD after stability test (n=3)					
		Cycle1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
F1	5.44 ± 0.02	5.26 ± 0.04	5.20 ± 0.01	5.24 ± 0.01	5.22 ± 0.03	5.27 ± 0.02	5.33 ± 0.02
F2	5.31 ± 0.02	5.27 ± 0.01	5.16 ± 0.02	5.21 ± 0.02	5.34 ± 0.01	5.33 ± 0.02	5.34 ± 0.04
F3	5.36 ± 0.02	5.41 ± 0.01	5.40 ± 0.02	5.31 ± 0.02	5.30 ± 0.02	5.30 ± 0.02	5.30 ± 0.02

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ISSN: 2354-8770 (Electronic)

Foam Height Test

The foam height and stability test results for all three formulations of garlic bulb extract shampoo are presented in Table 6. The garlic bulb extract shampoo formulations showed a decrease in foam height from an initial average of 39–41 mm, with foam height continuing to decline until the 15th minute. This reduction indicates that the addition of *Allium sativum* L. extract influenced foam stability, likely due to interactions between saponin bioactive compounds and the surfactants in the formulation. All garlic bulb extract shampoo formulations remained within the SNI 06-2692-1992 foam height standard of 13–220 mm, indicating that the shampoos met the required foam performance criteria. Foam formation and stability are primarily governed by surfactant type and concentration, making their balance essential for optimal foam characteristics.

Viscosity Test

The viscosity and stability test results for all three formulations of garlic bulb extract shampoo are presented in Table 7.

Table 6. Foam Height Test Evaluation Results of Garlic Bulbs Extract Shampoo

Foam Height Test	Time (min)	Mean ± SD after stability test (mm) n=3		
		F1	F2	F3
Before stability test	**0**	39.4 ± 1.2	38.0 ± 1.8	35.8 ± 1.3
	5	35.8 ± 1.3	24.3 ± 1.2	25.3 ± 0.6
	15	27.2 ± 1.6	23.9 ± 0.8	22.3 ± 0.6
Cycle 1	**0**	40.5 ± 1.0	34.5 ± 1.0	38.9 ± 3.0
	5	37.1 ± 2.6	26.0 ± 0.8	29.9 ± 0.7
	15	27.8 ± 0.7	24.9 ± 0.7	27.9 ± 1.0
Cycle 2	**0**	38.9 ± 2.7	34.0 ± 0.9	34.4 ± 2.1
	5	33.8 ± 0.9	22.8 ± 1.6	31.9 ± 1.5
	15	28.8 ± 0.7	21.7 ± 0.7	29.1 ± 1.4
Cycle 3	**0**	41.3 ± 4.0	35.8 ± 1.5	32.9 ± 1.3
	5	34.7 ± 1.2	22.9 ± 1.2	30.1 ± 1.1
	15	28.7 ± 1.5	20.7 ± 1.3	28.0 ± 1.0
Cycle 4	**0**	51.4 ± 1.4	36.7 ± 2.0	32.4 ± 1.4
	5	35.3 ± 1.2	26.0 ± 1.0	29.3 ± 0.7
	15	27.7 ± 0.9	22.8 ± 1.2	27.3 ± 1.1
Cycle 5	**0**	42.8 ± 0.7	34.2 ± 2.3	34.0 ± 1.9
	5	35.5 ± 0.7	25.3 ± 2.5	30.0 ± 1.9
	15	27.7 ± 1.3	22.1 ± 1.5	27.8 ± 0.7
Cycle 6	**0**	47.0 ± 0.9	35.5 ± 0.9	33.7 ± 2.7
	5	32.9 ± 1.4	25.5 ± 0.8	31.1 ± 2.0
	15	27.3 ± 1.3	23.2 ± 1.3	28.2 ± 0.5

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Table 7. Viscosity Test Evaluation Results of Garlic Bulbs Extract Shampoo

Formula	Mean ± SD before stability test (n=3)	Mean ± SD after stability test (n=3)					
		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
F1	1208 ± 4.0	1414.6 ± 14.0	1440 ± 4.0	1424 ± 4.0	1420 ± 4.0	1446.6 ± 6.1	1429.3 ± 6.1
F2	1381.3 ± 8.3	1414.6 ± 6.1	1444 ± 4.0	1434.6 ± 8.3	1432 ± 4.0	1464 ± 4.0	1434.6 ± 6.11
F3	1345.3 ± 18.9	1473.3 ± 6.1	1465.3 ± 6.1	1476 ± 4.0	1488 ± 4.0	1546.7 ± 2.3	1556 ± 4.0

The addition of plant extract can increase shampoo viscosity.[29] Viscosity values before and after stability testing showed no significant changes under varying storage conditions, indicating that the garlic bulb extract shampoos remained within the viscosity range required by SNI.

Homogeneity Test

The homogeneity and stability test results for all three formulations of garlic bulb extract shampoo are presented in Table 8.

Table 8. Homogeneity Test Evaluation Results of Garlic Bulbs Extract Shampoo

Formulation	Observation results Before Stability Testing	Observation results After Stability Testing (Cycle 1–6)
F1	Homogeneous	Homogeneous
F2	Homogeneous	Homogeneous
F3	Homogeneous	Homogeneous

The homogeneity test showed that all garlic bulb extract shampoo formulations remained homogeneous throughout all stability cycles. This indicates that the addition of the extract did not affect the physical stability of the preparation. Homogeneity is essential because it ensures even distribution of all ingredients, which directly influences the consistency and effectiveness of the shampoo. A non-homogeneous preparation may cause uneven active ingredient concentration and reduced product performance. Therefore, confirming good homogeneity ensures that the shampoo maintains optimal quality and functionality.

Antibacterial Activity of Garlic Bulb Extract Shampoo

The antibacterial activity of the garlic bulb (*Allium sativum* L.) shampoo against *Staphylococcus aureus* was evaluated using the well diffusion method. Each well was filled with 50 µL of the test solution. The shampoo formulations contained garlic extract at concentrations of 6%, 8%, and 10%. Clindamycin 10% was used as the positive control, while the shampoo base served as the negative control to ensure that the observed antibacterial activity originated solely from the added active ingredient. The results of the antibacterial activity test are presented in Table 9.

Table 9 shows that the shampoo base exhibited antibacterial activity due to ingredients with inherent antimicrobial properties, such as sodium lauroyl sarcosinate, citric acid, and phenoxyethanol. Sodium lauroyl sarcosinate acts as an antibacterial surfactant, citric acid lowers pH to create an unfavorable environment for bacterial growth, and phenoxyethanol functions as a preservative with effective antimicrobial action. Together, these components

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ISSN: 2354-8770 (Electronic)

contribute to the base shampoo's ability to inhibit microbial growth.

Table 9. Antibacterial Activity Test Results of Garlic bulbs Extract Shampoo

Test Parameters	Inhibition Zone Diameter (mm) ± SD n=3	Category
F1	19.80 ± 0.87	Intermediate
F2	23.65 ± 0.30	Susceptible
F3	25.73 ± 0.38	Susceptible
Control (+)	35.67 ± 0.49	Susceptible
Control (-)	15.53 ± 0.69	Intermediate

Statistical analysis using the Shapiro–Wilk and Levene tests confirmed that the inhibition zone diameter data met the assumptions of normality ($p > 0.05$) and homogeneity of variance (Levene's test, $p = 0.157$). One-way ANOVA revealed a significant difference among the treatment groups ($F = 608.880$, $p < 0.001$), indicating that garlic bulb extract concentration significantly affected antibacterial activity. The mean inhibition zone diameters for FI, FII, and FIII were 19.80 ± 0.87 mm, 23.65 ± 0.30 mm, and 25.73 ± 0.38 mm, respectively. Furthermore, Tukey's post hoc test demonstrated significant differences among all groups ($p < 0.05$), including between FI and FII, FI and FIII, and FII and FIII, as well as between each formulation and both the positive and negative controls. These findings indicate a concentration-dependent increase in antibacterial activity against *Staphylococcus aureus* with increasing garlic bulb extract concentration. Nevertheless, although all formulations exhibited notable antibacterial effects, none produced an inhibition zone comparable to that of the positive control, clindamycin, which showed the greatest antibacterial activity.

Based on the inhibition zone diameters and interpreted using the CLSI classification, Formulation I (19.80 mm) showed intermediate inhibition, whereas Formulation II (23.65 mm) and Formulation III (25.73 mm) demonstrated susceptible antibacterial activity against *Staphylococcus aureus*. Formulation II was identified as the optimal formulation because it met all physical stability criteria and produced strong antibacterial effects while using a more efficient extract concentration than Formulation III. These findings confirm that garlic bulb extract can be developed into a stable herbal shampoo with notable antibacterial properties, supported by the presence of active compounds such as alkaloids, flavonoids, saponins, and tannins, likely contributes to this effect through mechanisms including disruption of cell walls, inhibition of membrane function, and enzyme inactivation.

CONCLUSION

This study shows that garlic bulb (*Allium sativum* L.) extract can be formulated into a stable shampoo that meets all required physical quality parameters. Among the three formulations tested, Formulation II (8%) demonstrated the best overall performance, as it maintained good physical stability and provided efficient extract use. The antibacterial assay revealed inhibition zone diameters of 19.80 mm (F1), 23.65 mm (F2), and 25.73 mm (F3). Based on the CLSI criteria, F1 showed intermediate inhibition, whereas F2 and F3 exhibited susceptible antibacterial activity against *Staphylococcus aureus*. Considering both stability and antibacterial effectiveness, Formulation II was identified as the optimal formulation.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the

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content of this article will be borne by them.

Acknowledgments

The authors would like to express their sincere gratitude to Centre for Research and Community Development Universitas Pelita Harapan (UPH) for providing financial support for this research through institutional funding no. P-030-S-FIKes/XII/2024. Appreciation is also extended to the Faculty of Medicine and Health Sciences, UPH, for facilitating laboratory access and technical assistance during the experimental work. We thank the staff of the Pharmacy Study Program for their guidance and support throughout the study. Special thanks to the resource persons and academic colleagues who contributed valuable insights and expertise, as well as all individuals and organizations involved in ensuring the successful completion of this research.

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