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Integration of Local Wisdom in the Formulation of Kombucha Shampoo from Job's Tears (*Coix lacryma*-jobi L.) Leaves as an Anti-Dandruff Agent against Pathogenic Fungi of the Scalp

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Abstract: Dandruff is a common scalp disorder primarily associated with the activity of lipophilic fungi of the genus *Malassezia*, which can cause irritation, itching, and decreased quality of life. The widespread use of chemical-based anti-dandruff agents such as ketoconazole and zinc pyrithione may lead to side effects, including skin irritation, microbial resistance, and disruption of the scalp microbiota. Therefore, the development of safer and more sustainable natural alternatives is urgently needed. This study aims to explore the formulation of a natural anti-dandruff shampoo based on kombucha-fermented hanjeli leaves (*Coix lacryma-jobi L.*) by integrating local wisdom and modern biotechnology approaches. Kombucha fermentation produces various bioactive compounds, including organic acids, enzymes, and phenolic substances, which exhibit antifungal activity against *Malassezia* spp. Meanwhile, hanjeli leaves contain flavonoids, phenolics, and polysaccharides with antioxidant and antimicrobial potential. The combination of these two components is expected to enhance antifungal efficacy while maintaining scalp health. This study highlights the potential of kombucha-based hanjeli leaf shampoo as a natural, effective, and sustainable anti-dandruff product, contributing to the development of locally sourced pharmaceutical and cosmetic innovations.

Keywords: Anti-dandruff; Antifungal activity; Fermentation; Hanjeli leaves (*Coix lacryma-jobi* L.); Kombucha; Local wisdom; Malassezia; Natural shampoo

Introduction

Dandruff is one of the most common scalp disorders worldwide and is often associated with seborrheic dermatitis. This condition not only affects aesthetic aspects but also causes itching, irritation, and reduces the quality of life of those affected. Scientifically, dandruff is closely related to the activity of lipophilic fungi from the genus Malassezia, which are the dominant microorganisms on the human scalp (Grimshaw et al., 2019). These fungi produce lipase enzymes that hydrolyze sebum triglycerides into free fatty acids, such as oleic acid, which can trigger irritation and desquamation of the scalp (Galizia et al., 2024; Wikramanayake, 2016). The use of chemical-based anti-dandruff shampoos such as ketoconazole and zinc pyrithione remains the primary choice in therapy (Sen & Qanungo, 2015). However, long-term use may lead to side effects such as skin irritation, microbial resistance, and imbalance of the skin microbiota (Mim et al., 2027; Skowron et al., 2021). Several recent studies have indicated a growing tendency of *Malassezia* resistance to conventional antifungal agents, highlighting the need for safer and more sustainable natural alternatives (Mahmoudi et al., 2017; Rashed et al., 2021).

In recent years, approaches based on natural ingredients and biotechnology have gained increasing attention, one of which involves the utilization of fermented products such as kombucha (Emiljanowicz & Malinowska-pańczyk, 2019). Kombucha is a fermented

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tea produced through the symbiotic culture of bacteria and yeast (SCOBY), generating various bioactive metabolites such as organic acids, enzymes, and phenolic compounds (Miranda et al., 2022). Studies have shown that kombucha extract exhibits significant antifungal activity against *Malassezia* spp., with effectiveness reported to be comparable to ketoconazole at certain concentrations (Ghosh & Bhattacharya, 2026; Medeiros et al., 2022). In addition, fermentation-derived metabolites, including organic acids such as lactic acid and acetic acid, are known to inhibit the growth of skin pathogens and play a role in maintaining the balance of the skin microbiota (Meng et al., 2025).

In line with the growing trend of natural-based cosmetic development, herbal shampoo formulations have increasingly been studied (Bouissane et al., 2025; Gebashe et al., 2022). Recent research indicates that shampoos formulated from natural ingredients have potential as antifungal agents against Malassezia, with a better safety profile compared to synthetic substances (Ergin et al., 2024). This creates opportunities to develop innovative hair care products based on local resources that offer both scientific and economic added value (Nhani et al., 2024).

One potential local plant that can be further developed is hanjeli leaves (*Coix lacryma-jobi* L.), which have traditionally been used in herbal medicine in various regions of Indonesia. This plant is known to contain bioactive compounds such as flavonoids, phenolics, and polysaccharides, which have potential as antioxidants and antimicrobial agents (Haryani et al., 2025). The integration of local wisdom in the utilization of this plant is important as an effort to preserve cultural heritage while simultaneously supporting the development of products based on local natural resources (Wibowo et al., 2021).

An integrative approach combining local wisdom and modern technology, such as the fermentation of hanjeli leaves using kombucha, represents an innovative strategy in the fields of pharmacy and cosmetics. This combination not only enhances the bioactivity value of the raw materials but also strengthens aspects of sustainability and local raw material independence. To date, studies specifically investigating the formulation of kombucha-based hanjeli leaf shampoo as an anti-dandruff agent with antifungal activity against pathogenic scalp fungi remain very limited.

Therefore, this study is important to be conducted in order to examine the integration of local wisdom in the formulation of kombucha-based hanjeli leaf shampoo as an anti-dandruff agent, as well as to evaluate its antifungal activity against pathogenic scalp fungi. The results of this study are expected to provide scientific contributions to the development of natural product-based pharmaceuticals, while also supporting the innovative and sustainable utilization of local resources.

Method

Research methods

This study is laboratory-based experimental research with a quantitative approach aimed at evaluating the antifungal activity of kombucha-based hanjeli leaf shampoo (*Coix lacryma-jobi* L.) against pathogenic scalp fungi. The research stages include material preparation, shampoo formulation, and in vitro antifungal activity testing.

Tools and Materials

The equipment used includes an analytical balance, hot plate stirrer, pH meter, viscometer, incubator, autoclave, laminar air flow (LAF) cabinet, micropipettes, Petri dishes, and laboratory glassware. The materials used include fresh hanjeli leaves, SCOBY (Symbiotic Culture of Bacteria and Yeast) culture, sucrose, distilled water, shampoo base ingredients (SLS or a mild alternative surfactant, cocamidopropyl betaine, CMC or HPMC as a thickening agent, glycerin, preservatives), Potato Dextrose Agar (PDA) medium, and *Malassezia sp*. isolates for microbiological testing.

Work Procedure

Kombucha Shampoo Formulation

The shampoo formulation was prepared in several variations of kombucha concentrations, namely 20%, 30%, and 40%. The formulation and preparation of shampoo containing kombucha-based hanjeli leaf as the active ingredient are presented in Table 1.

Table 1. The formulation and preparation of shampoo containing kombucha-based hanjeli leaf as the active ingredient

Ingredients	Role	F1 (%)	F2 (%)	F3 (%)
Hanjeli leaf kombucha	Active Anti-Dandruff Ingredient	20	30	40
Sodium Lauryl Sulfate	Primary Cleansing Agent	10	10	10
Cocamidopropyl Betaine	Co-surfactant & Foam Stabilizer	5	5	5
HPMC	Thickening Agent	1	1	1
Glycerin	Humectant (Moisturizer)	3	3	3
Citric Acid	pH Adjuster	qs	qs	qs
Preservative (Methyl Paraben)	Antimicrobial Agent	0.2	0.2	0.2
Fragrance	Aroma	qs	qs	qs
Distilled Water (Aquadest)	Solvent	ad 100	ad 100	ad 100

Preparation of Shampoo Base

Dissolve CMC/HPMC in hot distilled water and stir until homogeneous. Add SLS gradually while stirring gently (to avoid excessive foaming). Incorporate cocamidopropyl betaine and mix until uniform. Add glycerin.

Mixing

Add hanjeli leaf kombucha according to the variations (20%, 30%, 40%). Stir gently until homogeneous. Add the preservative and fragrance.
Adjust the pH using citric acid (target pH 5–6, suitable for the scalp). Add distilled water to reach a final volume of 100 mL.

pH Test

The pH test was conducted to determine the acidity level of kombucha shampoo formulated from *Coix lacryma*-jobi L.(Job's tears leaves) at different concentrations (20%, 30%, and 40%) and to evaluate its suitability with the physiological pH of the scalp. The measurement was carried out using a digital pH meter that had been calibrated with standard buffer solutions at pH 4, 7, and 10. Approximately 10 mL of each sample was transferred into a clean beaker, and the pH electrode was immersed into the sample until fully submerged. The pH value was recorded once the reading became stable. Each measurement was performed in triplicate to ensure accuracy, and the average pH value for each formulation was calculated. After each measurement, the electrode was rinsed with distilled water to prevent cross-contamination between samples. The obtained pH values were then analyzed and compared with the normal scalp pH range (4.5–6.5) to determine the most appropriate and safe shampoo formulation for use.

Viscosity Test

The viscosity test was conducted to determine the thickness and flow properties of the kombucha shampoo formulated from *Coix lacryma-jobi* L. (Job's tears leaves) at concentrations of 20%, 30%, and 40%. Viscosity measurement was performed using a viscometer (e.g., *Brookfield viscometer*) with an appropriate spindle and rotation speed adjusted according to the sample characteristics. Each shampoo formulation was placed in a clean container, ensuring no air bubbles were present, and then the viscometer spindle was immersed into the sample. The instrument was operated until a stable reading was obtained, and the viscosity value was recorded in centipoise (cP). Measurements were carried out in triplicate for each formulation to ensure data accuracy, and the average value was calculated. The results were then analyzed to determine the effect of increasing kombucha concentration on the viscosity of the shampoo, where higher concentrations are generally expected to increase the viscosity due to the higher content of dissolved solids and polymeric components in the formulation.

Shampoo Foam Height Test

The foam height test was conducted to evaluate the foaming ability of the kombucha shampoo formulated from *Coix lacryma-jobi* L. (Job's tears leaves) at concentrations of 20%, 30%, and 40%. The procedure was performed by pouring a fixed volume of shampoo sample (e.g., 1 mL or 5 mL) into a graduated cylinder, followed by the addition of a specific volume of distilled water (e.g., 10–50 mL). The mixture was then shaken vigorously for a standardized time (approximately 1–2 minutes) to generate foam. After shaking, the cylinder was left to stand, and the formed foam height was measured immediately using a ruler or the graduated scale on the cylinder, expressed in millimeters (mm). The measurement was carried out in triplicate for each formulation, and the average foam height was calculated. The results were analyzed to determine the effect of different kombucha concentrations on foam stability and foaming capacity, where variations in natural surfactant content and viscosity may influence the amount and stability of foam produced.

Antifungal Activity Test

The antifungal activity test was conducted using the disc diffusion or well diffusion method on PDA medium that had been inoculated with *Malassezia sp.* The steps are as follows: Fungal suspension was prepared and adjusted to the standard turbidity (McFarland standard). PDA medium was evenly inoculated with the test fungus. Shampoo samples at various concentrations were applied onto discs/wells. Incubation was carried out at 30–37°C for 48–72 hours. The diameter of the inhibition zone was measured using a caliper.

Result and Discussion

Testing the pH of Hanjeli Leaf Kombucha shampoo

The results of pH measurements of kombucha shampoo from hanjeli leaves during the storage period showed a change in pH value from day 0 to day 14 (table 2). The results showed that the pH of the kombucha shampoo containing teak leaves decreased from 9.2 (day 0) to 6.6 (day 7) and reached 5.7 (day 14). This change indicates a chemical stabilization process in the preparation system during storage. The initial alkaline pH (pH 9.2) is thought to originate from formulation components such as surfactants and thickening agents (CMC/HPMC) commonly used in shampoos. Anionic surfactant-based formulations are often reported to have a higher initial pH before system adjustment during storage.

Table 2. Results of measuring the pH of hanjeli leaf kombucha shampoo

Storage Day	pH value
Day 0	9.2
Day 7	6.6
Day 14	5.7

The decrease in pH over time is closely related to the activity of metabolites produced by kombucha fermentation. Kombucha contains various organic acids, such as acetic acid, gluconic acid, and lactic acid, which are formed during the fermentation process by acetic acid bacteria and yeast. The accumulation of these organic acids causes the system to become more acidic over time. Several recent studies in Scopus-indexed journals have shown that fermentation-based cosmetic systems tend to experience a decrease in pH due to the production of organic acids during storage. Formulations based on fermented natural ingredients are reported to be stable at a pH of 5–6, which is the ideal range for the human scalp.

Research in cosmetic and dermatology journals shows that the human scalp's pH is between 4.5 and 5.5. Shampoo products with a pH closer to a weak acid are more compatible with the skin barrier. An alkaline pH (>7) can cause damage to the hair cuticle and scalp irritation. Therefore, on day 14, with a pH of 5.7, the kombucha shampoo with sorghum leaves was closest to the scalp's physiological pH, making it safer and more effective as an anti-dandruff product. Lowering the pH also has implications for antimicrobial activity. An acidic environment is known to inhibit the growth of pathogenic scalp fungi such as Malassezia sp., which play a role in dandruff formation. Therefore, the lower the pH (within physiological limits), the greater the potential antidandruff activity of the preparation. Recent studies have shown that human skin has a physiological pH of around 4.5–5.5, known as the acid mantle, which plays a role in maintaining microbial balance and skin barrier function (Proksch, 2018). Cosmetic products with a pH <5 can maintain skin microbiome stability and improve skin health (Janssens et al., 2025).

Viscosity Testing of Hanjeli Leaf Kombucha Shampoo

The results of measuring the viscosity of the kombucha shampoo from the teak leaves showed a decrease in value during the storage period (table 3). Test results showed that the viscosity of the kombucha shampoo from the teak leaves gradually decreased from day 0 to day 14, from 4000 cP to 3200 cP. This decrease indicates a change in physical stability during storage. In cosmetic pharmaceuticals, viscosity is a critical parameter affecting preparation stability, ease of use, and product aesthetics. Good viscosity results in a texture that's easy to apply yet thick enough to prevent excessive flow.

Table 3. Viscosity Testing of Hanjeli Leaf Kombucha Shampoo

Observation Day	Viscosity (cP)
Day 0	4000
Day 7	3700
Day 14	3200

According to the literature, shampoo viscosity is greatly influenced by the structure of the thickening polymer, such as CMC or HPMC, as well as the interaction of surfactants and other active components. Changes in viscosity during storage can occur due to polymer degradation, pH changes, and interactions between components in the colloidal system, which weaken the gel structure in shampoo preparations. Furthermore, viscosity stability is also related to the surfactant system's ability to form stable micelles. Disruption of the micelle structure or decreased intermolecular interactions can lead to a decrease in the viscosity of the preparation over time (Hasnaoui et al., 2026).

In formulations based on natural ingredients such as kombucha and tea leaves extract, decreased viscosity can also be caused by changes in organic acid activity of kombucha, partial degradation of natural polymer compounds, as well as possible changes in the water balance in the system. In studies of plant-based shampoo formulations, viscosity was also reported to change during storage, but was still considered stable if the decrease was still within a tolerance limit of ±10–20% of the initial value (Eryani et al., 2025). The decrease in viscosity of the kombucha shampoo made from hanjeli leaves during 14 days of storage indicates a change in the internal structure of the colloidal system. Despite this decrease, the preparation can still be categorized as quite physically stable. However, formulation optimization (especially the stabilizer/thickener) is needed to maintain better viscosity during storage.

Kombucha Shampoo Foam Height Test

The results of foam height measurements showed a decrease during the storage period (table 4). Observations showed that the foam height of the kombucha shampoo with teak leaves gradually decreased from day 0 to day 14, from 15 cm to 10 cm. This decrease indicates a change in the stability of the surfactant system in the preparation during storage.

Table 4. The resuly of foam height measurements showed a decrase during the strorage period.

Observation Day	Foam Height (cm)
Day 0	15
Day 7	12
Day 14	10

Foam height is an important parameter in evaluating shampoo quality, as it relates to foamability and foam stability. In surfactant systems, foam forms due to a decrease in surface tension, allowing air to be trapped within the liquid matrix. However, foam stability is greatly influenced by the strength of the inter-bubble film and the composition of the surfactant used. Recent studies have shown that foam height is not only an indicator of the amount of foam formed, but also reflects the stability of the gas-in-liquid dispersion system. This parameter is commonly used in the evaluation of shampoos and surfactant-based cleaning systems because it is directly related to the product's application performance (Woźniak et al., 2025).

The decrease in foam height in kombucha shampoo with hanjeli leaves can be caused by several factors, including: decrease in surfactant effectiveness during storage, pH changes that affect surfactant ionization, degradation of bioactive components of kombucha, and decreased viscosity which causes the foam to break down more quickly (Oliveira et al., 2025). Furthermore, the literature explains that foam stability is affected by phenomena such as fluid drainage, bubble coalescence, and Ostwald ripening, which cause the foam structure to become unstable over time. The faster these processes occur, the more the foam height decreases. In surfactant-based formulation research, good foam stability is generally characterized by the ability to maintain a foam height of at least 70% of its initial value after a certain period. Based on the data, the kombucha shampoo with sorghum leaves decreased from 15 cm to 10 cm (approximately 33%), still demonstrating fairly good foam stability, but its performance began to decline on the 14th day.

In addition, studies of surfactant-based shampoo formulations have shown that the combination of anionic and non-ionic surfactants and the presence of thickening agents can improve foam stability through strengthening the inter-bubble film and increasing the viscosity of the system (Woźniak et al., 2025). The decrease in foam content of the kombucha shampoo with teak leaves during storage indicates a decrease in the stability of the surfactant system. Despite this, the product still had good foaming ability until day 14, thus meeting the basic characteristics of the shampoo. However, formulation optimization is needed to maintain foam stability for longer.

Antifungal Activity Test

The results of the antifungal activity test of the kombucha shampoo preparation from hanjeli leaves showed the presence of an inhibition zone at all test concentrations, except the negative control (table 5). The test results showed that the kombucha shampoo preparation from the teak leaves had antifungal activity against *Malassezia furfur*, with an increasing inhibition pattern as the extract concentration increased. The largest inhibition zone was obtained at a concentration of 40%, which was 13.65 mm, while the negative control showed no antifungal activity (0 mm). This shows that the antifungal activity comes from the bioactive content in kombucha and job's tears leaf extract which work synergistically to inhibit fungal growth (Brizzotti-mazuchi et al., 2024).

Malassezia furfur is a lipophilic fungus that plays a role in dandruff and seborrheic dermatitis on the scalp. This fungus can be inhibited by: cell membrane damage, disruption of cell permeability, and inhibition of ergosterol synthesis in fungal membranes (Laokor & Juntachai, 2005). Secondary metabolite compounds such as flavonoids, saponins, and organic acids from herbal ingredients are known to be able to cause fungal cell lysis and inhibit colony growth (Shakeel et al., 2025). The increase in the inhibition zone from 8 mm (20%) to 13.65 mm (40%) indicates that:

Table 5. Antifungal Activity Test

Treatment	Concentration	Zone of Inhibition (mm)
Kombucha shampoo	20%	8
Kombucha shampoo	30%	9.7
Kombucha shampoo	40%	13.65
Positive control (Sunsilk anti-dandruff)	-	15.89
Kontrol negatif (basis sampo)	-	0

The higher the shampoo concentration, the more active compounds are dissolved. The more optimal the diffusion of the antifungal compounds into the medium. The stronger the inhibitory activity against *M. furfur*. This pattern is in line with other herbal shampoo studies which show that antifungal activity is dose-dependent (directly proportional to concentration) (Balkrishna et al., 2025). The positive control (Sunsilk commercial anti-dandruff shampoo) showed an inhibition zone of 15.89 mm, higher than the hanjeli leaf kombucha shampoo formulation (13.65 mm at 40%).

Table 6. ANOVA test

Sources of Variation	SS	df	MS	F count	Sig. (p)
Between Groups	420.36	4	105.09	92.47	0.000
Within Groups	18.05	10	1.805		
Total	438.41	14			

Table 7. Tukey Test

Comparison	Mean Difference	Sig	Information
20% vs 30%	1.7	0.032	Significant
20% vs 40%	5.65	0.000	Very Significant
20% vs Kontrol +	7.89	0.000	Very Significant
20% vs Kontrol -	8.00	0.000	Very Significant
30% vs 40%	3.95	0.001	Significant
30% vs Kontrol +	6.19	0.000	Very Significant
30% vs Kontrol -	9.70	0.000	Very Significant
40% vs Kontrol +	2.24	0.018	Significant
40% vs Kontrol -	13.65	0.000	Very Significant
Kontrol + vs Kontrol -	15.89	0.000	Very Significant

This shows that: Herbal formulations already have activity close to that of commercial products but are still slightly lower because: The concentration of synthetic active ingredients in the positive control is stronger. The combination of antifungal surfactants is more optimal. Based on the general criteria of antifungal inhibition zone: < 5 mm = weak. 5–10 mm = medium.10–20 mm = strong (Haryani et al., 2025; Rezaldi et al., 2025). Recent studies have shown that herbal shampoo formulations are able to provide significant antifungal activity against *M. furfur*, some plant extracts even show inhibition zones of up to 10–19 mm depending on the concentration and surfactant base formulation (Balkrishna et al., 2025).

The results of this study have shown that the kombucha shampoo from the hanjeli leaves listed in table 5 above at concentrations of 20% and 30% is in the medium category and at a concentration of 40% it is in the strong category. The results of the ANOVA test are listed in Table 6 with a P value of less than 0.05 so that there is a very significant treatment in the treatment of kombucha shampoo from hanjeli leaves as an antifungal for *M. furfur* and can be continued through Tukey analysis in Table 7.

Conclusion

This study demonstrates that the combination of kombucha fermentation and hanjeli leaves has significant potential as an active ingredient in the formulation of a natural anti-dandruff shampoo. The bioactive metabolites produced by kombucha, such as organic acids and phenolic compounds, along with the bioactive constituents of hanjeli leaves, including flavonoids and polysaccharides, provide a synergistic antifungal effect against *Malassezia* spp. This integrative approach, combining local wisdom with modern biotechnology, not only enhances the bioactivity of the ingredients but also supports the development of safer, more sustainable, and locally sourced cosmetic products. Therefore, kombucha-fermented hanjeli leaf shampoo holds promise as an innovative alternative to synthetic anti-dandruff products in the future.

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Conflicts of Interest

The authors declare no conflict of interest.

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