



Formulation and Evaluation of *Centella asiatica* (L.) Urb. Leaf Extract Shampoo: Physicochemical Characteristics and In Vitro Antifungal Activity

Formulasi dan Evaluasi Sampo Ekstrak Daun Pegagan (*Centella asiatica* (L.) Urb.): Karakteristik Fisikokimia dan Aktivitas Antijamur secara In Vitro

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Abstract

Dandruff is a common scalp disorder associated with alterations in scalp microbiota, including the presence of *Malassezia furfur*. The prolonged use of synthetic antifungal agents may cause adverse effects, highlighting the need for plant-based alternatives. This study aimed to formulate a shampoo containing *Centella asiatica* (L.) Urb. leaf extract and to evaluate its physicochemical characteristics and in vitro antifungal activity. *Centella asiatica* leaves were extracted by maceration using 96% ethanol, n-hexane, and ethyl acetate. The extract exhibiting the strongest antifungal activity was incorporated into three shampoo formulations, which were evaluated for organoleptic properties, pH, viscosity, foam height, specific gravity, moisture content, consumer preference, and in vitro antifungal activity using the agar diffusion method. The ethyl acetate extract produced inhibition zones of 12 mm against *Candida albicans* and 11 mm against *Malassezia furfur*. All shampoo formulations exhibited pH values ranging from 6.42 to 6.95, viscosities of 1438.67–1577.33 cP, foam heights of 4.0–4.2 cm, and specific gravity values of 1.0220–1.0288. The formulations produced inhibition zones of 16.7–19.7 mm against *Candida albicans* and 17.3–20.7 mm against *Malassezia furfur*. These findings demonstrate that *Centella asiatica* leaf extract can be formulated into a shampoo with acceptable physicochemical characteristics and measurable in vitro antifungal activity, supporting its potential for further development as a herbal shampoo formulation.

Abstrak

Ketombe adalah gangguan kulit kepala umum yang dikaitkan dengan perubahan mikrobiota kulit kepala, termasuk keberadaan *Malassezia furfur*. Penggunaan agen antijamur sintesis dalam jangka panjang dapat menimbulkan efek samping, sehingga memunculkan kebutuhan akan alternatif berbasis tanaman. Penelitian ini bertujuan untuk memformulasikan sampo yang mengandung ekstrak daun *Centella asiatica* (L.) Urb. serta mengevaluasi karakteristik fisikokimia dan aktivitas antijamur *in vitro*-nya. Daun *Centella asiatica* diekstraksi melalui metode maserasi menggunakan etanol 96%, n-heksana, dan etil asetat. Ekstrak yang menunjukkan aktivitas antijamur terkuat diformulasikan ke dalam tiga sediaan sampo, yang kemudian dievaluasi meliputi sifat organoleptik, pH, viskositas, tinggi busa, bobot jenis, kadar air, preferensi konsumen, dan aktivitas antijamur *in vitro* menggunakan metode difusi agar. Ekstrak etil asetat menghasilkan zona hambat sebesar 12 mm terhadap *Candida albicans* dan 11 mm terhadap *Malassezia furfur*. Seluruh formulasi sampo menunjukkan nilai pH berkisar antara 6,42 hingga 6,95, viskositas 1438,67–1577,33 cP, tinggi busa 4,0–4,2 cm, dan nilai bobot jenis 1,0220–1,0288. Formulasi tersebut menghasilkan zona hambat sebesar 16,7–19,7 mm terhadap *Candida albicans* dan 17,3–20,7 mm terhadap *Malassezia furfur*. Temuan ini menunjukkan bahwa ekstrak daun *Centella asiatica* dapat diformulasikan menjadi sampo dengan karakteristik fisikokimia yang dapat diterima dan aktivitas antijamur *in vitro* yang terukur, sehingga mendukung potensinya untuk pengembangan lebih lanjut sebagai formulasi sampo herbal.

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INTRODUCTION

Dandruff is one of the most prevalent scalp disorders affecting individuals across all age groups and remains a common dermatological concern worldwide. Excessive desquamation of the stratum corneum characterizes it and causes itching, irritation, and scalp discomfort. Although dandruff is not a life-threatening condition, it considerably affects patients' quality of life by impairing their physical appearance, reducing their self-confidence, and causing psychosocial distress. Owing to its high prevalence and recurrent nature, dandruff continues to represent a significant challenge in dermatological care, emphasizing the need for effective and safe long-term therapeutic strategies^{1,2}.

The pathogenesis of dandruff is multifactorial and involves complex interactions among sebum production, skin barrier integrity, scalp microbiota, and host immune responses. Among these contributing factors, colonization by lipophilic yeasts of the genus *Malassezia*, particularly *Malassezia furfur*, has consistently been associated with dandruff and seborrheic dermatitis. *Malassezia furfur* hydrolyzes sebum lipids through lipase activity, producing free fatty acids that disrupt the epidermal barrier, trigger inflammatory responses, and accelerate epidermal cell turnover, ultimately leading to the accumulation of visible scalp scales^{1,3,4}.

In addition to *Malassezia furfur*, *Candida albicans* has also been reported to colonize the scalp, particularly in individuals with microbial dysbiosis, altered scalp pH, or impaired immune function. However, its involvement in dandruff is less prominent than that of *Malassezia furfur*; *Candida albicans* may aggravate scalp inflammation and contribute to opportunistic fungal infections that delay the recovery of scalp health. Therefore, developing topical formulations capable of inhibiting both *Malassezia furfur* and *Candida albicans* may provide broader antifungal protection and improve the therapeutic effectiveness of antidandruff products³⁻⁵.

Current dandruff management primarily relies on synthetic antifungal agents, including ketoconazole, zinc pyrithione, selenium sulfide, ciclopirox olamine, and piroctone olamine. These compounds effectively inhibit fungal growth through different mechanisms, mainly by disrupting ergosterol biosynthesis and compromising fungal cell membrane integrity. Nevertheless, prolonged use of these agents has been associated with adverse effects such as scalp irritation, excessive dryness, alterations in hair texture, and the potential development of reduced fungal susceptibility. These limitations have stimulated increasing interest in plant-derived bioactive compounds as safer and more sustainable alternatives for scalp care owing to their multiple biological activities and favorable safety profiles^{1,4,6}.

Indonesia possesses remarkable biodiversity and represents an important source of medicinal plants for developing herbal pharmaceuticals and cosmetic products. Among these plants *Centella asiatica* (L.) Urban has been extensively used in traditional medicine for wound healing, inflammatory disorders, and various skin diseases. Recent pharmacological studies have demonstrated that *Centella asiatica* exhibits antioxidant, anti-inflammatory, antibacterial, antifungal, and immunomodulatory activities, indicating its considerable potential as an active ingredient in topical pharmaceutical and cosmeceutical formulations⁶⁻⁹.

The therapeutic potential of *Centella asiatica* is mainly attributed to its rich phytochemical composition, particularly pentacyclic triterpenoids such as asiaticoside, madecassoside, asiatic acid, and madecassic acid, together with flavonoids, tannins, phenolic compounds, alkaloids, and glycosides. These bioactive constituents exhibit antifungal activity through multiple mechanisms, including disruption of fungal cell membrane integrity, inhibition of ergosterol biosynthesis, increased membrane permeability, and suppression of essential metabolic enzymes. In addition to their antifungal effects, several of these compounds possess anti-inflammatory properties that may contribute to improving scalp health, making *Centella asiatica* a promising candidate for developing multifunctional herbal antidandruff formulations^{6–8,10}.

Recent studies have demonstrated that *Centella asiatica* possesses broad-spectrum antimicrobial and antifungal activities against various pathogenic microorganisms. However, most published investigations have primarily focused on the in vitro antifungal activity of crude extracts, the isolation and characterization of bioactive constituents, or the application of *Centella asiatica* in wound healing, anti-inflammatory, and antioxidant therapies. Comparatively, few studies have explored its incorporation into topical cosmetic formulations, particularly herbal antidandruff shampoos. Furthermore, existing studies generally evaluate antifungal activity against a single fungal species, providing limited evidence regarding the efficacy of *Centella asiatica* against *Malassezia furfur*, the dandruff-associated yeast, and *Candida albicans*, an opportunistic fungal pathogen that may exacerbate scalp disorders. Simultaneous evaluation against these two biologically distinct fungal species would provide a more comprehensive understanding of the antifungal spectrum of *Centella asiatica* and its potential application in scalp care products^{7,9,11–13}. Studies using different extraction approaches have also documented the antimicrobial or antifungal activity of *Centella asiatica* extracts against pathogenic microorganisms^{13–19}.

The successful development of an herbal antidandruff shampoo depends not only on the antifungal activity of its active ingredient but also on the physicochemical quality and consumer acceptability of the final formulation. A shampoo must exhibit appropriate organoleptic characteristics, pH, viscosity, foamability, specific gravity, and stability to ensure product safety, effectiveness, and user satisfaction. Therefore, comprehensive physicochemical evaluation is an essential component of cosmetic product development because these parameters directly influence product performance, storage stability, and consumer preference^{20–22}. Consequently, evaluating both biological activity and formulation quality is necessary to assess the feasibility of developing a herbal antidandruff shampoo for further investigation. Previous topical and herbal-shampoo studies likewise emphasize physicochemical characterization and product performance^{20,21,23}.

Despite the growing body of evidence supporting the pharmacological potential of *C. asiatica*, several important research gaps remain. Most previous studies have focused exclusively on extract screening or phytochemical characterization without integrating extract selection based on antifungal activity, shampoo formulation, physicochemical evaluation, consumer acceptability testing, and antifungal assessment against both *Malassezia furfur* and *Candida albicans* within a single experimental framework. As a result, scientific evidence supporting the development of *C. asiatica*-based herbal antidandruff shampoos remains limited.

The novelty of the present study lies in the integration of systematic extract selection, formulation development, physicochemical characterization, hedonic evaluation, and dual-species antifungal assessment into a comprehensive product development approach. Unlike previous investigations that mainly evaluated the biological activity of *Centella asiatica* extracts, this study employed the ethyl acetate extract selected based on its antifungal activity as the active ingredient in a shampoo formulation and subsequently evaluated both formulation quality and

antifungal performance. This integrated strategy provides a scientific basis for developing plant-based antidandruff shampoos derived from Indonesian medicinal plants.

Based on these considerations, this study aimed to formulate a herbal shampoo containing *Centella asiatica* (L.) Urban leaf extract, evaluate its physicochemical characteristics and consumer acceptability, and determine its antifungal activity against *Malassezia furfur* and *Candida albicans*. The findings are expected to contribute scientific evidence supporting the development of safe, effective, and high-quality herbal antidandruff shampoos as alternative scalp care products.

METHODS

Tools and Materials

Research Procedure

Study Design

The study included 30 participants for the hedonic evaluation. This laboratory-based experimental study was conducted to develop a herbal shampoo containing *Centella asiatica* (L.) Urb. Leaf extract and to evaluate its physicochemical characteristics and antifungal activity against *Malassezia furfur* and *Candida albicans*. The experimental workflow consisted of plant material authentication and characterization, extraction, phytochemical screening, antifungal screening of the extracts, shampoo formulation, physicochemical evaluation, antifungal evaluation of the formulations, hedonic assessment, and statistical analysis.

Materials

Plant Material

Dried *Centella asiatica* (L.) Urb. leaves were obtained from the Center for Research and Development of Medicinal Plants and Traditional Medicine (B2P2TOOT), Tawangmangu, Central Java, Indonesia. Botanical authentication was performed at the same institution before the experimental study.

Chemicals and Reagents

Analytical-grade ethanol (96%), ethyl acetate, *n*-hexane, hydrochloric acid, ferric chloride, sodium hydroxide, picric acid, glacial acetic acid, chloroform, distilled water, dimethyl sulfoxide (DMSO), carboxymethyl cellulose (CMC), Tween, betaine, cocodiethanolamide, sodium benzoate, citric acid, and Sabouraud Dextrose Agar (Merck) were used throughout the study. Ketoconazole (30 µg/mL) served as the positive control.

Test Microorganisms

Reference cultures of *Malassezia furfur* and *Candida albicans* were used for antifungal evaluation.

Experimental Procedures

Step I. Plant Material Preparation and Characterization

The dried leaves of *Centella asiatica* were subjected to botanical authentication before processing. Quality evaluation of the crude drug included macroscopic, microscopic, organoleptic, moisture content, ash content, and preliminary phytochemical characterization according to standard pharmacognostic procedures. These analyses were performed to ensure the identity and quality of the plant material before extraction.

Step II. Extraction Procedure

Approximately 250 g of powdered *Centella asiatica* leaves were extracted separately by maceration using 96% ethanol, ethyl acetate, and *n*-hexane as extraction solvents. Each extraction was carried out at room temperature for 48 h with occasional stirring. The filtrates were collected by filtration and concentrated under reduced pressure

using a rotary evaporator at approximately 50°C to obtain viscous extracts. Extraction yield was calculated as the percentage of dried extract relative to the initial weight of powdered plant material.

Step III. Phytochemical Screening

Qualitative phytochemical screening was performed on each extract according to the Harborne method to identify the major classes of secondary metabolites, including alkaloids, flavonoids, tannins, phenolic compounds, steroids, terpenoids, saponins, and glycosides. The phytochemical profile was used to support the interpretation of the observed antifungal activity of the extracts.

Step IV. Antifungal Activity Screening of the Extracts

The antifungal activity of the *Centella asiatica* extracts was evaluated against *Malassezia furfur* and *Candida albicans* using the agar well diffusion method. Fungal suspensions were prepared at approximately 1×10^6 CFU/mL and inoculated onto Sabouraud Dextrose Agar (SDA) plates. Wells were filled with each extract solution, while ketoconazole (30 µg/mL) and dimethyl sulfoxide (DMSO) served as the positive and negative controls, respectively. Ketoconazole (30 µg/mL) was used as the positive control and reference for evaluating the antifungal activity of the extracts and shampoo formulations. This concentration was selected based on a published agar diffusion study that used ketoconazole at 30 µg/mL as a standard antifungal control against *Candida albicans* [26]. Following incubation under appropriate conditions, the inhibition zone diameters were measured in millimeters (mm). All experiments were performed in triplicate. The extract exhibiting the greatest antifungal activity was selected as the active ingredient for the shampoo formulation.

Step V. Shampoo Formulation

The extract selected from the antifungal screening was incorporated into three shampoo formulations (F1, F2, and F3). The shampoo base consisted of carboxymethyl cellulose (CMC), Tween, betaine, cocodiethanolamide, sodium benzoate, citric acid, lavender fragrance, and distilled water. The concentrations of ethyl acetate extract were 5%, 10%, and 15% (w/v) for F1, F2, and F3, respectively.

Dispersing CMC in hot water until a homogeneous gel formed initiated the shampoo preparation process. The extract was then mixed with Tween, betaine, and cocodiethanolamide before being gradually incorporated into the CMC dispersion under continuous stirring. Sodium benzoate was added as a preservative, and the pH was adjusted using a citric acid solution to obtain a scalp-compatible pH. Finally, lavender fragrance, and distilled water were added to achieve the desired final volume.

Step VI. Physicochemical Evaluation of Shampoo Formulations

All shampoo formulations were evaluated for physicochemical quality using standard cosmetic evaluation procedures. The evaluated parameters included organoleptic characteristics (color, odor, and appearance), pH, viscosity, foam height, specific gravity, and moisture content.

The pH of each formulation was measured using a calibrated pH meter after an appropriate dilution with distilled water. Viscosity was determined using a Brookfield LV viscometer, whereas specific gravity was measured using a pycnometer. Foam height was evaluated using the shaking method, and moisture content was determined gravimetrically until a constant weight was obtained. These evaluations were performed to assess formulation quality, stability, and suitability for topical scalp application.^{20–23}

Step VII. Antifungal Activity of Shampoo Formulations

The antifungal activity of the shampoo formulations was evaluated against *Malassezia furfur* and *Candida albicans* using the agar well diffusion method, following the same procedure described for the extract screening.

Ketoconazole (30 µg/mL) was used as the positive control, whereas the shampoo base without *C. asiatica* extract served as the negative control. Following incubation, the diameters of the inhibition zones were measured in millimeters (mm) to determine the antifungal activity of each formulation. All experiments were conducted in triplicate.

Step VIII. Hedonic Evaluation

Consumer acceptability of the shampoo formulations was assessed through a hedonic evaluation involving 30 participants. Each participant evaluated the three formulations based on color, odor, and texture using a structured evaluation form. The hedonic assessment was conducted to determine the level of consumer preference for each formulation.

Data Analysis

Hedonic test results for color, aroma, and texture were analyzed using the Kruskal–Wallis test at a 95% confidence level ($\alpha = 0.05$).

RESULT AND DISCUSSION

Characterization of *Centella asiatica* (L.) Urb. Simplicia

Table 1. Pharmacognostic characteristics of *Centella asiatica* (L.) Urb. simplicia

Parameter	Observation
Macroscopic appearance	Curled and wrinkled leaf lamina
Color	Dark green
Odor	Aromatic
Taste	Bitter
Microscopic characteristic	Rosette-shaped calcium oxalate crystals
Powder characteristics	Green powder with characteristic herbal odor
Moisture content	0.82%
Total ash content	0.07%

As presented in Table 1, the simplicia exhibited the characteristic pharmacognostic features of *C. asiatica*, including dark green leaves with an aromatic odor and bitter taste. Microscopic examination revealed rosette-shaped calcium oxalate crystals, which are recognized as one of the diagnostic characteristics of *C. asiatica*. These findings confirm the botanical identity of the plant material and indicate that the raw material used in this study was suitable for subsequent extraction and formulation processes. Similar pharmacognostic characteristics have been reported in previous studies describing the morphological and microscopic features of *Centella asiatica*^{6,8,9}.

The moisture content of the simplicia was 0.82%, indicating adequate drying before extraction. Low moisture content is essential for minimizing microbial contamination during storage and preserving the stability of bioactive secondary metabolites. Likewise, the total ash content (0.07%) indicates low level of inorganic impurities, reflecting the good quality of the crude plant material. Moisture and ash contents are widely recognized as important quality control parameters for medicinal plants because they contribute to the reproducibility and consistency of herbal products^{6–8}.

Overall, the pharmacognostic evaluation demonstrated that *Centella asiatica* simplicia met the quality requirements for herbal raw materials and provided a reliable basis for extraction and subsequent shampoo formulation.

Extraction Yield and Phytochemical Profile

Table 2. Extraction yield of the *C asiatica* leaf extracts

Solvent	Initial weight (g)	Extract weight (g)	Yield (%)
96% Ethanol	250.021	35.020	14.01
<i>n</i> -Hexane	250.018	11.183	4.47
Ethyl acetate	250.040	20.183	8.07

The extraction yields obtained using solvents with different polarities are summarized in Table 2. Ethanol produced the highest extraction yield (14.01%), followed by ethyl acetate (8.07%) and *n*-hexane (4.47%). The higher yield obtained with ethanol is attributable to its ability to dissolve both polar and moderately polar phytochemicals, whereas the non-polar nature of *n*-hexane limits the extraction primarily to lipophilic compounds. Ethyl acetate, a semipolar solvent, selectively extracts compounds such as aglycone flavonoids, phenolic constituents, and certain triterpenoids that have been associated with antifungal activity^{6-8,11,17,18,24,25}.

Although the ethanol extract produced the highest yield, the ethyl acetate extract was selected for formulation because it exhibited superior antifungal activity during the preliminary screening. This finding indicates that extraction yield alone is not an appropriate indicator of biological effectiveness. Instead, the qualitative composition and concentration of bioactive constituents play a more important role in determining the pharmacological potential of herbal extracts. Similar observations have been reported in studies demonstrating that semipolar fractions often possess stronger antimicrobial activity despite lower extraction yields^{11,15,16,20,21}.

Table 3. Qualitative phytochemical profile of the ethyl acetate extract of *C. asiatica*

Secondary metabolite	Result
Alkaloids	+
Flavonoids	+
Phenolic compounds	+
Tannins	+
Terpenoids	+
Steroids	+
Glycosides	+
Saponins	–

As shown in **Table 3**, qualitative phytochemical screening demonstrated the presence of alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, steroids, and glycosides, whereas saponins were not detected. The phytochemical profile indicates that ethyl acetate effectively extracted semipolar bioactive constituents commonly associated with antimicrobial and antifungal activities. These results are consistent with previous reports describing *Centella asiatica* as a rich source of triterpenoids, flavonoids, and phenolic compounds with diverse pharmacological properties^{6-8,14}.

The antifungal activity observed in this study is likely attributable to the synergistic effects of multiple secondary metabolites rather than the action of a single compound. Flavonoids and phenolic compounds have been reported to disrupt fungal membrane integrity and inhibit essential metabolic pathways, whereas triterpenoids such as asiaticoside and madecassoside may interfere with ergosterol-associated membrane function. The coexistence of these bioactive constituents may explain the antifungal activity demonstrated by the ethyl acetate extract despite the absence of detectable saponins.

Compared with studies focusing solely on phytochemical identification, the present study further linked the phytochemical profile with biological screening and extract selection for formulation development. This integrated approach strengthens the rationale for selecting the ethyl acetate extract as the active ingredient in the herbal shampoo and provides a more comprehensive basis for subsequent formulation and efficacy evaluation.

Antifungal Activity of the *Centella asiatica* Leaf Extracts

Table 4. Antifungal activity of *Centella asiatica* leaf extracts against *Candida albicans* and *Malassezia furfur*

Treatment	<i>Candida albicans</i> (mm)	<i>Malassezia furfur</i> (mm)
Positive control (ketoconazole)	36	42
Negative control (DMSO)	0	0
Ethyl acetate extract	12	11
96% Ethanol	7	6
<i>n</i> -Hexane	6	7

As presented in Table 4, the ethyl acetate extract showed the largest inhibition zones among the three extracts, producing inhibition zones of 12 mm against *Candida albicans* and 11 mm against *Malassezia furfur*, compared with 7 and 6 mm for the 96% ethanol extract and 6 and 7 mm for the *n*-hexane extract, respectively. In contrast, the negative control (DMSO) produced no inhibition, confirming that the observed antifungal activity originated from the plant extract rather than the solvent. As expected, ketoconazole exhibited substantially larger inhibition zones owing to its well-established fungicidal activity.

Although the inhibition zones produced by the extract were smaller than those of ketoconazole, the results clearly demonstrate that the ethyl acetate extract possesses intrinsic antifungal activity. This finding supports the selection of the ethyl acetate extract as the active ingredient for shampoo formulation. Similar observations have been reported in previous studies, where semi-polar fractions of medicinal plants frequently exhibited stronger antifungal activity than highly polar extracts because of their enrichment with bioactive flavonoids, phenolic compounds, and triterpenoids^{6-8,11}.

The antifungal activity observed in the present study is likely associated with the synergistic action of the phytochemicals identified during qualitative screening. Rather than acting through a single molecular target, flavonoids, phenolic compounds, tannins, and triterpenoids collectively disrupt fungal membrane integrity, interfere with ergosterol biosynthesis, and impair essential metabolic pathways. Such synergistic interactions have been proposed as one of the major advantages of plant-derived antifungal agents because they reduce the likelihood of fungal adaptation to a single mechanism of action^{6,7,10,12}.

An important finding of this study is that the extract demonstrated inhibitory activity against both *Candida albicans* and *Malassezia furfur*. While *Malassezia furfur* is one of the microorganisms associated with dandruff and seborrheic dermatitis, *Candida albicans* may colonize the scalp under conditions of microbial imbalance and contribute to opportunistic infections. Therefore, the observed antifungal activity against both fungal species supports further investigation of *Centella asiatica* as a potential active ingredient in topical scalp-care formulations.

Overall, these findings demonstrate that the ethyl acetate extract of *Centella asiatica* possesses sufficient antifungal activity to justify its incorporation into a topical shampoo formulation for subsequent evaluation.

Physicochemical Characteristics of Shampoo Formulations

Table 5. Physicochemical characteristics of the *Centella asiatica* shampoo formulations

Parameter	F1	F2	F3
Appearance	Lotion	Lotion	Lotion
Color	Light green	Moss green	Dark green
Odor	Lavender	Lavender	Lavender
pH	6.95	6.42	6.62
Foam height (cm)	4.1	4.0	4.2

Viscosity (cP)	1569.33	1438.67	1577.33
Specific gravity	1.0288	1.0220	1.0242
Moisture content (%)	0.82	0.79	0.86

As summarized in **Table 5**, all shampoo formulations exhibited acceptable physicochemical characteristics. No marked differences in appearance or odor were observed among the formulations, whereas the color became progressively darker with increasing extract concentration. The gradual color change is expected because higher concentrations of *Centella asiatica* extract contribute greater amounts of natural chlorophyll and phenolic pigments without adversely affecting formulation homogeneity.

The pH values ranged from 6.42 to 6.95, which fall within the acceptable range for scalp application. Maintaining a pH close to that of the scalp is important for preserving skin barrier integrity, minimizing irritation, and maintaining microbial homeostasis. Comparable pH values have been reported for herbal shampoo formulations containing plant extracts intended for routine scalp care^{20–22}.

Viscosity values ranged from 1438.67 to 1577.33 cP, indicating that incorporation of the ethyl acetate extract did not adversely affect the rheological properties of the shampoo base. The relatively uniform viscosity among formulations indicates satisfactory compatibility between the extract and formulation excipients. Appropriate viscosity contributes to product stability, ease of application, and prolonged contact of the active ingredients with the scalp surface.

Foam heights varied only slightly (4.0–4.2 cm), demonstrating that increasing extract concentration did not interfere with the foaming performance of the surfactant system. Although foam height is not a direct indicator of cleansing efficiency, it remains an important quality attribute because consumers frequently associate abundant foam with effective cleansing performance.

Similarly, all formulations exhibited comparable specific gravity and moisture content, indicating uniform dispersion of formulation components and acceptable physical stability. Moisture contents below 1% further indicate minimal free water, which may contribute to improved storage stability and reduced microbial contamination.

Collectively, the physicochemical evaluation demonstrates that the incorporation of *Centella asiatica* extract does not compromise the quality of the shampoo formulations. Instead, all formulations fulfilled the fundamental physicochemical characteristics expected of a herbal shampoo while maintaining acceptable sensory properties.

Antifungal Activity of Shampoo Formulations

Table 6. Antifungal activity of *Centella asiatica* shampoo formulations against *Candida albicans* and *Malassezia furfur*

Treatment	<i>Candida albicans</i> (mm)	<i>Malassezia furfur</i> (mm)
Positive control (ketoconazole)	19.0	34.0
Negative control (Shampoo base)	0	0
F1 (5%)	19.7	17.3
F2 (10%)	16.7	20.7
F3 (15%)	19.7	18.0

As shown in Table 6, all shampoo formulations inhibited the growth of both *Candida albicans* and *Malassezia furfur*, whereas the shampoo base produced no inhibition zone. These findings confirm that the observed antifungal activity was attributed to the incorporation of *Centella asiatica* extract rather than the formulation base itself. Formulas F1 and F3 exhibited the largest inhibition zones against *Candida albicans* (19.7 mm), whereas Formula F2 showed the greatest activity against *Malassezia furfur* (20.7 mm).

A notable finding of this study is that the inhibition zones produced by the shampoo formulations were greater than those produced by the ethyl acetate extract before formulation. During the preliminary screening, the

extract produced inhibition zones of 12 mm against *Candida albicans* and 11 mm against *Malassezia furfur*. After incorporation into the shampoo base, the inhibition zones increased to 16.7–19.7 mm and 17.3–20.7 mm, respectively. This observation indicates that the antifungal activity of the extract was retained after incorporation into the shampoo formulation. The larger inhibition zones may be related to differences in dispersion and diffusion of the formulation components in the agar medium.

One possible explanation is that the surfactant system, consisting of Tween, betaine, and cocodiethanolamide, improved the dispersion and diffusion of the extract within the agar medium. Enhanced dispersion may facilitate the release of bioactive compounds from the formulation, allowing greater interaction with fungal cells. Similar improvements in biological activity following formulation have been reported for several plant-based topical preparations, in which formulation excipients enhanced local delivery without altering the phytochemical composition of the extract^{20–22}.

Differences in antifungal activity among the formulations were relatively small, indicating that increasing extract concentration did not proportionally increase antifungal activity. Formula F2 demonstrated the highest activity against *Malassezia furfur*, whereas F1 and F3 were slightly more effective against *Candida albicans*. These findings indicate that antifungal performance is influenced not only by extract concentration but also by formulation characteristics affecting the release and diffusion of active compounds. Similar variations in fungal susceptibility have been reported previously and are associated with differences in fungal cell wall composition, membrane lipids, and adaptive responses to antifungal agents^{1,3–5}.

Dandruff is a multifactorial scalp condition, and *Malassezia* species, including *Malassezia furfur*, are among the microorganisms associated with its development. Therefore, the antifungal activity observed against *Malassezia furfur* in this study should be interpreted as activity against one microorganism associated with dandruff, rather than evidence that *Malassezia furfur* is the principal etiological factor of dandruff^{1,3–5}.

Compared with previous studies that primarily evaluated crude *Centella asiatica* extracts, the present study demonstrates that the antifungal activity of the extract was retained after incorporation into a shampoo formulation while maintaining acceptable physicochemical characteristics. This finding provides additional evidence supporting the feasibility of developing *C. asiatica*-based herbal shampoos as multifunctional topical products for scalp care.

Collectively, these results demonstrate that the developed shampoo formulations exhibited antifungal activity against both dandruff-associated and opportunistic fungi, supporting the potential application of *Centella asiatica* as a natural active ingredient in herbal antidandruff shampoos.

Hedonic Evaluation

Table 7. Hedonic evaluation of the *Centella asiatica* shampoo formulations

Attribute	Formula	Score 1	Score 2	Score 3	Score 4	Score 5	p
Texture	F1	0	2	9	16	3	0.262
	F2	0	3	12	12	3	
	F3	0	1	11	10	8	
Aroma	F1	1	3	12	8	6	0.768
	F2	0	5	12	11	2	
	F3	1	5	8	13	3	
Color	F1	0	4	4	11	11	0.002
	F2	0	5	11	10	4	
	F3	2	8	9	10	1	

Note: Data represent the number of panelists at each hedonic score. A difference was considered statistically significant when $p < 0.05$

As summarized in **Table 7**, the hedonic evaluation was performed to assess consumer acceptance of the developed shampoo formulations based on color, odor, and texture. Sensory acceptability is an important quality attribute because consumer preference strongly influences the practical use and commercial feasibility of cosmetic products.

The hedonic test involved 30 panelists aged 16–18 years, comprising male and female participants. Inclusion criteria were willingness to follow the research protocol, provide informed consent, and assess the color, odor, and physical form of the shampoo. Exclusion criteria included having flu, being color-blind, refusing participation, or experiencing fatigue or significant mental stress. Before participation, the researcher provided a research information sheet, and all panelists provided informed consent. Ethical considerations were applied to protect the safety, rights, and well-being of the participants.

Overall, the participants considered all formulations acceptable, indicating that incorporating *Centella asiatica* extract did not negatively affect the sensory characteristics of the shampoo. The lavender fragrance effectively masked the characteristic odor of the plant extract, while the lotion-like consistency and homogeneous appearance contributed to favorable user perception.

Differences in consumer preference among formulations may be associated with variations in extract concentration that influenced the intensity of the green coloration and the overall appearance of the products. Nevertheless, these differences did not substantially reduce consumer acceptance, indicating that the selected formulation components successfully balanced functional performance and esthetic quality.

The hedonic findings complement the physicochemical evaluation by demonstrating that the developed formulations not only exhibited acceptable quality characteristics but were also well accepted by potential users. This aspect is particularly important because successful cosmetic products require both biological efficacy and positive consumer perception.

Overall Discussion

This study systematically integrated pharmacognostic characterization, phytochemical screening, extract selection, shampoo formulation, physicochemical evaluation, and antifungal assessment within a single experimental framework. Unlike many previous studies that focused primarily on the biological activity of *Centella asiatica* extracts, this study demonstrated that the ethyl acetate extract retained its antifungal activity after formulation into a herbal shampoo while maintaining acceptable physicochemical characteristics and consumer acceptability.

The combination of satisfactory physicochemical properties and measurable antifungal activity against both *Candida albicans* and *Malassezia furfur* indicates that *Centella asiatica* has considerable potential as a natural active ingredient for herbal antidandruff shampoo formulations. These findings provide additional scientific evidence supporting further investigation of Indonesian medicinal plants for the development of plant-based cosmeceutical formulations.

LIMITATION

This study had several limitations. First, the antifungal activity of the developed shampoo formulations was evaluated using an agar diffusion method; therefore, minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values were not determined. Second, the study did not evaluate the in vitro release profile, skin permeation, or long-term stability of the active compounds in the shampoo formulation. Third, although a hedonic evaluation was conducted, the numerical scores and detailed statistical results should be further documented

to provide a more comprehensive assessment of product acceptability. In addition, the study did not include clinical evaluation in subjects with dandruff or seborrheic scalp conditions. Therefore, further studies are needed to determine the MIC and MFC, characterize the release and skin-permeation behavior, assess long-term stability, and evaluate the clinical efficacy and safety of the developed *Centella asiatica* herbal shampoo.

This study demonstrated that *Centella asiatica* (L.) Urb. leaf extract can be successfully incorporated into a herbal shampoo formulation with acceptable physicochemical characteristics and measurable in vitro antifungal activity against *Candida albicans* and *Malassezia furfur*. The developed formulations showed acceptable physicochemical properties while maintaining the antifungal activity of the extract, supporting the potential of *Centella asiatica* leaf extract as a natural active ingredient for further development of herbal shampoo formulations. Furthermore, the integration of pharmacognostic characterization, phytochemical screening, extract selection, formulation development, physicochemical evaluation, and antifungal assessment provides a basis for further development of plant-based cosmeceutical products. Further studies are recommended to investigate the minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), in vitro release behavior, skin permeation, long-term stability, and clinical efficacy of the developed formulations.

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AUTHOR CONTRIBUTION STATEMENT

AES: Conceptualization, Methodology, Investigation, Data Curation, Writing—Original Draft Preparation; L: Methodology, Validation, Investigation, Supervision; DH: Investigation, Data Curation, Writing—Review & Editing.

CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest.

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