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Short Communication

Hybrid function of light fraction patchouli oil in hair care formulations for effective hair and anti-dandruff treatment

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Abstract

Hair issues, such as hair loss and dandruff, pose significant challenges in hair care. Patchouli oil, rich in bioactive components, has emerged as a promising candidate for addressing these concerns. The aim of this study was to investigate the hybrid functionality of fractionated patchouli oil in hair care formulations designed to promote hair growth and control dandruff caused by *Malassezia globosa*. Crude patchouli oil (CPO) was fractionated to enhance its efficacy, producing light fraction patchouli oil (LFPO), which was then characterized using gas chromatography-mass spectrometry (GC-MS) analysis. Hair tonic formulations containing three different LFPO concentrations (0.5%, 1.0%, and 1.5%) were developed and evaluated for stability, pH, viscosity, and antifungal activity against *M. globosa*. The results showed that LFPO contained 2.51% acid number, 0.70% ester number, 0.71 mg/kg iron content, and 25.88% patchoulol. The formulations exhibited stable physicochemical properties, with pH levels of 5.36–5.51 and viscosity ranging from 3.94 to 4.08 centipoise (cP), suitable for hair tonic applications. Formulation of 1.5% LFPO demonstrated the strongest antifungal activity, producing a 31.18±1.37 mm inhibition zone against *M. globosa*, surpassing ketoconazole (21.72±0.28 mm), suggesting potential as a natural antifungal agent. Histological analysis in rabbits revealed that 1.5% LFPO formulation reduced epidermal cell shedding, increased hair length by 41.6±0.35 mm after six weeks, and promoted dense hair follicle growth. This research provides a foundation for developing natural, effective, and stable hair care formulations. Despite these promising results, the efficacy and safety of LFPO formulations in humans remain unexplored. Therefore, a clinical human trial is necessary to assess skin tolerance, irritation risks, and long-term effects under real-world conditions.

Keywords: Patchouli oil, anti-dandruff, formulation hair tonic, *Malassezia globsa*, hair care

Introduction

Hair is identified as a fundamental aspect of personal identity and expression, serving aesthetic, cultural, and protective functions. However, hair is vulnerable to conditions including *Malassezia* spp. infection [1-4], notably *Malassezia globosa*, which is strongly associated with dandruff in

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humans and animals [5-7]. *Malassezia* spp. naturally resides on the human scalp, and its interaction with *Staphylococcus* spp., which breaks down sebum, promotes dandruff and seborrheic dermatitis [8-10].

In Indonesia, issues related to *M. globosa* present notable dermatological challenges, impacting scalp health [10-12]. Indonesia's warm and humid tropical climate is conducive to the proliferation of *M. globosa* [10]. Previous studies have shown a higher *M. globosa* presence on the scalps of individuals not wearing hijabs compared to those wearing hijabs and in 52% of patients with seborrheic dermatitis [10,12]. By metabolizing scalp oils, *Malassezia* spp. produce byproducts that can trigger inflammation, leading to scalp imbalance, irritation, itching, and redness [13-15]. Conditions associated with *Malassezia* spp. typically require prolonged treatment with azole antifungal drugs; however, the emergence of azole-resistant strains in humans and animals underscores the need to explore natural sources for alternative antifungal agents [13-15].

Indonesia's rich biodiversity includes approximately 40,000 plant species, such as patchouli [16]. Known locally as *nilam*, patchouli belongs to the Lamiaceae family and contains bioactive compounds such as saponins, terpenoids, flavonoids, and alkaloids [17]. Three main varieties are widely cultivated: Java patchouli (*Pogostemon hortensis*), Aceh patchouli (*Pogostemon cablin*), and patchouli soap (*Pogostemon heyneanus*), with Aceh patchouli having the highest oil yield, exceeding 3% [16]. Patchouli oil, extracted from the leaves via steam distillation, offers antimicrobial [18], antifungal [19], antiviral [17], antioxidant [20], antimutagenic [21], anticancer [22], anti-inflammatory [23], fixative [18], and aromatherapy properties [18]. Its primary constituents include sesquiterpene hydrocarbons (40–45%) and oxygenated hydrocarbons (52–57%) [17,24], featuring δ-guaiene, α-guaiene, α-patchoulene, seychellene, trans-caryophyllene, alloaromadendrene, 2-(1,4,4-trimethyl-cyclohex-2-enyl)-ethanol, α-gurjunene, and the main compound, patchouli alcohol [25].

Patchouli oil is commonly used in products such as creams, lotions, soaps, and perfumes, where it acts as a strong fixative and natural insect repellent [26,27]. Patchouli alcohol has demonstrated antimicrobial effects against various pathogenic bacteria, including *Escherichia coli, Pseudomonas aeruginosa, Bacillus proteus, Shigella dysenteriae, Typhoid bacillus*, and Staphylococcus aureus [28] Although a study demonstrated its antifungal activity against *Penicillium digitatum* [19], research on its antifungal properties remains limited, particularly studies on the antifungal effects of fractionated patchouli oil derived from crude patchouli oil (CPO) against *Malassezia sp*. and its potential to promote hair growth. Therefore, the aim of this study was to investigate antidandruff activity of fractionated patchouli oil at concentrations of 0.5%, 1.0%, and 1.5% in hair care formulations against *M. globosa*, along with the effects of these formulations on hair growth in male rabbits.

Methods

Study design and setting

The experimental study was conducted from March 2023 to March 2024, aiming to explore the hybrid functionality of fractionated patchouli oil in hair care formulations designed to promote hair growth and combat dandruff caused by the *M. globosa* fungus. Light fraction patchouli oil (LFPO) was extracted using rotary evaporator and the characterization of contents was made using gas chromatography-mass spectrometry (GC-MS). In addition, the study involved both in vivo and in vitro methodologies. The in vitro antifungal activity against *M. globosa* was evaluated using the cup-plate method at the Microbiology Laboratory, Faculty of Medicine, Universitas Syiah Kuala. For the in vivo hair growth test, male rabbits aged 1 to 2 years and weighing 1.5 to 2 kilograms were used and treated with three doses of formulations (0.5%, 1% and 1.5% of LFPO) for 42 days. The length of hair growth was measured repeatedly six times. Detailed study design and main steps are presented in **Figure 1**.

Extraction of light fraction patchouli oil

Leaves of patchouli (*P. cablin* Benth.) were obtained from Aceh patchouli plants aged five months to 2 years, with a voucher specimen deposited at the Atsiri Research Centre, Universitas Syiah

Kuala, Banda Aceh, Indonesia (ARC-012023). The fractionation was performed at a pressure of 200 kilopascals and a temperature range of 125°C to 160°C to produce LFPO. The samples were dried and distilled to obtain CPO using a stainless-steel kettle which was subsequently fractionated using a rotary evaporator, following the method outlined previously [18].

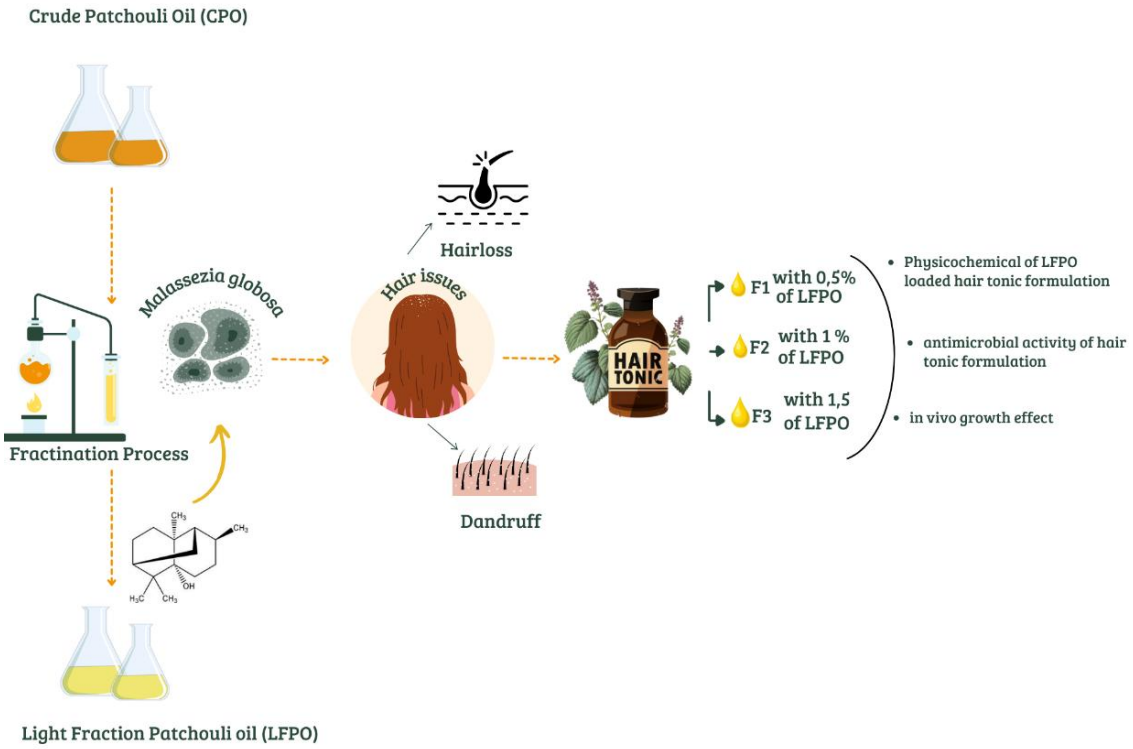

Figure 1. Study design of light fraction patchouli oil loaded hair tonic.

The characterization of light fraction patchouli oil involved testing for color, refractive index, specific gravity, ester number, iron content, and acid number [29]. GC-MS was utilized to identify the chemical components of LFPO using a Shimadzu GC-MS QP 2010 Ultra (Shimadzu, Kyoto, Japan). The peaks were cross-referenced with a mass spectrum database using specialized Chromeleon software version SR5 (Thermo Fisher Scientific, Waltham, USA) for the interpretation of mass spectral fragmentation patterns [30].

Hair care formulations

Four hair care formulations were prepared: a blank formulation without LFPO, designated as F0, along with formulations containing 0.5% LFPO (F1), 1% LFPO (F2), and 1.5% LFPO (F3). The chemical compounds utilized in these hair care formulations were aquadest, glycerin (20%), disodium EDTA (15%), dextrose (10%), benzalkonium chloride (5%), nitrogen (4,5%), polysorbate 80 (4%), panthenol (1%), sodium hydroxide (1%), PEG 400 (1%), and CMC-Na (0,8%). All chemical compounds were obtained from certified chemical distributors that provided safety profiles for each component, thereby minimizing the potential for irritation.

Homogeneity test and pH measurements

Homogeneity test was used to assess the distribution of particles in the tonic formulation of LFPO by applying a sample onto a glass slide following previous study [31]. The pH of the formulations was measured three times using a digital pH meter (Eutech PC 700 Meter With pH Electrode, USA) with measurements taken directly from the tonic without dilution. All measurements were conducted at 25°C and involved freshly prepared samples.

Viscosity and flow measurement

The viscosity and flow properties of the formulations were measured three times using a bob-cup Brookfield rheometer model LVDV-III Ultra (Brookfield Engineering Laboratories, Massachusetts, USA) equipped with a small sample adapter and utilizing freshly prepared

samples. The measurements were controlled using Brookfield software V3.1-1 (Brookfield Engineering Laboratories, Massachusetts, USA). Samples were subsequently tested at five different shear rates (revolutions per minute) to generate rheograms. All measurements were conducted at 32°C, reflecting the surface temperature of the skin.

Stability test

Physical stability of the formulations was evaluated through a cycling test, which involved heating and cooling cycles. Each of the four formulations underwent accelerated stability testing by being subjected to 24 hours in a refrigerator at 4±2°C, followed by 24 hours in an incubator at 40±2°C. This cycle was repeated for six times. The physical stability was assessed based on the average pH, viscosity, and homogeneity in the previous procedures after the completion of six cycles. All results for pH, viscosity, and homogeneity were presented in tabular form, accompanied by *p*-values for each evaluation to determine the statistical significance of the differences observed across the formulations and cycles.

Antimicrobial activity test: In vitro study

For this in vitro study, a standard isolate of *M. globosa* strain No. CBS 7966 (Indilab, Franklin Park, USA) was used. The cup-plate method was used to assess the antimicrobial activity by creating a solid agar layer in a Petri dish then a cylindrical hole in the agar contained an antifungal solution, which inhibited the growth of microorganisms in the surrounding area. Briefly, *M. globosa* was cultured on Sabouraud-dextrose agar media and incubated at 32°C for 96 hours. Following incubation, the growth was washed, and spectrophotometry was employed to measure the culture concentration at 560 nanometers. A sterile swab was dipped into the saline-washed culture and spread uniformly across a plate. After allowing the plate to dry for 20 minutes at room temperature, 6 mm wells were punched into the agar and filled with a 10 mg/mL extract solution. The plates were then incubated at 32°C for 96 hours, and the inhibition zones were recorded at the end of the incubation period [32]. Control measurements were obtained using the control solvent and ketoconazole served as the standard.

Hair growth assessment: In vivo study

The hair growth study utilizing different formulations were conducted on six male rabbits weighing between 1.8 kg and 2 kg. The rabbits underwent a one-week acclimation period to the environment, enclosures, and diet, receiving consistent water and uniform nutrition throughout.

Prior to treatment, fur was shaved from five designated areas on the rabbits' backs, with each area measuring 2×2 cm (each rabbits had five shaved areas). After 24-hours post-shaving, all rabbits received treatments corresponding to the marked areas. The positive control area was treated with 0.2 mL of 2% minoxidil while the negative control area received no treatment. The application areas included F1 (0.5% LFPO), F2 (1% LFPO), and F3 (1.5% LFPO), each treated with 0.2 mL of the respective formulation. The application was evenly spread across each shaved area using a sterile cotton swab to ensure uniform distribution. Treatments were administered over a period of 42 days, starting from the first day of treatment. Data collected included the length of hair growth measured on days 7, 14, 21, 27, 34, and 42 following the initiation of treatment.

Histological assessment of skin tissue and hair follicles

The histological assessment was also conducted on skin tissue and hair follicles (hair growth) using hematoxylin and eosin (H&E) staining at the end of the study. This followed the standard procedure to assess the structure of the rabbit skin after application of hair tonic.

Statistical analysis

All experiments were performed six times, and results were expressed as mean ± standard deviation (for data with a normal distribution) and the median (minimum-maximum) for non-normally distributed data. The normality of the data distribution was evaluated using the Shapiro-Wilk test. Single-factor analysis of variance determined significance at a 95% confidence interval (95%CI), with $p<0.05$ considered significant. Duncan's post hoc test was also applied, maintaining the threshold of $p<0.05$ for statistical significance. SPSS software version 22 (IBM, New York, USA) was employed for statistical analysis.

Results

Physicochemical properties of crude patchouli oil and light fractionpatchouli oil

In the present study, vacuum distillation in an aqueous medium yielded approximately 5% CPO from leaves, which was then purified to produce LFPO with a 65% yield. The physicochemical properties, including refractive index, specific gravity, acid number, ester number, iron content, and oil color were evaluated for both the CPO and LFPO.

Physicochemical properties analysis revealed that CPO had a slightly higher refractive index and specific gravity compared to light fraction (1.510 vs 1.506 and 0.962 g/mL vs 0.954 g/mL, respectively) (**Table 1**). Light fraction exhibited significantly lower acid number (2.51%) and ester number (0.70%) compared to CPO, indicating reduced rancidity and extended shelf life [33]. Additionally, the iron content decreased from 5.30 mg/kg in crude to 0.71 mg/kg in LFPO. The color change from reddish-brown in CPO to light yellow in LFPO further confirmed the purification effectiveness (**Table 1**).

Purification process used to produce LFPO significantly improved several key properties of patchouli oil. The results showed that LFPO met the standards set by SNI 06-2385-2006, exhibiting a lighter color, reduced impurities, and enhanced chemical characteristics. These findings highlight the effectiveness of vacuum distillation and purification in improving the quality of patchouli oil, establishing LFPO as the preferred active ingredient for hair tonic formulations targeting *M. globosa* compared to CPO.

Table 1. Physicochemical properties of crude patchouli oil (CPO) and light fraction patchouli (LFPO)

Properties	CPO	LFPO	Reference
Refractive index (nD20)	1.510	1.506	1.507–1.515
Specific gravity (g/mL, 25°C)	0.962	0.954	0.950–0.975
Acid number (%)	3.62	2.51	Max 8
Ester number (%)	2.80	0.70	Max 20
Fe content (mg/kg)	5.30	0.71	Max 25
Color	Reddish brown	Light yellow	Light yellow-reddish brown

GC-MS analysis

GC-MS analysis identified 26 chemical components in LFPO (**Table 2**). The primary constituents included patchouli alcohol (25.88%), α-guaiene (20.78%), δ-guaiene (17.84%), seychellene (8.67%), α-patchoulene (6.51%), trans-caryophyllene (5.13%), β-patchoulene (4.61%), γ-gurjunene (3.08%), viridiflorol (2.75%), and globulol (1.39%) (**Table 2**).

Table 2. Chemical composition of light fraction patchouli oil

Retention time (t_R)	Area (%)	Compounds	Retention time (t_R)	Area (%)	Compounds
3.306	0.09	α-pinene	13.246	17.84	δ-guaiene
3.698	0.23	β-pinene	13.637	0.29	α-panasinsene
4.153	0.02	Limonene	13.799	0.17	Cedren-13-ol acetate
9.413	0.19	δ-elemene	14.204	0.15	1,2-benzenedicarboxylic acid
10.450	0.03	β-elemene	14.511	0.1	Germacrene B
10.656	4.61	β-patchoulene	14.746	0.18	Isoaromadendrene epoxide
11.405	5.13	Trans-caryophyllene	15.723	0.97	Spathulenol
11.704	20.78	α-guaiene	15.961	1.39	Globulol
11.951	0.20	γ-selinene	16.216	0.32	Pogostol
12.025	0.13	Cadinene	16.687	2.75	Viridiflorol
12.170	8.67	Seychellene	16.824	0.15	Guaiyl acetate
12.427	6.51	α-patchoulene	17.167	25.88	Patchouli alcohol
12.468	3.08	γ-gurjunene	17.383	0.04	Longifolenaldehyde
Total				99.9	

Physicochemical properties and stability of hair tonic formulation

All dose groups (0 to 1.5% of LFPO) had pH ranging from 5.36 to 6.83 ($p<0.05$), remaining within the skin pH range of 4.5 to 7.0 (**Table 3**). Freshly prepared samples showed consistent viscosity values: 4.11 centipoise (cP) for F0 (0%), 3.97 cP for F1 (0.5%), 4.08 cP for F2 (1%), and 4.07 cP

for F3 (1.5%). After heating-cooling, viscosity fluctuated minimally, confirming formulation stability, with values from 4.14 (cP) for F0 to 3.97 cP for F2 and F3. Analysis indicated no significant differences ($p>0.05$) among groups (**Table 3**). This suggested that increasing the concentration of LFPO did not elevate viscosity. All formulations exhibited stable pH, minimal viscosity changes post heating-cooling, and consistent homogeneity. The formulations had a shelf life of 30 months if the seal remains intact [34], demonstrating robustness and reliability crucial for effective hair tonic development.

Table 3. Physicochemical properties of light fraction patchouli oil (LFPO)-loaded hair tonic formulations

Formulation	pH		Viscosity (centipoise)		Homogeneity	
	Freshly prepared	Heating-cooling	Freshly prepared	Heating-cooling	Freshly prepared	Heating-cooling
F0 (0% LFPO)	6.73±0.15^{c}	6.83±0.07^{a,b}	4.11±0.03^{b}	4.14±0.03^{c}	Homogenize	Homogenize
F1 (0.5% LFPO)	5.46±0.07^{a}	5.51±0.04^{b}	3.97±0.03^{a}	3.94±0.05^{a}	Homogenize	Homogenize
F2 (1% LFPO)	5.41±0.29^{a,b}	5.39±0.04^{a}	4.08±0.01^{a}	3.97±0.08^{b,c}	Homogenize	Homogenize
F3 (1.5% LFPO)	5.36±0.14^{a}	5.38±0.03^{a}	4.07±0.01^{a}	3.97±0.02^{b}	Homogenize	Homogenize

^{a,b,c} Different superscripts on the same line indicate a significant difference at $p<0.05$

Antimicrobial activity of hair tonic formulation

The mean inhibition zone for ketoconazole was 21.72 mm, establishing a benchmark for antifungal activity (**Table 4**). In contrast, the negative control group (F0) exhibited no inhibitory effect against *M. globosa*, confirming the absence of antifungal properties in this formulation (**Table 4**). F1, F2, and F3 formulations exhibited progressively higher inhibition zone values (28.54 mm, 29.37 mm, 31.18 mm for F1, F2 and F3, respectively) and F3 demonstrating the most potent antifungal activity (**Table 4**). The increasing inhibition zone values suggest a potential dose-dependent relationship. All formula were significantly different with both negative control and positive control groups. These results indicate that formulations F1, F2 and F3 possessed significant antifungal properties against the targeted microorganisms (**Table 4**).

Table 4. Antimicrobial activity of light fraction patchouli oil (LFPO)-loaded hair tonic formulations against *Malassezia globosa*

Samples	Inhibition zone (mm) (mean±SD, n=3)
Positive control (ketoconazole)	21.72±0.28^{b}
F0 (0% LFPO)	00.00±0.00^{a}
F1 (0.5% LFPO)	28.54±0.64^{c}
F2 (1% LFPO)	29.37±0.07^{c}
F3 (1.5% LFPO)	31.18±1.37^{d}

^{a,b,c} Different superscripts indicate a significant difference at $p<0.05$

In vivo hair growth effect

The hair growth on rabbits was evaluated to assess the effectiveness of hair tonic formulations. The mean increases in hair length over six weeks were measured and the results are presented in **Table 5** and **Figure 2**. F3 formulation demonstrated superior average hair length growth compared to F1 and F2. Minoxidil at a concentration of 2% served as a positive control for comparison, revealing longer hair growth compared to in the patchouli oil formulation groups. The representative photographs of hair length from each group after 6 weeks are presented in **Figure 3**. The results indicated that F3 formulation exhibited the highest mean growth value of 41.6 mm by the sixth week.

Table 5. Hair-promoting growth activity of light fraction patchouli oil (LFPO)-loaded hair tonic formulations over six weeks

Group	Hair growth (mm) (mean±SD, n=6)						
	Basal	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Positive control (minoxidil)	35.16±0.64^{b,c}	0.26±0.04^{a,b}	4.1±0.09^{a,b}	12.27±0.16^{a,b}	22.24±1.23^{a,b}	29.11±0.17^{b}	32.12±1^{b}
F0 (0% LFPO)	34.86±0.4^{a,c}	0±0^{a}	0.15±0.06^{a}	0.25±0.03^{a,b}	0.83±0.05^{a}	1.14±0.07^{a}	1.41±0.26^{a,b}

Group	Hair growth (mm) (mean±SD, n=6)						
	Basal	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
F1 (0.5% LFPO)	34.62± 0.78[a,c]	0±0[a]	0.32± 0.16[a]	9.3± 0.15[a,b]	15.62± 0.34[a]	19.08± 0.15[a]	21.7± 0.59[a,b]
F2 (1% LFPO)	33.7± 0.78[a,c]	0±0[a]	6.18± 0.13[a]	13.43± 0.5[a,b]	1903± 0.16[a]	21.31± 0.22[a]	25.4± 1.6[a,b]
F3 (1.5% LFPO)	34.88±1. 07[b,c]	0±0[a,b]	10.09±0. 08[a,b]	21.63±0. 39[b,a]	29.93±0. 53[b,a]	33.6±0. 36[b]	41.6±0. 35[b]

[a,b,c] Different superscripts indicate a significant difference at $p<0.05$.

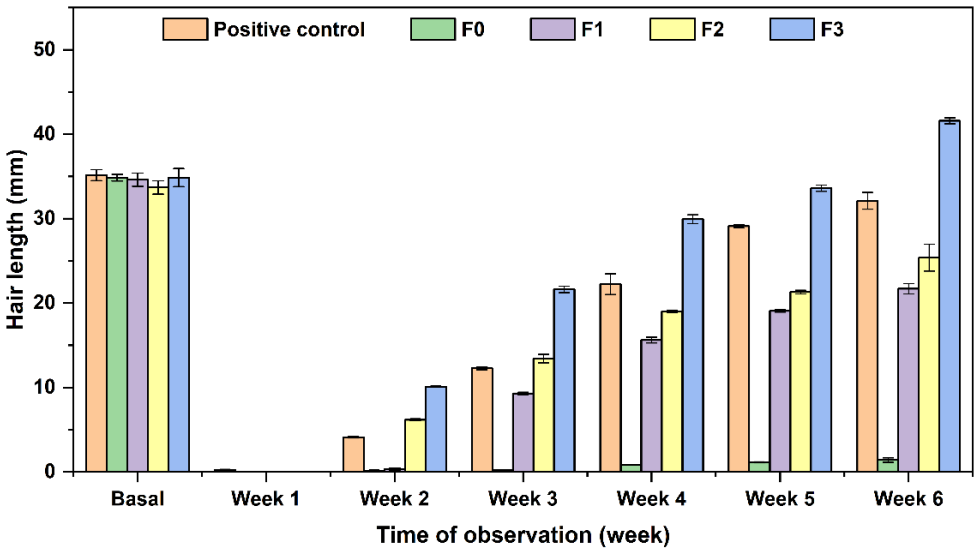

Figure 2. Mean changes of hair length using light fraction patchouli oil(LFPO)-loaded hair tonic formulations over six weeks. Basal refers to the original hair length of the rabbits before shaved.

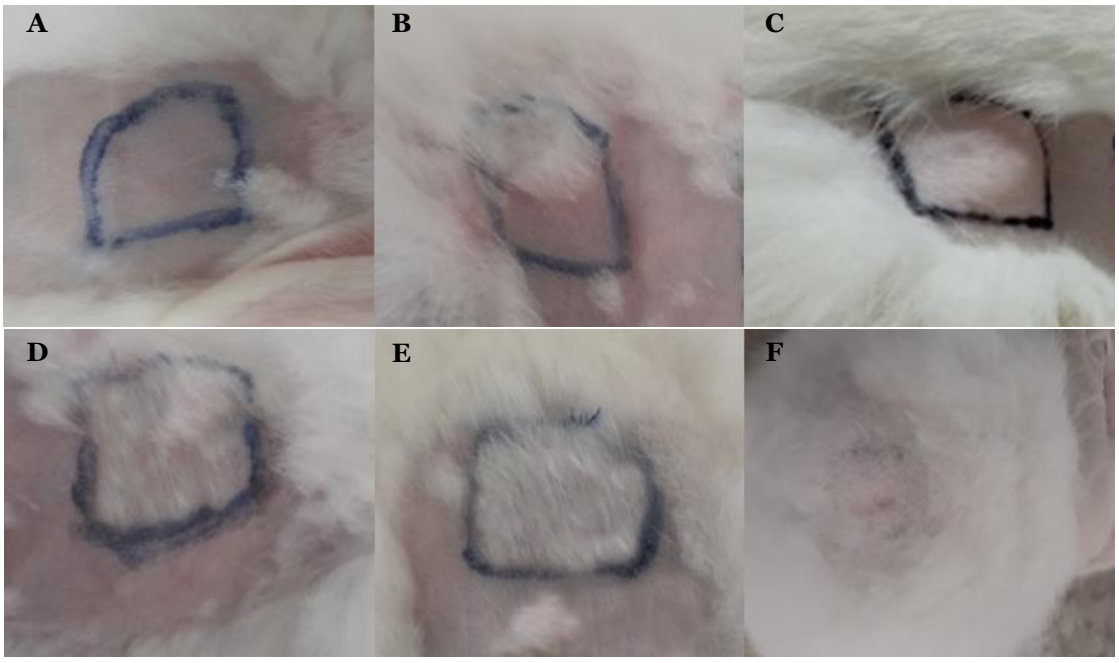

Figure 3. Hair growth-promoting effects of light fraction patchouli oil (LFPO)-loaded hair tonic formulations in rabbits over six weeks. (A) Initial shaved skin; (B) negative control (untreated); (C) positive control (2% minoxidil) and (D-F) groups treated with 0.5%, 1%, and 1.5% LFPO, respectively.

Histological assessment of skin tissue and hair follicles

The results of the histological analysis using H&E staining showed differences in the structure of the rabbit skin after the application of hair tonic containing various concentrations of LFPO for 21 days (**Figure 4**). In the group with a concentration of 0.5%, there was slight thickening of the

epidermis and lower hair follicle activity compared to the other groups (**Figure 4A**). This indicates that a low concentration of LFPO may be less optimal in stimulating hair follicle growth. Meanwhile, in the 1% group, the increase in hair follicle activity was more pronounced, with a more organized follicle structure and a healthier epidermal layer (**Figure 4B**).

In the 1.5% group, hair follicles showed the most significant regenerative activity, with increased epidermal thickness and denser dermal tissue (**Figure 4C**). This indicates that the 1.5% concentration provides the best stimulation for hair follicle growth and skin tissue regeneration. However, it should be noted that the use of higher concentrations of patchouli oil can have an irritating effect or reduce effectiveness if applied excessively. Therefore, these results provide important insights for determining the optimal concentration of LFPO in hair care products to stimulate healthy hair growth without causing side effects.

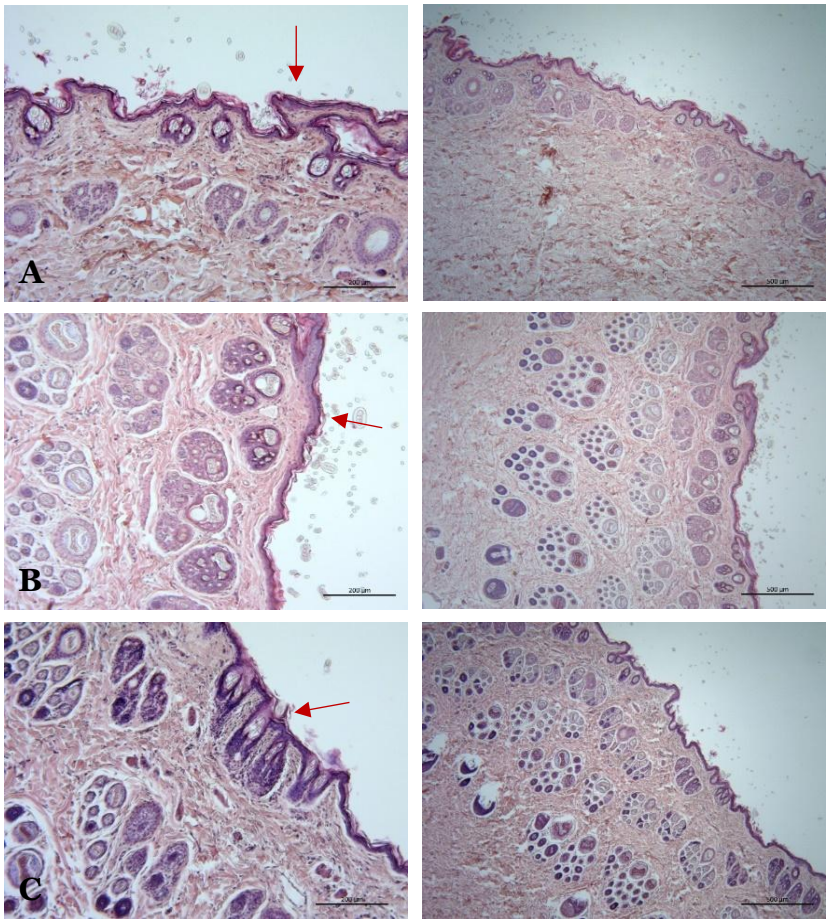

Figure 4. Histological analysis of rabbit skin using hematoxylin and eosin staining following application of hair tonic formulations containing 0.5% light fraction patchouli oil (A), 1% light fraction patchouli oil (B), and 1.5% light fraction patchouli oil (C) over a period of six weeks.

Discussion

M. globosa, a fungal infection, significantly contributes to dandruff, a common issue in Indonesia associated with various dermatological problems [35]. The concentration of patchouli alcohol from P. *cablin* was higher than that from *P. heyneanus* (14%) [36]. Recognized for its biological activities, patchouli alcohol exhibited antifungal properties against *Aspergillus* species and demonstrated antibacterial effects against all 127 tested strains, with inhibitory concentrations ranging from 1.5 to 200 µg/mL for Gram-positive bacteria and 25 to 768 µg/mL for Gram-negative bacteria [33,34]. LFPO also contained minor quantities of pinene, limonene, elemene, selinene, spathulenol, and cadinene, enhancing the complexity of its aroma and therapeutic potential [37]. Therefore, patchouli oil has demonstrated potential in aiding oily hair while providing an authentic aromatherapy experience, serving as a basis for anti-dandruff shampoo formulations in the present study [33]. The benefits of patchouli oil include antimicrobial [18],

antifungal [19], antiviral [17], antioxidant [20], antimutagenic [21], anticancer [22], anti-inflammatory [23], and fixative [18], in addition to its role in aromatherapy [18]. Given the presence of multiple strains of *Malassezia* resistant to azoles in humans and animals, there is a need to explore natural pharmaceutical resources as potential alternative antifungal agents. In the present study, three formulations of hair tonic based on liquid fraction of patchouli oil, F1 (28.54±0.64 mm), F2 (29.37±0.07 mm), and F3 (31.18±1.37 mm), were found to be more effective than ketoconazole (21.72±0.28 mm) in inhibiting the growth of *M. globosa*. In the present study, F3 (1.5% LFPO) exhibited the greatest antifungal activity against *M. globosa* with a 31.18±1.37 mm inhibition zone. GC-MS analysis identified patchouli alcohol (25.88%) as a primary compound in liquid fraction patchouli oil, which, along with other constituents like α-guaiene and δ-guaiene, likely contributed to the observed antifungal efficacy. Patchouli alcohol has been shown to be effective against various fungal species, such as *Aspergillus flavus, Aspergillus cinerea*, and *Aspergillus oryzae*, supporting its potential in scalp health applications [32,33]. Their mechanism of action is typically associated with the disruption of microbial membranes, causing interference with the proton motive force, electron transport, and active transport processes, which eventually leads to the coagulation of intracellular components [38]. The study conducted by Vu *et al*. [39] demonstrated that essential oils from *Mentha arvensis* (MIC 2.5 µl/ml) and *Piper betle* (MIC 1 µl/ml) effectively inhibit *Malassezia* species and synergistically eliminate *M. furfur*, likely by impairing organelle structure and function.

In the present study, F3 (1.5% LFPO) demonstrated slightly lower effectiveness than the positive control (2% minoxidil) but showed greater hair length growth compared to F1 (0.5% LFPO) and F2 (1% LFPO). Hair length data were analyzed using post hoc analysis, yielding a significance value of $p>0.05$, indicating no significant difference between treatment groups. All formulations were evaluated for pH, viscosity, and homogeneity in both freshly prepared and heating-cooling stages. Higher viscosity correlated with a thicker solution, which can lead to scalp crusting and dandruff [40]. The viscosity of F1 (0.5% LFPO), F2 (1% LFPO), and F3 (1.5% LFPO), remained low, resulting in a thinner solution with no significant difference. A high-quality hair tonic formulation yields a low-viscosity, liquid solution [37]. pH testing confirmed that formulations F0 (no LFPO), F1 (0.5% LFPO), F2 (1% LFPO), and F3 (1.5% LFPO), maintained stable, scalp-appropriate pH levels, minimizing irritation risks while meeting requirements for hair tonic application [31]. According to previous study [41], the formulation of hair tonic utilizing herbal oil possesses suitable physicochemical qualities when surfactants and stabilizers are incorporated at appropriate proportions, hence ensuring the consistency of the formulation during storage.

Increased epidermal cell shedding is associated with a higher risk of dandruff. Antifungal testing against *M. globosa* showed that patchouli oil in hair tonic formulations effectively inhibited this dandruff-causing fungus, reducing scalp itching and flaking [33]. Histological findings in rabbits treated with similar formulations revealed that higher concentrations of patchouli oil led to decreased cell shedding and greater hair length and thickness, with a dense arrangement of hair follicles observed in treated skin [31]. The present study is the first to examine patchouli oil's anti-dandruff effects in hair tonic formulations. The main limitation of this research is the brief testing period after the hair tonic is stored, both before and after opening the seal. Additionally, while promising results were observed in laboratory and animal models, the efficacy and safety of patchouli oil formulations have not yet been tested on human skin. Human trials are essential to evaluate potential skin irritation, allergic reactions, and long-term effects under real-world conditions. Further research should also explore additional plant extracts and other parts of the patchouli plant to enhance antifungal efficacy and develop new strategies for dandruff prevention.

Conclusion

Hair tonic formulations derived from Aceh patchouli oil demonstrated significant antifungal activity against *M. globosa*. The presence of a high percentage of patchouli alcohol (25.88%) and 26 identified bioactive compounds suggests that the oil has therapeutic and aromatic properties, potentially enhancing its effectiveness as a hair tonic. The formulations exhibited stable pH,

viscosity, and homogeneity, with formulations of 1–1.5% LFPO could serve as effective agents for improving scalp health and managing dandruff.

Ethics approval

Ethical approval for the present study was obtained from the Ethical Committee for Animal Research, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia (245/KEPH/VIII/2023).

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None to declare.

Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

Derived data supporting the results of this study are available from the corresponding authors on request.

Declaration of artificial intelligence use

We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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References

1. Pereira-Silva M, Martins AM, Sousa-Oliveira I, *et al.* Nanomaterials in hair care and treatment. Acta Biomater 2022;142:14-35.
2. Rhimi W, Theelen B, Boekhout T, *et al.* Conventional therapy and new antifungal drugs against *Malassezia* infections. Med Mycol 2021;59(3):215-234.
3. Rathie B, Theelen B, Laurence M, *et al.* Antimicrobial susceptibility testing for three *Malassezia* species. Microbiol Spectr 2023;11(4):e0507622.
4. Hadrich I, Khemakhem N, Ilahi A, *et al.* Genotypic analysis of the population structure in *Malassezia globosa* and *Malassezia restricta*. J Fungi 2023;9(2):263.
5. Sharma P, Rana T. Fungal diseases of goats. In: Rana T, editor. Principles of goat disease and prevention. Chichester: Wiley; 2023.
6. Sibi D, Silvanose CD, Jibin VG. Role of *Malassezia furfur* and *M. globosa* in dandruff and seborrheic dermatitis. J Clin Investigat Dermatol 2023;11(1):2.
7. Grimshaw SG, Smith AM, Arnold DS, *et al.* The diversity and abundance of fungi and bacteria on the healthy and dandruff affected human scalp. PLoS One 2019;14(12):e0225796.
8. Korassa YB, Saptarini NM, Mustarichie R, *et al.* Antifungal activity of Moringa (*Moringa Oleifera* Lamk.) seed oil from East Nusa Tenggara, Indonesia, against *Malassezia furfur* causes seborrheic dermatitis. J Hunan Univ Nat Sci 2023;50(5):11-20.

9. Kumari KU, Yadav NP, Luqman S. Promising essential oils/plant extracts in the prevention and treatment of dandruff pathogenesis. Curr Top Med Chem 2022;22(13):1104-1133.
10. Widaty S, Surachmiati L, Legiawati L, *et al.* Scalp microbiome of healthy women wearing hijab compared to those not wearing hijab: A cross-sectional study. Sci Rep 2023;13(1):11797.
11. Wikanto JR, Wijaya L, Astiarani Y. Haircare practice and dandruff problems among Indonesian medical students. J Gen Proced Dermatol Venereol Indones 2022;6(2):1.
12. Zoulba EN, Widaty S, Krisanty IAIA, *et al.* Identification of *Malassezia* species and the severity of seborrheic dermatitis on scalp in Dr. Cipto Mangunkusumo Hospital Jakarta. Adv Sci Lett 2018;24(9):6649-6652.
13. Iatta R, Puttilli MR, Immediato D, *et al.* The role of drug efflux pumps in *Malassezia pachydermatis* and *Malassezia furfur* defence against azoles. Mycoses 2017;60(3):178-182.
14. Kano R, Aramaki C, Murayama N, *et al.* High multi-azole-resistant *Malassezia pachydermatis* clinical isolates from canine *Malassezia* dermatitis. Med Mycol 2020;58(2):197-200.
15. Murayama N, Kano R. Azole and terbinafine susceptibility testing of *Malassezia pachydermatis* in Japan. J Vet Med Sci 2023;85(3):383-385.
16. Munanda Z, Mahyiddin Z, Muhammad S, *et al.* The impact of redistillation temperature using rotary vacuum evaporator on the quality of Aceh patchouli oil. J Patchouli Essent Oil Prod 2022;1(2):52-55.
17. Isnaini N, Khairan K, Faradhilla M, *et al.* A study of essential oils from Patchouli (*Pogostemon cablin* Benth.) and its potential as an antivirus agent to relieve symptoms of COVID-19. J Patchouli Essent Oil Prod 2022;1(2):27-35.
18. Muhammad S, Hisbullah H, Rahmi J, *et al.* Vacuum distillation of Aceh patchouli oil into hi-grade and crystal patchouli with rotary vacuum evaporator. J Patchouli Essent Oil Prod 2022;1(2):36-41.
19. Zulu L, Gao H, Zhu Y, *et al.* Antifungal effects of seven plant essential oils against *Penicillium digitatum*. Chem Biol Technol Agric 2023;10(1):82.
20. Lalthafamkimi L, Kumar A, Wann SB, *et al.* Metabolite bioprospection and expression analysis of patchoulol synthase gene in different in vitro systems of *Pogostemon cablin*: An important medicinal aromatic plant. Ind Crops Prod 2023;205:116689.
21. Fatima S, Farzeen I, Ashraf A, *et al.* A comprehensive review on pharmacological activities of pachypodol: A bioactive compound of an aromatic medicinal plant *Pogostemon Cablin* Benth. Molecules 2023;28(8).
22. Chang KF, Lai HC, Lee SC, *et al.* The effects of patchouli alcohol and combination with cisplatin on proliferation, apoptosis and migration in B16F10 melanoma cells. J Cell Mol Med 2023;27(10):1423-1435.
23. Deliana F, Illian DN, Nadirah S, *et al.* Therapeutic effects of patchouli (*Pogostemon cablin*) essential oil in relieving Eczema Symptoms in infants and toddlers: A literature review. J Patchouli Essent Oil Prod 2023;2(1):1-8.
24. Widyasanti A, Nurjanah S, Nurhadi B, *et al.* Optimization of the vacuum fractional distillation process for enhancing the α-Guaiene of patchouli oil with response surface methodology. Separations 2023;10(9):469.
25. Nour AH, Modather RH, Yunus RM, *et al.* Characterization of bioactive compounds in patchouli oil using microwave-assisted and traditional hydrodistillation methods. Ind Crops Prod 2024;208:117901.
26. Une VR, Bondarde MP, Some S. Formulation and development of water-based fragrance from patchouli essential oils using nonionic surfactant. Appl Nanosci 2022;12(7):2117-2125.
27. Deng W, Li M, Liu S, *et al.* Repellent screening of selected plant essential oils against dengue fever mosquitoes using behavior bioassays. Neotrop Entomol 2023;52(3):521-529.
28. Nurjanah S, Alhafidz Z, Maulani M, *et al.* Alpha-guaiene isolated from patchouli oil exhibits antifungal activity against four pathogenic fungi. F1000Res 2025;14:4.
29. Abram PH, Putri W, Nuryanti S, *et al.* Characterization of patchouli oil (*Pogostemon cablin* Benth.) production of Tinombala Village, Ongka Malino District, Parigi Moutong Regency. J Phys Conf Ser 2021;2126:012024.
30. Ginting B, Sufriadi E, Harnelly E, *et al.* Identification of volatile compounds contained in the therapeutic essential oils from *Pogostemon cablin*, *Melaleuca leucadendra*, and *Mentha piperita* and their purified fractions. J Adv Pharm Technol Res 2023;14(3):208-212.
31. Heroweti J, Wibowo DN, Ulya AN, *et al.* Physical stability test hair tonic combination cinnamon (*Cinnamomum burmanii*) essential oil and vco (virgin coconut oil) with cycling test method and irritation test on the rabbit. JFSP 2023;9(2):72-82.
32. Kumar Tiwari A, Mishra RK, Kumar A, *et al.* A comparative novel method of antifungal susceptibility for *Malassezia furfur* and modification of culture medium by adding lipid supplement. J Phytol 2011;3(3):44-52.
33. Muhammad S, Abdul Khalil HPS, Abd Hamid S, *et al.* Characterization of bioactive compounds from patchouli extracted via supercritical carbon dioxide (SC-CO2) extraction. Molecules 2022;27(18).

34. Songkro S, Isnaini N, Sungkharak S, *et al.* Characterization, antioxidant and antibacterial potentials of *Tamarindus indica* L. fruit pulp extract loaded o/w nanoemulsions. Braz J Pharm Sci 2022;58:e19373.
35. Saunte DML, Gaitanis G, Hay RJ. *Malassezia*-associated skin diseases, the use of diagnostics and treatment. Front Cell Infect Microbiol 2020;10:112.
36. Taufik M, Rizal S, Hernelly E, *et al.* The therapeutic potential of Aceh patchouli oil (*Pogostemon cablin* Benth.) in enhancing full-thickness wound healing in mice. Trop J Nat Prod Res 2024;8(1):5840-5844.
37. Akib NI, Adjeng ANT, Lakasa RP, *et al.* Physical stability of hair tonic contain ethanol extract galangal (*Alpinia galanga* L.) rhizome and aloe vera leaf filtrate (*Aloe vera* L.) Pharmauho 2020;6(2):67-74.
38. Muhammad S, Isnaini N, Indra I, *et al.* Patchouli essential oil: Unlocking antimicrobial potential for dandruff control. IOP Conf Ser Earth Environ Sci 2024;1356:012095.
39. Vu TX, Tran TB, Hoang CQ, *et al.* Chemical compositions and anti-*malassezia* properties of Vietnamese *Mentha arvensis* and *Piper betle* essential oils. Int J Agric Techn 2021;17(4):1619-1630.
40. Heroweti J, Novianto WD, Khoeriyah H, *et al.* Hair growth activity of hair tonic preparations containing cinnamon essential oil (*Cinnamomum burmanii*) and virgin coconut oil. J Ilmu Kefarmasian Indones 2023;21(2).
41. Satheeshan K, Author C, Seema B, *et al.* Development and evaluation of VCO based herbal hair tonic. J Pharmacogn Phytochem 2020;9(2):485-493.