Original Article

Anti-dandruff effects of butterfly pea flowers (*Clitoria ternatea*)-based shampoo: A pretest-posttest control study

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Abstract

Butterfly pea flower (*Clitoria ternatea*) may serve as an alternative anti-dandruff treatment; however, its effects on *Malassezia* spp. remain unexplored. The aim of this study was to explore the effects of *C. ternatea* as an herbal-based anti-dandruff treatment on *Malassezia* spp. DNA expression, plakoglobin levels, IL-8 levels, sebum levels, dandruff severity scores, adverse effects, and patient satisfaction. An experimental study with a pretest-posttest control design was conducted at the Outpatient Clinic of Dermatology and Venereology, Arifin Achmad Hospital, Pekanbaru, Indonesia, from November 2023 to January 2024. The flower of *C. ternatea* was used to formulate the shampoo. The study involved 70 female patients aged 18–25 with dandruff, who were divided into two groups: (a) experimental group using 20% *C. ternatea* shampoo and (b) control group using 2% ketoconazole shampoo. The present study found that 2% ketoconazole shampoo significantly reduced *Malassezia* spp. DNA expression compared to 20% *C. ternatea* shampooo (*Clitoria ternatea*: ΔCq=1.76±3.18; ketoconazole: ΔCq=3.77±2.90; *p*=0.008). No significant difference was observed in plakoglobin levels (*C. ternatea*: ΔCq=1.98±3.63; ketoconazole: ΔCq=2.50±2.36; *p*=0.427) or IL-8 levels (*C. ternatea*: ΔCq=3.46±4.00; ketoconazole: ΔCq=4.16 ± 3.62; *p*=0.459). *C. ternatea* significantly reduced sebum levels more than ketoconazole (*C. ternatea*: 1.16±0.98%; ketoconazole: 0.22±0.38%; *p*<0.001). Dandruff scores and patient satisfaction were similar for both shampoos (*p*=0.115 and *p*=0.336, respectively). Adverse effects were more common in the 2% ketoconazole shampoo group, affecting 21.2% of the patients. In conclusion, 2% ketoconazole shampoo is more effective in reducing *Malassezia* spp. DNA expression, while 20% *C. ternatea* shampoo offers better sebum control. Both shampoos are similarly effective in ameliorating dandruff severity and are well-tolerated, with fewer adverse effects reported for *C. ternatea*.

Keywords: *Clitoria ternatea*, anti-dandruff, *Malassezia* spp., herbal, treatment

Introduction

Dandruff is a manifestation of seborrheic dermatitis, presenting with localized lesions on the scalp, erythema, and flakes, leading to dryness and itching [1]. The global prevalence of dandruff

in 2015 was 50% in the general population, with females being the most commonly affected [1,2]. In Surabaya and Semarang, Indonesia, the prevalence is reported to be 48% and 24.1%, respectively [3,4]. Although comprehensive prevalence data for Indonesia is lacking, dandruff significantly impacts patients' quality of life [5,6]. Thus, effective treatment is needed for favorable clinical outcomes [7].

The exact pathogenesis of dandruff remains uncertain [8]. Studies have shown that increased colonization of *Malassezia* spp., a normal skin flora, in dandruff lesions induces inflammation and disrupts the epidermis [8-12]. *Malassezia* spp. hydrolyzes sebum triglycerides, releasing fatty acids such as oleic and arachidonic acids. This reaction consequently activates keratinocytes to produce proinflammatory cytokines (such as IL-1α, IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α)) and promote abnormal keratinocyte differentiation [1].

Current anti-dandruff treatments can cause scalp dryness with prolonged use and often require long-term treatment to prevent recurrence [1,13-15]. Natural ingredients, such as butterfly pea flower (*Clitoria ternatea*), may offer alternative treatments as an anti-dandruff [16,17]. Butterfly pea flower, known for its antifungal, antimicrobial, anti-inflammatory, and antioxidant properties, contains flavonoids known as cysteine-rich antifungal proteins (CRAFP) in its flowers, seeds, stems, and leaves [17,18]. While CRAFP has been studied against *Candida albicans*, its effects on *Malassezia* spp. in dandruff remain unexplored [18-20].

Dandruff treatment aims to ameliorate symptoms and achieve long-term remission by using both chemical and herbal anti-dandruff shampoos formulated specifically for the scalp [10,21]. Extensive research has focused on treatments targeting *Malassezia* spp., such as zinc pyrithione 1% (ZPT), an antifungal agent that has shown significant improvement in adherent scalp flaking scores [1]. Additionally, 2% ketoconazole shampoo is the predominant antifungal agent used targeting *Malassezia* spp., albeit associated with potential adverse effects such as allergic contact dermatitis and hair-related concerns [10,15]. Herein, the aim of this study was to explore the effects of *C. ternatea* as an herbal-based anti-dandruff treatment on *Malassezia* spp. DNA expression, plakoglobin levels, IL-8 levels, sebum levels, dandruff severity scores, adverse effects, and patient satisfaction.

Methods

Study design and setting
An experimental study using a pretest-posttest control group design was conducted at the Outpatient Clinic of Dermatology and Venereology, Arifin Achmad Hospital, Pekanbaru, Indonesia, from November 2023 to January 2024. The primary aim of the present study was to evaluate the effectiveness of *C. ternatea* flower extract shampoo compared to 2% ketoconazole shampoo in reducing *Malassezia* spp. DNA expression, plakoglobin levels, IL-8 levels, sebum levels, dandruff severity scores, adverse effects, as well as improving patient satisfaction in dandruff treatment. The present study was conducted over four weeks and involved 70 female patients aged 18–25 years with dandruff, divided into two groups: (a) experimental group, receiving 20% *C. ternatea* shampoo; and (b) control group, receiving 2% ketoconazole shampoo. DNA expression of *Malassezia* spp., plakoglobin levels, IL-8 levels, sebum levels, and dandruff severity scores were assessed before and after four weeks of shampoo application. Adverse effects and patient satisfaction were documented after four weeks of shampoo application. The protocol of the present study was reviewed and approved by the Ethical Committee of Health Research, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia (Approval number: 342/KEPK/USU/2023).

Production of *Clitoria ternatea* flower extract shampoo

Taxonomic determination of the plant specimen
Plant specimens collected from Tanah Karo Regency, North Sumatra, Indonesia, were transported to Medan, North Sumatra, Indonesia, for further analysis. The taxonomic identification of the plant specimen was carried out at the Laboratory of Plant Systematics for Herbarium Medanense (MEDA), Universitas Sumatera Utara, Medan, Indonesia (Reference

number: 115/MEDA/2023). The results confirmed that the specimen belongs to Kingdom Plantae, Division Dicotyledonae, Order Fabales, Family Fabaceae, Genus *Clitoria*, and Species *Clitoria ternatea* L.

Clitoria ternatea extraction
Fully bloomed, fresh, and blue-purple flowers of *C. ternatea* (11 kg) were harvested, rinsed with running water, and dried for 4 days at 50°C. The dried sample (3,041 grams) was crushed into fine powder. Maceration was carried out by immersing the fine powder (2,954 grams) into 20 L ethanol 96% in a closed container. The maceration lasted for five days at room temperature, where the sample was protected from direct sunlight and stirred for every 24 hours. Thereafter, the macerate was separated from the residue using a flannel cloth. The residue was re-macerated three times using the same procedure. Macerate obtained from each maceration was combined and evaporated in a rotary evaporator at 40–50°C for four days to produce an extract paste, which was subsequently evaporated using a water bath at 50°C to completely remove the ethanol. The maceration yielded a solid extract weighing 1.7 kg.

Preparation of C. ternatea shampoo
The *C. ternatea* shampoo was prepared with the following composition: 20% *C. ternatea* extract, 20% sodium lauryl sulfate, 10% glycerin, 0.5% carbopol 940, 0.1% Na-EDTA, 0.1% methylparaben, 5% propylene glycol, 0.25% menthol, and 0.5% citric acid, with distilled water added to a total volume of 100 mL. Initially, a thickening solution was prepared by dissolving methylparaben and propylparaben in hot water and dispersing carboxymethylcellulose sodium. The active ingredients (*C. ternatea* extract) were dissolved in propylene glycol with PEG-40 hydrogenated castor oil, and this solution was then mixed into the thickening solution. Thereafter, sodium lauryl sulfate was dissolved in water and added to the mixture, where the pH was adjusted with aqueous citric acid. Finally, melted menthol was added to the mixture to obtain the shampoo. The shampoo had a consistent and homogenous liquid (pH 5.23) with dark purple color and menthol scent.

Subject recruitment
Minimum sample size was estimated using the following parameters: standard deviation (s)=1.23 [9], desired clinical difference (X_1-X_2)=0.87 [9], type I error (α)=0.05 ($Z\alpha$=1.96), and type II error (β)=0.20 (Power 80%, $Z\beta$=0.842). The calculation required 32 patients for each group, totaling 64 patients. To account for potential dropouts, an additional 10% (six patients) was added, resulting in a minimum sample size of 70 patients, with 37 in the experimental group and 33 in the control group. A consecutive sampling method was employed, and no randomization was used to assign patients to the experimental and control groups.

Inclusion and exclusion criteria
Inclusion criteria were female patients aged 18–25 years with dandruff who had no scalp skin diseases (such as tinea capitis, psoriasis vulgaris, or atopic dermatitis), were not pregnant or breastfeeding, and had not used topical antifungal or anti-inflammatory agents and systemic corticosteroids or non-steroidal anti-inflammatory drugs (NSAIDs) for at least two weeks. Exclusion criteria included non-compliance with shampoo use or follow-up. Dropout criteria included the absence from post-experimental examinations or non-use of the shampoo for less than four weeks. Diagnosis of dandruff was based on clinical findings of white scales on the scalp and hair shaft, with or without mild inflammation or redness. Tinea capitis was identified through clinical manifestations, including single or multiple scaly patches with alopecia and patches of alopecia with black dots at follicular orifices, indicating broken hairs. Psoriasis vulgaris was diagnosed by observing well-defined, thick, silvery white scales, the presence of a smooth, red membrane with bleeding points beneath the scales when manually removed (Auspitz sign), and typical lesion sites (Köebner area) such as the scalp, elbows, knees, and gluteal region. Atopic dermatitis was diagnosed based on clinical presentations of erythematous papules, patches, or plaques located on the face (particularly the cheeks), scalp, trunk, and extremities.

Study procedures

Shampoo application

Patients were divided into two groups: 37 received 20% *C. ternatea* shampoo, and 33 received 2% ketoconazole shampoo. Shampoo was applied three times weekly for four weeks, using 5 mL each time (measured with a measuring spoon) applied to the hair and scalp, followed by adding water to create lather and leaving it on the hair and scalp for five minutes before rinsing. Patients were reminded of their shampooing schedule via WhatsApp messages by the author (T.S.D.I.S.A.).

Scalp scraping procedure

Scalp scrapings were collected from both the experimental and control groups before the initial shampoo application and after four weeks of treatment. Scrapings were obtained from dandruff on the scalp using two glass slides, scraping until slight pinpoint bleeding occurred. The scrapings were then swabbed with a swab moistened with buffered saline and placed into a tube containing Amies agar gel transport medium. The collected dandruff samples were processed at the Laboratory of Molecular Biology, Arifin Achmad Hospital, Pekanbaru, Indonesia, for further analysis.

DNA and RNA extraction

The DNA/RNA isolation was performed using Patho Gene-spin™ DNA/RNA Extraction Kit (Intron Biotechnology Inc, Seongnam, South Korea). A total of 300 µL of swab sample was transferred into a 1.5 mL tube, followed by the addition of 300 µL of lysis buffer, which was then mixed using a vortex for 15 seconds. The mixture was incubated at room temperature (15–25°C) for 10 minutes, after which 300 µL of binding buffer was added and homogenized with the vortex. The lysate was then added to a spin column placed in a provided 2 mL tube and centrifuged at 13,000 rpm for one minute. The liquid was discarded, and the column was returned to the same 2 mL tube. Subsequently, 500 µL of washing buffer A was added to the column and centrifuged for one minute at 13,000 rpm, followed by a second wash with 500 µL of washing buffer B. Buffers A and B used in the present study were prepared according to the kit manual. After the final wash, the column was placed into a new 1.5 mL RNase-free tube, and 50 µL of elution buffer was added directly to the spin column membrane. The column was incubated at room temperature for 1 minute and then centrifuged for 1 minute at 13,000 rpm. The isolated nucleic acids were either stored at -20°C or used directly for subsequent analysis.

cDNA synthesis

Complementary DNA (cDNA) synthesis from isolated total RNA was performed using ExcelRT™ Reverse Transcription Kit II (Smobio Technology Inc, Hsinchu, Taiwan) and Bio-Rad Thermal Cycler (Bio-Rad Laboratories Inc, Hercules, California, USA). Reagents and RNA samples were prepared as follows: for each sample, Mix A included 5 µL of total RNA (~30 ng), 1 µL of Oligo (dT)/Random Primer Mix, and 4 µL of diethyl pyrocarbonate (DEPC)-treated H2O, resulting in a final volume of 10 µL. Mix A was incubated at 70°C for 5 minutes and then placed on ice for at least 1 minute. Mix B was prepared with 4 µL of 5X reverse transcription (RT) buffer (deoxythymidine triphosphate (dTTP) or deoxyribonucleoside triphosphates (dNTP)), 5 µL of DEPC-treated H2O, and 1 µL of RTase/RI Enzyme Mix. Mix A and Mix B were combined, yielding a total volume of 20 µL. cDNA synthesis was performed with the following thermal cycling protocol: incubation at 25°C for 10 minutes, 42°C for 50 minutes, and termination at 85°C for 5 minutes. The synthesized cDNA was then stored at -20°C.

Real-Time quantitative PCR (RT-qPCR): Malassezia spp. DNA expression, plakoglobin levels, and IL-8 levels measurement

Malassezia spp. DNA expression, plakoglobin levels, and IL-8 levels were measured for both the experimental and control groups before the initial shampoo application and after four weeks of treatment. For RT-qPCR quantification of *Malassezia* spp., SensiFAST™ SYBR® No-ROX Kit (Bioline, Meridian Bioscience Inc, London, UK) and Bio-Rad CFX-96 PCR instrument (Bio-Rad Laboratories Inc, Hercules, California, USA) were used. Primers specific for *Malassezia* spp.

DNA, plakoglobin, and IL-8 were employed. The master mix for each target primer was prepared as follows: 10 μL of 2x SensiFAST SYBR® No-ROX Mix, 0.8 μL each of 10 μM forward and reverse primers, and DEPC-treated H2O to a final volume of 15 μL. This was aliquoted into RT-qPCR plate wells, with 5 μL of isolated sample for *Malassezia* spp. or 5 μL of cDNA for other primers added per well. The RT-qPCR protocol included polymerase activation at 95°C for 2 minutes, followed by 40 cycles of denaturation at 95°C for 5 seconds and annealing/extension at 60°C for 30 seconds, concluded with melting analysis.

Sebum levels measurement
Sebum levels were measured for both the experimental and control groups before the initial shampoo application and after four weeks of treatment. Sebum levels were assessed using Skin Analyzer Model SK-8 (ISEMECO, Shanghai, China) by positioning the probe on the dandruff-affected areas of the scalp, with the sebum concentration automatically displayed on the screen as a percentage.

Dandruff severity score
The dandruff severity score was measured for both the experimental and control groups before the initial shampoo application and after four weeks of treatment. The severity of dandruff was assessed using the Criteria for Clinical Severity Score [23] and Investigator Global Assessment [24]. Erythema was scored as follows: 0 for no redness, 1 for pale pink, 2 for pink, and 3 for bright red. Dandruff severity was rated with 0 for no flakes, 1 for flakes visible only upon scraping, 2 for visible scales, and 3 for layered scales. Lesion extent was categorized as 0 for no lesions, 1 for 1–30% of the scalp affected, 2 for 31–70%, and 3 for 71–100%. Pruritus was assessed with 0 for no itching, 1 for mild itching, 2 for itching causing discomfort, and 3 for extreme itching. The combined maximum score was 12, indicating severe symptoms, and the minimum score was 0, indicating no symptoms.

Patient satisfaction assessment
Patient satisfaction was measured for both the experimental and control groups only after four weeks of treatment. Satisfaction with 20% *C. ternatea* shampoo and 2% ketoconazole shampoo was assessed using a single question with a 5-point Likert scale, where 1 indicated "not satisfied," 2 indicated "less satisfied," 3 indicated "moderately satisfied," 4 indicated "satisfied," and 5 indicated "very satisfied."

Adverse effects documentation
Adverse effects were documented for both the experimental and control groups only after four weeks of treatment. Patients in both groups were interviewed about skin symptoms associated with shampoo use, such as itching, stinging, burning, hair loss, stiff hair, and dry scalp, occurring within the four weeks of shampoo application.

Study variables
Patients' data were documented, including age, dandruff duration, history of scalp skin diseases, and treatment history. Primary outcomes of the present study included *Malassezia* spp. DNA expression, plakoglobin levels, IL-8 levels, sebum levels, dandruff severity scores, adverse effects, and patient satisfaction. RT-qPCR using Bio-Rad CFX96 quantified *Malassezia* spp. DNA, plakoglobin, and IL-8 levels in scalp scrapings, with results expressed in terms of Quantification Cycle (Cq). Sebum levels were measured with Skin Analyzer Model SK-8 and expressed as a percentage. Dandruff severity was assessed using Criteria for Clinical Severity Score and Investigator Global Assessment, with a maximum score of 12. Patient satisfaction was evaluated using a single question with a 5-point Likert scale, where 1 indicated "not satisfied," 2 indicated "less satisfied," 3 indicated "moderately satisfied," 4 indicated "satisfied," and 5 indicated "very satisfied." Adverse effects were presented as categorical data with frequency and percentage.

Statistical analysis

SPSS version 25.0 software (IBM SPSS, Chicago, Illinois, USA) was employed for data analysis, with $p<0.05$ set as the statistical significance threshold. Continuous data were presented as mean and standard deviation (for normally distributed data) and median (minimum-maximum) for non-normally distributed data; categorical data were presented as frequency and percentage. Shapiro-Wilk test was utilized to assess data distribution normality. To compare the effects of shampoo treatment between experimental and control groups, paired t-tests and Wilcoxon signed-rank tests were used for normally and non-normally distributed data, respectively. Differences between the groups were assessed using independent t-tests and Mann-Whitney tests, depending on the data distribution. Spearman's rho correlation was applied to evaluate the correlation of dandruff severity scores between experimental and control groups.

Results

Characteristic of patients

Both groups were similar in age distribution, with 54% aged 18–21 and 46% aged 22–25. All patients in both groups reported having dandruff for less than 6 months. There were no cases of tinea capitis, seborrheic dermatitis, psoriasis vulgaris, or atopic dermatitis in either group. Additionally, neither group had previously used anti-dandruff shampoos or topical corticosteroids. All subjects were present during the follow-up phase and complied with the shampoo application, resulting in no dropouts.

Malassezia spp. DNA expression

The present study found that *Malassezia* spp. DNA expression decreased significantly in both experimental and control groups (**Table 1**). In the 20% *C. ternatea* shampoo group, the DNA expression changed from 21.51±1.67 Cq to 23.37±3.04 Cq ($p=0.001$). In the 2% ketoconazole shampoo group, it changed from 21.71±2.45 Cq to 25.50±1.78 Cq ($p<0.001$). The delta in DNA expression was 1.76±3.18 Cq for 20% *C. ternatea* shampoo group and 3.77±2.90 Cq for 2% ketoconazole shampoo group, with a significant difference between groups ($p=0.008$), indicating greater efficacy of 2% ketoconazole shampoo in reducing *Malassezia* spp.

Plakoglobin levels

Plakoglobin levels decreased significantly in both experimental and control groups (**Table 1**). In the present study, plakoglobin levels in the 20% *C. ternatea* shampoo group decreased from 34.75±3.05 Cq before use to 36.73±1.56 Cq after use ($p=0.002$). In the 2% ketoconazole shampoo group, the plakoglobin levels decreased from 35.19±2.46 Cq to 37.70±1.12 Cq ($p<0.001$). The delta in plakoglobin levels was 1.9±3.63 Cq for 20% *C. ternatea* shampoo group and 2.50±2.36 Cq for 2% ketoconazole shampoo group. While the 2% ketoconazole shampoo group exhibited a greater reduction, the difference between the two groups was not statistically significant ($p=0.427$, **Table 1**).

IL-8 levels

IL-8 levels in the 20% *C. ternatea* shampoo group increased from 29.55±3.00 Cq before use to 33.02±2.85 Cq after use ($p<0.001$, **Table 1**). In the 2% ketoconazole shampoo group, IL-8 levels increased from 29.96±3.31 Cq to 34.00±1.35 Cq ($p<0.001$). The delta in IL-8 levels was 3.46±4.00 Cq for 20% *C. ternatea* shampoo group and 4.16±3.62 Cq for 2% ketoconazole shampoo group. Although the delta was higher in the 2% ketoconazole shampoo group, the difference between the two groups was not statistically significant ($p=0.459$, **Table 1**).

Sebum levels

The sebum levels decreased significantly from 6.21±1.15% to 5.05±0.36% in the 20% *C. ternatea* shampoo group ($p<0.001$) (**Table 1**). For the 2% ketoconazole shampoo group, sebum levels reduced from 5.33±0.41% to 5.11±0.39% ($p=0.002$). The change in sebum levels (Δ) was 1.16±0.98% with 20% *C. ternatea* shampoo group and 0.22±0.38% with 2% ketoconazole shampoo group, with a significant difference between groups ($p<0.001$). *C. ternatea* shampoo resulted in a greater reduction in sebum levels compared to 2% ketoconazole (**Table 1**).

Dandruff severity scores

The median dandruff scores decreased significantly from 4 (range: 2–7) to 1 (range: 0–4) with 20% *C. ternatea* shampoo group ($p<0.001$, **Table 1**). For the 2% ketoconazole shampoo group, the median scores decreased from 4 (range: 1–7) to 0 (range: 0–2) ($p<0.001$). The Δ dandruff scores were 2.94±1.31 for 20% *C. ternatea* shampoo group and 3.45±1.45 for 2% ketoconazole shampoo group, with no statistically significant difference between the groups ($p=0.115$). A significant negative correlation was found between *Malassezia* spp. (**Figure 1**). DNA Cq values and dandruff scores, with a stronger correlation in the 2% ketoconazole shampoo group (Control group: $r=-0.677$, $p<0.001$; Experimental group: $r=-0.286$, $p=0.014$; **Figure 1**).

Table 1. Efficacy of 20% *Clitoria ternatea* shampoo on ameliorating dandruff and patients' satisfaction score

Variables		*Malassezia* spp. DNA expression (Cq), mean±SD	Plakoglobin levels (Cq) mean±SD	IL-8 (Cq), mean±SD	Sebum levels (%)	Dandruff severity scores, median (min-max)	Patient satisfaction score
20% *Clitoria ternatea*	Before	21.51±1.67	34.75±3.05	29.55±3.00	6.21±1.15	4 (2–7)	NA
	After	23.37±3.04	36.73±1.56	33.02±2.85	5.05±0.36	1 (0–4)	4 (3–5)
	Δvalue	1.76±3.18	1.98±3.63	3.46±4.00	1.16±0.98	2.94±1.31	NA
	p-value	0.001*	0.002*	<0.001*	<0.001*	<0.001*	NA
2% ketoconazole	Before	21.71±2.45	35.19±2.46	29.96±3.31	5.33±0.41	4 (1–7)	NA
	After	25.5±1.78	37.7±1.12	34±1.35	5.11±0.39	0 (0–2)	4 (2-5)
	Δvalue	3.7±2.90	2.5±2.30	4.16±3.62	0.22±0.38	3.45±1.45	NA
	p-value	<0.001*	<0.001*	<0.001*	0.002*	<0.001*	NA
p-value		0.008*	0.427	0.459	<0.001*	0.115	0.336

Δ: changed value before and after; NA: not applicable
*Significant at $p<0.01$

Patient satisfaction and adverse effects

The median for patient satisfaction was 4 for both the 20% *C. ternatea* and 2% ketoconazole shampoo groups, with no significant difference between the groups ($p=0.336$) (**Table 1**), among the 37 patients using 20% *C. ternatea* shampoo, 1 patient (2.7%) experienced hair loss. In the 2% ketoconazole shampoo group, adverse effects included stiff hair in 3 patients (9.1%), dry scalp in 2 patients (6.1%), burning or itching in 1 patient (3.0%), and hair loss in 1 patient (3.0%). No serious adverse effects were reported. Overall, 21.2% of patients in the 2% ketoconazole shampoo group experienced adverse effects (**Table 2**).

Table 2. Adverse effects: 20% *C. ternatea* shampoo compared to 2% ketoconazole shampoo

Adverse effects	20% *Clitoria ternatea*, n (%)	2% ketoconazole, n (%)
Itching, stinging, and burning	0	1 (3.03)
Hair loss	1 (2.7)	1 (3.03)
Stiff hair	0	3 (9.09)
Dry scalp	0	2 (6.06)

Discussion

In the present study, 2% ketoconazole shampoo demonstrated superior efficacy in reducing *Malassezia* spp. DNA expression, while 20% *C. ternatea* shampoo may offer better sebum control. Both treatments are similarly effective in reducing dandruff severity and are well-tolerated by patients, with minimal adverse effects reported for 20% *C. ternatea* shampoo.

The delta value (ΔCq) for the 2% ketoconazole shampoo group in the present study is higher (3.77±2.90 Cq) compared to the 20% *C. ternatea* shampoo group (1.76±3.18 Cq). A higher ΔCq indicates a greater reduction in DNA expression levels of *Malassezia* spp., suggesting that 2% ketoconazole shampoo is more effective ($p=0.008$). *C. ternatea* produces a range of secondary metabolites, including phenolic compounds, phenylpropanoids, saponins, terpenoids, alkaloids, tannins, and steroids, with flavonoids being the most prominent [27,28]. These flavonoids, particularly in the flowers of *C. ternatea,* are recognized for their potent antifungal activity [29,30]. This antifungal effect is primarily attributed to the defensin protein CTD-1 [30-32]. CTD-

1 protein features a 228 bp open reading frame and consists of 75 amino acids: 28 N-terminal amino acids form a signal peptide, and 47 amino acids form the mature peptide [32]. The mature peptide contains eight cysteine residues that create four disulfide bridges (Cys14-Cys35, Cys20-Cys41, Cys24-Cys43, Cys3-Cys47), which stabilize its structure [28,32]. Known as cysteine-rich antifungal protein (CRAFP), CTD-1 exhibits strong antifungal properties [28,32,33]. CRAFP displays lytic activity against various yeasts, including *Cryptococcus neoformans, Cryptococcus albidus, Cryptococcus laurentii, Candida albicans, Candida parapsilosis, Curvularia* sp., *Alternaria* sp., *Cladosporium* sp., *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Rhizopus* sp., and *Sclerotium* sp. [33-38].
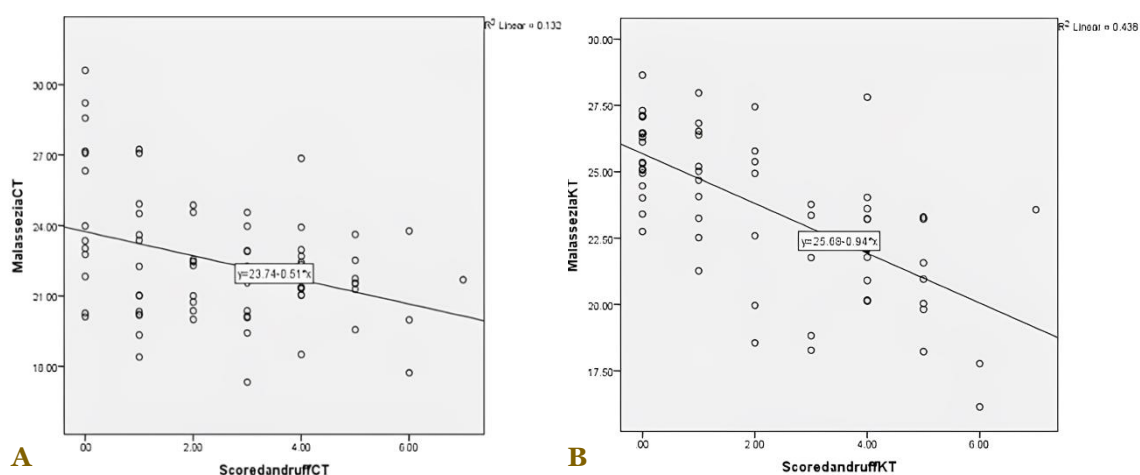

Figure 1. Correlation plot of dandruff severity scores between (A) 20% *Clitoria ternatea* group and (B) 2% ketoconazole group.

In the present study, 2% ketoconazole shampoo reduced plakoglobin levels more (2.50±2.36 Cq) than 20% *C. ternatea* shampoo (1.98±3.63 Cq), yet the difference was not statistically significant (p=0.427); thus, there is no strong evidence that ketoconazole is significantly more effective than *C. ternatea*. The extent of corneodesmosomal protein release in dandruff correlates with enzyme-mediated hydrolysis, including plakoglobin [39,40]. Rawlings *et al*. described that maintaining corneocyte function as a healthy skin barrier relies on the cadherin family of transmembrane glycoproteins, notably desmoglein 1 and desmocollin 1 [40]. These proteins link corneocytes to keratin filaments via corneodesmosomal proteins such as plakoglobin, desmoplakin, and plakophilin [39,40]. Corneocyte exfoliation in the skin is facilitated by hydrolytic enzymes in the stratum corneum that degrade corneodesmosomal proteins and glycoproteins, explaining the presence of plakoglobin in dandruff scales [39,40]. Key enzymes in the desquamation process include sphingoid hydrolase, ceramidase, glycosidases, serine proteases, cysteine proteases, and aspartic proteases, resulting in increased corneodesmosomal protein release, detectable in dandruff scales [39,40].

Group receiving 2% ketoconazole shampoo (4.16±3.62 Cq) resulted in a slightly higher reduction in IL-8 levels compared to the group receiving 20% *C. ternatea* shampoo (3.46±4.00 Cq); however, the difference is not statistically significant (p=0.459), indicating there is no strong evidence that 2% ketoconazole shampoo is more effective than 20% *C. ternatea* shampoo in reducing IL-8 levels. *C. ternatea* demonstrates significant anti-inflammatory potential, attributed to its steroid and flavonoid content [41]. This is supported by a study by Al-Khayri found that flavonoids exert anti-inflammatory effects by inhibiting enzyme secretion (e.g., lysozymes, β-glucuronidase) and arachidonic acid, which reduces inflammation [41]. Flavonoids such as quercetin and epigallocatechin 3-gallate modulate cytokines (IL-1β, TNF-α, IL-6, IL-8) and regulate pro-inflammatory gene expression (e.g., nuclear factor κB (NF-κB), activator protein 1 (AP-1)) and inhibit pro-inflammatory enzymes (nitric oxide [NO] synthase, cyclooxygenase-2 (COX-2), lipoxygenase) [41]. A study by Shyamkumar and Ishwar reported a significant inflammation reduction in rats with *C. ternatea* petroleum ether extracts at doses of 200 mg/kg

and 400 mg/kg [42]. A study by Indriyani found that *C. ternatea* ethanol extracts in cream form (0.5%, 1%, 1.5%) effectively reduced carrageenan-induced paw edema in rats [43].

The present study found that 20% *C. ternatea* shampoo (1.16±0.98%) reduced sebum levels more than 2% ketoconazole shampoo (0.22±0.38%), with a significant difference ($p<0.001$), indicating that *C. ternatea* is significantly more effective. *C. ternatea* exhibits anti-sebum effects primarily due to its potent antioxidant properties [44]. Antioxidants can inhibit pro-inflammatory cytokines, matrix metalloproteinases, and sebum production [18,44,45]. Kwack *et al.* demonstrated that antioxidants reduce sebum levels by downregulating proliferator-activated receptor (PPAR)-γ, stearoyl-CoA desaturase (SCD), and sterol regulatory element-binding proteins (SREBP) 1a and 1c in sebocytes [44]. Chauhan *et al.* showed that aqueous and ethanol extracts of *C. ternatea* exhibit potent antioxidant effects [18]. *C. ternatea* maintains cellular integrity and immune function by neutralizing reactive oxygen species (ROS), which disrupt skin homeostasis [18]. Rangkuti *et al.* found that a 5% *C. ternatea* flower extract cream significantly reduced inflammatory and non-inflammatory lesions in acne vulgaris (p<0.001), indicating its potential for treating other sebum-related skin conditions [45].

In the 20% *C. ternatea* group, one patient (2.7%) experienced hair loss, but overall, the minimal side effects indicate that *C. ternatea* is a safe topical treatment for dandruff. Supporting studies confirmed the safety of 20% *C. ternatea* for topical use [46,47]. A study by Daryanto found no irritation from a gel spray of *C. ternatea* seed extract, and Irawan *et al.* reported no irritation in a study of an ointment with *C. ternatea* flower extract [46,47]. Thus, no severe irritation or allergic reactions have been documented with topical *C. ternatea* use.

Although ketoconazole is highly effective in reducing dandruff symptoms, the present study found that 21.2% of patients using 2% ketoconazole shampoo experienced adverse effects: stiff hair in 3 patients (9.1%), dry scalp in 2 patients (6.1%), burning or itching in 1 patient (3.0%), and hair loss in 1 patient (3.0%). Aligned with the present study, Kubicki *et al.* reported a case of hair discoloration to reddish hues in a 61-year-old woman using 2% ketoconazole shampoo for three weeks, with pink to red particles observed on the hair shafts [48].

To the best of our knowledge, the present study is the first to explore the effect of *C. ternatea* extract as an anti-dandruff. A limitation of the present study is the four-week assessment period, which is insufficient for evaluating the long-term effectiveness and safety of 20% *C. ternatea* shampoo – longer usage is needed for a comprehensive assessment. Future research should investigate the effects of *C. ternatea* in dandruff remission and explore other plant parts and formulations of *C. ternatea* to enhance its antifungal activity and inhibition rate.

Conclusion

While 2% ketoconazole shampoo exhibited greater efficacy in decreasing *Malassezia* spp. DNA expression, 20% *C. ternatea* shampoo provided more effective sebum control. Both 2% ketoconazole and 20% *C. ternatea* shampoo showed comparable effectiveness in ameliorating dandruff severity and were well-tolerated by patients, with fewer adverse effects reported for 20% *C. ternatea* shampoo.

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

How to cite

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