Clinical efficacy of natural formulated shampoo in subjects with dandruff and seborrheic dermatitis

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Abstract

Backgrounds and aims: Dandruff is a common scalp condition characterized by flakes, pruritus and sometimes mild erythema. Despite the development of several medical treatments**,** the number of men and women who suffer from Dandruff and Seborrheic Dermatitis is increasing. We searched for potential therapeutics among those plant extracts that have been used traditionally in oriental medicine for treating Dandruff (D) and Seborrheic Dermatitis (SD).

Patients & Methods: 120 volunteers, who suffered from dandruff, were recruited into the study in 3 groups. The first group used Cepigene shampoo, the second group used Ketoconazole shampoo and the third group used vehicle. The samples were also cultured for fungi detection. For isolating the fungi and evaluating the rate of Dandruff and Seborrheic dermatitis, the subjects were sampled from their scalp in zero day of the study. Trypan blue assay was used to study the antifungal effects of cepigen and ketoconasole.

Results: There was a remarkable decrease in the scaling and itching of scalp after a weeklong treatment by Cepigene and Ketoconazole shampoo. Indeed, both products delivered a reduction in

ASF scores in comparison with those of controls. Trypane blue assay showed a dose-dependent decrease in the M. furfur and M. globosa viability following exposure to cepigen and ketoconazole.

Conclusion: This study supports the efficacy in treating Dandruff and Seborrheic Dermatitis with Cepigene shampoo enriched in appropriate chemical and herbal compounds.

Key words: Dandruff, Seborrheic Dermatitis, Cepigen shampoo, Antifungal

Running title: Anti- dandruff in Natural Formulated Shampoos

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Introduction

Dandruff (D) and Seborrheic Dermatitis (SD) are features of fungal infection of the scalp and occur in nearly half of the population regardless of gender and race (1). In addition to problems such as itching, burning and other clinical complications, psychological and social draw backs may well be experienced by the patients (2).

Despite the high prevalence of D and SD, little is known about its etiology; however, several factors (such as *malassezia* fungi, variation of hormone levels, nutritional deficiency and neurogenic factors) are associated with these conditions (1, 3, and 4). Additionally, in Iran, large number of cases with D or SD refers to clinics for treatments annually, but there is no standard research or available data for D or SD in this country.

The relationship between D and SD is controversial. Some may believe that the two conditions are distinct clinical entities, while others believe that D is the mildest, non-inflammatory form of SD. The involvement of *Malassezia* in the conditions has also been extensively debated; SD presents

clinically as scaling and inflammation on the areas of the body rich in sebaceous glands, such as face, scalp and the upper trunk, whereas D is a non-inflammatory scaling condition confined to the scalp. Lesions of the SD occur primarily on the eyebrows, nasolabial folds, cheeks, and sternal and interscapular regions (5).

A more causal link that seems to exist between D and SD is the proliferation of *Malassezia* species (e.g., *Malassezia furfur, Malassezia globosa* and *Malassezia ovalis*) found in normal dimorphic human flora (1, 3, and 4).

There are several ways for treatment of D and SD but Shampoos treatments are the most common means of managing hair and scalp conditions (6).

The number of men and women who suffer from Dandruff (D) or Seborrheic Dermatitis (SD) is increasing, despite the development of several medical treatments. Therefore, it is of great importance to develop novel therapies to prevent Dandruff (D) and Seborrheic Dermatitis (SD). In this respect, alternative medicine has attracted interest. Although it has not yet been incorporated into mainstream of medical care, due to limited scientific evidence and incomplete knowledge of the mechanisms involved, alternative medicine has become an increasingly attractive approach worldwide. The search for treatment results into few drugs of synthetic origin, but side effects associated with them cannot be neglected. Herbal cosmetics are now emerged as the appropriate solution to the current problem, natural products are fancy in cosmetics and about 1000 kinds of plant extract have been examined with respect to hair growth and still it is a fast growing segment with a vast scope of manifold expansion in coming years.

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Taking into account what said above, we searched for potential therapeutics among those plant extracts that have been used traditionally in oriental medicine for treating Dandruff (D) and Seborrheic Dermatitis (SD).

Materials & Methods

Study population

With local ethical approval and informed consent**,** 120 volunteers (60 men and 60 women) aged 18–75 years, who suffered from dandruff, were recruited into the study. Dandruff affection was based on the following assessment scale: 1 = no dandruff; 2 = single dandruff visible; 3 = slight dandruff affection; 4 = medium dandruff affection; 5 = strong dandruff affection; 6 = very strong dandruff affection.To standardize the hair and scalp condition prior to the study start, all subjects underwent a 2-week wash-out period with a standard cleansing shampoo. They were requested not to take anti-fungal drugs such as steroids and retinoids, as well as immune suppressive drugs concurrent with this research.

Test products

In this study, the subjects were divided into three groups of 40 subjects (20 men and 20 women). In group1, subjects were assigned to a treatment regimen that consisted of 10 ml syringes of a cepigen-containing shampoo (Table1) which the subject used three times per week for 7 week. In second group, subjects were assigned to a treatment that consisted of a Ketoconazole shampoo to be used at least three times per week for 7 weeks. In third group, subjects were assigned to a non-medicated treatment regimen which consisted of 10 ml syringes of basic shampoo (Table2) to be used three times per week as a control group. The samples were also cultured for fungi detection. For isolating the fungi and evaluating the rate of D and SD, the subjects were sampled from their scalp in zero day of the study.

Table1: List of ingredients used in Cepigene shampoo

Name	Features	Percent
Piroctone olamine	Also known as an Octopirox, and effective in the treatment of fungal infections.	0.5
Salicylic acid	Mono-hydroxy benzoic acid that is effective in reducing fever and pain. It has also anti-inflammatory properties.	1
α-bisabolol	Anti-inflammatory properties	0.1
Aloe vera	A medicinal plant with anti-inflammatory, antimicrobial, antidiabetic and immune-boosting properties.	3
Tea tree oil	Disinfection, anti-inflammatory, anti-cancer, anti-virus, anti-microbial and anti-fungal properties.	3
Rosemary	It has anti-fungal properties and enhances blood flow to the scalp.	2
Salvia officinalis	It extracts on the scalp causing dandruff control.	3
Lavender	Disinfection, anti-fungal, anti-bacterial and anti-inflammatory properties.	3
Thyme Extract	It enjoys anti-bacterial and anti-fungal properties, and contains antioxidant	3
Willow extract	Anti inflammatory activity and keratolytic properties.	1
Marshmallow extract		3

Table2: List of ingredients used in vehicle

Name	Percent
Disodium MEA Sulfosuccinate	30%
Coco Amidopropyl Betaine	30%
Sodium lauryl Ether Sulfat	70%
Amonium lauryl Ether Sulfat	70%
Lauryl polyglycosite	50%

Isolation of Dandruff

The samples were obtained by scrapping the skin surface and the scalp with a sterile blade and transferred to the laboratory (7).

Isolation and Detection of Fungi

Isolation of fungi was carried out *in vitro* to determine the type of fungus. Diagnosis of *Malassezia* was made after budding the yeast cells that were microscopically observed on skin scales stained with methylene blue. For counting the yeast cells, each slide was examined under high power field (hpf) of microscope and it was recorded as the number of observed yeast cell per hpf. All samples were also cultivated on Leeming and Notman medium and Sabouraud's dextrose agar culture media. The agar plates were incubated at 32°C for 2 weeks and evaluated for the existence of growth every day for one week. Identification of the isolated yeasts was based on the morphologic and physiologic tests, namely Tween assimilation profiles and Catalase reaction (8).

The fungal culture

Malassezia species were identified according to Guillot et al. (9). Using isolates from the primary cultures. *M. pachydermatis* is able to grow on Sabouraud's agar. Tween assimilation and Catalase test were applied for identification of other *Malassezia* species. Briefly, *Malassezia* yeast suspensions were prepared in sterile distillated water. Then they were adjusted to McFarland No. 2 turbidity, mixed with Mycobiotic agar with cyclohexamide and chloramphenicol, and poured into the plates. Four holes were made in the Mycoviotic agar by means of a 3-mm diameter punch and filled with 30 µl each of 20, 40, 60 and 80 Tweens, respectively. The agar plates, incubated at 32°C for 1 week, were examined each day for the existence of any growth around the wells containing Tween compounds (8). For catalase Test One drop of hydrogen peroxide solution was applied onto a glass slide of a yeast colony. Production of gas bubbles was considered a positive Catalase reaction (9).

Viability of Malassezia species after antifungal treatment

For measurements of antifungal cytotoxicity, *Malassezia* species (M. globosa and M. furfur) were inoculated at 1×10^6 cells/ml in Sabouraud broth. M. globosa and M. furfur in cell suspension were treated with the antifungal agents, 0.5%-1.5% of cepigen and 0.5-1.5% of Ketoconazole. After

variable incubation times in a shaking incubator at 180 rpm, *Malassezia* species cells were centrifuged at 2500 rpm for 15 minutes and then washed with 0.1 M PBS. For the assessment of cell cytotoxicity, cells were counted using a hemocytometer after stained with trypan blue dye (1:1) to exclude non-viable cells.

Adherent scalp flake scoring

Shampoos were compared for their efficacy in treating dandruff by comparing the effect of treatment on ASF scores as reported previously [12]. Briefly, scalp examinations were conducted at baseline and after 3 and 7 weeks of supervised use of the assigned shampoo. At each examination, subjects were assessed for ASF scores under a 200-W photographic type spotlight located to the rear of the grader to provide a uniform light source. A comb was used to part the hair into eight sections. These eight sections (octants) were assigned a grade ranging from 0 (no scaling) to 10 (very heavy scaling). The final flaking score is a composite of the grades for all eight octants, which results in a scale ranging from 0 to 80 points. Assessments were performed by a qualified grader trained by a board-certified dermatologist trained in the method (9).

Statistical analysis: Non parametric one-way analysis of variance (ANOVA) was performed with the Dunnett's test, using software Graphpad Prism. Each experiment was carried out in triplicate and repeated three to four times independently. $P < 0.05$ was considered significant. All data are expressed as means ± SD.

Results

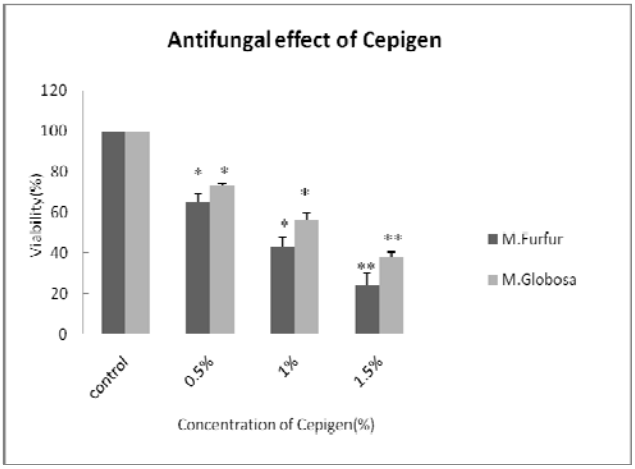
Fungal test results

The most commonly isolated *Malassezia* species was *M. globosa* (48.9%), followed by *M. furfur* (39.6%), *M. restricta* (6.5%), *M. sympodialis* (3.6%) and *M. japonica* (2.4%).

Antifungal effect of cepigen and Ketoconazole

The antifungal effects of cepigen and ketoconazole on the M. furfur and M. globosa viability were examined. M. furfur and M. globosa were treated with various concentrations of cepigen and ketoconazole (0.5%-1.5%) for 48 hours and the viability was measured. Trypane blue assay showed a dose-dependent decrease in the M. furfur and M. globosa viability following exposure to cepigen. As it is observed in Fig 1a, growth inhibition effects of cepigen in M. furfur starting at 0.5% and increased up to 1.5% (from 65% ± 4.1% for 0.5% to 24% ± 5.1% for 1.5% vs. control 100%, respectively; $P < 0.05$, one way ANOVA) . As shown in Fig. 1a, cepigen induced a significant reduction in the M. globosa viability (73± 2.47% for 0.5% to 38% ± 3.4% for 1.5% vs. control 100%, respectively; $P < 0.05$, one way ANOVA). In addition, ketoconazole induced significant inhibition of M. furfur and M. globosa viability, respectively (Fig. 1b). Although these data suggested a cytotoxic effects by cepigene and ketoconazole on these *Malassezia* species but anti-proliferative effect of cepigne is more than ketoconazole (But it is not significant).

Figre1a:



Figre1b:

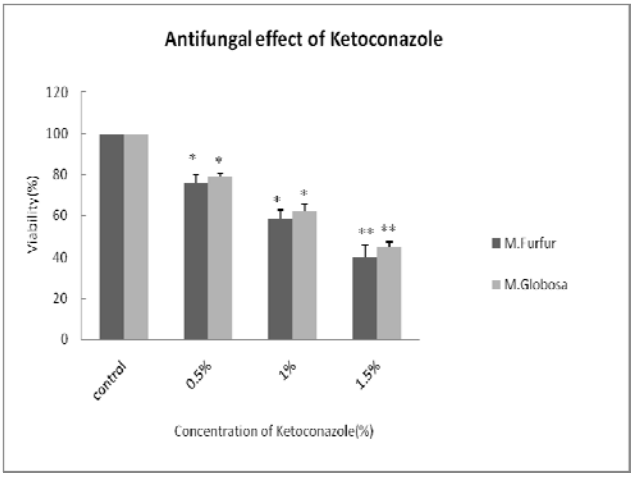


Figure1: The effects of cepigen and ketoconazole in inhibition of M.furfur and M.globosa viability. Cells were treated with different concentrations of cepigen and ketoconazole for 48 hr, and viability was assessed by Trypan blue. Cepigen (a) and ketoconazole (b) reduced viability of M.furfur and M.globosa in a dose dependent manner. Results (mean ± SD) were calculated as percent of corresponding control values. $^{*}P$ <0.05, $^{**}P$ <0.01 are significant. Statistical analysis was performed by ANOVA. Each point represents 4 repeats, each triplicate.

Reduction of scalp flaking following treatment with Cepigen shampoo

In this study, 80 subjects suffering from dandruff were assigned to a treatment regimen that required them to use the contents of a syringe containing 10 ml of a cepigen shampoo or Ketoconazole shampoo, three times per week for 7weeks. Dosing was standardized by using syringes to define the amounts of product. Another 40 subjects suffering from dandruff were assigned to an identical treatment regimen that used a non-medicated shampoo as control.

Adherent scalp flake scores were evaluated at the end of this period and compared with scores at baseline. As shown in table3, both products delivered a reduction in ASF scores in comparison with those of controls.

Table3: Decrease in flake appearance as adherent scalp flake score (ASFS)

Group study	Base line ASFS score	Week 3 ASFS score	Week 7 ASFS score
Group1	41±4	22±5	5±2
Group2	42±6	29±3	8±5

Effects of cepigen and ketoconazole shampoos on Dandruff (D) and Seborrheic Dermatitis (SD)

At week 2, the subjects receiving cepigen shampoo had a significantly greater reduction from baseline in scaling and itching of scalp compared with the subjects receiving vehicle (Fig. 2). Similarly, the subjects receiving the Ketoconazole shampoo had significantly greater reductions from baseline to week 4 in scaling and itching of scalp compared with the subjects receiving vehicle (Fig2). Indeed, cepigen shampoo therapy was significantly more efficacious than the ketoconazole shampoo therapy in decreasing the severity of scaling and itching at week 4.

There was a remarkable decrease in the scaling and itching of scalp after a weeklong treatment by Cepigene shampoo and Ketoconazole shampoo. Subsequently, there was considerable improvement in the healing of the D and SD lesions after the 7^{th} week and there was complete control of D and SD in all the patients in these groups (Fig 2).

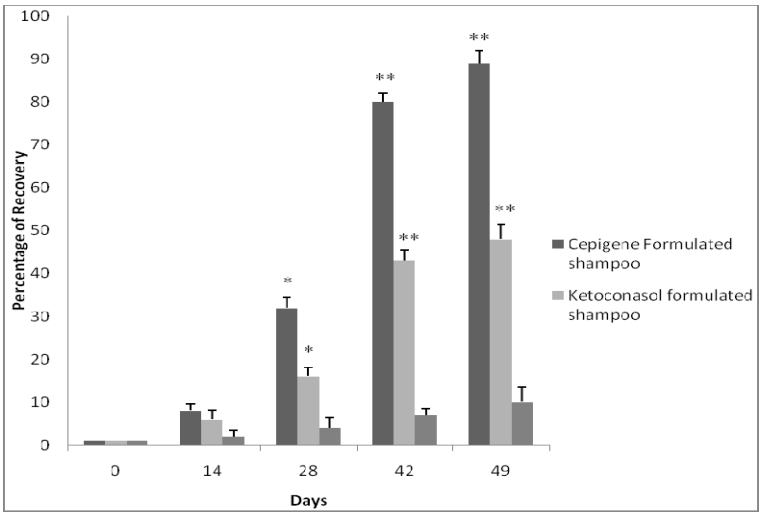


Figure2: percentage recovery of cepigen and ketoconazole shampoos on Dandruff (D) and Seborrheic Dermatitis (SD). The subjects receiving cepigen shampoo had a significantly greater reduction from baseline in scaling and itching of scalp compared with the subjects receiving vehicle.* *P* <0.05, ** *P* <0.01 are significant. Statistical analysis was performed by ANOVA. Each point represents 4 repeats, each triplicate.

Discussion

Dandruff (D) and Seborrheic Dermatitis (SD) are chronic, inflammatory skin disorders, affecting areas of the head and body where sebaceous glands are most prominent and active. The etiology of D and SD is unknown, though some hormones and the *Malassezia spp* are thought to be involved in the development of these disorders (10).

Malassezia species are lipid-dependent microorganisms that adapt to the narrow niche provided by sebum-rich skin. *Malassezia globosa* and *M. restricta* predominate on dandruff scalp. *Malassezia globosa* likely initiates dandruff formation due to its high lipase activity. *Malassezia restricta* and *M. globosa* require lipids. Due to its inability to metabolize its own lipids, *Malassezia* species relies on the lipids in sebum. Sebum is degraded to free fatty acids from triglycerides. Saturated fatty acids are consumed while unsaturated fatty acids are left behind: penetration of unsaturated fatty acids results in inflammation, irritation and scalp flaking. There remains a lack of correlation

between the number of *Malassezia* cells and the development and severity of D and SD and other forms of dermatitis. However, there may be a correlation between specific *Malassezia* metabolites and severity of infection, specifically irritating free fatty acid metabolites (11).

Washing hair with a regular shampoo can produce a small and transient effect on D and SD by removing loosely adherent scales (12).According to the association of the causing agents of D and SD with such factors as sex (hormonal changes), climate, culture, education level and economic conditions (nutrition and hair care), race (hair and skin type) and using drugs, the subjects selected for this study were evaluated and matched on all possible cases.

The purpose of this study was evaluating the anti- D and SD effects of Cepigene shampoo.

For this purpose, 120 subjects were selected and divided into three different groups. Each group was asked to use only one kind of shampoo during a period of 7 weeks. In this study, the first group was asked to use the Cepigenee shampoo. The second group was asked to use Ketoconazole shampoo and from the third group was asked to use vehicle. All subjects were asked to use their shampoo three times per week for 7 weeks. Each participant had to massage his scalp with 10 ml of shampoo for 5 minutes.

The results of using the vehicle in the third group after the 7^{th} week showed that only 10% of D and SD were reduced, and the vehicle was not effective. The 10% reduction was due to the use of shampoo at desired times. Since the vehicle contained detergents that reduce fat, 10% reduction in D and SD was due to decrease in the skin fat.

Regarding the Ketoconazole and Cepigene shampoos, the rate of decrease in D and SD in the second week was 8% and 10%; in the fourth week, 16% and 32%; in the sixth week, 43% and 80% and in the seventh week, 48% and 89%, respectively.

The rate of decrease in D and SD for the subjects that used Ketoconazle shampoo was suitable but for the subjects that used Cepigene shampoo was far better, Such that after the seventh week, the subjects in the first (Cepigene) group have been fully recovered.

Each of the Ketoconazole and Cepigene shampoos contained compounds that can treat D and SD by special mechanism:

Anti-fungal effect of Ketoconazole acts through inhibition of cytochrome P-450 enzyme (13). By inhibition of the cytochrome, production of ergosterol, which is a vital component of cell membrane of fungus, is inhibited (14, 15).

Azoles such as Ketoconazole have selective toxicity against fungi, because ergosterol synthesis, which is a sterol unique to fungal cell membrane, will interfere by azoles. Azoles with ergosterol inhibition disrupt the fungal cell membrane's permeability, and inhibit the activity of the yeast. This, in turn, results in fewer young cells that are made in the skin, and thus prevents the creation of scales (16, 17).

Piroctone olamine is an active combination in the treatment of D and SD that relieves inflammation of the scalp and reduces scale formation in the skin by fungi inhibition. Piroctone olamine is functionally similar to ciclopirox so that effects on the yeast cell division and transfer of materials (inhibition of sodium–potassium channel), and thus inhibits the fungus (18,19).

Salicylic acid is one of the active compounds that in treating D and SD. Salicylic acid is a kind of β-hydroxy acid that removes the hard shell and thick scalp through keratolytic activity, and thus is effective in treating D and SD (17).

Tea tree oil is an essential oil in the leaves of a tree in Australia, namely Melaleuca *alternifolia*, which is used for treating D and SD (20). This combination has disinfection, anti-inflammatory, anti-cancer, anti-virus and anti-microbial and anti-fungal properties (21-24).

Tea tree oil has combinations called terpenes that penetrate into the top layer of skin and scalp and cause disinfection in that area (25, 26). Terpinen-4-ol that is one of the *tea tree oil* components can reduce the inflammation caused by histamine inhibition released by monocytes. The effect of tea tree oil on the fungus also causes loss of potassium ions, and respiration inhibition. α-Pinene

inhibits mitochondrial activity and respiration and, subsequently, kills the yeast; s a result, the scalp D and SD can be treated (27, 28).

Rosemary oil has anti-microbial, anti-viral and anti-inflammatory activity. Its anti-microbial activity is because of compounds in *Rosemary* such as camphor (18.9%), verbenone (11.3%), α-pinene (9.6%), β-myrcene (8.6%), 1, 8-cineole (8.0%), and beta-caryophyllene (5.1%) (29-32).

Lavender has a variety of health benefits. *Levander* oil has disinfecting, anti-fungal, anti-bacterial and anti-inflammation properties, and reduces pain and itching (33).

Salvia officinalis is effective in the treatment of D, SD and hair loss (34). *Salvia officinalis* has anti-bacterial, anti-virus, anti-inflammatory and antioxidant properties due to the presence of acid rosmarinic in this plant (35, 36).*Thyme* contains carvacrol that is a monoterpenoid phenol and has a powerful antioxidant activity. It also has anti-microbial and anti-fungal activity (37,38).

Willow extract is frequently used in the treatment of painful rheumatological diseases but it has a powerful anti inflammatory activity, which can be effective in treating inflammation caused by fungi (39). *Aloe vera* is a medicinal plant with anti-inflammatory, antimicrobial, antidiabetic and immune-boosting properties (40). It can also be used as a biological vehicle and an anti-microbial and antifungal agent and for treatment of SD (41).*Marshmallow* extract (*Althaea officinalis*) is an herb that is effective in treating some diseases. This plant has strong anti-inflammatory properties (42).

Conclusion

The results of this study indicate the potential use of cepigene shampoo for the treatment of Dandruff and Seborrheic Dermatitis, which show a potent antifungal activity against the fungi that isolated from samples such as M.furfur and M.globosa. These findings will be applied to further analysis of antifungal activity of compounds derived from natural sources.

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