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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Comparative study on the effects of a new antifungal lotion (*Artemisia sieberi* essential oil) and a clotrimazole lotion in the treatment of pityriasis versicolor

Étude comparative d'une nouvelle lotion antifongique à base d'huile essentielle d'*Artemisia sieberi* et d'une lotion au clotrimazole dans le traitement du pityriasis versicolor

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KEYWORDS
Pityriasis versicolor;
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Artemisia sieberi

Summary
Objective. — To determine the efficacy of Mycoderm lotion (*Artemisia sieberi* essential oil) in treating Pityriasis versicolor and comparing its effects with clotromazole lotion.
Material and methods. — Sixty-eight patients, 34 males and 34 females, were enrolled in this study. The disease was confirmed by standard mycological method; direct microscopic examination. The patients randomly divided into two groups, which treated with Mycoderm and clotrimazole lotions twice daily for two weeks and then followed up for two weeks.

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Results. — The results showed 71% improvement in clotrimazole group and 91.9% in Mycoderm group after two weeks of the treatment, representing significant statistical difference between two groups ($p < 0.05$). The rate of improvement was shown 67.7% and 100% in clotrimazole and Mycoderm groups after four weeks of the treatment, respectively ($p < 0.001$). Moreover, at the end of treatment, 51.6% and 70.3% of the clotrimazole and Mycoderm groups were cured, respectively.
Conclusion. — It is concluded that Mycoderm lotion, as a new antifungal agent, could be used in treating patients with Pityriasis versicolor into the future.
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MOTS CLÉS
Pityriasis versicolor ;
Malassezia furfur ;
Mycoderm ;
Clotrimazole ;
Artemisia sieberi

Résumé
Objectif. — Comparer l'effet de la lotion Mycoderm (huile essentielle d'*Artemisia sieberi*) à celui de la lotion à base de clotrimazole dans le traitement du pityriasis versicolor.
Matériel et méthodes. — Soixante huit patients (34 hommes et 34 femmes) ont participé à cette étude. L'infection a été contrôlée par les méthodes mycologiques habituelles (examen microscopique direct). Les patients ont été répartis de façon aléatoire en deux groupes distincts, l'un traité par Mycoderm, l'autre par la lotion au clotrimazole deux fois par jour pendant deux semaines puis suivis cliniquement pendant deux semaines.
Résultats. — Ils montrent l'amélioration de 71 % des patients du groupe clotrimazole et de 91,9 % des patients du groupe Mycoderm après deux semaines de traitement ; la différence est significative ($p < 0,05$). L'amélioration est de 67,7 % et de 89,2 % ($p < 0,001$) et la guérison complète est de 51,6 % et de 70,3 % respectivement dans le groupe clotrimazole et dans le groupe Mycoderm après quatre semaines.
Conclusion. — La lotion Mycoderm pourrait être utilisée comme nouvel antifongique dans le traitement des patients atteints de pityriasis versicolor.
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Introduction

The fungi are common causes of skin infections around the world. The risk of fungal infections of the human body skin during the whole life is 10—20% that Pityriasis versicolor is one of the most common infections among them [4]. Pityriasis versicolor is a common, benign, superficial cutaneous fungal infection usually characterized by hypopigmented or hyperpigmented macules and patches on the face, neck, chest and back [7]. Different *Malassezia* species, *M. globosa*, *M.furfur*, *M. sympodialis, M. restricta* have been isolated from patients with PV, and the causal agent of PV is still the matter of discussion [4]. Disease may chronically recur in patients with a predisposing condition [9]. Recently new oral and topical drugs are used for the treatment of Pityriasis versicolor, including imidazoles and aminoacids. In some cases, these drugs show failure to treatment, side effects and high relapse of disease [5]. For these reasons, the widespread efforts were made to identify natural agents to combat these opportunistic infections. Herbal medicines have been used since ancient times as drugs for treatment of a range of diseases. *Artemisia sieberi* Besser subsp. *sieberi* (*A. sieberi*) is a fairly large species belonging to the family of Compositeae, with the common Persian name of ''dermane'', includes 34 species that are found wild all over Iran [8]. The main components of the essential oil include α- and β-thujone (44%), limonene and 1.8-cineole (15%), comphor (23%), camphen (6%) and α-pinen (5%) [10]. In Iran, *A. sieberi* is used in traditional folk remedies for its antiseptic, analgesic (pain-relieving) and reducing-cough properties [25]. Numerous studies in the literature have reported the antibacterial, antiparasitic and antifungal activity of essential oils isolated from various species of *Artemisia* [13,17,18,24]. *Artemisia sieberi* essential oil and its derivatives are serving as therapeutic regimens against schistosomiasis disease caused by the protozoan species *Schistosoma japonicum*, *S. mansoni* and *S. haematobium*, which cause 1.5 million disabilities each year [23]. We demonstrated an in vitro good efficacy of *A. sieberi* essential oil on various dermatophytes and *Candida* species in previous study [14]. The objectives of this study were to provide a drug with the least side effects from internal resources (native pharmaceutical drugs) that can economically compete with the available chemical remedies in the present pharmaceutical markets and to determine the effect of topical application of natural lotion (Mycoderm) containing *A. sieberi* essential oil on the symptoms of Pityriasis versicolor in compare with synthetic lotion (clotrimazole).

Materials and methods

Essential oil

Standard *A. sieberi* Besser subsp. *sieberi* essential oil was obtained from Barij Essence Pharmaceutical Company, Kashan, Iran.

Preparation of Mycoderm lotion

Materials: *A. sieberi* essential oil (3% V/V), gallic acid (0.1% W/V, *Merck, Darmstadt, Germany*), propylene glycol (40% V/V, *Merck, Darmstadt, Germany*) and ethyl alcohol (75% V/V, *Daru Pakhsh, Tehran, Iran*).

Procedure: 10% *A. sieberi* essential oil was added to alcohol, the mixture was shaked along with talc powder for 3 hours and filtered. The proper amount of this mixture

was taken and mixed with gallic acid (as antioxidant), propylene glycol (as carrier) and alcohol. The prepared lotion was analyzed by qualitative and quantitative methods [3].

Patients

The present study was a randomized clinical trial. A total of 68 patients, 34 males and 34 females, suffering from Pityriasis versicolor, which have not been received antifungal drugs, were enrolled in this study. All patients examined by dermatologists from the department of dermatology, Imam-Khomeini hospital. The diagnosis of disease was confirmed according to standard laboratory method (direct microscopic examination). Occupationally, 16 (23.5%) cases were housewives, 16 (23.5%) students, 11 (16.2%) employees, 10 (14.7%) workers and the rest had free jobs. The range of patient's age was from six to 61-years-old, with the average of 28.4-years-old. Thirty-one and 37 patients received clotrimazole (*Daru Pakhsh, Tehran, Iran*) and Mycoderm lotions twice daily for two weeks, respectively. Also, 34 patients with Pityriasis versicolor, as positive controls, were run in experiments and received placebo including gallic acid, propylene glycol and ethyl alcohol. It is necessary to mention that a letter of satisfaction was received from all patients understudy. Clinical and laboratory findings were detected two weeks after starting of treatment and followed up the next two weeks.

Statistical analysis

The data were analyzed based on the statistical tests of Chi-square, Will kakson and *t*-Student using SPSS software (Version 12). A p value less than 0.05 were considered statistically significant.

Results

As shown in Table 1, the most frequent clinical sign in two groups was pink/brown macules. The clinical findings showed improvement two weeks after starting treatment, but many of cases had recurrence of symptoms after two weeks follow-up. A prominent improvement of clinical findings had been occurred in patients during two weeks after treatment with Mycoderm lotion and this improvement was continued after two weeks follow up. It was obvious that two weeks after treatment, the improvement rates in clotrimazole and Mycoderm groups was 71% and 91.9%, respectively, whereas four weeks after treatment, the improvement rate was 67.7% in clotrimazole group and 100% in Mycoderm group ($p < 0.001$). The results represented that the efficacies of both drugs from the statistical point of view is significant ($p < 0.007$), but in clotrimazole group a decrease of 3.3% in improvement was observed after two weeks. This decrease has not seen in Mycoderm group. The improvement rate of clotrimazole group was 71% two weeks after treatment. This amount was decreased to 67.7% after the next two weeks follow up. The improvement rate in clotrimazole lotion group had decreased 3.3%, although this result was not significant, but it showed the recurrence of disease. In Mycoderm group, the improvement rate was 91.9% two weeks after treatment and reached to 100% after two weeks follow up. This result showed 8.1% improvement in the course of disease. Comparing the efficacies of clotrimazole and Mycoderm lotions two weeks after treatment of Pityriasis versicolor showed no significant difference, whereas the results of two groups after two weeks follow up indicated significant difference in favor of Mycoderm group ($p < 0.001$). In general, there were no improvement effects in clotrimazole (32.3%) and Mycoderm (10.8%) groups versus complete improvement in clotrimazole (51.6%) and Mycoderm (70.3%) groups (Table 2). Of 34 patients with Pityriasis versicolor receiving placebo (positive controls), only three patients showed partial clinical cure.

Discussion

In the past few decades, a worldwide increase in the incidence of fungal infections has been observed as well as a rise in the resistance of some species of fungus to different antifungal drugs used in medicinal practice. The majority of clinically

Table 1 Frequency distribution of clinical signs and laboratory results of Pityriasis versicolor by clotrimazole and Mycoderm lotions at different times of study (No., %).
Évolution des signes cliniques et des résultats de laboratoire au cours du traitement par la lotion au clotrimazole ou par Mycoderm (No., %).

	Clotrimazole lotion (No. 31)			Mycoderm lotion (No. 37)		
	Start[a]	2 W[b]	4 W[c]	Start[a]	2 W[b]	4 W[c]
Sign						
White macules	2 (6.5)	0 (0)	2 (6.5)	3 (8.1)	1 (2.7)	1 (2.7)
Pink/brown macules	26 (83.8)	11 (35.5)	10 (28.6)	28 (75.7)	6 (16.2)	1 (2.7)
White patches	1 (3.2)	1 (3.2)	1 (3.2)	0 (0)	0 (0)	0 (0)
Pink/brown patches	2 (6.5)	0 (0)	1 (3.2)	6 (16.2)	0 (0)	0 (0)
Laboratory Test						
Positive	31 (100)	9 (29)	10 (32.3)	37 (100)	3 (8.1)	0 (0)
Negative	0 (0)	22 (71)	21 (67.7)	0 (0)	34 (91.9)	37 (100)
p value	-	<0.05	<0.05	-	<0.05	<0.05

[a] Start: Start of treatment.
[b] 2 W: Two weeks after treatment.
[c] 4 W: Four weeks after treatment.

Table 2 Frequency distribution of improvement rate of Pityriasis versicolor by clotrimazole and Mycoderm lotions (No., %).
Répartition du taux d'amélioration clinique en fonction du traitement par la lotion au clotrimazole ou par Mycoderm (No., %).

Clinical cure	Clotrimazole lotion	Mycoderm lotion	Total
No.	10 (32.3)	4 (10.8)	14 (20.6)
Partial	5 (16.1)	7 (18.9)	12 (17.6)
Complete	16 (51.6)	26 (70.3)	42 (61.8)

used antifungals have various drawbacks in terms of toxicity, efficacy and cost, and their frequent application has led to the emergence of resistant strains. Regarding to above points, using natural products with antimicrobial effects may represent a reasonable approach to protect human exposed to pathogenic agents. The herbal essential oil have been known to show growth inhibitory or killing activity against a wide variety of microorganisms including viruses, mycoplasma, chlamydia, bacteria, fungi, protozoans and harmful insects such as mites [6]. Previous studies have indicated that *A. sieberi* essential oil have antiparasitic activities against *Plasmodium falciparum*, *Schistosoma* and *Coccidia* species [1,11,18]. In a study conducted by Yashphe et al. [26], the antibacterial activity of the *Artemisia* essential oil was determined against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The oil exhibited a good inhibitory activity against different genera of bacteria with the presence of terpenoids in its essence. Literature studies showed that there is not enough research about antifungal activity of essential oil of *A. sieberi*. The results of the present study showed the positive efficacy of Mycoderm lotion in treating Pityriasis versicolor ($p < 0.001$). The efficacy of Mycoderm lotion, two weeks after treating Pityriasis versicolor was 91.9% versus 71% with clotrimazole, and four weeks after treatment was 67.7% and 100% in clotrimazole and Mycoderm groups, respectively. Khosravi et al. [14] demonstrated the inhibitory efficacy of *A. sieberi* essential oil on *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis*, *M. gypseum* and *Candida albicans*. Kordali et al. [16] investigated the chemical composition and antifungal activity of the essential oils from three Turkish *Artemisia* species (Asteraceae): *Artemisia absinthium*, *Artemisia santonicum* and *Artemisia spicigera*. The results showed that all the oils had potent inhibitory effects over a very broad spectrum against *Aspergillus ochraceus*, *Candida pseudotropicalis* and *Fusarium moniliforme*. Kalemba et al. [12] showed a good inhibitory antifungal activity of this essential oil against *Candida albicans, Rhodotorula rubra* and *Aspergillus fumigatus*. In another study, *Cryptococcus neoformans* and *Saccharomyces cerevisiae* were completely inhibited by *A. sieberi* essence [19,21]. Our study showed that at the end of treatment, 51.6% of the clotrimazole group and 70.3% of the Mycoderm group were cured. It is suggested that the higher antifungal activity of *A. sieberi* essential oil than clotrimazole was associated with terpenoids (α- and β-thujone, camphor and 1.8-cineole as main components), which are very active against different fungi [2]. The mode of its action has been attributed to its potential to induce a state of oxidative stress through the free radical cascade generated by the endoperoxide function, to the ability of the free radical to alkylate protein and to disrupt the depolarization of the mitochondrial membrane potential [20]. The increasing frequency of drug-resistance *Malassezia* species stimulated the medical researcher to look for efficacious antimicrobial options that are inexpensive, available and lack of side effects [15,22]. Considering that the growth of *M. furfur* is apparently inhibited by the essential oil of *A. sieberi*, this would suggest that this essential oil could be useful as a topical agent, a twice-daily application, in the treatment of Pityriasis versicolor.

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References

[1] Allen PC, Lydon J, Danforth HD. Effects of components of Artemisia annua on coccidia infections in chickens. Poult Sci 1997;76:1156–63.
[2] Amaral JA, Ekins A, Richards SR, Knowles R. Effect of selected monoterpenes on methane oxidation, denitrification, and aerobic metabolism by bacteria in pure culture. Appl Environ Microbiol 1998;64:520–5.
[3] Barry BW. Dermatological formulation, 14. Marcel Dekker Inc; 1983. 1–10.
[4] Crespo Erchiga V, Ojeda Martos A, Vera Casano A, Crespo Erchiga A, Sanchez Fajardo F. *Malassezia globosa* as the causative agent of pityriasis versicolor. Br J Dermatol 2000;143:799–803.
[5] De Silva Barros ME, Hamdan JS. Determination of susceptibility/resistance to antifungal drugs of *Trichophyton mentagrophytes* isolates by a macrodilution method. Can J Microbiol 2005;51:983–7.
[6] Elgayyar M, Draughon FA, Golden DA, Mount JR. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J Food Protect 2001;64:1019–24.
[7] Faergemann J. *Pityrosporum* species as a cause of allergy and infection. Allergy 1999;54:41341–9.
[8] Ghahraman A. Iran flora. Research institute of forests; 2003.
[9] Gupta AK, Kohli Y, Faergemann J, Summerbell RC. Epidemiology of *Malassezia* yeasts associated with pityriasis versicolor in Ontario, Canada. Med Mycol 2001;39:199–206.
[10] Javidnia K, Miri R, Khoshneviszadeh M, Edraki N, Javidnia A, Nasiri A. Constituents of the volatile oil of *Artemisia sieberi* Besser. from Iran. First Seminar of Medicinal & Natural Products chemistry Shiraz, Iran. May 10–11, 2005.
[11] Jefford CW. Why artemisinin and certain synthetic peroxides are potent antimalarials. Implication for the mode of action. Curr Med Chem 2001;8:1803–26.
[12] Kalemba D, Kusewicz D, Swiader K. Antimicrobial properties of the essential oil of *Artemisia asiatica* Nakai. Phytother Res 2002;16:288–91.
[13] Khanuja SPS. Antimicrobial composition and method for producing the same. US patent 6,423,741, 2002.
[14] Khosravi AR, Shirani D, mahmoudi M. Evaluation of *Artemisia sieberi* essence application in treatment of pet animals with dermatophytosis. J Vet Res 2003;58:293–5.
[15] Kontoyiannis DP, Lewis RE. Antifungal drug resistance of pathogenic fungi. Lancet 2002;359:1135–44.
[16] Kordali S, Cakir A, Mavi A, Kilic H, Yildirim A. Determination of the chemical composition and antioxidant activity of the

essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of turkish *Artemisia absinthium*, *A. dracunculus*, *A. santonicum*,and *A. spicigera* essential oils. J Agric Food Chem 2005;53:1408.
[17] Kumar S. Method for the use of alpha arteether as an anti-bacterial and anti-fungal agent. US patent 6,127,405, 2000.
[18] Kumar TRS, Khanuja SPS, Jain DC. A simple microbiological assay for the stereospecific differentiation of *a* and *b* isomers of arteether. Phytother Res 2000;14:644—6.
[19] Li W, Mo W, Shen D. Yeast model uncovers dual roles of mitochondria in action of artemisinin. PLoS Genet 2005; 1:e36.
[20] Olliaro PL, Haynes RK, Meunier B, Yuthavong Y. Possible modes of action of the artemisinin-type compounds. Trends Parasitol 2001;17:122—6.
[21] Ross SA, Jacob M. Antifungal activity of artemisinin derivatives. J Nat Prod 2005;68:1274—6.
[22] Sanglard D. Resistance of human fungal pathogens to antifungal drugs. Cur Opin Microbiol 2002;5:379—85.
[23] Shuhua X. Recent investigations of arteether, a novel agent for the prevention of *Schistosomiasis japonica*, *mansoni* and *haematobia*. Acta Trop 2002;82:175—81.
[24] Srivastava S. Bioprospecting potent plant compounds targeted to inhibit cell wall biosynthesis and DNA replication in Mycobacterium smegmatis. Indore: Devi Ahilya Vishwavidyalaya; 2002, Ph.D thesis.
[25] Tadjbakhsh H. History of human and veterinary medicine in Iran. lion, France: Merial Co; 2003.
[26] Yashphe J, Segal R, Breuer A, Gabi EN. Antibacterial activity of *Artemisia herba-alba*. J Pharma Sci 2006;68:924—5.