J Herbmed Pharmacol. 2018; 7(1): 18-21.

http://www.herbmedpharmacol.com doi: 10.15171/jhp.2018.04

Journal of Herbmed Pharmacology

Inhibitory effect of plant essential oils on *Malassezia* strains from Iranian dermatitis patients

Ali Reza Naeini[1], Mehdi Nazeri[2], Hojjatollah Shokri[3*]

[1]Member of the Faculty of Medicine and Medicinal Plants Research Center, Shahed University, Tehran, Iran
[2]Department of Medical Parasitology and Mycology, Faculty of Medicine, Kashan University of Medical Sciences, Kashan, Iran
[3]Department of Pathobiology, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 3 September 2017
Accepted: 3 December 2017

Keywords:
Anti-*Malassezia* activity
Medicinal plants
Cuminum cyminum
Lavandula stoechas
Ketoconazole

ABSTRACT

Introduction: The genus *Malassezia* is an important skin resident of human. The present study aimed to analyze *in vitro* activity of the essential oils of *Lavandula stoechas*, *Cuminum cyminum* and *Artemisia sieberi* against clinical strains of *Malassezia* species.
Methods: A total of 47 *Malassezia* strains, including *Malassezia furfur*, *Malassezia globosa* and *Malassezia obtuse*, were used in this study. A disk diffusion technique was selected for testing the susceptibility of *Malassezia* strains to the essential oils.
Results: All the essential oils showed *in vitro* activity against *Malassezia* strains, with *M. furfur* and *M. obtusa* being the highest and lowest susceptible of the strains, respectively. The highest antifungal activity was associated with the essential oil of *C. cyminum* (mean ± SD: 50.0 ± 0.0 mm), followed by *L. stoechas* (mean ± SD: 46.8 ± 3.1 mm) and *A. sieberi* (mean ± SD: 36.9 ± 5.7 mm). The inhibition zone ranges were 12.5 to 15.6 mm (mean ± SD: 14.4 ± 1.6 mm) for ketoconazole and 11.6 to 13.3 mm (mean ± SD: 12.4 ± 0.9 mm) for fluconazole. Although all the antifungal drugs were found to possess good antifungal activities against *Malassezia* strains, their effects were lower than the activities shown by the essential oils tested ($P < 0.05$).
Conclusion: These results indicated that the essential oils tested, especially the one from *C. cyminum*, inhibited the growth of clinical strains of *Malassezia*, implying its potential use in the treatment of *Malassezia* infections. This indicates that this plant may be useful in preparation of new drugs.

Implication for health policy/practice/research/medical education:
The essential oils of *C. cyminum, L. stoechas* and *A. sieberi* showed promising antifungal effects against *Malassezia* strains from patients with *Malassezia dermatitis* as important fungal pathogens. Hence, these essential oils, especially *C. cyminum,* could be used to develop an effective therapy against skin infections caused by *Malassezia* strains.
Please cite this paper as: Naeini AR, Nazeri M, Shokri H. Inhibitory effect of plant essential oils on *Malassezia* strains from Iranian dermatitis patients . J Herbmed Pharmacol. 2018;7(1):18-21. doi: 10.15171/jhp.2018.04.

Introduction

Members of *Malassezia* genus are the normal mycoflora on human cutaneous surfaces. They colonize the regions containing sebaceous glands like the head, neck and shoulders of humans (1). The genus of *Malassezia* has been divided into 14 various species based on the last taxonomic revision (2). *Malassezia* species are associated with a wide spectrum of clinical signs, such as Tinea versicolor, Folliculitis and Seborrheic dermatitis. It also causes systemic infections in immune-compromised patients (3). The conditions that cause *Malassezia*-related infections in humans are not fully understood but researchers have been able to determine the role of different factors including genetic and environmental factors, imbalance in skin normal biota, immune suppression and profuse sweating as agents for these infections (4).

The antifungal therapies with different drugs, especially azoles, are generally successful in controlling the yeast overgrowth, but treatment failure and rapid recurrences are common (5). Therefore, considering the limitations of currently available antifungal drugs, the search for natural effective drugs is justified.

Iranian plants, such as *Lavandula stoechas*) (known as Ostokhodos), *C. cyminum* (known as Ziree) and

***Corresponding author**: Dr. Hojjatollah Shokri, Associate Professor; Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Imam Khomeini Street, 24th Aftab, Amol, Iran.
POB: 46168-49767, Tel: 0121 2271057, Fax: 0121 2271054,
Email: hshokri@ut.ac.ir

Artemisia sieberi (known as Dermane) have been used for centuries as herbal remedies by local or regional subjects (6,7). Several reports indicated the inhibitory activities of essential oils of the above-mentioned plants against different fungal agents (8-10). This study evaluated the antifungal activity of *L. stoechas*, *C. cyminum* and *A. sieberi* essential oils against clinical strains of *Malassezia* from the skin of patients with *Malassezia* dermatitis.

Materials and Methods

Malassezia identification

Forty-seven clinical strains of *Malassezia* were included in this study. Strains of *Malassezia* were maintained on modified Dixon's agar (Difco, USA) containing 6 g peptone (Oxoid), 20 g ox bile (Oxoid), 36 g malt extract, 2 mL glycerol, 2 mL oreic acid and 12 g agar at 32°C for 7 days. The yeasts were identified based on microscopic morphology and physiological characteristics, including catalase reaction, Tween assimilation and esculin hydrolysis tests to distinguish among different *Malassezia* strains (11).

Medicinal plants

Three herbal genera, such as *L. stoechas*, *C. cyminum* and *A. sieberi*, were sleeted according to the Iranian traditional practices (Table 1). Botanical identification was carried out in the herbal medicine laboratory of Shahed University (Tehran, Iran).

Disc-based testing

Anti-*Malassezia* susceptibility testing by disc diffusion method was carried out based on the Clinical & Laboratory Standards Institute (CLSI) guidelines (CLSI document M44-A2) and manufacturer's instructions (12). For disc testing, the modified Dixon's medium was used. The yeast colonies were mixed in 5 mL of sterile distilled water, and the turbidity was adjusted to yield 1×10^5 -1×10^6 cells/mL (0.5 McFarland standard). Yeast cells were inoculated on the agar surface using a sterile cotton swab. Subsequently, paper discs moistened with undiluted essential oils were placed on the media. In addition, discs containing ketoconazole and fluconazole (10 µg/disc, Master group, England) were placed on the media. The media were incubated at 32°C for 4 days and the zones of inhibition around the discs were measured. Disc moistened with sterile distilled water was considered as control. Each treatment was replicated twice for each sample.

Statistical analysis

Two-tailed paired student's *t* test was used for analyzing the anti-*Malassezia* activities of the essential oils. A *P* value less than 0.05 was considered to be statistically significant.

Results

As shown in Table 2, all three essential oils had inhibitory activities against different *Malassezia* strains at first screening. The zone diameters of yeast growth inhibition of the essential oils were between 15 and 50 mm. The highest antifungal activity was associated with the essential oil of *C. cyminum* (mean ± SD: 50.0 ± 0.0 mm), followed by *L. stoechas* (mean ± SD: 46.8 ± 3.1 mm) and *A. sieberi* (mean ± SD: 36.9 ± 5.7 mm). There were no significant differences among the essential oils tested against clinical strains of *Malassezia* ($P > 0.05$). *A. sieberi* essential oil indicated lower anti-*Malassezia* activity than the others. For 46 clinical strains of *Malassezia*, the inhibition zone ranges were 12.5-15.6 mm (mean ± SD: 14.4 ± 1.6 mm) for ketoconazole and 11.6-13.3 mm (mean ± SD: 12.4 ± 0.9 mm) for fluconazole. There was no significant difference between the standard antifungal drugs tested against *Malassezia* strains ($P > 0.05$).

Discussion

The antimicrobial activity of herbal plants has attracted much interest from scientists as a result of the growing problem of drug resistance among pathogenic fungi. For this reason, the current study was done for investigating the importance of herbal essential oils as anti-*Malassezia* agents. The results showed that the essential oils of *L. stoechas*, *C. cyminum* and *A. sieberi* had inhibitory activities against different *Malassezia* strains. The zone diameters of growth inhibition of the essential oils ranged from 15 to 50 mm. The highest antifungal activity was associated with the essential oil of *C. cyminum* (mean ± SD: 50.0 ± 0.0 mm), followed by *L. stoechas* (mean ± SD: 46.8 ± 3.1 mm) and *A. sieberi* (mean ± SD: 36.9 ± 5.7 mm). There were no significant differences among the essential oils tested against *Malassezia* strains. Several publications on antifungal activity of the essential oils against *Malassezia* species were reported in the literature (4,13,14). Our results are consistent with those found by Naeini et al (15) who reported *C. cyminum* being the most active essential oil with a mean inhibition zone of 48.3 mm against different *Malassezia* strains. Vijayakumar et al (16) observed inhibition zones of 5.3-30 mm by different herbal plants against *Malassezia* strains. The inhibitory and fungicidal activities of *C. cyminum* are related to the mark vacuolation in the cytoplasm, isolation of fibrillar layer of cell wall, disruption of plasma and nuclear membranes and a large swelling in the mitochondrial matrix (17).

Table 1. Some characteristics of the tested plants

Scientific name	Voucher No.	Family	Local name	Medicinal use	Major components
Lavandula stoechas	101	Labiatae	Ostokhodos	Antispasmodic, carminative, wound healing	Fenchone, camphor, cineole
Cuminum cyminum	1172	Apiaceae	Ziree	Carminative, antidiarrhoeaic, antispasmodic	α-Pinene, cineole, linalool
Artemisia sieberi	1559	Compositeae	Dermane	Anti-septic, anti-infective	α-Thujone, camphor, β-thujone

Table 2. Antifungal activity (growth inhibition zone, millimeter) of the herbal essential oils and reference antifungal drugs against clinical strains of *Malassezia*

Fungal strains	Antifungals				
	Lavandula stoechas (Mean ± SD)	*Cuminum cyminum* (Mean ± SD)	*Artemisia sieberi* (Mean ± SD)	Ketoconazole (Mean ± SD)	Fluconazole (Mean ± SD)
Malassezia furfur	46.7 ± 8.2	50 ± 0.0	43.3 ± 14.1	15.6 ± 5.9	11.6 ± 3.7
Malassezia globosa	50 ± 0.0	50 ± 0.0	35 ± 14.1	15 ± 7.6	12.4 ± 2.5
Malassezia obtusa	43.7 ± 12.5	50 ± 0.0	32.5 ± 11.9	12.5 ± 5.0	13.3 ± 1.5

In the present study, *A. sieberi* essential oil indicated lower anti-*Malassezia* activity than the others. In agreement with our results, Khosravi et al (14) showed that *A. sieberi* essential oil had inhibitory activity against different *Malassezia* strains, including *M. pachydermatis*, *M. globosa*, *M. restricta*, *M. sloofiae*, *M. furfur*, *M. nana*, *M. obtuse* and *M. sympodialis*. Previous studies also demonstrated inhibitory activity of *A. sieberi* essential oil against both yeasts (*Candida* spp., *Rhodotorula* spp., *Cryptococcus* spp. and *Saccharomyces* spp.) and filamentous fungi (*Fusarium* spp. and *Aspergillus* spp.) (18,19). In an experimental study conducted by Khosravi et al (20), anti-*Malassezia* activity of *A. sieberi* essential oil was demonstrated. The results exhibited the improvement rates of 71% and 91.9% in clotrimazole and *A. sieberi* essential oil groups after 2 weeks of the treatment ($P<0.05$). Also, 4 weeks after treatment, the definitive cure rates were achieved approximately 51.6% for patients receiving clotrimazole and 70.3% for patients receiving *A. sieberi* essential oil. It is suggested that the major components of *A. sieberi* essential oil, including α-and β-thujone, camphor and 1,8-cineole, can be responsible for inhibiting the growth of saprophytic and pathogenic fungi. The exact mechanisms of antifungal activity are associated with oxidative stress induction, protein alkylation and depolarization disorder of the mitochondrial membrane (21).

Although no report was found concerning the effect of *L. stoechas* essential oil against *Malassezia* strains but several investigators studied on other fungi. In this context, Zuzarte et al (10) demonstrated the inhibitory effect of *L. stoechas* against *Cryptococcus* spp., *Candida* spp., *Aspergillus* spp. and dermatophytes. Also, the antifungal activity of *L. stoechas* essential oil from various regions of Algeria was previously evaluated against *Candida albicans*, *Aspergillus niger* and *A. flavus* (22).

In this study, the mean inhibition zones were found to be 14.4 and 12.4 mm for ketoconazole and fluconazole, respectively. No statistically significant difference was observed between the standard antifungal drugs tested against *Malassezia* strains. In agreement with our observations, Strippoli et al (23) exhibited that ketoconazole was the most active drug against *Malassezia* strains. According to Miranda et al (24), these pathogenic yeasts were susceptible to all drugs tested, especially ketoconazole and fluconazole. Fluconazole is an effective antifungal drug for treating *Malassezia* dermatitis (25). Our results exhibited that fluconazole has lower activity than another azole. This finding is consistent with that of Velegraki et al (26), who showed low activity of fluconazole for *Malassezia* strains. Prophylactic therapies using fluconazole can be led to frequent recurrence of *Malassezia* infections. Although the antifungal drugs were found to possess good antifungal activity against *Malassezia* strains, their effects were lower than the activities shown by the essential oils tested. Previous studies exhibited that combination of various components in each essential oil has higher antifungal activity than each component and studies also suggested that the sum of essential oil components can help in potentiating synergistic effect (27,28).

Conclusion

In summary, the present study results showed that clinical strains of *Malassezia* had different susceptibilities to antifungal agents, with *M. obtusa* being the lowest susceptible of the strains. In addition, we demonstrated that the essential oils of *L. stoechas*, *C. cyminum* and *A. sieberi* had inhibitory activities against clinical strains of *Malassezia*, being *C. cyminum* essential oil as the most active herbal oil. These results could be used for the design of new antifungal agents with effective clinical importance.

Authors' contributions

All authors contributed to the study. They conducted the experiment, analyzed and discussed results and prepared the manuscript for publication equally. All read and confirmed its publication.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

Ethical issues have been observed by the authors.

Funding/Support

This research has been supported by a research grant from the Amol University of Special Modern Technologies, Amol, Iran (Grant No. D-178).

References

1. Coutinho SD, Fedullo JD, Correa SH. Isolation of Malassezia spp. from cerumen of wild felids. Med Mycol. 2006;44(4):383-7. doi: 10.1080/13693780500411006.
2. 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(4):799-803.
3. Pooja A, Arun N, Maninder K. Screening of plant essential oils for antifungal activity against *Malassezia furfur*. Int J Pharm Pharm Sci. 2013;5(2):37-9.
4. Nazeri M, Ata-Bakhshian R, Taghizadeh M, Talaee R, Mahboubi M. Antifungal activity of herbal extracts against *Malassezia* species. Iran J Dermatol. 2015;18(71):10-5.
5. Faergemann J, Djarv L. Tinea versicolor: treatment and prophylaxis with ketoconazole. Cutis. 1982;30(4):542-5, 50.
6. Avicenna. Al-Qanun fi al Tibb (The Canon of Medicine). Persian Edition by Sharaf-Kandi AR. 1st ed. Tehran: Soroush Press; 1985.
7. Tadjbakhsh H. History of Human and Veterinary Medicine in Iran. Lion: Merial Co; 2003.
8. Angioni A, Barra A, Coroneo V, Dessi S, Cabras P. Chemical composition, seasonal variability, and antifungal activity *of Lavandula stoechas* L. ssp. stoechas essential oils from stem/leaves and flowers. J Agric Food Chem. 2006;54(12):4364-70. doi: 10.1021/jf0603329.
9. Salari S, Khosravi AR, Katiraee F, Ayatollahi Mousavi SA, Shokri H, Nikbakht Borujeni GH. Evaluation of inhibitory effects of *Cuminum cyminum* oil on the fluconazaole resistant and susceptible *Candida albicans* isolated from HIV patients in Iran. J Am Sci. 2012;8(5):54-60.
10. Zuzarte M, Gonçalves MJ, Cavaleiro C, Cruz MT, Benzarti A, Marongiu B, et al. Antifungal and anti-inflammatory potential of *Lavandula stoechas* and *Thymus herba-barona* essential oils. Ind Crops Prod. 2013;44(Suppl C):97-103. doi: 10.1016/j.indcrop.2012.11.002.
11. Guillot J, Gueho E, Lesourd M, Midgley G, Chevrier G, Dupont B. Identification of *Malassezia* species. A practical approach. J Med Mycol. 1996;6(2):103-10.
12. Clinical Laboratory Standards Institute. Method for antifungal disk diffusion susceptibility testing in yeasts. Wayne, M44-A: CLSI; 2002.
13. Naeini A, Eidi S, Shokri H. Fungitoxicity of Zataria multiflora essential oil against various *Malassezia* species isolated from cats and dogs with *Malassezia dermatitis*. Afr J Microbiol Res. 2011;5(9):1057-61.
14. Khosravi AR, Shokri H, Fahimirad S. Efficacy of medicinal essential oils against pathogenic *Malassezia* sp. isolates. J Mycol Med. 2016;26(1):28-34. doi: 10.1016/j.mycmed.2015.10.012.
15. Naeini AR, Nazeri M, Shokri H. Antifungal activity of *Zataria multiflora, Pelargonium graveolens* and *Cuminum cyminum* essential oils towards three species of *Malassezia isolated* from patients with pityriasis versicolor. J Mycol Med. 2011;21(2):87-91. doi: 10.1016/j.mycmed.2011.01.004.
16. Vijayakumar R, Muthukumar C, Kumar T, Saravanamuthu R. Characterization of *Malassezia furfur* and its control by using plant extracts. Indian J Dermatol. 2006; 51(2):145-8. doi: 10.4103/0019-5154.26942.
17. Khosravi AR, Minooeianhaghighi MH, Shokri H, Emami SA, Alavi SM, Asili J. The potential inhibitory effect of *Cuminum cyminum, Zziphora clinopodioides* and *Nigella sativa* essential oils on the growth of *Aspergillus fumigatus* and *Aspergillus flavus*. Braz J Microbiol. 2011;42:216-24.
18. Kalemba D, Kusewicz D, Swiader K. Antimicrobial properties of the essential oil of *Artemisia asiatica* Nakai. Phytother Res. 2002;16(3):288-91. doi: 10.1002/ptr.856.
19. Kordali S, Kotan R, Mavi A, Cakir A, Ala A, 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, Artemisia santonicum,* and *Artemisia spicigera* essential oils. J Agric Food Chem. 2005;53(24):9452-8. doi: 10.1021/jf0516538.
20. Khosravi AR, Shokri H, Darabi MH, Kashani A, Mansouri P, Naser A. Comparative study on the effects of a new antifungal lotion (*Artemisia sieberi* essential oil) and a clotrimazole lotion in the treatment of pityriasis versicolor. J Mycol Med. 2009;19(1):17-21. doi: 10.1016/j.mycmed.2008.12.001.
21. Olliaro PL, Haynes RK, Meunier B, Yuthavong Y. Possible modes of action of the artemisinin-type compounds. Trends Parasitol. 2001;17(3):122-6.
22. Benabdelkader T, Zitouni A, Guitton Y, Jullien F, Maitre D, Casabianca H, et al. Essential oils from wild populations of Algerian *Lavandula stoechas* L.: composition, chemical variability, and in vitro biological properties. Chem Biodivers. 2011;8(5):937-53. doi: 10.1002/cbdv.201000301.
23. Strippoli V, Piacentini A, D'Auria FD, Simonetti N. Antifungal activity of ketoconazole and other azoles against *Malassezia furfur* in vitro and in vivo. Infection. 1997;25(5):303-6.
24. Miranda KC, de Araujo CR, Costa CR, Passos XS, de Fatima Lisboa Fernandes O, do Rosario Rodrigues Silva M. Antifungal activities of azole agents against the *Malassezia* species. Int J Antimicrob Agents. 2007;29(3):281-4. doi: 10.1016/j.ijantimicag.2006.09.016.
25. Shahid J, Ihsan Z, Khan S. Oral fluconazole in the treatment of pityriasis versicolor. J Dermatolog Treat. 2000;11(2):101-3. doi: 10.1080/09546630050517496.
26. Velegraki A, Alexopoulos EC, Kritikou S, Gaitanis G. Use of fatty acid RPMI 1640 media for testing susceptibilities of eight *Malassezia* species to the new triazole posaconazole and to six established antifungal agents by a modified NCCLS M27-A2 microdilution method and Etest. J Clin Microbiol. 2004;42(8):3589-93. doi: 10.1128/jcm.42.8.3589-3593.2004.
27. Gill AO, Delaquis P, Russo P, Holley RA. Evaluation of antilisterial action of cilantro oil on vacuum packed ham. Int J Food Microbiol. 2002;73(1):83-92.
28. Mourey A, Canillac N. Anti-Listeria monocytogenes activity of essential oils components of conifers. Food Control. 2002;13(4):289-92. doi: 10.1016/S0956-7135(02)00026-9.