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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Antifungal activity of *Zataria multiflora*, *Pelargonium graveolens* and *Cuminum cyminum* essential oils towards three species of *Malassezia* isolated from patients with pityriasis versicolor

Activité antifongique des huiles essentielles de Zataria multiflora, Pelargonium graveolens et Cuminum cyminum sur trois espèces de Malassezia isolées de patients atteints de pityriasis versicolor

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Antifungal activity;
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Zataria multiflora;
Pelargonium graveolens;
Cuminum cyminum;
Essential oil

Summary

Objective. – To investigate the anti-*Malassezia* activities of *Zataria multiflora*, *Pelargonium graveolens* and *Cuminum cyminum* essential oils (EOs) against different pathogenic *Malassezia* species isolated from patients with pityriasis versicolor (PV).

Patients and methods. – The EOs were obtained by water-distillation using a Clevenger-type system. Anti-*Malassezia* activity against *Malassezia* species was carried out using disk diffusion method in Dixon agar.

Results. – The main oil components were carvacrol (61.3%) and thymol (25.2%) for *Z. multiflora*, α pinene (30%) and limonene (21%) for *C. cyminum* and citronelol (28.2%) and geraniol (22.1%) for *P. graveolens*. The three *Malassezia* species showed a similar susceptibility to the three plants tested, *C. cyminum* (mean value: 48.3 mm) being the most active, followed by *Z. multiflora* (mean value: 28.1 mm) and *P. graveolens* (mean value: 26.1 mm).

Conclusion. – This study indicated that *Z. multiflora*, *P. graveolens* and *C. cyminum* EOs have considerable anti-*Malassezia* activities, deserving further investigation for clinical applications for the treatment of PV.

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MOTS CLÉS

Activité antifongique ;
Malassezia ;
Zataria multiflora ;
Pelargonium graveolens ;
Cuminum cyminum ;
 Huile essentielle

Résumé

Objectif. – Examiner l'activité antifongique des huiles essentielles (EO) de *Zataria multiflora*, *Pelargonium graveolens* et *Cuminum cyminum* sur trois espèces de *Malassezia* isolées de patients atteints de *pityriasis versicolor* (PV).

Patients et méthodes. – L'EO a été obtenue par distillation selon le système de Clevenger. L'activité antifongique sur les espèces de *Malassezia* a été étudiée par la méthode de diffusion par disque dans le milieu solide de Dixon.

Résultats. – Les composants principaux étaient : carvacrol (61,3 %) et thymol (25,2 %) pour *Z. multiflora*, pinène (30%) et limonène (21 %) pour *C. cyminum* et citronelol (28,2%) et geraniol (22,1 %) pour *P. graveolens*. Les trois espèces de *Malassezia* ont montré une bonne sensibilité aux trois plantes essayées : *C. cyminum* (valeur moyenne : 48,3 mm) a été la plus active, suivie par *Z. multiflora* (valeur moyenne : 28,1 mm) et par *P. graveolens* (valeur moyenne : 26,1 mm).

Conclusion. – Cette étude a indiqué que les OE de *Z. multiflora*, *P. graveolens* et *C. cyminum* présentent une activité antifongique particulièrement intéressante sur les *Malassezia* testées ; de plus amples investigations seront nécessaires pour leur application au traitement du PV.

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Introduction

Malassezia species have been recognized as members of the microbiological flora of human skin that cause skin diseases such as pityriasis versicolor (PV), seborrheic dermatitis (SD), folliculitis and also otitis externa, atopic dermatitis and even fatal sepsis [3,9,14]. As most of *Malassezia* species show lipid-dependency, lipolytic enzymes including lipase and phospholipase are necessary for them to obtain useful lipids from the environment. Consequently, these enzymes are thought to play an important role in the growth and pathogenicity of *Malassezia* [24].

Malassezia-associated skin diseases are the major classes of superficial and cutaneous mycoses caused by different *Malassezia* species and usually subjected to topically antifungal treatment. It has been reported that various antifungal agents including fluconazole, itraconazole, ketoconazole, voriconazole and terbinafine are active against *Malassezia* species. In some cases, these drugs show failure to treatment, side-effects and high relapse of disease. However, the in vitro/in vivo relationship of the anti-*Malassezia* activity of antifungal drugs or the pathogenetic role of each *Malassezia* species in the development of *Malassezia*-associated skin diseases remains to be answered [16].

Herbal plants have been widely used to extend the shelf life of foods and in folk medicine. It is known that most of their properties are due to the EOs. They contain as products of their secondary metabolism. Several studies on the antifungal activities of the EOs against different pathogenic fungi have been reported [1,8,17,25]. However, there is only limited information in the literature on the antifungal activity of EOs towards human fungal pathogens. Previous reports demonstrated the in vitro susceptibility of *Malassezia* species to *Melaleuca alternifolia* (tea tree oil), *Curcuma xanthorrhiza* and *Artemisia abrotanum* [4,10,19]. In the present study the antifungal activities of three herbal EOs including *Zataria multiflora*, *Pelargonium graveolens* and *Cuminum cyminum* were examined against different pathogenic *Malassezia* species isolated from patients with PV.

Materials and Methods**Organisms**

Three different *Malassezia* species, *M. furfur* (18 strains), *M. globosa* (seven strains) and *M. obtusa* (four strains) isolated from patients with PV were selected as test microorganisms. They were obtained from the Culture Collection of Mycology Center of Kashan University of Medical Sciences (Kashan, Iran).

Medicinal plants

In this study three plant genera *Z. multiflora* (Labiatae; known as Avishan; voucher no. 1106), *P. graveolens* (Geraniaceae; known as Geranium; voucher no. 1356) and *C. cyminum* (Apiaceae; known as Ziree; voucher no. 1172) were chosen on the basis of traditional information regarding the treatment of various skin diseases in Iran. The identification of medicinal plants was performed in the laboratory of Herbarium of Pharmacognosy, School of Medicine, Shahed University of Medical Sciences (Tehran, Iran).

Preparation of essential oils

The EOs were obtained from 40 g fresh plants by steam distillation using Clevenger system during three hours. The aqueous phase was extracted with dichloromethane (3 × 50 mL). The organic phase was dried with sodium sulphate, filtered and evaporated until dryness. The EOs were solubilized in ethyl acetate for gas chromatography and mass spectrometry (GC/MS) analysis.

Gas chromatography and mass spectrometry analysis

GC/MS analysis was carried out in a Hewlett Packard 5890 gas chromatograph fitted with a HP₁ fused silica column (polydimethyl siloxane, 25 × 0.2 mm i.d., film thickness 0.33 μm), interfaced with a Hewlett Packard mass selective

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