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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Efficacy of medicinal essential oils against pathogenic *Malassezia* sp. isolates



Efficacité des huiles essentielles médicinales sur des isolats pathogènes de Malassezia sp.

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KEYWORDS

Dog;
Atopic dermatitis;
Malassezia;
Malassezia pachydermatis;
Essential oils

Summary

Objectives. – The purposes of this study were to evaluate the distribution pattern and population size of *Malassezia* species in dogs with atopic dermatitis (AD) and the inhibitory efficacy of *Zataria multiflora*, *Thymus kotschyianus*, *Mentha spicata*, *Artemisia sieberi*, *Rosmarinus officinalis* and *Heracleum persicum* essential oils against pathogenic *Malassezia* isolates.

Methods. – The samples were collected from 5 different anatomical sites of 33 atopic dogs and cultured onto modified Dixon agar (MDA) and Sabouraud dextrose agar (SDA) media. The essential oil extraction was performed by steam distillation using Clevenger system. Anti-*Malassezia* efficacy of medicinal essential oils and standard drugs was evaluated using broth microdilution method.

Results. – A total of 103 yeast colonies were isolated from dogs with AD. Eight different *Malassezia* species were identified as follows: *Malassezia pachydermatis* (81.4%), *M. globosa* (7.8%), *M. restricta* (3.9%), *M. slooffiae* (2.9%), *M. furfur* (1%), *M. nana* (1%), *M. obtusa* (1%) and *M. sympodialis* (1%). The most and least infected sites were: anal (21.2%) and ear (10.6%) respectively. *M. pachydermatis* was the most frequent *Malassezia* species isolated from both skin and mucosa of dogs with AD. Antifungal susceptibility test revealed the inhibitory efficacy of essential oils on pathogenic *Malassezia* isolates with minimum inhibitory concentration (MIC⁹⁰) values ranging from 30 to 850 µg/mL. Among the tested oils, *Z. multiflora* and *T. kotschyianus* exhibited the highest inhibitory effects ($P < 0.05$).

Conclusion. – The essential oils of *Z. multiflora* and *T. kotschyianus* showed strong antifungal activity against pathogenic *Malassezia* species tested.

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MOTS CLÉS

Chien ;
Atopique ;
Dermatite atopique ;
Malassezia sp. ;
Malassezia
pachydermatis ;
Huiles essentielles

Résumé

Objectif. — Les buts de cette étude étaient d'évaluer la distribution corporelle et l'abondance d'espèces de *Malassezia* chez des chiens ayant une dermatite atopique (DA) et l'effet inhibiteur des huiles essentielles de *Zataria multiflora*, de *Thymus kotschyanus*, de *Mentha spicata*, d'*Artemisia sieberi*, de *Rosmarinus officinalis* et de *Heracleum persicum* contre des isolats de *Malassezia* pathogènes.

Matériel et méthodes. — Les échantillons ont été recueillis de 5 sites anatomiques différents chez 33 chiens atopiques et cultivés sur gélose Dixon modifiée (MDA) et sur gélose dextrose de Sabouraud (SDA). L'extraction des huiles essentielles a été faite par distillation à la vapeur en utilisant le système de Clevenger. L'effet d'anti-*Malassezia* d'huiles essentielles médicinales et de médicaments de référence a été évalué en utilisant la méthode de microdilution en milieu liquide.

Résultats. — Cent trois colonies de levures ont été isolées chez des chiens avec une DA. Huit espèces différentes de *Malassezia* ont été identifiées comme suit : *Malassezia pachydermatis* (81,4%), *M. globosa* (7,8%), *M. restricta* (3,9%), *M. slooffiae* (2,9%), *M. furfur* (1%), *M. nana* (1%), *M. obtusa* (1%) et *M. sympodialis* (1%). Les sites les plus et les moins infectés ont été l'anus (21,2%) et l'oreille (10,6%), respectivement. *M. pachydermatis* était l'espèce de *Malassezia* la plus fréquemment isolée tant de la peau que des muqueuses des chiens avec une AD. L'étude de la sensibilité antifongique a révélé un effet inhibiteur des huiles essentielles sur *Malassezia* avec une concentration inhibitrice minimale (MIC⁹⁰) entre 30 et 850 µg/mL. Parmi les huiles évaluées, *Z. multiflora* et *T. kotschyanus* ont montré les plus hauts effets inhibiteurs ($p < 0,05$). Les huiles essentielles de *Z. multiflora* et de *T. kotschyanus* ont montré une forte activité antifongique contre les espèces de *Malassezia* évaluées.

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Introduction

Fungal infections in animals with atopic dermatitis (AD) have been reported, in particular in dogs, as a genetically-predisposed inflammatory and pruritic skin disease [31]. The skin and ears of dogs with AD are commonly colonized or infected with *Malassezia* species [32]. Previous studies demonstrated that cell-mediated and humoral reactivities to *Malassezia* contribute to the pathogenesis of AD in dogs [18,25]. Chen et al. [6] showed that the majority of atopic dogs with *Malassezia* dermatitis have a greater IgE response than normal dogs, suggesting an IgE-mediated immune response may be clinically important in the pathogenesis of the disease. Malasseziosis occurs in dogs of various breeds [2] in both sexes [34].

By 2005, 12 distinct species of *Malassezia* has been identified (*M. furfur*, *M. globosa*, *M. sympodialis*, *M. pachydermatis*, *M. obtusa*, *M. restricta*, *M. slooffiae*, *M. dermatis*, *M. equi*, *M. nana*, *M. japonica* and *M. yamatensis*) and since that time, 2 more species has been detected [14,16]. The only non lipid-dependent species, *M. pachydermatis*, is considered as an opportunistic secondary pathogen affecting the canine skin surface and is the most common yeast isolated from otitis externa in dogs [17].

The development of resistance in common fungal pathogens and emergence of new fungal pathogens intrinsically resistant to the currently available antibiotics demonstrates the urgent importance of identifying novel antifungal agents. There will be an increasing need for fungal inhibiting substances from plants. Traditional medicinal plants represent a reservoir of pharmacologically active substances or drugs [12]. Essential oils from several plant species are able to control microorganisms related to skin [1], dental caries [5] and food spoilage [13]. The medicinal plants of *Zataria multiflora*, *Thymus kotschyanus*, *Mentha spicata*, *Artemisia*

sieberi, *Rosmarinus officinalis* and *Heracleum persicum* were selected on the basis of traditional information regarding to the treatment of various diseases in Iran [26]. The aims of this study were to identify *Malassezia* species isolated from the skin and mucosa of atopic dogs and to assess the antifungal activity of 6 herbal essential oils against pathogenic *Malassezia* isolates.

Materials and methods**Animals**

Thirty-three privately owned dogs of different breeds, ages and genders were clinically examined. The animals were diagnosed to be atopic as they all fulfilled the clinical criteria published by Prelaud et al. [35] and had relevant detailed history. According to the criteria, any dog with the appearance of first signs, between ages of 6 months and 3 years, of cheilitis, bilateral cranial erythematous pododermatitis, erythema of pinnae and corticosteroid-sensitive pruritis were clinically diagnosed with AD and included in this study. These dogs had no history of any medication for a month prior to sample collection. They were of age varying from 9 months to 7 years (mean age: 3.1 years old), of different breeds and both sexes (14 females and 19 males). All the experiments were performed based on the Veterinary Research Ethics and this study was approved by the Society for the Protection of Cruelty to Animals (SPCA) in Iran. More information about age, breed and gender of dogs are given in Table 1.

Sampling and fungal culture

Samples were collected from 5 anatomical sites of each dog including mouth, external ear canal, interdigital, groin and anal regions. From each site, 3 sterile cotton swabs and a

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