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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;
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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 kotschyanus*, *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. sloofiae* (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. kotschyanus* exhibited the highest inhibitory effects ($P < 0.05$).

Conclusion. — The essential oils of *Z. multiflora* and *T. kotschyanus* 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 kotschymanus*, 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. kotschymanus* ont montré les plus hauts effets inhibiteurs ($p < 0,05$). Les huiles essentielles de *Z. multiflora* et de *T. kotschymanus* 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-pre-disposed 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 kotschymanus*, *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

Table 1 Details of breed, age and sex of dogs with atopic dermatitis.*Détails de race, âge et sexe des chiens avec une dermatite atopique.*

Number	Breed (no.)	Age (average years)	Sex
1	Terrier (17)	3.7	8 male + 9 female
2	Terrier- Poodle (1)	5.5	1 male
3	Terrier-Shih- Tzu (3)	3.1	1 male + 2 female
4	Yorkshire Terrier (1)	2	1 male
5	Shih-Tzu (5)	9.5	4 male + 1 female
6	Pug (2)	5.7	1 male + 1 female
7	Maltese (1)	5	1 female
8	Japanese Spaniel (1)	9	1 male
9	Lhasa Apso (1)	8	1 male
10	Bulldog (1)	5	1 male

tape strip (except for external ear canal) in dimensions of $1 \times 1 \text{ cm}^2$ were applied. The cotton tip of the swab was rolled and rubbed firmly on the skin area for 30 s, and the tape strip was applied and pressured with the blunt end of the scalpel for 30 s to collect enough amount of sample.

Two swabs were streaked onto modified Dixon agar (MDA) (Merck Co., Darmstadt, Germany) and Sabouraud dextrose agar (SDA) (Merck Co., Darmstadt, Germany) media with chloramphenicol (0.5%) and cycloheximide (0.5%). In addition, the adhesive tape strip was cultured by pressuring it in the middle of the plates of MDA and SDA. To avoid contaminants, plates were sealed with paraffin. All the plates were incubated at 32 °C for 10–14 days, and from day 4 were observed daily for detecting *Malassezia* growth. The yeast colonies were cream to light brown. Every single colony was stained with Giemsa or lactophenol cotton blue for microscopic observation. Subsequently, the identification of *Malassezia* colony was based on the ability to use certain polyoxyethylene sorbitan esters (Tweens 20, 40, 60 and 80), catalase reaction, cremophor EL assimilation test, splitting of esculin and precipitate production on MDA [14,16]. For cytological study, the third swab was rolled on a clean glass

slide and the smear was first fixed with methanol and then stained using Giemsa for microscopic observation.

Medicinal plants and essential oil extraction

Z. multiflora, *T. kotschyanus*, *M. spicata*, *A. sieberi*, *R. officinalis* and *H. persicum* were chosen in this study. These plants were collected from different regions of Iran. Botanical identification was performed at the Herbarium of Pharmacognosy Department, School of Pharmacy, Shaheed Beheshti University of Medical Sciences, Iran. Details of the plants and voucher botanic specimens are given in Table 2.

Essential oils were isolated by water distillation for 3 h from air-dried material and seeds, using a Clevenger type apparatus, according to the procedure described in the European Pharmacopoeia [9]. The oils were stored at -4°C in sealed brown vials until use.

Broth microdilution method

Minimum inhibitory concentrations (MICs) of 6 medicinal essential oils were determined by broth microdilution method [7]. In brief, stock solutions of essential oils (3400 $\mu\text{g/mL}$) were prepared in sterile modified Dixon broth containing DMSO 1%. Serial dilutions of stock solution of essential oils were prepared for a final oil concentration ranging from 1700 $\mu\text{g/mL}$ to 7.5 $\mu\text{g/mL}$ in sterile, flat-bottom 96-well microtiter plates. The suspension was adjusted to make a yeast concentration of 1×10^5 cell/mL by counting with a hemacytometer. Volumes of 100 μL of the dilutions and 100 μL of the inoculum were dispensed into the wells. For essential oils, aliquots of 100 μL of prepared samples were distributed from column 2 to 11 microtiter plates. For each isolate, negative (only medium) and positive (medium plus yeast suspension without antifungal addition) controls were included. The plates were incubated at 30 °C for 48 h with daily monitoring. After 48 h, the reading of the drug and oil tests was performed in microplate reader (Asys Hitech GmbH, Eugendorf, Austria). The MICs of the essential oils, itraconazole (Janssen, Beerse, Belgium), fluconazole (Darus-pakhsh Co., Tehran, Iran) and amphotericin B (Sigma Chemical Co., St. Louis, MO) were defined as the first well with a significant growth reduction (90%; MIC^{90}) when compared to that of the positive control. These experiments were performed in duplicate as well.

Table 2 Some characteristics of the tested plants [28].*Caractéristiques des plantes étudiées [28].*

Scientific name	Voucher no.	Family	Local name	Medicinal use	Main constituent
<i>Artemisia sieberi</i>	1559	Compositae	Dermane	Antiseptic, anti-infective, anti- <i>Malassezia</i>	α -thujone, β -thujone
<i>Heracleum persicum</i>	1312	Apiaceae	Golpar	Flavoring, digestive, antiseptic	Anethole, terpinolene
<i>Menta spicata</i>	1491	Labiatae	Naena	Digestive, carminative	Carvone, limonene
<i>Rosmarinus officinalis</i>	1449	Labiatae	Eclil Kohi	Digestive, additive	Pinene, cineole, Linalool
<i>Thymus kotschyanus</i>	1616	Labiatae	Avishan	Anti-infective, expectorant, coughing	Thymol, carvacrol
<i>Zataria multiflora</i>	1106	Labiatae	Avishan	Anti-infective, expectorant, coughing	Thymol, carvacrol

Statistical analysis

Student's *t* test was applied to determine the significance of difference between different study groups (*Malassezia* species, anatomical sites, breeds, gender and essential oils). A *P* value less than 0.05 was statistically considered as significant.

Results

Malassezia species were isolated from all dogs with AD (100%). A total of 103 fungal isolates were collected. As shown in Table 3, the most frequent *Malassezia* species were *M. pachydermatis* (81.6%), followed by *M. globosa* (7.8%) and *M. restricta* (3.9%). Regarding to the anatomical sites, the most frequent site was anal (28.2%), followed by interdigital (25.2%), mouth (19.4%), groin (18.5%) and external ear canal (8.7%) (Table 3). There were significant differences between frequency of yeast isolates and anatomical sites ($P < 0.05$).

As illustrated in Table 4, the most frequent *Malassezia* species were associated with Terrier dogs (no. 18; 54%), followed by Shih-Tzu (no. 5; 15%) and Pug (no. 2; 8%) breeds. No significant difference was observed in *Malassezia* prevalence among various breeds as well as between male (57.6%) and female (42.4%) dogs.

The antifungal activity of medicinal essential oils is illustrated in Table 5. Data confirmed the inhibitory efficacy of essential oils against pathogenic *Malassezia* isolates with MIC₉₀ values ranging from 30 to 850 µg/mL. *Z. multiflora* essential oil showed the best antifungal activity, with MIC₉₀ values ranging from 30 to 80 µg/mL. *M. nana* isolate was the most susceptible one (30 µg/mL), while *M. sloofiae* isolates showed the highest MIC₉₀ value (80 µg/mL) ($P < 0.05$). The inhibitory activity of *T. kotschyanus* essential oil ranged from 30 to 110 µg/mL. MIC₉₀ values were close or similar to those found for *Z. multiflora* essential oil; however, *Z. multiflora* essential oil was more active than *T. kotschyanus*, *M. nana* and *M. restricta* were the most and least susceptible isolates to *T. kotschyanus* ($P < 0.05$). Inhibitory activity of *M. spicata* essential oil was detected at concentrations ranging from 65 to 250 µg/mL. Based on MIC₉₀ results, this essential oil was less active than *Z. multiflora* and *T. kotschyanus* but similar to *A. sieberi* essential oil (MIC₉₀ values: 50–250 µg/mL). *M. nana* and *M. globosa* were the most susceptible isolates to *M. spicata* and *A. sieberi*, respectively ($P < 0.05$). The antifungal activity of *H. persicum* (MIC₉₀ values: 75–580 µg/mL) and *R. officinalis* (MIC₉₀ values: 100–850 µg/mL) essential oils were the lowest of all as judged by their MIC₉₀ ranges. *M. nana* was the most susceptible isolate to both *H. persicum* and *R. officinalis* oils ($P < 0.05$). In addition, itraconazole (MIC₉₀ value: 2.8 µg/mL) and amphotericin B (MIC₉₀ value: 3 µg/mL) showed

Table 3 Relative frequency of pathogenic *Malassezia* isolates from various anatomical sites of atopic dogs.
Fréquence relative de Malassezia isolé des différents sites anatomiques de chiens atopiques.

Site	Yeast								
	<i>M. pachydermatis</i> No. (%)	<i>M. globosa</i> No. (%)	<i>M. restricta</i> No. (%)	<i>M. sloofiae</i> No. (%)	<i>M. furfur</i> No. (%)	<i>M. nana</i> No. (%)	<i>M. obtusa</i> No. (%)	<i>M. sympodialis</i> No. (%)	Total No. (%)
Ear	7 (77.8)	0 (0)	0 (0)	1 (11.1)	1 (11.1)	0 (0)	0 (0)	0 (0)	9 (8.7)
Mouth	16 (80)	2 (10)	1 (5)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)	20 (19.4)
Interdigital	19 (73.1)	2 (7.7)	2 (7.7)	2 (7.7)	0 (0)	0 (0)	1 (3.9)	0 (0)	26 (25.2)
Groin	16 (84.2)	2 (10.5)	1 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	19 (18.5)
Anal	26 (89.7)	2 (6.9)	0 (0)	0 (0)	0 (0)	1 (3.5)	0 (0)	0 (0)	29 (28.2)
Total	84 (81.4)	8 (7.8)	4 (3.9)	3 (2.9)	1 (1)	1 (1)	1 (1)	1 (1)	103 (100)

Table 4 Relative frequency of pathogenic *Malassezia* isolates from various breeds of atopic dogs.
Fréquence relative de Malassezia sp. isolées des différentes races de chiens atopiques.

Breed	Yeast								
	<i>M. pachydermatis</i> No. (%)	<i>M. globosa</i> No. (%)	<i>M. restricta</i> No. (%)	<i>M. sloofiae</i> No. (%)	<i>M. furfur</i> No. (%)	<i>M. nana</i> No. (%)	<i>M. obtusa</i> No. (%)	<i>M. sympodialis</i> No. (%)	Total No. (%)
Terrier (no. 18)	46 (85.2)	4 (7.8)	1 (2)	1 (2)	0	1 (2)	1 (2)	0	54 (100)
Mixed terrier (no. 4)	4 (66.7)	1 (16.7)	1 (16.7)	0	0	0	0	0	6 (100)
Shih-Tzu (no. 5)	11 (73.3)	2 (13.3)	2 (13.3)	0	0	0	0	0	15 (100)
Pug (no. 2)	8 (100)	0	0	0	0	0	0	0	8 (100)
Maltese (no. 1)	6 (100)	0	0	0	0	0	0	0	6 (100)
Japanese Spaniel (no. 1)	5 (83.3)	0	0	0	1 (16.7)	0	0	0	6 (100)
Lhasa Apso (no. 1)	1 (50)	0	0	0	0	0	0	1 (50)	2 (100)
Bulldog (no. 1)	3 (50)	1 (16.7)	0	2 (33.3)	0	0	0	0	6 (100)
Total (no. 33)	84 (81.4)	8 (7.8)	4 (3.9)	3 (2.9)	1 (1)	1 (1)	1 (1)	1 (1)	103 (100)

Table 5 The minimum inhibitory concentration (MIC⁹⁰) of some medicinal essential oils and standard antifungal drugs against pathogenic *Malassezia* isolates (μg/mL).Concentrations inhibitrices minimales (MIC⁹⁰) de quelques huiles essentielles médicinales et de médicaments antifongiques de référence contre des isolats de *Malassezia* (μg/mL).

Agent	Yeast								Average total
	<i>M. pachydermatis</i> (No. 84)	<i>M. globosa</i> (No. 8)	<i>M. restricta</i> (No. 4)	<i>M. sloofiae</i> (No. 3)	<i>M. furfur</i> (No. 1)	<i>M. nana</i> (No. 1)	<i>M. obtusa</i> (No. 1)	<i>M. sympodialis</i> (No. 1)	
<i>Zataria multiflora</i>	60	50	40	80	35	30	60	30	48.1
<i>Thymus kotschyanus</i>	100	80	110	80	60	30	80	60	75
<i>Mentha spicata</i>	150	250	85	100	125	65	85	100	120
<i>Artemisia sieberi</i>	80	50	100	150	250	110	150	85	121.9
<i>Rosmarinus officinalis</i>	360	410	350	420	260	100	850	250	375
<i>Heracleum persicum</i>	580	250	320	480	125	75	560	250	330
Amphotericin B	4	2	2	4	2	2	4	4	3
Fluconazole	8	12	4	8	12	2	8	8	7.8
Itraconazole	2	4	4	2	2	2	2	4	2.8

more inhibitory activity than fluconazole (MIC₉₀ value: 7.8 μg/mL) against each *Malassezia* isolate. The most susceptible isolates to amphotericin B and itraconazole were *M. furfur* and *M. nana* (MIC₉₀ value: 2 μg/mL), and the least susceptible isolates to fluconazole were *M. furfur* and *M. globosa* (MIC₉₀ value: 12 μg/mL).

Discussion

AD has been known as a skin disorder with an uncertain etiology. The occurrence of sensitization to fungal allergens in different allergic diseases has been investigated previously [21,36]. *Malassezia* species are normal flora of the skin of dogs and Type-1 hypersensitivity reacts to *M. pachydermatis* extract in atopic dogs with *Malassezia* dermatitis [6]. *Malassezia* dermatitis is common in dogs of any age or gender, although certain breeds are reported to be predisposed, including Basset Hound, West Highland White Terrier, Dachshund, Cocker Spaniel, Poodle, English Setter and Shih-Tzu [3]. In this study, the majority of dogs with positive *Malassezia* isolate were Terrier dogs. In addition, we found no significant correlation between age and isolation of *Malassezia*. However, the most frequent isolation was associated with dogs with age varying from 2 to 6 years old, which is almost in accordance with Nardoni et al. [30] findings that *Malassezia* was isolated from dogs of age ranging 1–5 years old. The results of our study showed generally higher prevalence of *Malassezia* species in male dogs (57.6%) than in females (42.4%). This fact may be explained by the assumption that androgens cause higher production of sebum, which is one of the predisposing factors for development of latent infection.

In the present study, *Malassezia* species were isolated from 100% of atopic dogs. *M. pachydermatis* was the predominant isolate (81.6%), followed by *M. globosa* (7.8%) and

M. restricta (3.9%). Previous studies reported the isolation of lipid-dependent *Malassezia* species from dogs [10,14,30], which is in accordance with our findings. According to the findings of Nardoni et al. [29], 100% of atopic dogs showed positive reactions with *M. pachydermatis* from different body sites. Although in the present study, *M. pachydermatis* was isolated from 81.6% of dogs, other *Malassezia* species, such as *M. globosa*, *M. furfur* and *M. restricta*, have been isolated from the skin and mucosal samples as well. Ultrastructural evaluation of skin from dogs with AD has shown that intercellular lipid lamellae are disorganized and markedly heterogenous when compared to normal skin [33]. There is a widening of intercellular space as well as abnormal release of altered skin lipids that is exacerbated in lesional skin after specific allergen challenge [23]. For this reason, lipid-dependent *Malassezia* species are also recovered from skin of dogs with AD. It seems that *M. furfur* is not considered as mycoflora of the animals' skin and it is transmitted from the owner to the animals. The potential for human exposure to this organism is therefore quite great. While no evidence has shown that dogs represent an overt health concern to immunocompromised humans, the increasing incidence of immune suppression in humans suggests that a survey of the zoonotic potential of this organism is relevant to modern hospital hygiene practices [26].

In dogs with allergic or seborrheic skin diseases, the homeostasis of the local cutaneous microenvironment is disrupted by inflammation and increased levels of moisture or sebum [2,27]. Under these conditions, the number of *M. pachydermatis* organisms on the skin and in the ear canals may increase dramatically, making it possible to readily identify the organism with rapid cytologic screening [24]. Therefore, the correlation between *M. pachydermatis* presence and atopic signs has been previously investigated [8,20].

In this study, the most infected site was anal (21.2%), followed by interdigital (17.2%), mouth (16.2%), groin (13.1%) and external ear canal (10.6%). Previous studies showed the isolation of *Malassezia* species from the skin of the mouth region, chin, external ear canal, anal sacs and from vagina and rectum [4,15]. Nardoni et al. [29] demonstrated that ear, anal cavity and groin were the predominant sites of *Malassezia* isolation. These differences may be due to different geographical locations, health conditions, nutritional regimes and other predisposing factors.

Herbal medicinal oils are natural components with known antimicrobial, anti-inflammatory and anticancer activities [37]. We selected 6 medicinal essential oils to evaluate anti-*Malassezia* activity. Among tested oils, *Z. multiflora* (MIC90 value: 48.1 µg/mL) and *T. kotschyianus* (MIC90 value: 75 µg/mL) were more active than the other essential oils. The essential oils of *Z. multiflora* and *T. kotschyianus*, possessing the strongest antifungal properties against various *Malassezia* species, contain a high percentage of phenolic compounds, such as carvacrol and thymol [19,22]. It seems reasonable that their mechanism of action would therefore be similar to other phenolics; this is generally considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force (PMF), electron flow, active transport and coagulation of cell contents [11]. Although *M. nana* was the most susceptible *Malassezia* species to most essential oils tested, the mechanism of its susceptibility was still not understood. However, the MIC90 values of essential oils against *M. nana* are a significant finding that requires new investigations. In our study, amphotericin B and itraconazole were found to be more active than fluconazole against *Malassezia* isolates. Although MIC90 values of essential oils were high when compared to those of the antifungal drugs, these results were of interest as we were dealing with an essential oil and not a pure product. With attention to this point that antifungal resistance are increasing with time, these natural agents may be used to treat animals suffering from *Malassezia* infections.

Conclusions

In summary, *M. pachydermatis* was the most predominant *Malassezia* isolate on the skin and mucosa of dogs with AD. In addition, *Z. multiflora* and *T. kotschyianus* essential oils remarkably inhibited the growth of pathogenic *Malassezia* yeasts and they may be used as anti-*Malassezia* agents in the treatment of infected animals. Further studies are needed to investigate the exact correlation between *Malassezia* antigens and their colonization in dogs with AD.

Disclosure of interest

The authors declare that they have no competing interest.

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