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SHORT COMMUNICATION/COURTE COMMUNICATION

In vitro and *in vivo* antifungal activity of some essential oils against feline isolates of *Microsporum canis*

Activité antifongique de quelques huiles essentielles de plantes in vitro *et* in vivo *sur* Microsporum canis *isolé chez le chat*

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Summary The treatment of dermatophytoses due to *Microsporum canis* is cumbersome and relapses can occur. Volatile essential oils (EOs) obtained from plants would seem to represent suitable tools to contrast mycoses both in human and animals. The anti-*M. canis* activity of some EOs chemically characterized was evaluated both *in vitro* and *in vivo*. Eleven feline isolates of *M. canis* were tested by microdilution against EOs extracted from *Thymus serpillum*, *Origanum vulgare*, *Rosmarinus officinalis*, *Illicium verum* and *Citrus limon*. A mixture composed by 5% *O. vulgare*, 5% *R. officinalis* and 2% *T. serpillum*, in sweet almond oil was administered to seven infected, symptomatic cats. *T. serpillum* and *O. vulgare* showed the lowest MICs, followed by *I. verum*, *R. officinalis* and *C. limon*. The assay performed on mixture showed that antimycotic activity of each component was enhanced. Four out of seven treated cats recovered both clinically and culturally. *T. serpillum* and *O. vulgare* EOs showed a strong antifungal activity. Preliminary data suggest a possible application in managing feline microsporiasis. Considering the potential zoonotic impact of this infection, the use of alternative antimycotic compounds would be of aid to limit the risk of environmental spreading of arthrospores.

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MOTS CLÉS
Microsporum canis ;
Chats ;
Huiles essentielles ;
CMI ;
Activité *in vitro* ;
Traitement *in vivo*

Résumé Le traitement des teignes à *Microsporum canis* est une tâche difficile et parfois décevante, les animaux rechutent souvent. Dans cet objectif, les huiles essentielles des plantes pourraient représenter une véritable alternative dans le traitement des teignes chez les hommes et les animaux. L'activité antifongique des huiles essentielles de *Thymus serpillum*, *Origanum vulgare*, *Rosmarinus officinalis*, *Illicium verum* et *Citrus limon*, caractérisées par chromatographie gazeuse avec détecteur à spectrométrie de masse, a été evaluée sur 11 souches de *M. canis* d'origine féline avec le test de microdilution. *O. vulgare* et *R. officinalis* en pourcentage de 5 % et *T. serpillum* en pourcentage de 2 % ont été utilisés en mélange pour le traitement local de sept chats atteints de teigne à *M. canis*. *T. serpillum* et *O. vulgare* ont montré les concentrations minimales inhibitrices inférieures. Quatre parmi sept chats traités ont été guéris cliniquement et mycologiquement. Les huiles essentielles de *T. serpillum* et d'*O. vulgare* ont montré une puissante activité antifongique. Les résultats de cette étude ont montré que les huiles essentielles analysées pourraient être utilisées comme une alternative en attendant les résultats du laboratoire et pour limiter la dissémination d'éléments fongiques zoonotiques dans l'environnement.

Introduction

Dermatophytoses are regarded as major cause of morbidity-associated mycoses worldwide and several causative agents are zoonotic fungi.

The treatment is not always effective and relapses can occur; furthermore the fungal resistance to antimycotic drugs is an increasing problem [4]. For these reasons, there is a need of alternative treatments and, in this view, volatile essential oils (EOs) obtained from aromatic plants would seem to represent suitable tools to contrast mycoses both in human and animals.

Antifungal activity of EOs has been demonstrated both against pathogenic fungi causing mycoses [1,3,21] and against fungi causing food spoilage and producing mycotoxins [13]. The mode of action of EOs seems to be related to a damage in fungal cell membrane, leading to an impairment of the biosynthesis of ergosterol [7,14,16].

The antimycotic effects depend on botanical species and on chemotype, so far EOs examined for their biological activity should be analyzed to ascertain the different amounts of the active compounds. Data dealing with the use of such mixtures are scarce in veterinary literature and there is a general request of putative compounds to be locally administered in infected animals, to clean the host as well as to control the spread of fungal elements into the environment.

In the present paper, the antimycotic activity of EOs extracted from some Mediterranean autochthonous plants was evaluated *in vitro* against *Microsporum canis* isolated from infected cats; moreover the results of the *in vivo* administration of an active EOs' mixture to some infected cats are reported.

Material and methods

Essential oils: for all *in vitro* studies, *Citrus limon* s.90660, *Illicium verum* s.70487, *Origanum vulgare* s.60326, *Rosmarinus officinalis* s.60734, *Thymus serpillum* s.90017 and sweet almond oil s.70312 of EOs were employed. EOs solutions were prepared in sweet almond oil (*Prunus dulcis* Mill. Flora Srl, Lorenzana, Pisa, Italy). All the EOs were supplied by Flora Srl (Lorenzana, Pisa, Italy). Volatile constituents of each EOs were identified by GC-MS analysis.

Gas chromatography-mass spectrometry analysis: The EOs were analyzed by GC-MS in order to investigate the relation between chemical composition and biological activity as antifungal agents. GC-MS analysis was accomplished as previously described [15] with a Varian CP-3800 gas 3 °C/min. Identification of the constituents was based on comparison of the retention time with those of authentic samples, comparing their linear indices relative to a series of *n*-alkanes (C8—C23). Further identifications were also made possible by the use of a homemade library of mass spectra built up from pure substances and components of known oils, and MS literature data (NIST 2000, ADAMS).

In vitro assay

The *in vitro* antimycotic activity of EOs was evaluated on 11 clinical isolates of *M. canis*. The dermatophytes were cultured by affected cats and maintained on a selective dermatophyte media (Mycobiotic agar®, Difco, USA). The effectiveness of EOs was assessed by means of a microdilution test carried out as previously described using a semisolid malt extract medium (MEA) with 1% agar [12]. Portions of approximately 1 mm^3 non-sporulating mycelia from Sabouraud dextrose agar were used as fungal inocula in 24-wells plates (Pbi International, Milano, Italy). Stock solutions at 20% of all the chemically defined EOs (*T. serpillum*, *O. vulgare*, *R. officinalis*, *C. limon* and *I. verum*) were diluted in sweet almond oil to obtain concentrations ranging from 0.01 to 10%. The dilutions were chosen considering that EOs *in vivo* maximum dilution for dermatologic administration is 20%. In detail 10%, 7.5%, 5%, 2.5%, 1%, 0.05%, 0.025% and 0.01% dilutions were checked. After preliminary results about the effectiveness of each single EO, some of them were selected to perform a mixture for a further *in vivo* trial. Therefore eight further *M. canis* isolates, obtained from feline, were also tested against a mixture composed by 5% *O. vulgare*, 5% *R. officinalis* and 2% *T. serpillum*, in sweet almond oil. EOs were chosen on the basis of their *in vitro* efficacy (*T. serpillum* and *O. vulgare*) and for their dermo-equilibrant, anti-inflammatory topical actions (*R. officinalis*). The mixture was checked at dilutions starting