Journal de Mycologie Médicale (2014) 24, e101—e107

Available online at ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com

ORIGINAL ARTICLE/ARTICLE ORIGINAL

Potential effects of *Trachyspermum copticum* essential oil and propolis alcoholic extract on *Mep3* gene expression of *Microsporum canis* isolates

Effets potentiels de l'huile essentielle de Trachyspermum copticum *et de l'extrait alcoolique de propolis sur l'expression du gene* Mep3 *d'isolats de* Microsporum canis

A.R. Khosravi [a,*], H. Shokri [b], N. Sohrabi [a]

[a] *Mycology research center, faculty of Veterinary medicine, university of Tehran, 14155-6453 Tehran, Iran*
[b] *Faculty of veterinary medicine, Amol university of special modern technologies, Amol, Iran*

Received 19 October 2013; received in revised form 25 February 2014; accepted 7 March 2014
Available online 17 April 2014

KEYWORDS
Dermatophytosis;
Microsporum canis;
Trachyspermum copticum;
Propolis;
Mep3 gene;
RT-PCR

Summary
Objective. — The purpose of this study was to evaluate the effects of *Trachyspermum copticum (T. copticum)* essential oil and propolis alcoholic extract on growth and transcription of *Mep3* gene of *Microsporum canis* (*M. canis*) strains.
Methods. — The antifungal activity was assayed by broth macrodilution method. Fungal isolates were grown in soy peptone liquid medium and treated with *T. copticum* oil and propolis extract. Total RNAs of *M. canis* were subjected to reverse transcription-polymerase chain reaction (RT-PCR). Specific primers of *Actin* and *Mep3* genes were used.
Results. — The results revealed that MIC values of *T. copticum* oil against *M. canis* strains were ranged from 0.2—30.5 μg/mL, with 42.3% of the strains inhibited at 0.9 μg/mL. In addition, MIC values of propolis extract against *M. canis* strains were ranged from 0.2—488.2 μg/mL, with 34.6% of the strains inhibited at 0.9 μg/mL. RT-PCR analysis of *Mep3* and *Actin* expression showed DNA fragments of 661 and 690 bp amplified in all isolates before treatments with *T. copticum* essential oil and propolis extract. Both *T. copticum* and propolis completely inhibited the expression of *Mep3* gene.

* Corresponding author.
E-mail address: khosravi@uf.ac.ir (A.R. Khosravi).

http://dx.doi.org/10.1016/j.mycmed.2014.03.003

Conclusion. — We reported for the first time that *T. copticum* and propolis inhibits the expression of *Mep3* gene in *M. canis* strains in relation to a remarkable inhibition in protease production by the fungus.

MOTS CLÉS
Dermatophytosis ;
Microsporum canis ;
Trachyspermum copticum ;
Propolis ;
Gène *Mep3* ;
RT-PCR

Résumé
Objectif. — Le but de cette étude était d'évaluer les effets d'huile essentielle de *Trachyspermum copticum* (*T. copticum*) et de l'extrait alcoolique de propolis sur la croissance et la transcription du gène *Mep3* d'isolats de *Microsporum canis* (*M. canis*).
Matériel et méthodes. — L'activité antifongique a été testée par la méthode de macrodilution en milieu liquide. Les isolats fongiques ont été cultivés en milieu liquide soja-peptone et traités par l'extrait de propolis et par l'huile essentielle de *T. copticum*. L'ARN total de *M. canis* a été soumis à la réaction en chaîne par polymrase avec transcription inverse (RT-PCR). Les primers spécifiques d'*actine* et des gènes *Mep3* ont été utilisés.
Résultats. — Les résultats ont révélé que les valeurs de CMI de l'huile de de *T. copticum* contre *M. canis* variaient de 0,2 à 30,5 μg/mL, avec 42,3 % des souches inhibées à 0,9 μg/mL. En outre, les CMI d'extrait de propolis contre *M. canis* variaient de 0,2 à 488,2 μg/mL, avec 34,6 % des souches inhibées à 0,9 μg/mL. L'analyse de RT-PCR de l'expression de *Mep3* et d'*actine* a montré des fragments d''ADN de 661 et 690 paires de bases amplifiés chez tous les isolats avant les traitements avec l'huile de *T. copticum* et d'extrait de propolis. Tant *T. copticum* que propolis ont complètement inhibé l'expression du gène *Mep3*.
Conclusion. — Nous avons montré pour la première fois que *T. copticum* et propolis inhibent l'expression du gène *Mep3* dans les souches de *M. canis* en rapport avec une inhibition remarquable de la production de protéase par le champignon.

Introduction

Dermatophytes are fungi that have the ability to invade keratinized structures, such as the superficial cornified skin layers, hair, and nails, causing a superficial cutaneous infection called dermatophytosis [32]. *Microsporum canis* (*M. canis*) is the main agent of dermatophytosis in dogs and cats [11,25] and causes a zoonosis that has increased in several countries [15]. The cat, considered as the natural host and the main reservoir of *M. canis*, is the principal source of human contamination [10,25]. In addition, the existence of feline asymptomatic infection and the lack of efficient immunoprophylaxis are responsible for the high frequency of endemic *M. canis* dermatophytosis in catteries [9].

Pathophysiological mechanisms of dermatophytosis, including *M. canis* infection, are poorly understood. Among potential fungal virulence factors, attention has been paid to proteases for their potential role in the nutrition of the fungi [25], in tissue invasion [1], and in the control of host defense mechanisms [4]. Given the ability of dermatophytes to invade and to be essentially confined to keratinized structures, it can be assumed that keratinolytic proteases might be significant virulence factors. Therefore, the characterization of proteases seems to be a major step for a better understanding of dermatophytic infection pathogenesis and subsequently of the host-fungus relationship. Recently, the present authors have purified and characterized two *M. canis* keratinases, a 31.5-kDa subtilisin-like protease [21] and a 43.5-kDa metalloprotease [3] produced in vitro in a minimal feline keratin-enriched medium. Despite the fact that the *M. canis* 31.5-kDa subtilisin-like protease was produced in vivo in naturally infected cats and in experimentally infected guinea pigs [20], this protease does not appear as a major antigen in *M. canis* infection in these natural and experimental hosts [19]. However, cellular and humoral immune responses to a crude *M. canis* exoantigen containing mainly the 31.5- and 43.5-kDa keratinases were demonstrated in both animals, suggesting that the 43.5-kDa metalloprotease could be a valuable candidate for further immunological studies, including vaccination trials.

Research on different natural products possessing antimicrobial properties may be useful in reducing the risk of various diseases. Propolis is a natural product of plant resins collected by honeybees (*Apis mellifera*) from various plant sources. Its chemical composition is quite complex since more than 300 compounds, such as polyphenols, phenolic aldehydes, sequiterpene quinines, coumarins, amino acids, steroids, and inorganic compounds, have been identified in propolis samples. Propolis has cytotoxic [17], antitumor [13], antifungal [18], antiprotozoan [7] and anti-HIV [5] activity. *Trachyspermum copticum* (*T. copticum*, Umbelliferae) is an annual plant which grows in Iran. Some biological effects of this oil such as antiviral [6], anti-inflammatory [30], antifungal [24], and antioxidant activity [2] have been confirmed. Carvacrol, γ-terpinene and p-cymene were reported as main components of Iranian oil [27]. The purpose of this paper was to evaluate the inhibitory effects of *T. copticum* essential oil and propolis alcoholic extract on growth and *Mep3* gene expression of *M. canis* strains.