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Short communication

Interactions between Clotrimazole and selected essential oils against *Malassezia pachydermatis* clinical isolates

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Abstract

The aim of this study was to investigate interactions between conventional antifungal drug and essential oils against isolates of *Malassezia pachydermatis.* Antifungal activity of *Cinnamomum cassia*, *Melaleuca alternifolia*, *Mentha piperita*, *Origanum vulgare* and *Syzygium aromaticum* essential oils were tested against 19 strains of *M. pachydermatis* isolated from healthy dogs and reference strain *M. pachydermatis* CBS 1879. The checkerboard assay was used to search for interactions. Synergism was observed for the combination of clotrimazole with *Melaleuca alternifolia* essential oil, *Mentha piperita* and *Origanum vulgare*. The combinations of *Cinnamomum cassia* and *Syzygium aromaticum* essential oils with clotrimazole showed indifferent effect. Additive antimicrobial activity was observed for the combination of clotrimazole with *Syzygium aromaticum* and *Melaleuca alternifolia* essential oils against reference strain. The obtained results showed synergistic interactions between essential oils and clotrimazole which could improve effectiveness of this antifungal drug.

Key words: antimycotic, antifungal, synergism, clotrimazole, essential oil, checkerboard

Introduction

Malassezia yeasts are lipophilic microorganisms known for more than a century as part of the natural skin microflora of humans and almost all domestic and wildlife animals. Since the 1980s these yeasts have been considered as potential agents of occasional systemic infections (Boekhout et al. 2010). The most common zoophilous species, often isolated in domestic animals, is *Malassezia pachydermatis*. This yeast is opportunistic and is often associated with inflammation of the external auditory canal and various types of dermatitis in domestic animals (Cabañes 2014). Many types of antimycotic drugs, characterized by different mechanisms of action, are used for treatment of yeast infections. Azole antimycotics are one of the most widely used antifungals in clinical practice. As well as in human medicine, in veterinary medicine fungal diseases may exhibit resistance to classic antimycotics, justifying the search for new alternative therapies and special interest is focused on the use of essential oils. They have a wide range of antifungal properties and are environmentally friendly (biodegradable and do not leave toxic residues) (Abdel-Kader et al. 2011).

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Table 1. Fractional inhibitory concentration indices (FICI) of clotrimazole combined with selected essential oils against isolates of *M. pachydermatis.*

Combinations	*M. pachydermatis* CBS 1879	*M. pachydermatis* (isolated from dogs)
Clotrimazole + *Cinnamomum cassia*	1.6 (I)	1.38 (I)
Clotrimazole + *Melaleuca alternifolia*	0.56 (A)	0.2 (S)
Clotrimazole + *Mentha piperita*	0.06 (S)	0.17 (S)
Clotrimazole + *Origanum vulgare*	0.5 (S)	0.1 (S)
Clotrimazole + *Syzygium aromaticum*	0.8 (A)	1.4 (I)

A – additive effect, I – indifferent effect, S – synergistic effect

The aim of this study was to determine the effects of the combinations of essential oils and conventional antifungal drugs against *M. pachydermatis* clinical isolates.

Materials and Methods

To test the antimicrobial interactions between antimycotic and essential oil we used azole antimycotic clotrimazole (Sigma-Aldrich, St. Louis, USA) and five essential oils, *Cinnamomum cassia*, *Melaleuca alternifolia*, *Mentha piperita*, *Origanum vulgare* and *Syzygium aromaticum* (Calendula, Nová Lubovňa, Slovak Republic).

As fungal strains, we used isolates of *Malassezia pachydermatis*, which were obtained from healthy dogs. From 20 examined dogs we isolated 19 samples of *M. pachydermatis*. Samples were taken from the predilection sites of malassezia occurrence – the external auditory canal of both ears, the skin surface, the interdigital space, and the perineal area. Prior to antimicrobial assay, each sample was inoculated onto a specific selective medium and afterwards, samples were phenotypically identified according to Kaneko et al. (2007). Reference strain of *M. pachydermatis* CBS 1879 was purchased from Fungal Biodiversity Centre, Utrecht, Netherlands.

For testing the antimicrobial interactions, we used a modified broth microdilution checkerboard method according to Verma (2007). We used combinations of clotrimazole (concentrations ranging from 0.0625 μg/mL to 32 μg/mL) with essential oils at the following concentrations: *C. cassia* (0.156 - 20 mg/mL), *S. aromaticum* (0.156 - 20 mg/mL), *M. piperita* (0.4 - 50 mg/mL), *O. vulgare* (0.4 - 50 mg/mL) and *M. alternifolia* (0.4 - 50 mg/mL). Concentrations of clotrimazole and essential oils were selected based on the results of previous microdilution testing where we determined the MIC. In checkerboard method we used lower concentrations than the MIC of essential oils. The fractional inhibitory concentration index (FICI) is used to calculate the interactions between two substances.

$$FICI = \frac{\text{MIC of essential oil in combination}}{\text{MIC of essential oil separately}} + \frac{\text{MIC of antimycotic in combination}}{\text{MIC of antimycotic alone}}$$

The combination of two substances acts synergistically when FICI ≤ 0.5, additively if 0.5 <FICI <1, indifferently if 1 <FICI <4 and antagonistically when FICI ≥ 4 (Iten et al. 2009).

Results and Discussion

By checkerboard method (Table 1), the combination of clotrimazole with *M. alternifolia* was synergistic against strains of *M. pachydermatis* isolated from dogs (FICI = 0.2). CLT in combination with *M. piperita* showed also synergistic effect (FICI = 0.17), same was for *O. vulgare* + CLT combination which exhibited synergistic interaction (FICI = 0.1). The combination of C. *cassia* + CLT determined indifferent effect (FICI = 1.3). Same result was shown for CLT in combination with *S. aromaticum* essential oil (FICI = 1.4). Results of the combinations on the reference strain *M. pachydermatis* CBS 1879 are shown in Table 1.

Several publications on antifungal activity of the essential oils against *Malassezia* species were reported in the literature and many of them state good antifungal activity of *O. vulgare*, *M. piperita*, *M. alternifolia* and C. *cassia* EOs (Pistelli et al. 2012, Arora et al. 2013, Nardoni et al. 2014). Váczi et al. (2018) used modified disc diffusion method for testing the antifungal activity of 14 selected essential oils. They found out that essential oils obtained from clove, cinnamon and oregano exhibited an excellent effectiveness against *M. pachydermatis* growth. Data obtained by Khosravi et al. (2016) confirmed the inhibitory efficacy of 6 medicinal essential oils against *Malassesia* isolated from dogs. Devkatte et al. (2005) in a study on 38 herbal essential oils as potential inhibitors of *Candida albicans* growth

reported that cinnamon oil was the best and had the impact against 4 candida strains. Padalia et al. (2017) in their study investigated the effects of the cinnamon oil alone and in the combination with azole antifungals and other essential oils against 3 *Candida* species. The cinnamon oil alone showed inhibitory antifungal activity against all studied *Candida* strains. Cinnamon oil in combination with clotrimazole showed indifferent effect (FICI 1.125). Essential oils are natural plant products which contain lot of different components with antimicrobial properties and interactions between these constituents and conventional antifungal drugs may lead to antagonistic, additive or synergistic effects. Our results showed that essential oils can increase the efficacy of clotrimazole against the *Malassezia* strains.

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