NATURAL PRODUCT RESEARCH, 2018
https://doi.org/10.1080/14786419.2018.1468325

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SHORT COMMUNICATION

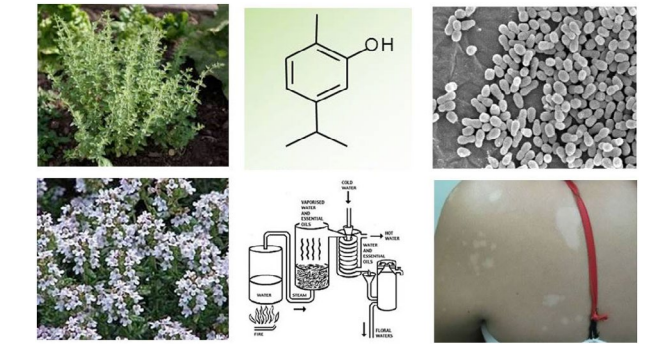
Chemical characterization and antifungal activity of *Origanum vulgare*, *Thymus vulgaris* essential oils and carvacrol against *Malassezia furfur*

Vittorio Vinciguerra[a], Florencia Rojas[b], Viviana Tedesco[c], Gustavo Giusiano[b] and Letizia Angiolella[c]

[a]Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF) University of Tuscia-Viterbo, L.go dell'Università, Viterbo, Italy; [b]Departamento de Micologia, Instituto de Medicina Regional, Universidad Nacional del Nordeste, CONICET, Resistencia, Argentina; [c]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Roma, Italy

ABSTRACT

The composition of the essential oils (EOs) of *O. vulgare* L. EO and *T. vulgaris* EO, were analyzed by GC and GC–MS. Antifungal activities of the EOs and its main component, carvacrol, were evaluated against 27 clinical isolates of *Malassezia furfur*. Minimum inhibitory concentrations (MICs) were measured according to the broth microdilution protocols by Clinical and Laboratory Standards Institute (CLSI) modified for *Malassezia* spp. EOs and carvacrol showed low MIC values ranged 450–900 µg/ml against *M. furfur*. No differences in EOs antifungal activity were observed in sensitive to resistant fluconazole isolates. The antifungal activity obtained showed *O. vulgare* EO, *T. vulgaris* EO and carvacrol, their compound, as potential antimicrobial agents against *M. furfur*, yeast associated with human mycoses.



ARTICLE HISTORY
Received 28 December 2017
Accepted 19 April 2018

KEYWORDS
Origanum vulgare; *Thymus vulgaris*; carvacrol; essential oil; fluconazole; *Malassezia furfur*; drug-resistance

CONTACT Letizia Angiolella letizia.angiolella@uniroma1.it

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2018.1468325.

1. Introduction

Malassezia genus includes a group of lipophilic and mostly lipid-dependent yeasts recognized as members of the normal skin microbiome of both human and other homoeothermic organisms (Cabañes 2014). Malassezia is an opportunistic yeast and under certain conditions, it may cause pityriasis versicolor and folliculitis, can be associated with seborrheic dermatitis or exacerbate several skin diseases such as atopic dermatitis (Saunders et al. 2012, Angiolella et al. 2018). Usually, are treated with topical and systemic antifungal therapy, but not always with successful outcomes (Ashbee 2007). The essential oils (EOs) are complex hydrophobic mixtures of different compounds extracted from plants by distillation. Many of them, including two of the most common edible plants used in traditional medicine, as *Origanum* and *Thymus* possess antimicrobial activity against different microorganisms. The EO from *O. vulgare* L. possess strong inhibitory effects against a variety of phytopathogenic fungi and do not seems to cause the development of resistance in microorganisms (Llana-Ruiz-Cabello et al. 2015). The *O. vulgare* EO alone or in combination with *Mirtus communis* or *Rosmarinus officinalis* EOs show high antimicrobial activity against *Salmonella thyphy murium* (Fadil et al. 2017). Boruga et al. (2014), showed the strong antimicrobial activity of *Thymus vulgaris* EO against both bacteria and fungi. Carvacrol is a monoterpene phenol and a major component of EO extract from oregano and other plants belonging to the Labiatae family (Lambert et al. 2001). This monoterpene is considered nontoxic for humans and it has been studied for its extensive pharmacological proprieties, including antifungal and antibacterial effects (Nostro and Papalia 2012). There is a lack of knowledge about the activity of EOs against *Malassezia* genus. Nenoff et al. (1996) demonstrated that the tea tree oil inhibits the growth of *M. furfur* and Hammer et al. (2000) against *Malassezia* spp. Khosravi et al. (2016) described the antimicrobial activity of some EOs against *Malassezia* spp.

Aims of this study were the chemical characterization of the *Origanum vulgare and Thymus vulgaris* EOs and the investigation of the antifungal activities of these EOs compared to one of their components, carvacrol, against clinical isolates of *Malassezia furfur*.

2. Results and discussion

The general chemical profile of the OVEO and TVEO expressed as the percentage content of the individual components is summarised in Table S1. Twenty-two components from OVEO and 27 from TVEO were identified. OVEO was characterised by high contents of thymol (45.43%) and ɣ-Terpinene (23.69%) and by similar contents of minor components ranging 12.41–1.27%. Fourteen components were present with percentage <1. Szczepanik et al. (2018), reported similar number of constituent of *O. vulgare* L. EO but a different percentage of main compounds; in particular, higher content of carvacrol. TVEO was characterized by high contents of p-Cymene (36.36%) and Thymol (24.35%) and minor components ranging 7.5–1.17%. Thirteen components were present with percentage <1. In contrast, Ballester-Costa et al. (2017), reported Linalool (44%) as the main component of TVEO followed by terpineol-4 (11.84%), γ-terpinene (8.91%) and β-myrcene (6.89%). In this study, the percentage of carvacrol present in OVEO and TVEO was 0.52% and 2.91%, respectively. That differs with Texteira et al. (2013) who report 14.1% of carvacrol in OVEO. Differences in the EOs main compounds percentages and yield could be explained by the geographical distribution. Climatic and edaphic conditions, and other environmental variables, including the

geographical location of the collection site can influence the EO composition (Garzoli et al. 2015). Because in OVEO and TVEO there are low percentages of carvacrol, in this study we wanted to compare the antifungal activity of pure carvacrol to that of EOs. The effect of π-cymene, a main compound of EO, on the membrane potential was less pronounced than the effect of carvacrol due the lack of the hydroxyl group (Ultee et al. 2002). So the π-cymene alone do not have antimicrobial activity. Carvacrol has extensively been tested as an antimicrobial agent in food to control Gram-positive and Gram-negative pathogens (Hyldgaard et al. 2012). Due to *M. furfur* requirements to growth, scarce data are published about susceptibility testing of this yeast, especially regarding to EOs antimicrobial activity. MIC range, geometric mean, MIC_{50} and MIC_{90} values obtained with OVEO, TVEO, carvacrol and fluconazole are summarized in Table S2. Fluconazole was included in this study in order to observe the activity of EOs compared to one of the most widely used antifungal agents against this yeast. Low activity of fluconazole with MIC ≥ 32 µg/ml values was obtained in about 32% of the isolated tested, while about 46% of isolates showed MICs values in a range between 4 and 16 µg/ml. In contrast, no differences in EOs and carvacrol activities were observed in sensitive and resistant fluconazole isolates (Table S2). In this study, TVEO was less active than OVEO while, Giordani et al. (2004) reports the most efficiency of the TVEO respect to OVEO against *C. albicans*. Others EOs were tested by other authors against *M. furfur* showing a high variability of their antimicrobial activity. Khosravi et al. 2016, testing *Z. multiflora* and *T. kotschyanus* EOs against ten *M. furfur* isolates reported MIC_{90} values ranging from 30 to 850 µg/m. Higher results, with MIC values between 556.2 and 4450.0 µg/ml, were obtained by Nenoff et al. (1996), using the Tea Tree Oil against this yeast. Lee and Lee (2010), described the antifungal activity of a hundred eight EOs against *M. furfur* evaluated by disc diffusion methods. Seventeen EOs show inhibitory activity against *M. furfur* at 2 mg/mL. More recently, Pinto et al. 2017, reported the activity of *Thapsia villosa* EO against one isolate of *M. furfur* with a MIC value of 2.5 µl/ml. In this work, carvacrol, a constituent of EO, was most active compared to OVEO and TVEO. These results demonstrate a better antimicrobial activity of a single EOs compound even though EOs are usually a complex mixture of different ones. Percentages of isolates with high MIC values to OVEO, TVEO and carvacrol, was 27, 37 and 11%, respectively. Similar antifungal activity of carvacrol were reported by Lima et al. (2013), with MICs of 128 and 256 µg/mL but testing *Candida albicans* isolates, and Nóbrega et al. (2016) against *Cryptococcus neoformans* with MIC of ranged from 25 to 81 µg/mL. It is not simple compare these results because these studies have been performed with different essential oils, different microorganisms, different methods (disk diffusion method CLSI M2-A6) or with broth microdilution method (CLSI M27-A3) and different number of isolates.

3. Experimental (provided as supplementary material)

4. Conclusion

For the first time, this study reported the antifungal activity of *O. vulgare* and *T. vulgaris* EOs and carvacrol against 27 clinical isolates of *M. furfur*. EOs and carvacrol were more actives against resistant or dose dependent to fluconazole *M. furfur* isolates. Its potential as

antifungal agents against *Malassezia* can be considering in a future. Chronic and recurrent skin disorders associated with *Malassezia* could have a therapeutic alternative.

Acknowledgements

The authors thank "Cooperativa Comunale Logistica Industriale Integrata" for the donation to support at the research.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Letizia Angiolella http://orcid.org/0000-0002-0473-5587

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