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RESEARCH ARTICLE

Comparison between the vapor-phase-mediated anti-*Candida* activity of conventional and organic essential oils

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Abstract

Essential oils (EOs) are known for their antimicrobial activities against a broad range of microorganisms. In this study we investigated if EOs obtained from plants grown by organic farming are more potent than those obtained from conventional farming, or vice versa. Therefore, the aim of this study was to compare pairwise the inhibitory vapor-phase-mediated antimicrobial activity of 33 certified organic EOs and as many equivalent EOs without such certification against two human pathogenic *Candida* species using the vapor-phase-mediated susceptibility assay. Overall, *C. glabrata* is more susceptible than *C. albicans* to EOs, but we could not show a significant difference in EO antimicrobial activity between certified organic and without certification.

Keywords: Essential oil, vapor-phase, antimicrobial, *Candida*, organic, vapor-phase-mediated susceptibility assay

Introduction

An essential oil (EO) is composed of several components that are principally derived from the methylerythritol phosphate -, mevalonic acid -, or shikimate pathways (Dewick, 2008). Natural variation in EO composition between harvests is expected, but is usually limited. However, some botanical species yield EOs with substantial intra-species differences in composition, which are referred to as chemotypes of that species. Because chemotypes can have a different biological activity, it is necessary to specify the chemotype of an EO when applicable (Baser & Buchbauer, 2016; Franchomme & Pénoël, 1996).

A common distinction between EOs is, whether they are obtained from plants grown by organic or conventional farming. Different countries may have different requirements or procedures to certify a product as organic (EU, 2007, 2008). Organic farming is considered superior over conventional farming by most consumers. One of the main reasons for consumers to favor organic products (besides lifestyle) is to avoid pesticides, although even in organic farming a limited number of pesticides are allowed. Furthermore, organic products are often believed to be more nutritious and/or healthier than conventional products. However, most research on this matter is inconclusive (Baranski et al., 2014; Hole et al., 2005; Michael & David, 2017; Seufert & Ramankutty, 2017; Smith-Spangler et al., 2012; Tuomisto, Hodge, Riordan, & Macdonald, 2012; Wilcox, 2011).

EOs are well-known for their activity against fungal pathogens such as *Candida albicans* and *Candida glabrata* (Palmeira-de-Oliveira et al., 2009; Sharifi-Rad et al., 2017). Recently, we showed that EOs can have potent vapor-phase-mediated anti-*Candida* activities, and can therefore be considered a novel class of antifungals with distinct characteristics (A. F. Feyaerts et al., 2018). *C. albicans* and *C. glabrata* are the *Candida* species most commonly

isolated from humans. While belonging to the same genus, they are genetically and phenotypically very different, and mostly employ species-specific virulence factors (Brunke & Hube, 2013; Adam F. Feyaerts et al., 2017; Mathé & Van Dijck, 2013).

In this study, a pairwise comparison was performed between the inhibitory vapor-phase-mediated antimicrobial activity (iVMAA) of 33 organic EOs (oEOs) and 33 equivalent conventional EOs (cEOs) against the two *Candida* species.

Materials and Methods

Material

The EOs used in this work (Table 1) and their chemical analysis were acquired from a commercial source (Pranarôm International, Belgium). Half of the EOs were certified organic and the other half were obtained from the same plant species, chemotype, and plant part, but were conventional. The composition of the EOs used in this study has been published (Feyaerts et al., 2018). The detection of organochlorine and organophosphorus pesticides was performed using GC-MS-XSD and GC-MS-FPD, respectively, using the internal multi-residue method validated according to NF V03-110. The maximum residue limit according to EU-legislation was never exceeded.

Table 1. List of essential oils

Number	Essential oil	Part of the plant	Lot numbers of pair oEO – cEO
1	*Cananga odorata* extra/totum	Flowers	OF10390 – OF9867
2	*Cedrus atlantica*	Wood	OF10992 – OF10799
3	*Chamaemelum nobile*	Flowers	OF10863 – OF11255
4	*Cinnamomum cassia*	Twigs	OF10584 – OF10588
5	*Citrus aurantium ssp amara*	Leaves	OF10467 – OF11484
6	*Citrus limon*	Peel	OF11188 – OF11178
7	*Citrus paradisii*	Peel	OF9436 – OF9722
8	*Citrus reticulata*	Peel	OF3457 – OF10644
9	*Citrus sinensis*	Peel	OF9238 – OF11321
10	*Cupressus sempervirens* variety *stricta*	Twigs	OF10218 – OF10846
11	*Cymbopogon martinii* variety *motia*	Aerial Parts	OF10011 – OF9950
12	*Eucalyptus globulus*	Leaves	OF10646 – OF11274
13	*Eucalyptus radiata ssp radiata*	Leaves	OF10865 – OF10720
14	*Eugenia caryophyllus*	Flower buds	OF9948 – OF10583
15	*Helichrysum italicum ssp serotinum*	Flowering tops	OF9441 – OF10622
16	*Lavandula angustifolia ssp angustifolia*	Flowering tops	OF10864 – OF10951
17	*Lavandula latifolia*	Flowering tops	OF10007 – OF9693
18	*Lavandula x burnatii* clone *grosso*	Flowering tops	OF1743 – OF6689
19	*Lavandula x burnatii* clone *super*	Flowering tops	OF10869 – OF10745
20	*Litsea citrata*	Fruits	OF10225 – OF11261
21	*Melaleuca alternifolia*	Leaves	OF11248 – OF10388

Table 1. List of essential oils (cont.)

Number	Essential oil	Part of the plant	Lot numbers of pair oEO – cEO
22	*Melaleuca cajuputi*	Leaves	OF10662 – OF11405
23	*Melaleuca quinquenervia* chemotype cineole	Leaves	OF10731 – OF10956
24	*Mentha arvensis*	Aerial parts	OF10883 – OF9728
25	*Mentha piperita*	Aerial parts	OF10867 – OF11594
26	*Myrtus communis* chemotype myrtenyl acetate	Leaves	OF9391 – OF10882
27	*Origanum compactum*	Flowering tops	OF11283 – OF10299
28	*Origanum majorana*	Flowering tops	OF10217 – OF9776
29	*Pinus sylvestris*	Needles	OF11339 – OF2115
30	*Pogostemon cablin*	Flowering tops	OF10211 – OF9954
31	*Rosmarinus officinalis* chemotype cineole	Flowering tops	OF10655 – OF10408
32	*Salvia officinalis*	Flowering tops	OF10880 – OF9241
33	*Thymus satureioides*	Flowering tops	OF10106 – OF10589

oEO = organic EO; cEO = conventional EO; ssp = subspecies

Microorganisms

C. albicans SC5314 (Gillum, Tsay, & Kirsch, 1984) and *C. glabrata* ATCC 2001 were maintained on YPD agar plates composed of 10 g/L yeast extract (Merck), 15 g/L Difco™ agar (Becton, Dickinson & Co.) and 20 g/L bactopeptone (Oxoid). Prior to experiments, the strains were grown overnight at 35°C on plates containing 47 g/L Sabouraud agar (Sigma-Aldrich).

Preparation of the cell inocula

The cell density of overnight propagated cells resuspended in 1x phosphate-buffered saline, containing 0.20 g/L potassium chloride (VWR International), 0.24 g/L potassium dihydrogen phosphate (Merck), 1.44 g/L disodium hydrogen phosphate (Merck) and 8 g/L sodium chloride (Sigma-Aldrich), was estimated by measuring the optical density at 600 nm (OD_{600}). The cell suspension was prepared in Roswell Park Memorial Institute 1640 (RPMI) medium (Sigma-Aldrich) in accordance with CLSI guidelines (CLSI, 2012). Briefly, the RPMI medium was buffered with 3-(N-morpholino) propanesulfonic acid (MOPS; Sigma-Aldrich), and filter-sterilized over a 0.20 µm non-pyrogenic Nalgene™ filter (Fisher Scientific).

Vapor-phase-mediated susceptibility (VMS) assay

The VMS was set up and performed as described before (Feyaerts et al., 2018). Briefly, a 200 µL inoculum containing 5×10^3 cells was added to each well of a polystyrene 96-well microtiter plate (Greiner Bio-One), except for wells H1 and H12 which served as blanks and contained 200 µL medium. Next, 20 µL of the EOs to be tested was added on top of the cell suspension in wells D/E3-4 and D/E9-10. The microtiter plate was covered with a lid and statically incubated for 24 hours at 35°C while limiting air draughts. After resuspending the cells, the OD_{600} was measured with a multi-well plate reader (Synergy H1, BioTek Germany). Wells in which growth was visually absent ($OD_{600} \leq 0.07$) were counted, excluding wells to which the EO was added and blanks, and categorized to determine the iVMAA, with a higher category corresponding with a stronger iVMAA. If no iVMAA was detected,

category zero was assigned. All EOs with an iVMAA larger than zero against at least one of the two *Candida* species were tested in 3 independent experiments.

Statistical analysis

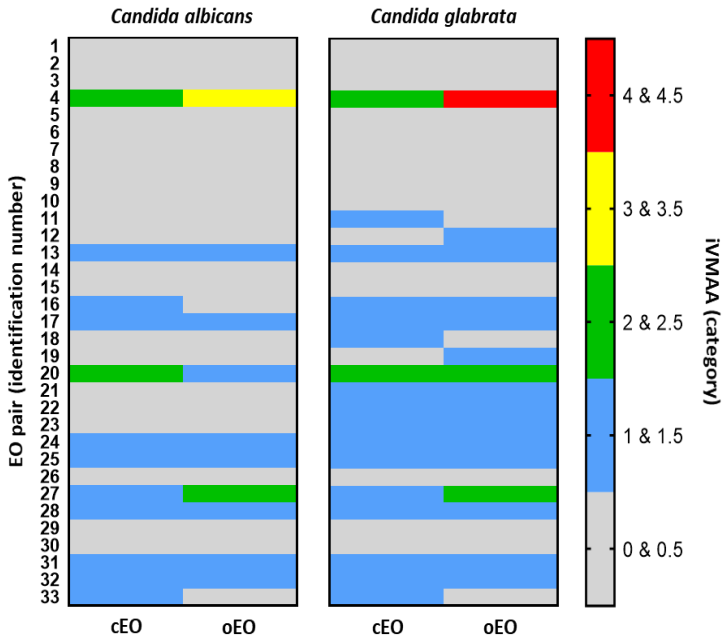
GraphPad Prism version 7.04 was used for statistical analysis. Figures show categorized averages of the biological repeats. The population-wide susceptibility of both *Candida* species to EOs was compared using the Wilcoxon matched-pairs signed rank test.

Results and Discussion

The 33 EO pairs shown in table 1 represent all possible organic-conventional EO combinations of the previously described EO collection (A. F. Feyaerts et al., 2018). The iVMAA of these EOs was determined against *C. albicans* and *C. glabrata* using the VMS assay.

Only for a few EOs we found intra-pair differences in iVMAA against one or both *Candida* species under study (**Figure 1**). For instance, *Cinnamomum cassia* oEO (**Table 1**; pair 4) has a higher iVMAA compared to *Cinnamomum cassia* cEO against both *C. albicans* ($iVMAA_{cEO}$ = 2.5; $iVMAA_{oEO}$ = 3.5) and *C. glabrata* ($iVMAA_{cEO}$ = 2.5; $iVMAA_{oEO}$ = 4.5). In contrast, *Thymus satureioides* oEO (**Table 1**; pair 33) has a lower iVMAA compared to *Thymus satureioides* cEO against both *C. albicans* ($iVMAA_{cEO}$ = 1; $iVMAA_{oEO}$ = 0.5) and *C. glabrata* ($iVMAA_{cEO}$ = 1; $iVMAA_{oEO}$ = 0.5).

Figure 1. Categorized average inhibitory vapor-phase-mediated antimicrobial activity of EO pairs against two *Candida* species



oEO = organic EO; cEO = conventional EO; iVMAA = inhibitory vapor-phase-mediated antimicrobial activity

Despite these apparent individual differences, a pairwise comparison including all EOs pairs did not show a significant difference between the iVMAA of cEO and oEO against *C. albicans* [p = 0.51; n_{pairs} = 33; sum of signed ranks (W) = 64], against *C. glabrata* (p = 0.91; n_{pairs} = 33; W = -14), or against both *Candida* species combined (p =

0.74; n_{pairs} = 66; W = 94). Hence, our results, which are based on a large set of EO pairs, show that the iVMAA of an EO likely does not depend in general on whether it is certified as "organic" or not.

Additionally, we showed that the overall iVMAA of cEOs ($\underline{p} < 0.0001$; n_{pairs} = 33; W = 371), oEOs ($p < 0.0001$; n_{pairs} = 33; W = 441), and cEOs and oEOs combined ($p < 0.0001$; n_{pairs} = 66; W = 1616) is significantly higher against *C. glabrata* than against *C. albicans*. This is in line with a previous report showing that *C. glabrata* is significantly more susceptible to the VMAA of EOs and their components (n = 212) compared to *C. albicans* (Feyaerts et al., 2018).

Together, we conclude that the iVMAA of an EO generally does not depend on whether the EO is certified organic or not. However, it is possible that our study failed to detect a difference in iVMAA between the two categories of EOs because of several reasons. (i) Some EOs lacking a certified organic label might have been derived from plants grown under organic conditions. While this seems unlikely considering that organic farming requires extra measures and costs, which producers try to recover by obtaining an organic label that assures a higher selling price, we cannot exclude it. (ii) It is also possible that our sample did not show a difference due to selection bias, despite the inclusion of all possible oEO-cEO pairs available in our large EO collection. (iii) We may have missed a difference because it was too small to detect because *e.g*. our sample lacks power. However, this raises the question of whether such a minimal difference would be relevant. (iv) Lastly, it is possible that a we did not observe a difference because we only tested two yeast species using a single antimicrobial test. Therefore, we encourage similar studies using multiple micro-organisms and different assays.

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