

# Antifungal activity of *Thymus* oils and their major compounds

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## ABSTRACT

The increasing recognition and importance of fungal infections, the difficulties encountered in their treatment and the increase in resistance to antifungals have stimulated the search for therapeutic alternatives. Essential oils have been used empirically. The essential oils of *Thymus* (*Thymus vulgaris*, *T. zygis* subspecies *zygis* and *T. mastichina* subspecies *mastichina*) have often been used in folk medicine. The aim of the present study was to evaluate objectively the antifungal activity of *Thymus* oils according to classical bacteriological methodologies – determination of the minimal inhibitory concentration (MIC) and the minimal lethal concentration (MLC) – as well as flow cytometric evaluation. The effect of essential oils upon germ tube formation, an important virulence factor, was also studied. The mechanism of action was studied by flow cytometry, after staining with propidium iodide. The chemical composition of the essential oils was investigated by gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS). The antifungal activity of the major components (carvacrol, thymol, *p*-cymene and 1,8-cineole) and also possible interactions between them were also investigated. The essential oils of *T. vulgaris* and *T. zygis* showed similar antifungal activity, which was greater than *T. mastichina*. MIC and MLC values were similar for all the compounds tested. At MIC values of the essential oils, propidium iodide rapidly penetrated the majority of the yeast cells, indicating that the fungicidal effect resulted primarily from an extensive lesion of the cell membrane. Concentrations below the MIC values significantly inhibited germ tube formation. This study describes the potent antifungal activity of the essential oils of *Thymus* on *Candida* spp., warranting future therapeutical trials on mucocutaneous candidosis.

**Key words:** *Candida* spp., *Thymus* spp., essential oil, antifungal activity, flow cytometry

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## Introduction

Superficial mycoses, often recurrent and recalcitrant, are an important cause of morbidity. Their incidence and severity has increased recently, particularly in patients with impaired immunity from the use of cytotoxic drugs, immunosuppressive therapy, in intensive care units and following treatment with broad-spectrum antimicrobials, as well as with immunosuppressive infections, such as human immunodeficiency virus (HIV). The small number of available antifungals and the increase in antifungal resistance have stimulated the search for alternatives. We previously reported the antifungal activity of

several compounds not classically considered as antifungals, such as ibuprofen,<sup>1</sup> benzydamine and local anaesthetics.<sup>2</sup> Our research currently involves the testing of essential oils as potential antimicrobial agents. Finding healing power in plants is a traditional and ancient concept.<sup>3</sup> However, since the advent of potent synthetic antibiotics in the 1950s, the use of plant derivatives as antimicrobials has become almost nonexistent.<sup>3</sup> In the past decade the use of plant derivatives, as well as other alternative medical treatments, has enjoyed great popularity. The public is becoming increasingly aware of problems inherent in overprescription and overuse of conventional antibiotics, while scientists realized that the effective lifespan of any

antibiotic is being increasingly shortened. The limited knowledge concerning antimicrobial activity and the mechanism of action of plant extracts has led us to address such issues. Standardization of both the methods of extraction and the assays for *in vitro* testing is required so that research in this area can be systematic and objective, and the interpretation of results validated. Previous studies on the antimicrobial activity of the essential oils of some *Thymus* spp., most of them possessing a large amount of phenolic monoterpenes, showed activity against viruses,<sup>4</sup> bacteria<sup>5,6</sup> and fungi.<sup>7-9</sup> Although still far from being elucidated from a scientific aspect, the mechanisms suggested for the toxicity to microorganisms have included enzymatic inhibition by the oxidized compounds.<sup>7</sup>

The genus *Thymus* (Lamiaceae), widely distributed on the Iberian Peninsula, is a taxonomically complex group of aromatic plants traditionally used for medicinal purposes because of their antiseptic, antispasmodic and antitussive properties. *Thymus* essential oils show a widespread chemical polymorphism. Several chemotypes of various Portuguese *Thymus* spp. have been identified by our team.<sup>10-16</sup> The objective of our present research is to determine the antifungal activity of chemically well-defined specific chemotypes of *T. vulgaris* (carvacrol type), *T. zygis* subsp. *zygis* (thymol type) and *T. mastichina* subsp. *mastichina* (1,8-cineole type) on *Candida* species. These are the most commonly used commercially available oil types. The antifungal activities of the major components of these oils were determined. The corresponding mechanisms of activity were evaluated according to the classical method and also by flow cytometry. Our study showed a marked anti-*Candida* activity of these compounds, supporting further therapeutical trials on mucocutaneous candidosis.

## Materials and methods

### Strains

Seven clinical strains isolated from recurrent cases of vulvo-vaginal candidosis [two of *C. albicans* (M1, H37), one of *C. krusei* (H9), one of *C. tropicalis* (H18), one of *C. guillermoidii* (Mat23) and two of *C. glabrata* (H16, H30)] as well as three type strains from the American Type Culture Collection (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13803 and *C. parapsilosis* ATCC 90018), showing different resistance patterns to classic antifungals (fluconazole and amphotericin B), were used.

### Essential oils and reference products

The essential oil of *T. vulgaris* was acquired from Segredo da Planta (Produtos Naturais e Biológicas, Lda, Lisbon, Portugal). Essential oils of *T. Zygis* subsp. *zygis* and *T. mastichina* subsp. *mastichina* were obtained from aerial parts of plants grown at Direcção Regional de Agricultura de Trás-os-Montes, Mirandela, Portugal.

Authentic samples of carvacrol (Fluka, Sintra, Portugal, 99% purity), thymol (BDH, Portugal, 99.5% purity), *p*-cymene (Fluka-Portugal, 99.5% purity) and 1,8-cineole (Merck, Paço de Arcos, Portugal, 99.5% purity) were also used.

### Essential oil isolation

For isolation of the essential oil, air-dried plant material was submitted to water distillation for 3 h using a Clevenger-type apparatus according to the European Pharmacopoeia method.<sup>17</sup>

### Essential oils analysis

The oils were analysed by gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS) using fused silica capillary columns with two different stationary phases (SPB-1 and SupelcoWax 10, 30 m × 0.20 mm i.d., film thickness 0.2 µm), as reported previously.<sup>18</sup> The constituents of the essential oils were identified on the basis of their gas chromatography retention index (RI), determined by linear interpolation relative to retention times of a series of *n*-alkanes, and by matching their 70-eV mass spectra with our own data and reference libraries. Relative amounts of individual components were calculated based on peak areas without using correction factors.

### Antifungal activity

Minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC) were determined according to the NCCLS protocol M27-A macrodilution method.<sup>19</sup> FUN-1 staining, followed by cytometric analysis, was used as an alternative method to evaluate the antifungal activity.<sup>20</sup> In metabolically active cells, FUN-1 (a membrane-permeant fluorescent probe) is converted into orange/red cylindrical intravacuolar structures that are best detected by epifluorescence microscopy. By contrast, yeast cells with impaired metabolism do not form cylindrical intravacuolar structures, the dye remaining in the cytoplasm in a diffuse pattern, which can be detected by an increase in intracellular green-yellow fluorescence by flow cytometry.<sup>19</sup> In brief, blastoconidia (10<sup>6</sup>/mL of *C. albicans* M1 and of *C. krusei* H9) were incubated with 0.5 µM of FUN-1 for 30 min, after treatment with the essential oils or its main components for 1 h. The intensity of fluorescence of treated and non-treated cells (control) was determined at FL2 (575 nm), using a Coulter XL MCL flow cytometer (Beckman-Coulter Corp., Hialeah, FL, USA). A staining index was calculated as the ratio between the intensity of fluorescence of treated and non-treated cells. The increase in the intensity of fluorescence of treated cells compared to non-treated cells indicated a metabolic disturbance and corresponds to a staining index > 1.<sup>20</sup>

The ability to form germ tubes was assessed in a germ tube formation assay. In brief, isolates of two strains of *C. albicans* (M1 and ATCC 10231) were inoculated in Sabouraud glucose

broth (SGB) and incubated for 16–24 h at 35 °C, with shaking. Cells were collected by centrifugation and washed twice with phosphate-buffered saline (PBS) pH 7.4 and then resuspended in the same buffer. Cell suspensions were then adjusted to match the turbidity of 1.0 McFarland standard and germ tube formation was induced by incubation in RPMI 1640 culture medium (Sigma, St Louis, MO, USA) for 3 h at 37 °C in the presence of MIC and subinhibitory concentrations of essential oils or their components. Cells were examined using phase contrast microscopy ( $\times 400$ ) and the percentage of cells presenting germ tubes determined. These experiments were repeated twice per isolate.

### Mechanism of action

The mechanisms of action were investigated using flow cytometry analysis following serial incubation times (5, 10, 15 and 30 min) with increasing concentrations of the oils or their respective components and staining the yeast cells (M1 and H9) with propidium iodide, to evaluate the lesion of the membrane.<sup>1,2</sup> *Blastoconidia* ( $10^6/\text{mL}$ ) were incubated with 1  $\mu\text{g}$  of propidium iodide for 30 min and the intensity of fluorescence determined by flow cytometry at FL3 (625 nm).

### Synergistic effect

The effect of different combinations of the major components of *Thymus* oils (thymol/carvacrol, thymol/*p*-cymene, thymol/1,8-cineole, carvacrol/*p*-cymene, carvacrol/1,8-cineole, *p*-cymene/1,8-cineole) was assayed with *C. albicans* M1 and *C. krusei* H9, according to the checkerboard method.<sup>21</sup> Hypothetical interactions occurring between the different components were determined following the calculation of the fractional inhibitory concentration (FIC) and the fractional inhibitory index (FIX). Synergistic, indifferent and antagonistic interactions were defined, respectively, by an FIX of  $< 0.5$ ,  $0.5-4.0$  or  $> 4.0$ .<sup>21</sup>

## Results

The qualitative and quantitative analytical compositions of the three *Thymus* oils are detailed in Table 1. Over 95% of the essential oils was identified in each sample. The main components of *T. vulgaris* oil were carvacrol (70.3%) and *p*-cymene (11.7%). In the case of *T. zygis* the major components were thymol (39.6%) and *p*-cymene (21.2%). Thus, the phenolic monoterpenes (carvacrol or thymol) and *p*-cymene are the most abundant constituents of the essential oils of these two *Thymus*, in contrast to the oil of *T. mastichina*, in which the phenolic compounds were not detected and *p*-cymene only represents 0.7%. Conversely, the oil of *T. mastichina* is characterized by a large amount of 1,8-cineole (67.4%).

*T. zygis* and *T. vulgaris* exhibited a more potent antifungal activity than *T. mastichina*. These findings are probably due to the reduced antifungal activity of 1,8-cineole (the main component

**Table 1** Composition of the essential oils of *Thymus vulgaris*, *T. zygis* and *T. mastichina* determined by gas chromatography

Compound	Percentage in samples		
	<i>T. vulgaris</i>	<i>T. zygis</i>	<i>T. mastichina</i>
$\alpha$ -Thujene	0.3	1.0	0.2
$\alpha$ -Pinene	0.9	0.9	3.0
Camphene	0.4	0.5	0.7
Oct-1-en-3-ol	0.1	0.6	–
3-Octanone	–	0.5	–
Sabinene	–	–	2.4
$\beta$ -Pinene	0.3	0.4	4.0
Myrcene	1.7	3.0	1.2
$\alpha$ -Phellandrene	0.1	0.2	–
$\Delta$ -2-Carene	–	–	0.1
$\alpha$ -Terpinene	0.6	1.2	0.2
<i>p</i> -Cymene	11.7	21.2	0.7
Limonene	0.5	1.7	1.0
$\beta$ -Phellandrene	0.3	–	–
1,8-Cineole	–	t	67.4
<i>E</i> - $\beta$ -Ocimene	t	0.2	0.4
$\gamma$ -Terpinene	3.2	7.9	0.8
<i>cis</i> -Sabinene hydrate	0.2	–	0.8
<i>cis</i> -Linalool oxide	–	–	0.1
<i>trans</i> -Linalool oxide	–	0.1	0.1
2,5-Dimethylstyrene	t	0.2	–
Terpinolene	0.1	0.2	0.1
<i>trans</i> -Sabinene hydrate	0.2	–	–
Linalool	2.2	5.5	4.3
$\alpha$ -Campholenal	–	–	0.2
<i>cis</i> - <i>p</i> -Menth-2-en-1-ol	–	0.2	0.2
Camphor	–	0.3	0.2
<i>trans</i> - <i>p</i> -Menth-2-en-1-ol	–	0.1	0.1
Isopulegol	–	–	0.1
Pinocarvone	–	–	0.2
Borneol	–	1.0	1.3
<i>p</i> -Cymene-8-ol	t	0.2	–
Terpinene-4-ol	0.4	1.0	0.8
$\alpha$ -Terpineol	0.2	0.4	3.5
Myrtenol	–	–	0.2
Fenchyl acetate	–	–	0.2
Linalyl acetate	–	t	–
Bornyl acetate	–	–	0.2
Thymol	0.6	39.6	–
Carvacrol	70.3	2.4	–
$\alpha$ -Terpenyl acetate	–	–	0.9
$\alpha$ -Copaene	–	0.1	–
$\beta$ -Bourbunene	–	0.1	0.1
<i>E</i> -Caryophyllene	2.9	3.6	0.7
$\alpha$ -Humulene	0.1	0.1	–
Germacrene D	–	–	0.2
$\alpha$ -Muurolole	–	0.1	–
$\beta$ -Bisabolene	0.1	–	–
$\gamma$ -Cadinene	–	0.1	–
<i>cis</i> -Calamelene	–	t	–
$\delta$ -Cadinene	–	0.2	–
Elemol	–	–	0.2
Caryophyllene oxide	0.6	1.0	0.3
Abietatriene	t	–	–
Total identified	98.1	95.9	97.0

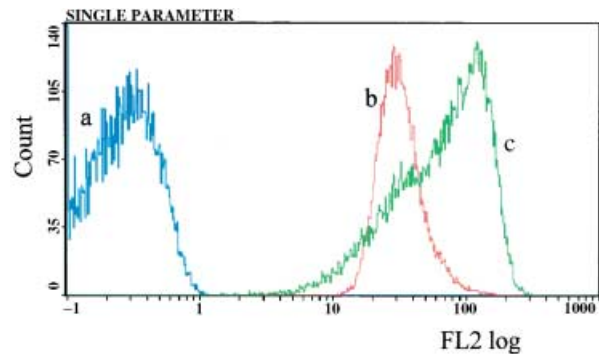
t, trace ( $< 0.05\%$ ).

Compounds listed in order of their elution from the SPB-1 column.

**Table 2** MICs and MLCs of different species of *Thymus*, their major components, Fluconazole (FLU) and Amphotericin B (Anf B) for 10 *Candida* strains

Strains	<i>Thymus vulgaris</i>		<i>Thymus zygis</i>		<i>Thymus mastichina</i>		Thymol		Carvacrol		<i>p</i> -Cymene		1,8-Cineole		FLU		Anf B	
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
<i>C. albicans</i> ATCC 10231	0.16–0.32	0.32	0.16–0.32	0.32	2.5	2.5	0.16	0.32	0.16	0.32	0.16–0.32	0.32	5.0–10.0	10.0	10.0	10.0	10.0	S
<i>C. albicans</i> H37	0.16	0.32	0.16–0.32	0.32	1.25–2.5	2.5	0.16	0.32	0.16	0.32	0.08–0.16	0.32	5.0–10.0	10.0	10.0	10.0	10.0	R
<i>C. albicans</i> M1	0.16–0.32	0.32	0.32	0.32	2.5	5.0	0.16	0.32	0.16	0.32	0.16–0.32	0.32	5.0–10.0	10.0	10.0	10.0	10.0	S
<i>C. tropicalis</i> ATCC 13803	0.16–0.32	0.32	0.16–0.32	0.32	2.5–5.0	5.0	0.16–0.32	0.32	0.16	0.32	0.63	0.63	5.0–10.0	10.0	10.0	10.0	10.0	S
<i>C. tropicalis</i> H18	0.32	0.32	0.32	0.32	5.0–10.0	5.0	0.16–0.32	0.32	0.16	0.32	0.16–0.32	0.32	5.0–10.0	10.0	10.0	10.0	10.0	R
<i>C. glabrata</i> H16	0.16	0.32	0.32	0.32	1.25–2.5	5.0	0.16	0.32	0.16	0.32	0.08–0.16	0.16	5.0–10.0	10.0	10.0	10.0	10.0	R
<i>C. glabrata</i> H30	0.32	0.32	0.32	0.32	2.5	5.0	0.32	0.32	0.16	0.32	0.32	0.32	10	20.0	10.0	10.0	10.0	R
<i>C. krusei</i> H9	0.08–0.16	0.32	0.16–0.32	0.32	1.25–2.5	2.5	0.16–0.32	0.32	0.16	0.32	0.08–0.16	0.16	5.0–10.0	10.0	10.0	10.0	10.0	R
<i>C. guilliermondii</i> MAT 23	0.16	0.16	0.16	0.16	1.25	1.25	0.16	0.16	0.08–0.16	0.16	0.08–0.16	0.16	5.0–10.0	10.0	10.0	10.0	10.0	S
<i>C. parapsilosis</i> ATCC 90018	0.16–0.32	0.32	0.32	0.32	2.5–5.0	5.0	0.32	0.32	0.16	0.32	0.16	0.32	10.0–20.0	20.0	20.0	20.0	20.0	S

MICs were determined by a macrodilution method and expressed in  $\mu\text{L/mL}$  (v/v). Phenotype to Flu and Anf B determined following the rules of the NCCLS M27-A protocol.



**fig. 1** Flow cytometry of *Candida albicans* M1 (shown as a representative strain) cells stained with FUN-1 and analysed at FL2 (575 nm) showing: (a) autofluorescence of non-treated (viable) cells; (b) fluorescence of non-treated (viable) cells stained with  $0.5 \mu\text{M}$  of FUN-1 for 30 min; and (c) fluorescence of yeast cells treated with MIC level of *Thymus vulgaris* ssp. *vulgaris* (shown as an representative example) during 1 h and stained with  $0.5 \mu\text{M}$  of FUN-1 for 30 min.

of *T. mastichina*) in comparison to the corresponding activity of carvacrol, thymol and *p*-cymene, as shown in Table 2. For these three compounds the MLC within one dilution corresponds, in most cases, to MIC values (Table 2). Flow cytometry proved to be an excellent alternative procedure to study the antifungal activity of these compounds, with an excellent correlation with the classic method, and allowing a considerably shorter incubation time (1 h vs. 48 h). Fungal cells treated with the MIC values of the essential oils or their respective components and stained by FUN-1 showed a marked increase in green–yellow fluorescence, indicating a metabolic disturbance (fig. 1).

Germ tube formation was significantly inhibited, an effect that was already evident at concentrations lower than the MIC values of the essential oils or their components, particularly in the case of *T. mastichina* and cineole (Table 3).

Increasing concentrations of the different assayed compounds induced the progressive staining of yeast cells by propidium iodide. After incubation for 15 min at the MIC, over 90% of the cells are stained with propidium iodide, corresponding to cell death resulting from a primary lesion of the cell membrane.

No antagonistic effect was detected when assaying the different combinations of the compounds tested. In terms of synergistic activity the most synergetic combinations were thymol/1,8-cineole and thymol/*p*-cymene, which resulted in values of FIX of 0.125, corresponding to a decrease in the MIC values of around three dilutions (Table 4).

## Discussion

The results underline the importance and the need for research into the potential use of essential oils in the management of fungal infections. The relatively small number of antifungals available for the treatment of mycoses, most of them fungistatic, together with emerging resistance, led us to search for

**Table 3** Percentage of germ tube formation by two strains of *Candida albicans* incubated with MIC and subinhibitory concentrations of *Thymus* (*vulgaris*, *zygis* and *mastichina*) and their major components (carvacrol, 1,8-cineole, thymol and *p*-cymene). Mean results of two consecutive experiments

	<i>C. albicans</i> M1	<i>C. albicans</i> ATCC10231
Control	88	76
<i>Thymus vulgaris</i> (µL/mL)		
0.32	25	26
0.16	48	49
0.08	63.5	55.5
<i>Thymus zygis</i> (µL/mL)		
0.32	4.5	4.5
0.16	39.5	15.5
0.08	64	37
<i>Thymus mastichina</i> (µL/mL)		
2.5	0	0
1.25	0	0
0.63	0.5	1
Thymol (µL/mL)		
0.16	59.5	40
0.08	78.5	49
0.04	85	42
Carvacrol (µL/mL)		
0.16	45	32
0.08	63	41.5
0.04	70	54
1,8-Cineole (µL/mL)		
10.0	0	0
5.0	0	2
3.3	1	4
<i>p</i> -Cymene (µL/mL)		
0.32	0.5	–
0.16	14	1
0.08	47	13
0.04	–	43

**Table 4** Checkerboard assay of combinations of the four major components of *Thymus* (thymol, 1,8-cineole, *p*-cymene and carvacrol)

	Fractional inhibitory index (FIX)		Outcome
	<i>C. albicans</i> M1	<i>C. krusei</i> H9	
Thymol/Cineole	0.125	0.125	Synergy
Thymol/ <i>p</i> -Cymene	0.125	0.125	Synergy
Thymol/Carvacrol	0.500	0.500	Indifferent
<i>p</i> -Cymene/Carvacrol	0.500	0.500	Indifferent
Carvacrol/Cineole	0.250	0.250	Synergy
<i>p</i> -Cymene/Cineole	0.250	0.250	Synergy

MIC was determined by the macrodilution technique.

therapeutic alternatives combining low cost and low toxicity, such as essential oils. Essential oils are a mixture of several volatile compounds. It was decided to avoid the use of microtitre plastic plates because of the possibility of interaction

between the essential oils and the plastic. In addition, to prevent evaporation of the essential oils, the glass tubes used in the macrodilution assay were closed with parafilm.

The essential oils of *Thymus* spp., particularly *T. vulgaris* and *T. zygis*, as well as their main components, carvacrol, thymol and *p*-cymene, showed a marked fungicidal activity. We determined the activity of the different components of the essential oils to find out the responsibility of each in the antifungal activity. Carvacrol and *p*-cymene were found to be the most active, which helps to explain the stronger activity of *T. vulgaris* and *T. zygis* in comparison to *T. mastichina*. These are important findings as they facilitate the use of single components rather than a mixture, giving more predictability and probably fewer side-effects. Flow cytometry analysis has the advantage of assessment of antimicrobial activity with a short incubation time.

FUN-1 proved to be a useful probe to evaluate yeast metabolism;<sup>25,26</sup> metabolically active yeast cells convert FUN-1 into red cylindrical intravacuolar structures, while metabolically disturbed cells show an increase in yellow fluorescence, without the formation of cylindrical intravacuolar structures. We have previously described an excellent correlation between the susceptibility testing of fluconazole using NCCLS protocol and flow cytometry with FUN-1.<sup>1,2</sup> The shorter incubation time is a considerable advantage, particularly in the case of essential oils to prevent evaporation of the volatile compounds.

Germ tube formation, a change in morphogenesis that plays a crucial role in terms of pathogenicity,<sup>22–24</sup> was also significantly inhibited by the *Thymus* oils. Curiously, a greater inhibition was found with *T. mastichina* and cineole (its respective main component) compared to the other tested species, at concentrations well below the MIC.

The cytometric approach, as well as being extremely useful for antimicrobial susceptibility testing, provides important keys for the understanding of the corresponding mechanisms of activity.<sup>27</sup> Propidium iodide only penetrates cells with severe damage of the membrane, i.e. cells that are already dead. Permeation to propidium iodide, particularly following a short incubation period as was the case in this study, indicates that the mechanism of action of the drug involves a primary lesion of the cell membrane, with inherent killing of the cell.<sup>1,2</sup> In our previous studies, a similar rapid permeation of propidium iodide by yeast cells exposed to fungicidal concentrations of ibuprofen or local anaesthetics was validated with complementary methodologies. Thus, such cells showed: (a) rapid leakage of potassium, (b) rapid lysis of yeast spheroplast when exposed in an osmotically protective medium, as well as severe cell membrane alterations with fracturing, and (c) solubilization of the membranes observed by electron microscopy.<sup>1,2</sup> This indicates that the fungicidal effect results from direct damage to the cell membrane rather than from metabolic impairment leading to secondary membrane damage. Such an effect is in accordance with the biochemical nature of the compounds assayed – monoterpenes – which most probably act as a solvent of the

cell membrane. Flow cytometry provides the possibility to evaluate the occurrence of this antifungal effect, following short incubation times. Thus the essential oils of *Thymus* spp., especially *T. zygis* and *T. vulgaris*, showed a potent fungicidal activity against *Candida* spp., resulting from direct damaging of the cytoplasmic membrane. Components such as carvacrol, thymol and *p*-cymene showed similar fungicidal activity against *Candida* species. In addition, the combination of thymol/*p*-cymene and thymol/1,8-cineole also resulted in a significant synergistic antifungal effect. Such findings might also have a considerable clinical interest for the treatment of superficial candidosis, with reduction in potential side-effects. Our results support the concept that essential oils might be useful in the clinical management of candidosis, particularly mucocutaneous presentations such as vulvovaginal candidosis, without significant side-effects. They appeared to possess a fast and potent fungicidal ability against described isolates of *Candida*.

Clinical trials are under way to evaluate the practical relevance of our *in vitro* results.

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