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Antifungal activity of essential oil from *Mentha spicata* L. and *Mentha pulegium* L. growing wild in Sardinia island (Italy)

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Abstract

This study aims to evaluate the antifungal activity of *Mentha. spicata* L. and *Mentha pulegium* L. from Sardinia and to assess their efficacy on virulence factors for *Candida albicans*, particularly on the inhibition of the germ tube formation. The major compounds of the essential oils were carvone (62.9 %) for *M. spicata* and pulegone (86.2 %) for *M. pulegium*. The essential oil from *M. spicata* showed a more preeminent effect against *Cryptococcus neoformans* and the dermatophytes *Trichophyton rubrum* and *T. verrucosum* (0.32 μL/mL). Both oils were very effective in inhibiting *C. albicans* germ tube formation, at doses well below their MIC (0.16μL/mL).

Keywords: *Mentha spicata* L., *Mentha pulegium* L., essential oil, antifungal activity, *Candida albicans*.

1. Introduction

Fluconazole and other conventional antifungals agents are usually associated with resistance for *Candida* spp., *Cryptococcus* spp., *Aspergillus* spp. and dermatophytes in addition to the issues associated with undesirable side effects, narrow activity spectrum and a small number of targets (Pfaller MA 2012; Fuentefria et al. 2018). Therefore, the development of new treatments for fungal infections is urgent. Many aromatic plants and their essential oils have been proved to possess antimycotic properties and may be potentially used as antifungal agents. The genus *Mentha* (Lamiaceae family) comprises about 25 species and hybrids and has a great importance, both medicinal and commercial. In Sardinian folk medicine *Mentha spicata* L. and *Mentha pulegium* L. are used as anti-inflammatory, digestive skin lenitive and against stomach and intestinal pain and helminthiasis (Maxia et al. 2008). The objective of our present research was to evaluate the antifungal activity of *M. spicata* and *M. pulegium* essential oil against widely spread pathogenic fungal strains that cause superficial skin infections in humans. In the present study, besides the antifungal effect of the oil against several yeasts (several *Candida* strains and *Cryptococcus neoformans*), dermatophytes (*Trichophyton* spp., *Epidermophyton floccosum*, and *Microsporum* spp.), and *Aspergillus* strains, we also aimed to elucidate a possible mode of action particularly on *Candida albicans*. For that, the effect of the oil on the inhibition of the germ tube formation, an important virulence factor, was evaluated. In fact, the hyphal form is important in the infection process as it promotes tissue penetration and immune system avoidance (Su et al. 2018).

2. Results and discussions

The results concerning the qualitative and quantitative analysis of the essential oils are presented in Table S1, where the components are listed in order of elution from a HP-5 column. The major compounds were carvone (62.9 %), limonene (8.5 %), 1,8-cineole (7.1 %), cis-carveol (4.5 %) and *neo*-dihydro carveol (3.0 %), for *M. spicata* and pulegone (86.2 %), piperitenone (3.3 %), iso-menthone (2.8 %) and cis-isopulegone (2.0 %) for *M. pulegium*. Commercially exploited *M. spicata* plants are always rich in carvone and related compounds; on the other hand, wild populations are very variable (Şarer et al. 2011). For example, some oils had carvone or related compounds as major constituents, while others were rich in menthone, isomenthone, piperitone, piperitone oxide, piperitenone oxide, linalool, *trans*-sabinene

hydrate and 1,8-cineole (Sivropoulou et al. 1995; Sokovic et al. 2009; Şarer et al. 2011; Arantes et al. 2017). Several studies have investigated the chemical composition of the *M. pulegium* essential oil grown in different geographical regions which reveal a great variability in their chemical profile: oils with high content of pulegone and oils with low levels of pulegone but rich in menthone/isomenthone, or in piperitonone/piperitone, or in piperitone (Sivropoulou et al. 1995; Lorenzo et al. 2002; El-Ghorab AH 2006; Ait-Ouazzou et al. 2012; Abdelli et al. 2016; Brahmi et al. 2016). The variation in the essential oil content and composition of *M. pulegium* plants were related to a variety of factors, such as genotypes of *Mentha* species, environmental conditions, season, plant age and different plant parts.

Although both oils are rich in monoterpene ketones *M. spicata* has high levels of carvone and *M. pulegium* of pulegone which can influence their biological properties.

Some reports have already stated that *M. spicata* and *M. pulegium* have an antimicrobial potential, being *M. spicata* oil the most active (Adam et al. 1998; Hajlaoui et al. 2009; Soković et al. 2009; Morteza-Semnani et al. 2011; Stringaro et al. 2018). The activity of this essential oil can be explained by its composition. Indeed, some reports have demonstrated the antimicrobial effect of carvone against several fungi strains (Adam et al. 1998; Kostik et al. 2015). The lack of methods standardization for the evaluation of antifungal activity of essential oils makes the comparison of results difficult. The principal variables are: the strains, the inoculum size, the growth phase of the microorganism chosen to be tested, the culture medium and its pH value, the incubation time and temperature (Palmeira-de-Oliveira et al. 2009). Our results (MICs) were obtained according to the Clinical and Laboratory Standards Institute (CLSI) reference protocols.

The antifungal activity of both essential oil against human and animal pathogens is presented in Table S2. In general, the oil of *M. spicata* is more active than the oil of *M. pulegium*, particularly against *Cryptococcus neoformans* (MIC=0.32 µL/mL) and the dermatophytes *Trichophyton rubrum* and *T. verrucosum* (MIC=0.32 µL/mL).

Some essential oils showed anti-dermatophytic activity, particularly against several *Trichophyton* strains (*T. rubrum; T. mentagrophytes; T. tonsurans; T. erinacei; T. schoenleinii; T. soudanense*) and some efforts are being made to establish the chemical composition/antifungal activity relationships. For example, oils containing high amounts of terpenic phenols, namely thymol and carvacrol and phenylpropanoids (such as eugenol, cinnamaldehyde and benzyl benzoate), act as better inhibitors of fungal growth, showing that the presence of a phenol function in the molecules framework increases their antifungal properties (Lopes et al. 2016). However, it is not always easy to correlate these parameters. For example the sesquiterpene α-bisabolol was demonstrated to inhibit the growth of several dermatophyte at very low concentrations: *T. tonsurans* (MIC = 2–8 µg/mL), *T. mentagrophytes* (MIC = 2–4 µg /mL), *T. rubrum* (MIC ≤1 µg/mL) and *M. canis* (MIC = 0.5–2.0 µg g/mL) (De Lucca et al. 2011). Other representative terpenic compounds of essential oils with important anti-dermatophytic activity are: citral, geraniol, geranial, neral, linalool, limonene, *cis*-ocimene, α-pinene, 1,8-cineole, myrcene, γ-terpinene, *p*-cymene and α-terpinene (Miron et al. 2014; Lopes et al. 2016)

Another species of *Mentha*, *M. piperita*, was moderately active against *T. rubrum* (MIC 576 µg/mL) (Khan and Ahmad 2011).

There are few studies on the effect of essential oils on *T. verrucosum*. Our results show that *M. spicata* has better activity than the oils of *Angelica major*, *Myrtus communis*, *Thapsia villosa*, *Foeniculum vulgare* and *Ocimum basilicum* (Bouzabata et al. 2015; Cavaleiro et al. 2015; Cabral et al. 2017; Pinto et al. 2017; Piras et al. 2018) and similar activity than the oils of of *Ocimum sanctum* and *Santolina semidentata* (Gomes et al. 2015; Piras et al. 2018). Both oils are less effective against *Candida* spp. (*C. albicans*, *C. tropicalis, C. krusei, C. guillermondii, C. parapsilosis*) and *Aspergillus* spp. (A. *niger, A. fumigates, A. flavus)*. Overall, the oils showed both fungistatic and fungicidal effects against most of the strains tested since the MIC values were similar to MLC ones.

Essential oils considered to be potent antifungals against *Candida* strains have usually in common high contents of phenols (Palmeira-de-Oliveira et al. 2009). *Origanum* spp and *Thymus* spp oils are the most cited as being rich in those compounds. Their activity has been largely evaluated (Manohar et al. 2001; Cetin et al. 2011; Delic et al. 2013; Ksouri et al. 2017). However, some non-phenolic essential oils, such as, *Cymbopogum citratus, Sygygium aromaticum* and *Rosmarinus officinalis,* demonstrated to inhibit the growth of several *Candida* strains at low concentrations (Khan and Ahmad 2012; Sivamani et al. 2014)

Studies on the effect of *Mentha* spp essential oils on *C. albicans* germ tube formation are scarce. To the best of our knowledge only the oil of *Mentha* x *piperita* and its major compounds (menthol, carvone, menthone) were evaluated.

The authors concluded that *M. piperita* oil and its main compounds reduced the transition of *C. albicans* from yeast to the invasive hyphal form at sub-inhibitory concentrations (Samber et al. 2014).

The effects of sub-inhibitory concentrations of *M. spicata* and *M. pulegium* oils on the inhibition of germ tube formation are presented in Table S3. Both oils inhibited the germ tube formation at concentrations well below their respective MIC, with *M. spicata* being more effective and attaining over 80 % inhibition at 0.16 µL/mL (MIC/8). The activity of the oils may be explained by their chemical composition. Indeed, carvone and pulegone have been described as effective natural compound for inhibiting germ tube formation in *C. albicans* (Samber et al. 2014; Boni et al. 2016). Interestingly, although a phenolic essential oil, like *Thymus vulgaris*, show potent antifungal activity it is less effective in inhibiting *C. albicans* germ tube formation than *M. spicata* and *M. pulegium* (Pina-Vaz et al. 2004).

Fluconazole (MIC=1µg/mL), the antifungal drug of choice in the clinic for the management of candidiasis failed to inhibit this feature even at concentrations much higher than its MIC. Indeed, even at 0.2 mg/mL, that corresponds to fluconazole's MICx200 (Table S4), the antifungal drug was completely ineffective.

These results are quite interesting, since filamentation (dimorphic transition from yeast to filamentous form) in *C. albicans* is a very important virulence factor due to its association with resistance and disseminative candidiasis and it seems that filamentation inhibition *per se* is sufficient to treat this disease (Saville et al. 2006; Dadar et al. 2018).

3. Conclusions

Our results demonstrated that the essential oil from *M. spicata* showed a more preeminent effect against *Cryptococcus neoformans* and the dermatophytes *Trichophyton rubrum* and *T. verrucosum* (0.32 μL/mL).

This study is the first report that shows the essential oil effect on the germ tube formation of *Candida albicans*: *M. spicata* decreases germ tube formation up to 80 % at concentrations eight times lower than MIC while *M. pulegium* is able to decrease tube formation about 40 % at concentrations eight times lower than MIC. In conclusion, this study highlights the antifungal activity of two widely used spices and complies with the antifungal uses described in folk medicine.

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SUPPLEMENTARY MATERIAL

Antifungal activity of essential oil from *Mentha spicata* L. and *Mentha pulegium* L. growing wild in Sardinia island (Italy)

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Abstract

This study aims to evaluate the antifungal activity of *Mentha. spicata* L. and *Mentha pulegium* L. from Sardinia and to assess their efficacy on virulence factors for *Candida albicans*, particularly on the inhibition of the germ tube formation. The major compounds of the essential oils were carvone (62.9 %) for *M. spicata* and pulegone (86.2 %) for *M. pulegium*. The essential oil from *M. spicata* showed a more preeminent effect against *Cryptococcus neoformans* and the dermatophytes *Trichophyton rubrum* and *T. verrucosum* (0.32 µL/mL). Both oils were very effective in inhibiting *C. albicans* germ tube formation, at doses well below their MIC (0.16µL/mL).

Keywords: *Mentha spicata* L., *Mentha pulegium* L., essential oil, antifungal activity, *Candida albicans*.

Experimental

Plant materials

Aerial parts of *M. spicata* and *M. pulegium* were collected in July 2016 in Sarrabus-Gerrei subregion (Sardinia, Italy), in a small valley, in a riverbed (geographical coordinates 39°12'56.91"N, 9°23'40.38"E; altitude: 238 m above sea level). Andrea Maxia, Pharmaceutical Botanist of University of Cagliari, performed the botanical identification by Pignatti (1982). The updated binomial nomenclature was confirmed by Conti et al. (2005).

M. spicata and *M. pulegium* voucher specimens were deposited at the Herbarium of the Botanical Garden of Cagliari, University of Cagliari (CAG 1070b and CAG 1072b respectively). The plants were air dried at 40°C with forced ventilation for two days and then subjected to distillation.

Essential oil isolation and analysis

Isolation of essential oils by hydrodistillation were performed in a Clevenger-type apparatus for 3 h (European Pharmacopoeia 1997).

Analyses of the oils were carried out by both gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). GC analyses were performed using a gas chromatograph (Agilent 7890A, Palo Alto, CA, USA), equipped with a 30 m × 0.25 mm i.d. with 0.25 µm stationary film thickness HP-5 capillary column (Agilent J&W). The following temperature program was used: from 60 ºC to 246 ºC at a rate of 3 ºC min^{-1} and then held at 246 ºC for 20 min (total analysis time 82 min). Other operating conditions were the following: carrier gas helium (purity ≥ 99.9999 % – Air Liquide Italy); flow rate, 1.0 mL min^{-1}; injector temperature, 250 ºC; detector temperature, 300 ºC. Injection of 1 µL of diluted sample (1:100 in *n*-hexane, w/w) was performed with 1:20 split ratio, using an autosampler (Agilent, Model 7683B).

GC-MS analyses were carried out using a gas chromatograph (Agilent 6890N) equipped with a 30 m × 0.25 mm i.d. with 0.25 µm stationary film thickness HP-5ms capillary column (Agilent J&W) coupled with a mass selective detector having an electron ionization device, EI, and a quadrupole analyzer (Agilent 5973). The temperature program and the chromatographic operating conditions

(except detector) were the same used for GC-FID. The MS conditions were as follows: MS transfer line temperature 240 °C; EI ion source temperature, 200 °C with ionization energy of 70 eV; quadrupole temperature 150 °C; scan rate, 3.2 scan s^{-1} at *m/z* scan range, (30 to 480). To handle and process chromatograms and mass spectra was used the software MSD ChemStation (Agilent, rev. E.01.00.237). Compounds were identified by comparison of their mass spectra with those of NIST02 library data of the GC/MS system and Adams libraries spectra (NIST/EPA/NIH Mass spectral library 2005; Adams RP 2007) or those of pure compounds whenever possible. The results were further confirmed by comparison with the compounds elution order with their retention indices on semi-polar phases reported in the literature (Adams RP 2007). Retention indices of the components were determined relative to the retention times of a series of *n*-alkanes (two standard mix C_8–C_{20} and C_{21}–C_{40}) with linear interpolation (Van Den Dool and Kratz 1963). Percentage of individual components was calculated based on GC peak areas without FID response factor correction.

Antifungal activity

Fungal strains

The antifungal activity of the essential oils of *M. spicata L.* and *M. pulegium L.* were evaluated against yeasts and filamentous fungi: two clinical *Candida* strain isolated from recurrent cases of vulvovaginal (*C. krusei* H9, *C. guilliermondii* MAT23); three *Candida* type strains from the American Type Culture Collection (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13803 and *C. parapsilopsis* ATCC 90018); one *Cryptococcus neoformans* type strain from the Colección Espanõla de Cultivos Tipo (*C. neoformans* CECT 1078); three dermatophyte clinical strains isolated from nails and skin (*Epidermophyton floccosum* FF9, *Trichophyton mentagrophytes* FF7 and *Microsporum canis* FF1), and four dermatophyte type strains from the Colección Espanõla de Cultivos Tipo (*T. mentagrophytes* var. *interdigitale* CECT 2958, *T. rubrum* CECT 2794, *T. verrucosum* CECT 2992, and *M. gypseum* CECT 2908). All strains were stored in Sabouraud

dextrose broth with 20 % glycerol at -80 ºC and subcultured in Sabouraud dextrose agar (SDA) or Potato dextrose agar (PDA) before each test, to ensure optimal growth conditions and purity.

Antifungal activity

A macrodilution broth method was used to determine the minimal inhibitory concentrations of the oil (MICs) according to the Clinical and Laboratory Standards Institute (CLSI) reference protocols M27-A3 (CLSI 2008), M27-S3 (CLSI 2008) and M38-A2 (CLSI 2008) for yeasts and filamentous fungi, respectively. Briefly, inoculum suspensions were prepared at appropriate densities in RPMI 1640 broth (with L-glutamine, without bicarbonate, and the pH indicator phenol red) from SDA or PDA cultures and distributed into 12×75 mm glass test tubes. Inoculum densities were confirmed by viability counts on SDA. The serial doubling dilution of the volatile extracts was prepared in dimethyl sulfoxide (DMSO), with concentrations ranging from (0.16 to 5.00) µL/mL. Final concentration of DMSO never exceeded 1 %. Oil-free growth controls and DMSO control tubes, were also included. The test tubes were incubated aerobically at 35 ºC for 48 h/72 h (*Candida* spp. and *Cryptococcus neoformans*) or at 30 ºC for 7 days (dermatophytes). MIC values were determined as the lowest concentration of the oil causing full growth inhibition. Quality control was performed by testing fluconazole with the reference strains *C. parapsilopsis* ATCC 22019 and *C. krusei* ATCC 6258 and the results were within the predetermined limits. To measure minimal lethal concentrations (MLCs), 20 µL samples were taken from each negative tube, plus the first tube showing growth (to serve as a growth control) after MIC reading to SDA plates and incubated at 35 ºC for 48 h/72 h (*Candida* spp. and *Cryptococcus neoformans*) or at 30 ºC for 7 days (dermatophytes). MLC values were determined as the lowest concentration of the oil causing fungal death. All experiments were performed in triplicate and repeated whenever the results of each triplicate did not agree. A range of values is presented when different results were obtained.

Germ tube inhibition assay

In order to determine the effect of the essential oils on the yeast-mycelium transition, the suspensions of *C. albicans* strains ATCC 10231, from overnight cultures in SDA (Sabouraud

dextrose agar; Becton-Dickinson) were prepared in NYP medium (N-acetylglucosamine [Sigma; 10^{-3} mol/L], Yeast Nitrogen Base [Difco; 3.35 g/L], proline [Fluka; 10^{-3} mol/L], NaCl [4.5 g/L], and pH 6.7±0.1. The suspensions were adjusted to obtain a density of yeast cell suspensions at (1.0±0.2) x 10^6 CFU/mL and distributed into glass test tubes in a volume of 990 µL. Each dilution of essential oil was added into the cell suspension tubes, in 10 µL volumes, to obtain appropriate sub-inhibitory concentration. After incubation at 37 ºC without agitation for 3 h, the treated and untreated yeast cells were counted for germ tube formation under light microscope and the percentage of germinating cells was calculated. Germ tubes were considered when the germinating protuberance was at least as long as the diameter of the blastopore. The results are presented as mean ± standard deviation (SD) of three independent experiments.

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Table S1: Composition of *M. spicata* L. and *M. pulegium* L. essential oil.

R_I	Compound	Identification	*M. spicata*	*M. pulegium*
932	α-pinene	MS, R_I, Inj	0.5	0.3
972	sabinene	MS, R_I, Inj	0.5	0.1
976	β-pinene	MS, R_I, Inj	0.8	0.2
990	myrcene	MS, R_I, Inj	0.3	0.2
995	3-octanol	MS, R_I	tr	1.2
~~1023~~	~~o-cymene~~	~~MS, R_I~~	~~0.1~~	~~tr~~
1028	limonene	MS, R_I, Inj	8.5	0.7
1030	1,8-cineole	MS, R_I, Inj	7.1	0.1
1057	γ-terpinene	MS, R_I, Inj	0.1	-
1100	linalool	MS, R_I, Inj	0.2	-
1104	*n*-nonanal	MS, R_I	0.2	-
1119	trans-p-mentha-2,8-dien-1-ol	MS, R_I	0.1	-
1152	menthone	MS, R_I, Inj	-	1.0
1163	iso-menthone	MS, R_I, Inj	0.4	2.8
1165	δ-terpineol	MS, R_I	0.4	-
1174	*cis*-isopulegone	MS, R_I	-	2.0
1175	terpinen-4-ol	MS, R_I, Inj	0.8	-
1182	neoiso-menthol	MS, R_I, Inj	-	0.6
1190	α-terpineol	MS, R_I, Inj	0.3	0.2
1194	*neo*-dihydro carveol	MS, R_I, Inj	3.0	-
1196	*trans*-dihydro carvone	MS, R_I	0.9	-
1219	*trans*-carveol	MS, R_I	0.2	-
1232	*cis*-carveol	MS, R_I, Inj	4.5	-
1237	pulegone	MS, R_I, Inj	1.5	86.2
1242	carvone	MS, R_I, Inj	62.9	-
1285	bornyl acetate	MS, R_I, Inj	0.2	-
1308	iso-menthyl acetate	MS, R_I, Inj	-	0.2
1312	neoiso-isopulegyl acetate	MS, R_I	0.4	-
1328	iso-dihydro carveol acetate	MS, R_I	0.6	-
1338	*trans*-carvyl acetate	MS, R_I	0.1	-
1339	piperitenone	MS, R_I	-	3.3
1363	*cis*-carvyl acetate	MS, R_I, Inj	0.7	-
1383	β-bourbonene	MS, R_I	1.4	-
1391	β-elemene	MS, R_I	0.1	-
1398	*Z*-jasmone	MS, R_I	0.2	-
1417	β-caryophyllene	MS, R_I, Inj	0.5	0.3
1427	β-copaene	MS, R_I	0.1	-
1451	*Z*-jasmonyl acetate	MS, R_I	0.2	-
1451	α-humulene	MS, R_I, Inj	-	0.5
1461	*cis*-cadina-1(6),4-diene	MS, R_I	0.2	-

1464	*cis*-muurola-4(14),5-diene	MS, R_I	0.2	-
1479	germacrene D	MS, R_I	0.4	-
1486	phenyl ethyl 2-methylbutanoate	MS, R_I	0.1	-
1503	germacrene A	MS, R_I	0.5	-
1521	*trans*-calamenene	MS, R_I	0.2	-
1575	spathulenol	MS, R_I	0.1	-
1580	caryophyllene oxide	MS, R_I, Inj	0.1	-
1613	1,10-di-epi-cubenol	MS, R_I	0.1	-
	Total identified		99.3	99.8
	Monoterpene hydrocarbons		10.7	1.4
	Oxygen containing monoterpenes		84.7	96.4
	Sesquiterpene hydrocarbons		3.6	0.8
	Phenylpropanoids		0.1	-
	Others		0.2	1.2

R_I, retention index determined on a HP-5 fused silica column relative to a series of n-alkanes (C8–C26); Identification has been realized by comparing mass spectra (MS), retention indices (R_I) and by injection of authentic compound (Inj). tr: trace, i.e., percentage lower than 0.1 %.

Table S2: Antifungal activity (MIC and MLC) of *M. spicata L.* and *M. pulegium L.* essential oil for *yeasts*, dermatophyte and *Aspergillus* strains.

Strains	*M. spicata*		*M. pulegium*	
	MIC	**MLC**	**MIC**	**MLC**
Candida albicans ATCC 10231	1.25	1.25	1.25	1.25
Candida tropicalis ATCC 13803	1.25	1.25	1.25	1.25
Candida krusei H9	1.25	1.25	1.25	1.25
Candida guillermondii MAT23	1.25	1.25	1.25	1.25
Candida parapsilosis ATCC 90018	1.25	1.25	1.25	2.5
Cryptococcus neoformans CECT 1078	0.32	0.64-1.25	0.64	1.25
Trichophyton mentagrophytes FF7	0.64	0.64	2.5-1.25	2.5-1.25
T. mentagrophytes var. *interdigitale* CECT 2958	0.64	1.25	2.5	2.5
T. rubrum CECT 2794	0.32	0.64	1.25	1.25
T. verrucosum CECT 2992	0.32	0.64	1.25	1.25
Microsporum canis FF1	0.64	0.64	1.25	1.25
M. gypseum CECT 2905	0.64	0.64	2.5-1.25	2.5-1.25
Epidermophyton floccosum FF9	0.64	0.64	1.25	1.25
Aspergillus niger ATCC16404	0.64	2.5	1.25	5
*A. fumigatus*ATCC 46645	0.64	2.5	1.25	2.5
A. flavus F44	1.25	5	1.25	2.5-5

MIC and MLC were determined by a macrodilution method and expressed in µL/ml (V/V).

Table S3: Influence of sub-inhibitory concentrations of *M. spicata L.* and *M. pulegium L.* essential oil on germ tube formation of *Candida albicans* ATCC 10231.

M. spicata		*M. pulegium*	
Control[(a)]	100	Control[(a)]	100
MIC/32 (Conc.)	85.5±2.16 (0.04)	MIC/32 (Conc.)	94.4±1.6 (0.04)
MIC/16 (Conc.)	68.5±8.1 (0.08)	MIC/16 (Conc.)	87.2±3.5 (0.08)
MIC/8 (Conc.)	18.2± 2.7 (0.16)	MIC/8 (Conc.)	57.7± 1.2 (0.16)
MIC/4 (Conc.)	0±0 (0.32)	MIC/4 (Conc.)	2.6±1.2 (0.32)
MIC/2 (Conc.)	0±0 (0.64)	MIC/2 (Conc.)	0±0 (0.64)
MIC (Conc.)	0±0 (1.25)	MIC (Conc.)	0±0 (1.25)

The results are expressed as mean ± standard deviation of a minimum of three independent experiments perfomed in duplicate

[a]Untreated samples including 1% DMSO are considered as control, with 100% filamentation.

[b]Absolute concentration in µl mL^{-1}

Table S4: Influence of sub-inhibitory concentrations of fluconazole on the germ tube formation of *Candida albicans* ATCC 10231.

	Fluconazole
Control[a]	100
0.200[b]	89.10 ± 5.09
0.128	90.04 ± 9.94
0.004	98.86 ± 6.07
0.002	100.5 3± 7.81
0.001	101.00 ± 5.27

The results are expressed as mean ± standard deviation of a minimum of three independent experiments perfomed in duplicate.
[a]Untreated samples including 1% DMSO are considered as control, with 100% filamentation.
[b]Absolute concentration of fluconazole in mg/mL.