Antifungal activity and chemical composition of the essential oil from the aerial parts of two new *Teucrium capitatum* L. chemotypes from Sardinia island, Italy.

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Abstract

The chemical composition of two populations of *Teucrium capitatum* L. one from the coastline and the other one from the mountainous area of Sardinia (Italy) was assessed. Two chemotypes were identified: limonene/α-pinene/(E)-nerolidol chemotype predominant in the coastline, and limonene/α-pinene/α-trans-bergamotene/humulene epoxide II chemotype common in plants growing in the mountainous area. In addition, our results showed that the sample growing in the coastline had a more promising antifungal activity. Furthermore, this sample was highly effective in inhibiting *C. albicans* germ tube formation, at doses well below its MIC. Overall, this study shows that the edaphoclimatic characteristics play an important role on the essential oil composition and biological activity of *Teucrium capitatum* L.

Keywords: Antifungal activity, chemotypes, essential oil, germ tube formation, *Teucrium capitatum* L.

1. Introduction

Although fungal infections are socially and politically neglected, they cause about 11.5 million life-threatening infections and are responsible for 1.5 million deaths per year. Despite the recent advances in fungal diagnosis and antifungal development, most of the global population still lacks access to these newly developed formulas (Tudela and Denning, 2017). Fungal infections are mainly cutaneous and usually non-life threatening. However, some infections can become systemic and require access to high-level medical skill, otherwise they can be fatal. Nevertheless, the antifungal arsenal is very limited and because of the disseminated use, is becoming ineffective due to the development of fungal resistance (Perlin et al. 2017). In this context, essential oils (EOs) appear as a very promising antifungal agent (Nazzaro et al. 2017). Many studies have reported several biological activities, including antifungal, particularly in plants belonging to the Asteraceae, Apiaceae and Lamiaceae families (Alves-Silva et al. 2016; Edris 2007; Pinto et al. 2013b, 2013a, 2009, 2006; Valente et al. 2013; Zuzarte et al. 2018). *Teucrium* L. (Lamiaceae) is an aromatic genus mainly found in the Mediterranean region, however, some species can be found scattered across Europe, North Africa and temperate parts of Asia. In Sardinia this genus is represented by 11 taxa (Bartolucci et al. 2018; Conti et al. 2005; Pignatti 1982). In Sardinian traditional medicine some *Teucrium* taxa are used as antiseptic, as cicatrising agents and to treat skin diseases (Sanna et al. 2006). The present study aimed to (1) compare the composition of the EOs obtained by hydrodistillation of the flowering aerial parts of two population of *T. capitatum* L. and (2) evaluate the antifungal

activity of both samples against yeasts and dermatophyte strains and, concomitantly, to elucidate the underlying mechanism of action by disclosing the effect of the EO on germ tube formation on *C. albicans*.

2. Results and Discussion

2.1 Chemical composition

The flowering aerial parts of *T. capitatum* collected in two different regions of Sardinia, in the coastline area, Porticciolo (P) and in mountainous area, Gennargentu (G) were submitted to hydrodistillation for 4 h using a Clevenger-type apparatus; All the oils were light yellow with a pleasant smell. The yields (w/w) were as follows: 0.6 % and 0.5%, respectively. The results concerning the qualitative and quantitative analysis of the EOs are presented in Table S2. The sample P was characterized by high amounts of hydrocarbon and oxygenated monoterpenes (62.8 % and 11.4 %, respectively) and by a low percentage of hydrocarbon sesquiterpenes (3.8 %). Sample G was rich in hydrocarbon monoterpenes and sesquiterpenes (40.2 % and 37.8 %, respectively) with low amounts of oxygenated sesquiterpenes and monoterpenes (10.0 % and 4.1 %). Although both samples were characterized by high amounts of limonene (20.6 % and 17.2 % , respectively) and α-pinene (20.4 % and 12.5 %, respectively), sample P was also rich in (E)-nerolidol (16.7 %), β-pinene (7.6 %) and myrcene (7.5 %) whereas sample G was characterized by significant amounts of α-*trans*-bergamotene (12.2 %), humulene epoxide II (9.2 %) and δ-cadinene (7.7 %).

These results showed the existence of two chemotypes of *T. capitatum* depending on the growth conditions: one characterized by limonene/α-pinene/(E)-nerolidol predominant in plants growing in the coastline area (chemotype P) and other rich in limonene/α-pinene/α-trans-bergamotene/humulene epoxide II found in specimens collected in the mountainous area of Sardinia (chemotype G). The chemical variability of this species has been widely reported. The oil from *T. capitatum* growing in Portugal showed a very high chemical variability even within samples from the same region (Antunes et al. 2004), thus suggesting that genetic factors might play an important role in the chemical composition of the essential oils of *T. capitatum*. Indeed, Boulila et al. (2008) shown that the composition of EOs from Tunisian samples depends both on the edaphoclimatic conditions as well as the genetic background of the species, e.g. plants from the semi-arid climate were rich in myrcene or α-pinene/germacrene D, depending if they were tetraploid or diploid, respectively. A study from *T. capitatum* growing in Turkey identified β-pinene, β-caryophyllene, α-pinene, caryophyllene oxide, myrcene and germacrene D (Çakir et al. 1998). EO from samples growing in Iran had similar composition to the Turkish one (Eikani et al. 1999). Another Iranian sample was rich in limonene, di-2,4-tetr-butylphenol and *p*-cymene (Vahdani et al. 2011). Samples from Corsica show a high chemical polymorphism, with some samples being characterized by α-pinene, β-pinene and *p*-cymene (Cozzani et al. 2005), while others are rich in α-pinene, β-pinene, α-thujene, terpinene-4-ol, limonene, sabinene, *p*-cymene (Djabou et al. 2013, 2012). In samples collected from Crete, Greece, the EO was characterized by carvacrol, caryophyllene, torreyol and caryophyllene oxide (De Martino et al. 2010; Menichini et al. 2009). EO obtained from *T. capitatum* growing in Algeria was characterized by T-cadinol, germacrene D and β-pinene (Kerbouche et al. 2015). While, the essential oil of plants collected from the northeast of Algeria was characterized by germacrene D, bicyclogermacrene, β-pinene, carvacrol and spathulenol (Belmekki et al., 2013). Samples collected from Morocco had a very distinct chemical composition being rich in Endo-borneol, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-(1s-cis)-naphthalene, bornyl acetate and alpha-terpineol (El Amri et al. 2017). Our study contributed to reinforce the chemical variability of this species, since (E)-nerolidol found in the coastline samples and trans-bergamotene and humulene epoxide II common in the samples collected in the mountainous region, are vestigial or even absent in most samples from the Mediterranean basin. Indeed, (E)-nerolidol is only found in vestigial amounts in the essential oil of *T. capitatum* collected in Portugal (Antunes et al. 2004).

2.2 Antifungal activity

Both EOs had a similar activity against dermatophytes and *C. neoformans* (MIC = 0.27 and 0.54 mg/mL) being the EO from *T. capitatum* collected in the coastline slightly more effective. Regarding the anti-candidal activity of the samples, similarly to dermatophytes, the coastline sample was more effective in inhibiting the growth of all tested strains (MIC = 1.05 – 2.10 mg/mL) (Table S3). For most tested strains, both EOs had an MLC at the same value of MIC therefore suggesting a fungicidal effect. The activity of both essential oils can be attributed to its major compounds, indeed α-pinene, limonene, β-pinene and myrcene have been widely reported to be antimicrobial (Hammer et al. 2003; Leite et al. 2007; Pinto et al. 2013b; Sekine et al. 2007; Sonboli et al. 2006; Tampieri et al. 2005; Yousefzadi et al. 2008). However, the coastline sample was more effective than the mountainous one, which might be due to the presence of (E)-nerolidol, as this compound has several studies reporting its antifungal activity, as reviewed elsewhere (Chan et al. 2016).

The antimicrobial properties of *Teucrium* spp. has been widely reported. Indeed, Djabou et al. (2013) showed that several species of genus *Teucrium* inhibited the growth of foodborne pathogens and infectious bacteria, including a multidrug resistant strain of *Enterobacter aerogenes*. The EO from *T. capitatum* growing in Algeria had a poor antimicrobial activity against a wide range of bacteria and yeasts (Belmekki et al. 2013; Kerbouche et al. 2015). EO isolated from *T. polium* growing in Iran had a weak inhibitory effect on the growth of several pathogenic bacteria (Vahdani et al. 2011). Two subspecies, *T. polium* subsp. *aurum* and *T. polium* subsp. *polium,* shown a promising activity against both Gram positive and negative bacteria (El Atki et al. 2019).

2.3 Germ tube inhibition of C. albicans

The capacity to inhibit germ tube formation in *C. albicans*, an important virulence factor for this pathogenic yeast, was carried out on the most promising EO (Sample P, from the coastline of Sardinia) in order to disclose the putative mechanism of action underlying the anti-candidal activity of the EO (Figure S2). Our results show that the EO totally inhibited the germ tube formation at concentrations 2 times lower than the MIC. In addition, at MIC/4 (0.27 mg/mL) the EO still inhibits this feature by 73.7 %. For the best of our knowledge no reports on the capacity to inhibit the germ tube formation by *Teucrium* spp. were ever conducted. The isolated compound α-pinene, a major compound of the essential oil, was able to decrease the germ tube formation (Alves et al. 2019) thus suggesting that activity of the essential oil might be attributed to the its presence. The capacity of both pinene isomers and limonene to inhibit this feature in *C. albicans* was also reported by Raut et al. (2013). Another study also reported that β-pinene is able to inhibit the germ tube formation (Halbandge et al. 2017).

3. Experimental

See supplementary material

4. Conclusion

This study highlights the importance of edaphoclimatic factors on the chemical variability of the essential of *Teucrium capitatum*, by emphazing to distinct chemical profiles between samples growing in the coastline and mountains of Sardinia island. In addition, this study also validates the uses of this species on the Sardinian traditional medicine by showing a promising antifungal activity particularly against dermatophytes and *C. neoformans*. In addition, the sample from the coastline showed a strong effect on inhibiting the germ tube formation by *C. albicans*, an important virulence factor for this pathogenic yeast.

Disclosure statement

We declare the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Alves-Silva JM, Zuzarte M, Gonçalves MJ, Cavaleiro C, Cruz MT, Cardoso SM, Salgueiro L. 2016. New Claims for Wild Carrot (Daucus carota subsp. carota) Essential Oil. Evid-Based Compl Altern Med. 2016:1-10.

Alves M, Gonçalves MJ, Zuzarte M, Alves-Silva J, Cavaleiro C, Cruz MTT, Salgueiro L. 2019. Unveiling the antifungal potential of two Iberian thyme essential oils: effect on C. albicans germ tube and preformed biofilms. Front Pharmacol. 10: 446-456.

Antunes T, Sevinate-Pinto I, Barroso JG, Cavaleiro C, Salgueiro LR. 2004. Micromorphology of trichomes and composition of essential oil of Teucrium capitatum. Flavour Frag J. 19: 336-340.

Bartolucci F, Peruzzi L, Galasso G, Albano A, Alessandrini A, Arenghi NMG, Astuti G, Bacchetta G, Ballelli S, Banfi E, et al. 2018. An updated checklist of the vascular flora native to Italy. Plant Biosyst. 152: 179-303.

Belmekki N, Bendimerad N, Bekhechi C, Fernandez X. 2013. Chemical analysis and antimicrobial activity of Teucrium polium L. essential oil from Western Algeria. J Med Plant Res. 7: 897-902.

Boulila A, Béjaoui A, Messaoud C, Boussaid M, 2008. Variation of Volatiles in Tunisian Populations of Teucrium polium L. (Lamiaceae). Chem Biodivers. 5: 1389-1400.

Çakir A, Duru ME, Harmandar M, Ciriminna R, Passannanti S. 1998. Volatile Constituents of Teucrium polium L. from Turkey. J Essent Oil Res. 10: 113-115.

Chan WK, Tan LTH, Chan KG, Lee LH, Goh BH. 2016. Nerolidol: A Sesquiterpene Alcohol with Multi-Faceted Pharmacological and Biological Activities. Molecules. 21(5): 529-569.

Conti F, Abbate G, Alessandrini A, Blasi C. 2005. An annotated checklist of the Italian vascular flora. Palombi.

Cozzani S, Muselli A, Desjobert JM, Bernardini AF, Tomi F, Casanova J. 2005. Chemical composition of essential oil of Teucrium polium subsp.capitatum (L.) from Corsica. Flavour Frag J. 20: 436-441.

De Martino L, Formisano C, Mancini E, Feo VD, Piozzi F, Rigano D, Senatore F. 2010. Chemical Composition and Phytotoxic Effects of Essential Oils from Four Teucrium Species. Nat Prod Comm. 5(12): 1969-1976.

Djabou N, Lorenzi V, Guinoiseau E, Andreani S, Giuliani MC, Desjobert JM, Bolla JM, Costa J, Berti L, Luciani A, Muselli A. 2013. Phytochemical composition of Corsican Teucrium essential oils and antibacterial activity against foodborne or toxi-infectious pathogens. Food Control, 30: 354-363.

Djabou N, Muselli A, Allali H, Dib MEA, Tabti B, Varesi L, Costa J. 2012. Chemical and genetic diversity of two Mediterranean subspecies of Teucrium polium L. Phytochemistry. 83: 51-62.

Edris AE. 2007. Pharmaceutical and therapeutic Potentials of essential oils and their individual volatile constituents: a review. Phytother Res. 21: 308-323.

Eikani MH, Goodarznia I, Mirza M. 1999. Comparison between the Essential Oil and Supercritical Carbon Dioxide Extract of Teucrium polium L. J Essent Oil Res. 11: 470-472.

El Amri J, Badaoui KEl, Haloui Z. 2017. The chemical composition and the antimicrobial properties of the essential oil extracted from the leaves of Teucrium capitatum L. Asian J Pharm Clin Res. 10: 112.

El Atki Y, Aouam I, El Kamari F, Taroq A, Lyoussi B, Oumokhtar B, Abdellaoui A. 2019. Phytochemistry, antioxidant and antibacterial activities of two Moroccan Teucrium polium L. subspecies: Preventive approach against nosocomial infections. Arab J Chem. 13(2): 3866-3874.

Halbandge SD, Mortale SP, Jadhav AK. 2017. Differential Sensitivities of various growth modes of Candida albicans to sixteen molecules of plant origin. J Pharmacogn Phytochem. 6(4): 306-318.

Hammer KA, Carson CF, Riley TV. 2003. Antifungal activity of the components of Melaleuca alternifolia (tea tree) oil. J Appl Microbiol. 95: 853-860.

Kerbouche L, Hazzit M, Ferhat MA, Baaliouamer A, Miguel MG. 2015. Biological Activities of Essential Oils and Ethanol Extracts of Teucrium polium subsp. capitatum (L.) Briq. and Origanum floribundum Munby. J Essent Oil Bear Pl. 18: 1197-1208.

Leite AM, Lima EO, Souza EL, Diniz M FFM, Trajano VN, Medeiros IA. 2007. Inhibitory effect of beta-pinene, alpha-pinene and eugenol on the growth of potential infectious endocarditis causing Gram-positive bacteria. Rev Bras Ciênc Farm. 43: 121-126.

Menichini F, Conforti F, Rigano D, Formisano C, Piozzi F, Senatore F. 2009. Phytochemical composition, anti-inflammatory and antitumour activities of four Teucrium essential oils from Greece. Food Chem. 115: 679-686.

Nazzaro F, Fratianni F, Coppola R, Feo VD. 2017. Essential Oils and Antifungal Activity. Pharmaceuticals. 10(4): 86-106.

Perlin DS, Rautemaa-Richardson R, Alastruey-Izquierdo A. 2017. The global problem of antifungal resistance: prevalence, mechanisms, and management. Lancet Infect Dis. 17: 383-392.

Pignatti S, 1982. Flora D'Italia. Edagricole.

Pinto E, Gonçalves MJ, Hrimpeng K, Pinto J, Vaz S, Vale-Silva LA, Cavaleiro C, Salgueiro L. 2013a. Antifungal activity of the essential oil of Thymus villosus subsp. lusitanicus against Candida, Cryptococcus, Aspergillus and dermatophyte species. Ind Crop Prod. 51: 93-99.

Pinto E, Hrimpeng K, Lopes G, Vaz S, Gonçalves MJ, Cavaleiro C, Salgueiro L. 2013b. Antifungal activity of Ferulago capillaris essential oil against Candida, Cryptococcus, Aspergillus and dermatophyte species. Eur J Clin Microbiol Infect Dis. 32: 1311-1320.

Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. 2009. Antifungal activity of the clove essential oil from Syzygium aromaticum on Candida, Aspergillus and dermatophyte species. J Med Microbiol. 58: 1454-1462.

Pinto E, Pina-Vaz C, Salgueiro L, Gonçalves MJ, Costa-De-Oliveira S, Cavaleiro C, Palmeira A, Rodrigues A, Martinez-De-Oliveira J. 2006. Antifungal activity of the essential oil of Thymus pulegioides on Candida, Aspergillus and dermatophyte species. J Med Microbiol. 55: 1367-1373.

Raut JS, Shinde RB, Chauhan NM, Mohan Karuppayil S. 2013. Terpenoids of plant origin inhibit morphogenesis, adhesion, and biofilm formation by Candida albicans. Biofouling. 29: 87-96.

Sanna C, Ballero M, Maxia A. 2006. Le piante medicinali utilizzate contro le patologie epidermiche in Ogliastra (Sardegna centro-orientale). Atti della Società Toscana di Scienze Naturali- Memorie Serie B. 113: 73-82.

Sekine T, Sugano, M, Majid A, Fujii Y. 2007. Antifungal Effects of Volatile Compounds from Black Zira (Bunium persicum) and Other Spices and Herbs. J Chem Ecol. 33: 2123-2132.

Sonboli A, Babakhani B, Mehrabian AR. 2006. Antimicrobial activity of six constituents of essential oil from Salvia. Z Naturforsch C. 61(3-4): 160-164.

Tampieri MP, Galuppi R, Macchioni F, Carelle MS, Falcioni L, Cioni PL, Morelli I. 2005. The inhibition of Candida albicans by selected essential oils and their major components. Mycopathologia. 159: 339-345.

Tudela JLR, Denning DW. 2017. Recovery from serious fungal infections should be realisable for everyone. Lancet Infect Dis. 17(11): 1111-1113.

Vahdani M, Faridi P, Zarshenas MM, Javadpour S, Abolhassanzadeh Z, Moradi N, Bakzadeh Z, Karmostaji A, Mohagheghzadeh A, Ghasemi Y. 2011. Major Compounds and Antimicrobial Activity of Essential Oils from Five Iranian Endemic Medicinal Plants. Pharmacogn J. 3: 48-53.

Valente J, Zuzarte M, Gonçalves MJ, Lopes MC, Cavaleiro C, Salgueiro L, Cruz MT. 2013. Antifungal, antioxidant and anti-inflammatory activities of Oenanthe crocata L. essential oil. Food Chem Tox. 62: 349-354.

Yousefzadi M, Sonboli A, Ebrahimi SN, Hashemi SH. 2008. Antimicrobial activity of essential oil and major constituents of Salvia chloroleuca. Z Naturforsch C. 63: 337-340.

Zuzarte M, Alves-Silva JM, Alves M, Cavaleiro C, Salgueiro L, Cruz MT. 2018. New insights on the anti-inflammatory potential and safety profile of Thymus carnosus and Thymus camphoratus essential oils and their main compounds. J Ethnopharmacol. 225: 10-17.

SUPPLEMENTARY MATERIAL

Antifungal activity and chemical composition of the essential oil from the aerial parts of two new *Teucrium capitatum* L. chemotypes from Sardinia island, Italy.

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Abstract

The chemical composition of two populations of *Teucrium capitatum* L. one from the coastline and the other one from the mountainous area of Sardinia (Italy) was assessed. Two chemotypes were identified: limonene/α-pinene/(E)-nerolidol chemotype predominant in the coastline, and limonene/α-pinene/α-trans-bergamotene/humulene epoxide II chemotype common in plants growing in the mountainous area. In addition, our results showed that the sample growing in the coastline had a more promising antifungal activity. Furthermore, this sample was highly effective in inhibiting *C. albicans* germ tube formation, at doses well below its MIC. Overall, this study shows that the edaphoclimatic characteristics play an important role on the essential oil composition and biological activity of *Teucrium capitatum* L.

Keywords: Antifungal activity, chemotypes, essential oil, germ tube formation, *Teucrium capitatum* L.

Experimental

Plant material and essential oil isolation

The flowering aerial parts of samples were collected in July 2015. Two populations (Table S1 and Figure S1) of *Teucrium capitatum* L. (synonym *Teucrium capitatum* L. subsp. *capitatum,*

Bartolucci et al. 2018) were picked up in the coastline in Porticciolo - Alghero, Sassari, and in the mountainous area of Gennargentu - Arzana, Nuoro. The voucher specimens were identified by the botanist Dr. Alfredo Maccioni using dichotomous identification keys of the Flora d'Italia (Pignatti 1982) and each *taxa* was deposited at the Herbarium of University of Cagliari (Herbarium CAG) - Sardinia (Italy): *T. capitatum* (Porticciolo - Alghero, Sassari), HerbCAG n. 1123a; *T. capitatum* (Gennargentu -Arzana, Nuoro), HerbCAG n.1123b.

Plant materials were air-dried at 40 °C with forced ventilation for two days in an oven (FD 115, BINDER) at the Laboratory of Plant Biology and Pharmaceutical Botany of the University of Cagliari, Sardinia (Italy). Isolation of EOs by hydrodistillation were performed in a Clevenger-type apparatus for 4 h (Council of Europe 2010). Each EO from every wild population was obtained by mixing the ten sampled individuals of each population. The EOs were stored at 4 °C in the dark until the chemical analyses.

Essential oil analysis

Analyses of the EOs 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 analyser (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 (Adams 2007; NIST/EPA/NIH 2015) 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 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 Dec. 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 EOs were evaluated against several yeasts *Candida krusei* H9*, C. guilliermondii* MAT23, *C. albicans* ATCC 10231, *C. tropicalis* ATCC 13803,*C. parapsilopsis* ATCC 90018 and *Cryptococcus neoformans* CECT 1078, as well as dermatophytes, *Epidermophyton floccosum* FF9, *Trichophyton mentagrophytes* FF7, *Microsporum canis* FF1*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 assess the antifungal potential of the EOs according to the Clinical and Laboratory Standards Institute (CLSI) reference protocols M27-A3 (CLSI, 2008a) and M38-A2 (CLSI, 2008b) for yeasts and filamentous fungi, respectively. Briefly, inoculum suspensions were prepared in RPMI from overnight cultures and dispersed into glass test tubes containing serial double dilutions of the EOs (0.13 to 4.2 mg/mL) made in DMSO. Final concentration of DMSO never exceeded 1 %. Negative (non-inoculated medium) and positive control (inoculated medium with DMSO at 1 %) were 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). Minimum inhibitory concentration (MIC) values were determined as the lowest concentration of the EO causing complete growth inhibition. To measure minimal lethal concentrations (MLCs), 20 µL samples were taken from each negative tube and plated in SDA and incubated as described above. Minimum lethal concentrations (MLC) values were determined as the lowest concentration where no growth was observed. Quality control was performed by testing fluconazole with the reference strains. All experiments were performed in triplicate. A range of values is presented when different results were obtained.

Germ tube inhibition assay

The effect of the EOs on the yeast-mycelium transition, an important virulence factor of *C. albicans* was carried out as previously described (Pinto et al. 2009). 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 made in duplicate.

References

Adams RP. 2007. Identification of essential oil components by gas chromatography/mass spectroscopy. Allured Publishing Corporation, Carol Stream, Illinois, USA.

Bartolucci F, Peruzzi L, Galasso G, Albano A, Alessandrini A, Arenghi NMG, Astuti G, Bacchetta G, Ballelli S, Banfi E, et al. 2018. An updated checklist of the vascular flora native to Italy. Plant Biosyst. 152: 179-303.

CLSI, 2008a. Reference Method for Broth Dilution antifungal susceptibility testing of yeasts; Approved Standard M27-A3 - Third Edition (ISBN 1-56238-666-2). Clin. Lab. Stand. Inst. 940 West Val. Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

CLSI, 2008b. Reference Method for Broth Dilution antifungal susceptibility testing of filamentous fungi; Approved Standard M38-A2 - Second Edition (ISBN 1-56238-668-9). Clin. Lab. Stand. Inst. 940 West Val. Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

Council of Europe 2010. European Pharmacopoeia 7th Edition. Directorate for the Quality of Medicines & HealthCare of the Council of Europe, Strasbourg, France.

NIST/EPA/NIH 2015. Mass spectral library.

Pignatti S. 1982. Flora d'Italia. Bologna: Edagricole. Italy.

Pinto E, Vale-Silva L, Cavaleiro C, Salgueiro L. 2009. Antifungal activity of the clove essential oil from Syzygium aromaticum on Candida, Aspergillus and dermatophyte species. J. Med. Microbiol. 58, 1454–1462. https://doi.org/10.1099/jmm.0.010538-0

van Den Dool H, Dec Kratz P. 1963. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. J. Chromatogr. A 11, 463–471. https://doi.org/10.1016/S0021-9673(01)80947-X

Table S1. Population data**.**

Species	**Location**	**Coordinates**	**Substrate**	**Distance from sea (m)**	**Precipitation mean (June 2015)**	**Elevation mean (m a.s.l)**	**Bioclimatic area**
Teucrium capitatum L. **(chemotype P)**	Porticciolo - Alghero (Sassari) – Sardinia, Italy	N 40°45'14.3" E 08°09'29.1"	Middle Permian Triassic successions - Red Sandstone	54.8	30 mm	10	Pluvi-seasonal-oceanic Mediterranean climate
Teucrium capitatum L. **(chemotype G)**	Gennargentu - Arzana (Nuoro) – Sardinia, Italy	N 39°56'20.3" E 9°19'51.4"	Phyllites	32833.6	30 mm	1200	Oceanic Temperate - submediterranean climate

Table S2. Composition of *Teucrium capitatum* L. (chemotype P, Porticciolo and chemotype G, Gennargentu) essential oils.

R_I	**Compound**	*T. capitatum* **(chemotype P)**	*T. capitatum* **(chemotype G)**
930	α-thujene	-	1.3
938	**α-pinene**	**20.4**	**12.5**
958	thuja-2,4(10)-diene	1.6	-
976	sabinene	1.0	1.2
980	**β-pinene**	**7.6**	**4.5**
992	**myrcene**	**7.5**	2.4
1027	o-cymene	1.3	0.6
1032	**limonene**	**20.6**	**17.2**
1051	(E)-β-ocimene	1.5	-
1062	γ-terpinene	-	0.5
1128	α-campholenal	1.0	-
1141	trans-pinocarveol	1.0	-
1147	trans-verbenol	0.6	-
1164	pinocarvone	1.1	-
1169	para-mentha-1,5-dien-8-ol	1.6	-
1178	terpinen-4-ol	-	1.1
1195	myrtenal	2.1	-
1220	trans-carveol	0.9	-
1245	carvone	1.8	0.8
1286	isobornyl acetate	1.3	-
1351	α-terpinyl acetate	-	2.2
1418	(E)-caryophyllene	2.3	0.9
1437	**α-trans-bergamotene**	-	**12.2**
1459	(E)-β-farnesene	-	0.9

1489	trans-muurola-4(14),5-diene		1.7
1491	α-selinene	0.7	-
1493	α-muurolene	-	0.9
1499	γ-patchoulene	-	0.9
1502	(Z)-α-bisabolene	-	2.4
1508	germacrene A	-	1.0
1514	γ-cadinene	-	2.1
1523	**δ-cadinene**	0.8	**7.7**
1544	α-calacorene	-	1.5
1560	**β-calacorene**	-	**5.6**
1566	**(E)-nerolidol**	**16.7**	0.8
1606	**humulene epoxide II**	-	**9.2**
Total identified		**94.7**	**92.1**
Hydrocarbon monoterpenes		**62.8**	**40.2**
Oxygenated monoterpenes		**11.4**	**4.1**
Hydrocarbon sesquiterpenes		**3.8**	**37.8**
Oxygenated sesquiterpenes		**16.7**	**10.0**

R_I, retention index determined on a HP-5 fused silica column relative to a series of n-alkanes (C_8–C_{26}).

Table S3. Minimum inhibitory concentrations (MIC) and minimum lethal concentration (MLC) of *Teucrium capitatum* L. (chemotype P, Porticciolo and chemotype G, Gennargentu) essential oils for yeasts and dermatophyte.

Strains	*T. capitatum* (chemotype P)		*T. capitatum* (chemotype G)	
	MIC [a]	**MLC** [a]	**MIC** [a]	**MLC** [a]
Candida albicans ATCC 10231	2.1	2.1	4.2	>4.2
Candida tropicalis ATCC 13803	2.1	2.1	4.2	4.2
Candida krusei H9	2.1	2.1	≥4.2	>4.2
Candida guillermondii MAT23	1.05	2.1	2.1	4.2
Candida parapsilosis ATCC 90018	2.1	4.2	>4.2	>4.2
Cryptococcus neoformans CECT 1078	0.27	0.54	0.54	1.05
T. mentagrophytes FF7	0.54	0.54	0.54	0.54
T. mentagrophytes var. *interdigitale* CECT 2958	0.54	0.54-1.05	0.54	2.1
Trichophyton rubrum CECT 2794	0.27	0.54	0.54	0.54
T. verrucosum CECT 2992	0.54	0.54	0.54	1.05
Microsporum canis FF1	0.27	0.27	0.27	0.54
M. gypseum CECT 2905	0.54	0.54	0.54	0.54
Epidermophyton floccosum FF9	0.27	0.27	0.27	0.27

[a] MIC and MLC were determined by a macrodilution method and expressed in mg/mL (w/v).

Figure S1. Population of *Teucrium capitatum* L. and chemotype locations in Sardinia - chemotype P, Porticciolo and chemotype G, Gennargentu.

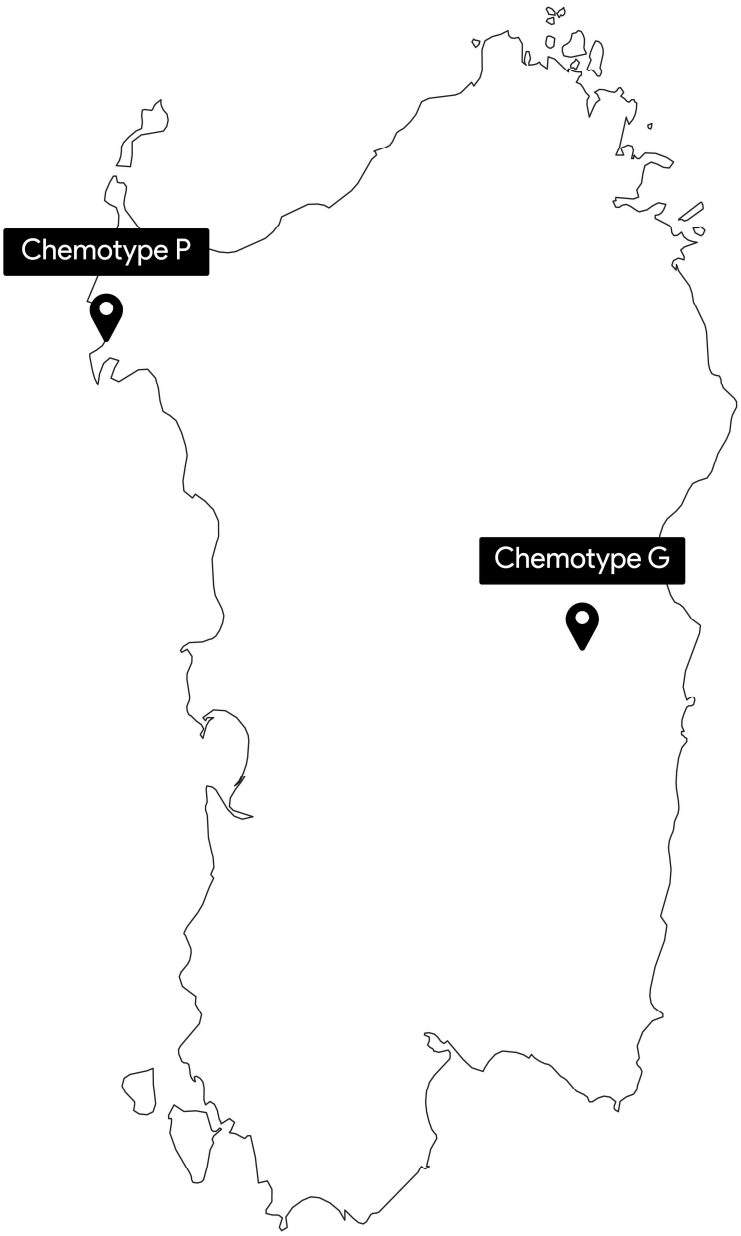

Figure S2. Influence of sub-inhibitory concentrations of *Teucrium capitatum* L. (chemotype P) essential oil on germ tube formation of *Candida albicans* ATCC 10231. Percentage of filamentous cells against control cells (DMSO treated cells, 100%, dashed line). Results are expressed as mean ±standard deviation of a minimum of three independent experiments performed in duplicate.