Chemical composition and biological activity of essential oil of *Teucrium scordium* L. subsp. *scordioides* (Schreb.) Arcang. (Lamiaceae) from Sardinia Island (Italy)

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ABSTRACT

The aim of this study is to demonstrate the antifungal, anti-inflam- matory and anti-migratory potential of the essential oil of *Teucrium scordium* subsp. *scordioides* (Schreb.) Arcang, a plant widely used in traditional medicine in Sardinia. The oil was rich in germacrene D (25.1%), d-cadinene (12.9%) and alloaromadendrene (11.3%). The yeast *Cryptococcus neoformans* and the dermatophytes *Trichophyton rubrum, T. mentagrophytes* var. *interdigitale* and *Epidermophyton floc- cosum* were the most susceptible fungi to the action of the oil. In lipopolysaccharide (LPS)-stimulated macrophages, the oil was able to decrease nitric oxide production by ca. 30% at 1.25 lL/mL, with- out affecting cell viability. In the scratch wound assay, it allowed for ca. 36% of wound closure after 18 h, thus showing anti-migratory properties. Overall, this study highlights the potential of this species to mitigate fungal infections associated with an inflammatory response. Furthermore, we also reported for the first time its anti-migratory capacity, thus suggesting anticancer properties.

KEYWORDS

Teucrium scordium L. subsp. *scordioides* (Schreb.) Arcang.; essential oil; antifungal activity; anti-inflammatory activity; dermatophytes; cell migration

Introduction

Teucrium L. (Lamiaceae) is a large and highly polymorphic genus that includes more than 300 species distributed in Europe, North Africa and temperate parts of Asia, although more prevalent in the Mediterranean region (Bini Maleci et al. 1995). Plants of this genus have been described as important sources of essential oils, iridoid glyco- sides, phenolic and polyphenolic compounds, evidencing the medicinal interest of the plants of this genus (Semiz et al. 2016; Belarbi et al. 2018; Frezza et al. 2018; Farahbakhsh et al. 2020; Maccioni et al. 2020). Several traditional uses of *Teucrium* spp. have been recently reviewed (Candela et al. 2021). In Sardinia 11 *Teucrium* taxa are described and several of them are widely used in Sardinian traditional medicine as cic- atrizing agents, antiseptic, antibacterial, antifungal, tonics, among several other pur- poses (Maccioni et al. 2021). Particularly, *Teucrium scordium* subsp. *scordioides* (Schreb.) Arcang is used as antiseptic and anthelmintic (Atzei 2003); however, the scientific val- idation of these claims is still lacking. Interestingly, the anticancer effect of *T. scordium* subsp. *scordioides* has been reported for phenolic extracts (Stankovic et al. 2011), but there is still no studies in the literature reporting this activity for the essential oil. Therefore, the aim of this study is to characterize the essential oil of *T. scordium* subsp. *scordioides* as well as to demonstrate its antifungal, anti-inflammatory and anti-migra- tory potential.

1. **Results and discussion**

1.1. Chemical composition

The results concerning the qualitative and quantitative analysis of the essential oil are presented in Table S1, where the components are listed in order of elution from a HP- 5 column. The oil

was characterized by a very high percentage of hydrocarbon sesqui- terpenes (67.6%) and oxygenated sesquiterpenes (21.6%). The main compounds include germacrene D (25.1%), d-cadinene (12.9%), alloaromadendrene (11.3%), a-cadinol (6.2%), germacrene D-4-ol (6.0%), a-pinene (4.9%), c-cadinene (4.7%) and a-epicadinol (4.7%).

This composition is very distinct from the oils obtained from plants from other regions. Indeed, the essential oil from plants collected in Sicily, Italy, are rich in caryo- phyllene oxide (25.8%), a-pinene (19.4%) and b-pinene (8.5%) (Gagliano Candela et al. 2021) while those from Serbia are characterized by menthofuran (11.9%), (Z)-octadec- 9-enoic acid (11.5%), and hexadecanoic acid (6.4%) (Radulovi´c et al. 2012). Other stud- ies address the composition of *T. scordium*, however, they fail to mention the subspe- cies, making the comparison to the present study difficult. Indeed, the oil from the aerial parts of *T. scordium* growing in North Iran was characterized by b-caryophyllene, (E)-b-farnesene, caryophyllene oxide, 1,8-cineole and b-eudesmol (Morteza-Semnani et al. 2007). In another study, samples from Serbia and Montenegro had a distinct composition, with a- and b-pinene being the major compounds (Kovacevic et al. 2001).

2.2 Antifungal activity

The antifungal effect of the essential oil is summarized in Table S2. Our results showed that Cryptococcus neoformans was the most susceptible yeast (MIC ¼ 0.32 lL/mL). The dermatophytes Trichophyton mentagrophytes var. interdigitale, T. rubrum and Epidermophyton floccosum were the most susceptible filamentous fungi (MIC ¼ 0.32 lL/mL) followed by T. mentagrophytes, Microsporum canis and M. gypseum with MIC ¼ 0.64 lL/mL.

To the best knowledge of the authors, there are no studies in the literature assess- ing the antimicrobial effect of T. scordium essential oil; indeed only two studies assessed this effect using non-volatile extracts that were ineffective against C. albicans (Tatjana et al. 2011; Stankovi´c et al. 2012).

Currently available therapies are often associated with problems related with drug safety, undesirable side effects, narrow activity spectrum and a small number of tar- gets (Fuentefria et al. 2018), as well as the emergence of resistant strains (Martinez- Rossi et al. 2018; Mourad and Perfect 2018). Indeed, dermatophytes from the genus Trichophyton have been reported to show resistance to terbinafine and fluconazole, the two most widely used antifungals to control dermatophytosis (Arendrup et al. 2021). Resistance to all the classes of antifungals has also been reported for C. neofor- mans (Bermas et al. 2020). In this scenario plant extracts, despite having lower antifun- gal activity, can emerge as effective alternatives/complements. Indeed, these extracts are able to act on multiple cell targets, an important feature when considering micro- organisms that are intrinsically or became resistant to conventional therapies. Several studies have demonstrated the effectiveness of essential oils in fungal infections

(Zuzarte et al. 2011; Lopes et al. 2017) such as Teucrium capitatum (MIC ¼ 0.32 — 0.64 lL/mL for dermatophytes and C. neoformans) (Maccioni et al. 2020), T. polium subsp. geyrii (MIC ¼ 2.45 lL/mL for C. albicans) (Roukia et al. 2013) and Santolina impressa (MIC ¼ 0.32 lL/mL against C. neoformans, Epidermophyton floccosum and Trichophyton rubrum (Alves-Silva et al. 2019). These activities are similar to the

reported activity of T. scordium subsp. scordioides. Also, its major compounds, namely germacrene D, d-cadinene, a-cadinol, epi-a-cadinol and a-pinene have been reported to inhibit the growth of several pathogenic fungi (Schmidt et al. 2007; Chang et al. 2008; Ho et al. 2011; Takao et al. 2012; Pinto et al. 2013; Lawson et al. 2020), thus sug- gesting that the activity of the oil might be attributed to their presence in the mixture.

2.3 Anti-inflammatory properties

Since the successful colonization of the host tissues by pathogenic fungi is fuelled by inflammation, an antifungal drug concomitantly presenting anti-inflammatory activity can be a valuable therapeutic strategy to fight fungal infections. Therefore, we also assessed the anti-inflammatory potential of the essential oil using an *in vitro* model of inflammation, specifically macrophages stimulated with the Toll-like receptor 4 agonist lipopolysaccharide (LPS), and the effect on NO production was analysed by measuring the accumulation of nitrites in the culture medium. NO is a well-established marker of inflammation and inhibition of its production upon activation with an inflammatory stimulus, such as LPS, might be a useful strategy to disclose new anti-inflammatory drugs. Our results show that pre-treatment with 1.25 lL/mL of the essential oil decreased the nitrite production evoked by LPS by *ca.* 30% (Figure S1A), without affecting macrophages viability (Figure S1B), thus suggesting and validating the safety profile of the essential oil at concentrations presenting pharmacological activity. Although we cannot state that the anti-inflammatory effect of the oil is superior to standard anti-inflammatory drugs, such as diclofenac, it is interesting to notice that for the concentration of the oil used in our experimental conditions (1.25 lL/mL), the per- centage of nitic oxide inhibition is similar to that achieved by 1.591 mg/mL diclofenac without presenting as much toxicity (79.5% vs 94.5% macrophages viability for diclofe- nac and the essential oil, respectively). The reported activity is similar to other essen- tial oils, even from different species, e.g., the essential oil from *Distichoselinum tenuifolium* decreases NO production by 40% at 1.25 lL/mL (Tavares et al. 2010). Although the anti-inflammatory potential of several *Teucrium* spp. has been widely reported (Barrachina et al. 1995; Puntero et al. 1997; Mukarram Shah 2015), the pre- sent study is pioneer in assessing the anti-inflammatory activity of *T. scordium* subsp. *scordioides* essential oil.

Regarding its major compounds, the anti-inflammatory potential of germacrene D, a-cadinol, epi-a-cadinol, a- and b-pinene has been already reported (Baylac 2003; Tung et al. 2011; Rufino et al. 2014; Coté et al. 2017), thus suggesting their involve- ment in the pharmacological activity of the oil. Since several essential oils exert their anti-inflammatory activity by inhibiting the pro-inflammatory transcription factor NF-jB (de Lavor et al. 2018) it will be of relevance to further explore the involvement of this signaling pathway on the anti-inflammatory activity of *Teucrium scordium* subsp. *scordioides* essential oil.

2.3. Cells migration assay

Cell migration was carried out using the scratch wound assay as reported by Martinotti and Ranzato (Martinotti and Ranzato 2019) by making a scratch on a cell monolayer and capturing images at regular intervals by microscopy.

The essential oil (1.25 lL/mL) decreased the capacity of the cells to migrate after the scratch (Figure S2A and S2B), thus suggesting its putative anti-migratory proper- ties. Importantly, the essential oil was devoid of toxicity (Figure S2C), thus validating its safety profile.

The anticancer properties of the genus *Teucrium* have been widely reported as reviewed elsewhere (Milutinovi´c and Cvetkovi´c 2020). Regarding *T. scordium* subsp. *scordioides* the anticancer properties have only been reported for a phenolic extract (Stankovic et al. 2011). Concerning cell migration, no studies have been conducted with this taxon; however several studies showing the anti-invasive and anti-migratory capacities of several *Teucrium* species have been reported (Kandouz et al. 2010; Haïdara et al. 2011; Tafrihi et al. 2014; Zivanovic et al. 2016; Tafrihi and Nakhaei Sistani 2017; Abdallah et al. 2018; Guesmi et al. 2018; Sheikhbahaei et al. 2018). The anti-inva- sive and anti-migratory properties of germacrene D, a-pinene and b-

eudesmol have also been shown (Kummer et al. 2015; Ben Sghaier et al. 2016; Kang et al. 2016; Huang et al. 2019; Schepetkin et al. 2020), reinforcing that the reported activity might be due to the presence of these compounds.

3 Experimental section

See Supplementary data

4 Conclusions

The present study shows, for the first time, the biological properties of the essential oil from *T. scordium* subsp. *scordioides*, particularly the antifungal and anti-inflamma- tory activities. Indeed, the oil was able to inhibit the growth of *Cryptococcus neofor- mans* and several dermatophytes. Furthermore, the essential oil decreased the production of nitric oxide in LPS-stimulated macrophages. Although the essential oil shows weaker activity than the standard antifungal or anti-inflammatory drugs, it exerts at the same time and at the same concentration antifungal and anti-inflamma- tory effects, which highlights its interest for the pharmaceutical industry due to this dual effect. Our results also showed that the essential oil possesses anti-migratory properties, which must be properly explored in an oncology context. Importantly, at pharmacological relevant concentrations, the oil was devoid of toxicity towards macro- phages and fibroblasts, thus highlighting its safety.

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SUPPLEMENTARY MATERIAL

Chemical composition and biological activity of essential oil of *Teucrium scordium* L. subsp. *scordioides* (Schreb.) Arcang. (Lamiaceae) from Sardinia Island (Italy)

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The aim of this study is to demonstrate the antifungal, anti-inflammatory and anti-migratory potential of the essential oil of Teucrium scordium subsp. scordioides (Schreb.) Arcang, a plant widely used in traditional medicine in Sardinia. The oil was rich in germacrene D (25.1 %), □-cadinene (12.9 %), alloaromadendrene (11.3 %). The yeast Cryptococcus neoformans and the dermatophytes Trichophyton rubrum, T. mentagrophytes var. interdigitale and Epidermophyton floccosum were the most susceptible fungi to the action of the oil. In lipopolysaccharide (LPS)-stimulated macrophages, the oil was able to

decrease nitric oxide production by ca. 30% at 1.25 μL/mL, without affecting cell viability. In the scratch wound assay, it allowed for ca. 36% of wound closure after 18h, thus showing anti-migratory properties. Overall, this study highlights the potential of species to mitigate fungal infections associated with an inflammatory response. Furthermore, we also reported for the first time its anti- migratory capacity, thus suggesting anticancer properties.

Keywords: *Teucrium scordium L.* subsp. *scordioides* (Schreb.) Arcang., essential oil, antifungal activity, anti-inflammatory activity, dermatophytes, cell migration.

Experimental

Plant material and essential oil isolation

The aerial parts of *T. scordium* subsp. *scordioides* (Schreb.) Arcang. were collected in Laconi, Oristano, coordinates (N 39°51'42.3097", E 09°08'24.8734"). A voucher specimen was deposited at the Herbarium of University of Cagliari (Herbarium CAG): *T. scordium* subsp. *scordioides* (TssLa), HerbCAG n.1120. The sample was identified by Dr. Alfredo Maccioni, Department of Life and Environmental Sciences, University of Cagliari. Plant materials were dried in air forced ventilation oven (FD 115, BINDER) at 30°C for two days (Rangari 2007) at the Laboratory of Plant Biology and Pharmaceutical Botany of the University of Cagliari, Sardinia (Italy). Identification of the taxon was carried out according to Flora d'Italia (Pignatti 2003) and Flora Europea (Tutin et al. 1972), with nomenclature standardized by Conti et al. (2005) and Bartolucci et al. (2008). Isolation of essential oils by hydrodistillation were performed in a Clevenger-type apparatus for 4 h in accordance with the European Pharmacopoeia (Europe 2010). The oil was stored at 4°C in the dark until the chemical analyses.

Essential oil analysis

GC-FID analysis. Quantitative analysis of the oil was performed on a Agilent 7890A GC equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm i.d. with a 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). Quantification of constituents was calculated

by integration of GC-FID peak areas without using the response correction factors.

GC-MS analysis. Qualitative analysis of the oil was 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 2005; Adams 2007) or those of pure compounds whenever possible. The results were further confirmed by comparing their 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).

Antifungal activity

Fungal strains

The antifungal activity of the essential oil was evaluated against yeasts and filamentous fungi. 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), one *Cryptococcus neoformans* type strain

from the Colección Espanõla de Cultivos Tipo (*C. neoformans* CECT 1078) and 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). 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 (MIC) and the minimum lethal concentration (MLC) of the oil according to the Clinical and Laboratory Standards Institute (CLSI) reference protocols M27-A3 (CLSI 2008b), and M38-A2 (CLSI 2008a) for yeasts and filamentous fungi, respectively. Briefly, to test tubes 10 µL of essential oil diluted in DMSO (5 – 0.08 µL/mL) were added, and then 990 µL of RPMI containing fungi was added and incubated at the appropriate temperature for the required time. After MIC determination, 10 µL of each negative tube was plated in SDA and incubated accordingly. The MIC was the lowest concentration in which no growth was observed in the inoculated test tubes, whereas the MLC was the lowest concentration where no growth was observed after inoculation in SDA of all the negative tubes. A negative (non-inoculated medium) and a positive (inoculated medium with 1% DMSO) controls were also included. Fluconazole was used to assess the purity of the tested strains. All experiments were performed in triplicate.

Anti-inflammatory activity

Cell culture

RAW 264.7, a mouse leukemic macrophage cell line obtained from the American Type Culture Collection (ATCC TIB-71), was cultured as previously reported by our group

(Zuzarte et al. 2018).

Nitric oxide production

NO production was measured by quantifying the accumulation of nitrites in culture supernatants, using the Griess reagent (Green et al. 1982). Cells (0.3 x 10^6 cells/well) were cultured in 48-well culture plates. After an overnight stabilization, macrophages were pre-treated for 1h with 1.25 – 0.08 µL/mL of the essential oil diluted in culture medium from a stock solution made in DMSO or with 1.591 µg/mL Diclofenac and then activated with 50 ng/mL of LPS during 24h. Positive (LPS-stimulated macrophages) and negative controls (untreated macrophages) were performed. After this time period of incubation, equal volumes of culture supernatants and Griess reagent [1:1 of 0.1% (w/v) N-(1-naphthyl) ethylenediaminedihydrochloride and 1% (w/v) sulphanilamide containing 5% (w/v) H_3PO_4] were mixed and incubated for 30 min, in the dark. The absorbance at 550 nm was registered in an automated plate reader (SLT, Austria) and nitrite concentration was determined from a sodium nitrite standard curve. DMSO at the maximum concentration used (0.4%) was already demonstrated by our group to be devoid of anti-inflammatory and cytotoxicity effects (data not shown).

Values shown as mean ± SEM of at least three independent experiments made in duplicate.

Cell migration

Cell culture

NIH 3T3, a mouse embryonic fibroblast cell line (ATCC CRL-1658), was cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Ref 31600-083) with 25 mM glucose, 3.7 g/L of sodium bicarbonate, 100 U/mL of penicillin and 100 µg/mL of streptomycin supplemented with 10% heat inactivated foetal bovine serum (FBS). Cells were sub-divided when reached 70-80% confluency. Cell morphology was controlled using an inverted light microscope.

Cell migration assay

Cell migration was carried out using the scratch wound assay as reported by Martinotti and colleagues (Martinotti and Ranzato 2019) with slight modifications. Briefly, 3T3 fibroblasts were seeded at 2.5 x 10^5 cells/mL in 12-well plates. After 24h of growth, a scratch was done in the cell monolayer using a pipette tip. Detached cells were removed by washing cells with sterile PBS 1x. DMEM with 2% serum was added to all plates, in the presence or absence of 1.25 µL/mL of the essential oil diluted in culture medium from a stock solution made in DMSO. Using phase-contract microscope, images were acquired 0, 12 and 18h post-scratch, and the wound area was measured using ImageJ/Fiji software. Results presented were obtained using the following equation

$$wound\ closure\ (\%) = \frac{A_{t=0h} - A_{t=xh}}{A_{t=0h}} \times 100$$

Where $A_{t=0h}$ is the area of the wound 0h after the scratch and $A_{t=xh}$ is the area at the different time post-scratch (0h, 12h and 18h). Values shown as mean ± SEM of at least three independent experiments made in duplicate.

Cell viability

The effect of different concentrations of the essential oil on the viability of both macrophages and fibroblasts was carried out using the resazurin reduction assay. Briefly, macrophages (0.6 x 10^6 cells/mL), or fibroblasts (1.25 x 10^5 cells/mL) were seeded in 48-well plates. After an overnight stabilization, 1.25 – 0.08 µL/mL of the essential oil diluted in culture medium from a stock solution made in DMSO was added for 24h. For macrophages 1.591 µg/mL of diclofenac was also used to disclose its effect on cell viability. At the end of the experiment, the medium was removed and fresh medium containing resazurin (1:10) was added for 4h. The absorbance at 570 nm with a reference filter 620 nm was registered in an automated plate reader (SLT, Austria). Cell viability was determined using the following equation:

$$Cell\ viability\ (\%) = \frac{Abs_{Exp}}{Abs_{CT}} \times 100$$

Where Abs_{Exp} is the absorbance (difference between 570 and 620 nm) in the different experimental conditions and Abs_{CT} is the absorbance in control cells (no essential oil). Values shown as mean ± SEM of at least three independent experiments made in duplicate.

Statistical analysis

Results are shown as mean ± SEM and an one-way ANOVA followed by Tukey's comparison test, with a p-value of 0.05, was carried out to determine statistical difference.

Table S1. Composition of *Teucrium scordium* subsp. *scordioides* essential oil.

RT_{EXP}	**RI_{EXP}**	**RI_{LIT}**	**Compound**	**Quantitative percentage**
5.2346	938	939	□-pinene	4.9
6.3275	980	979	□-pinene	2.8
7.8576	1031	1029	Limonene	0.8
8.1461	1040	1037	(Z)-□-ocimene	2.0
21.5974	1376	1376	□-copaene	2.5
21.9559	1384	1388	□-bourbonene	1.0
22.1964	1390	1388	□-cubebene	0.9
23.3592	1418	1419	(E)-caryophyllene	3.0
24.0587	1437	1434	□-trans-bergamotene	0.4
25.0248	1461	1460	Alloaromadendrene	11.3
25.7242	1477	1479	□-muurolene	0.7
25.9035	1482	1485	germacrene D	25.1
26.2663	1490	1493	trans-muurola-4(14),5-diene	0.5
26.3843	1493	1500	Bicyclogermacrene	1.7
26.6335	1499	1500	□-muurolene	2.0
26.9964	1508	1507	(Z)-□-bisabolene	0.5
27.1975	1514	1513	□-cadinene	4.7
27.5690	1524	1523	□-cadinene	12.9
28.0630	1537	1538	□-cadinene	0.4
29.5144	1575	1575	germacrene D-4-ol	6.0
30.5242	1600	1602	Ledol	0.5
31.4772	1627	1628	1-epi-cubenol	0.5
32.0018	1641	1640	□-epi-cadinol	4.7
32.1592	1645	1646	□-muurolol	0.8
32.2685	1648	1650	□-eudesmol	2.3
32.4696	1654	1654	□-cadinol	6.2
33.7198	1687	1689	Shyobunol	0.6
Total identified				99.7
Hydrocarbon monoterpenes				10.5
Hydrocarbon sesquiterpenes				67.6
Oxygenated sesquiterpenes				21.6

RT_{EXP}: Experimental retention time determined on a HP-5 fused silica;

RI_{EXP}: Experimental retention indexes determined on a HP-5 fused silica column relative to a series of n-alkanes (C_8–C_{40});

RI_{LIT}: Literature retention indexes as published by (Adams 2007).

Table S2. Antifungal activity (MIC and MLC) of *Teucrium scordium* subsp. *scordioides* for dermatophytes and yeasts

	T. scordium* subsp. *scordioides		**Fluconazole**	
	MIC[a]	**MLC[a]**	**MIC[b]**	**MLC[b]**
Trichophyton rubrum CECT 2794	0.32	0.64	128	≥128
T. mentagrophytes var. *interdigitale* CECT 2958	0.32	2.5	128	≥128
Epidermophyton floccosum FF9	0.32	0.64	16	16
T. mentagrophytes FF7	0.64	0.64	16-32	32-64
Microsporum canis FF1	0.64	1.25	128	128
M. gypseum CECT 2905	0.64	2.5	128	>128
T. verrucosum CECT 2992	2.5	>2.5	128	>128
Cryptococcus neoformans CECT 1078	0.32	0.32	16	128
Candida albicans ATCC 10231	>5	>5	1	>128
C. tropicalis ATCC 13803	>5	>5	4	>128
C. krusei H9	>5	>5	64	64-128
C. guillermondii MAT23	>5	>5	8	8
C. parapsilosis ATCC 90018	>5	>5	<1	<1

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

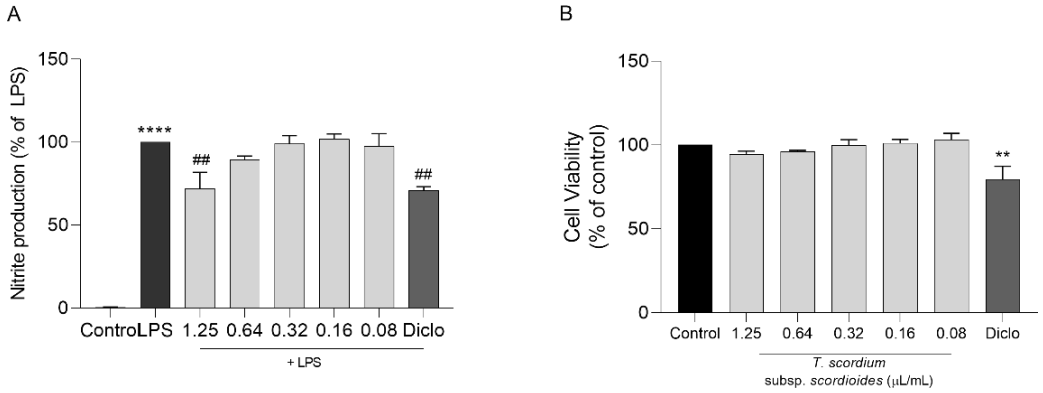

Figure S1. The essential oil from *T. scordium* subsp. *scordioides* decrease NO production in LPS-stimulated macrophages (A) without affecting cell viability (B). Diclofenac (Diclo) was used as positive control at a concentration of 1.591 µg/mL. (**p<0.01, ****p<0.0001 when compared to the control, ##p<0.01 when compared to LPS after ANOVA followed by Tukey's multiple comparison test)

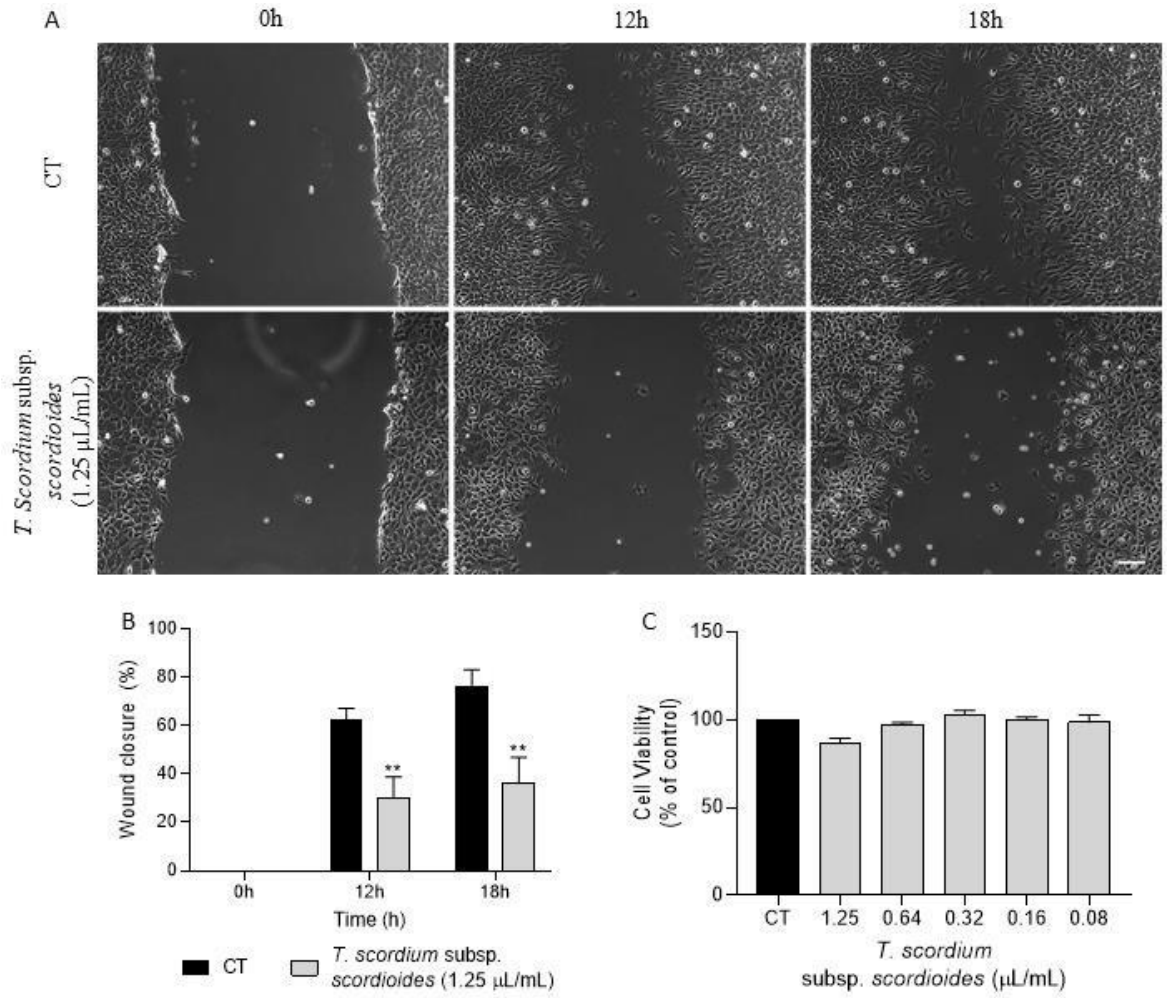

Figure S2. The essential oil from *T. scordium* subsp. *scordioides* decreases cell migration in 3T3 fibroblasts (A and B) without affecting cells viability (C). (**$p<0.01$ when compared to the control after 2-way ANOVA followed by Sidak's multiple comparison test). Scale bar: 100 µm

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