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New insights on the antifungal activity of essential oil of *Salvia desoleana* Atzei et Picci, an endemic plant from folk medicine of Sardinia, Italy

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This work reports the results concerning the antifungal activity of the essential oil obtained from *Salvia desoleana*, an endemic plant from folk medicine of Sardinia Island, Italy. Chemical analysis of *S. desoleana* essential oil isolated by hydrodistillation was carried out by gas chromatography (GC-FID) and gas chromatography–mass spectrometry (GC-MS). The essential oil contains high amounts of oxygenated monoterpenes and sesquiterpene hydrocarbons, being linalyl acetate (21.0%), α -terpinyl acetate (17.3%), 1,8-cineole (6.7%), linalool (3.6%), sclareol (3.5%) and germacrene D (22.1%) the main compounds. The oil was more active against the yeast *Cryptococcus neoformans* and the dermatophyte *Trichophyton rubrum* with MIC values of 0.16 μ L/mL and 0.32 μ L/mL, respectively. The oil revealed an important inhibitory effect on the germ tube formation in *C. albicans*. It was able to achieve about 40% of inhibition of filamentation at concentrations as low as 0.08 μ L/mL. These findings add significant information to the biological activity of the essential oil of *S. desoleana*, specifically to its antifungal properties, thus justifying and reinforcing the use of this plant in traditional medicine.

Keywords: Antifungal activity, Ethnomedicine, Essential oil, Germ tube, *Salvia desoleana*

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Salvia desoleana Atzei & Picci is an endemic Sardinian plant used in traditional medicine: leaves decoction used as antipyretic and compresses made with this plant have antiseptic and anti-inflammatory effects^{1,2}. Besides its traditional use, some articles on the chemical composition and biological activity of *S. desoleana* have been published³⁻⁵. Ceschel *et al.*³ tested the antimicrobial and the anti-inflammatory properties of *S. desoleana* essential oil on porcine buccal mucosa. Peana *et al.*⁴ found that the oil had a depressant action on the central nervous system in mice and an anti-inflammatory activity in rats. Sokovic *et al.*⁵ investigated the antifungal activities of *S. desoleana* essential oil and its three main components against micromycetes.

Due to the growing interest in the production of essential oil, the cultivated fields are supplanting wild biomass. Tests in the laboratory and in the field have

implemented the knowledge of large-scale cultivation of *S. desoleana*. Studies in open field have shown that plants are very resistant to dryness and require little fertilizer. Studies have shown that after three years of cultivation the plants reach an average height of 150 cm, with a weight ranging from 1200 grams to almost 2000 grams (which correspond to about 400-700 grams of dry material) for each plant^{6,7}. New biological properties have recently been demonstrated for its essential oil, notably antioxidant and antiviral properties^{8,9}.

Considering the traditional use of this plant in Sardinia, the aim of this study is to validate the antifungal potential of the essential oil of cultivated *S. desoleana*.

Methodology

Plant materials

The plants of *S. desoleana*, used for the present study, have been cultivated in Planta Medica

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greenhouse in the Laboratory of Plant Biology and Pharmaceutical Botany of the University of Cagliari, Italy, starting from seed.

After 28 days of seed germination in the growth chamber, seedlings were transplanted in small peat pots and kept in the greenhouse with a temperature of about (20-22) °C and under controlled irrigation. After three years, the whole aerial part of the plants were collected, dried in a forced ventilation stove with controlled temperature and humidity. The plant material was subjected to hydrodistillation. Voucher specimen (CAG1086b) was deposited in the *Herbarium Karalitanum* (Università di Cagliari, Viale S. Ignazio 13, Cagliari).

Essential oil isolation and analysis

Isolation of essential oil by hydrodistillation was performed in a Clevenger-type apparatus for 3 h¹⁰.

The essential oil sample was analyzed by using a gas chromatograph equipped with a flame ionization detector (GC-FID) to obtain the quantitative composition and by gas chromatography coupled to mass spectrometry (GC-MS) for constituents identification using the procedure described in Piras *et al.*¹¹. Constituents of the sample were identified by comparing mass spectra and linear retention indices (RI) with those reported in literature¹² or those of pure compounds whenever possible.

Fungal strains

The antifungal activity of the essential oil from leaves of *S. desoleana* was evaluated against yeasts and filamentous fungi: *Candida krusei* H9, *C. guilliermondii* MAT23, *C. albicans* ATCC 10231, *C. tropicalis* ATCC 13803, *C. parapsilopsis* ATCC 90018, *Cryptococcus neoformans* CECT 1078, *Epidermophyton floccosum* FF9, *Trichophyton mentagrophytes* FF7, *Microsporium canis* FF1, *T. mentagrophytes* var. *interdigitale* CECT 2958, *T. rubrum* CECT 2794, *T. verrucosum* CECT 2992 and *M. gypseum* CECT 2908.

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-A3¹³, M27-S3¹⁴ and M38-A2¹⁵ for yeasts and filamentous fungi, as previously described¹⁶.

Germ tube inhibition assay

The effect of sub-inhibitory concentrations of the essential oil on yeast-to-hypha transition, an important virulence factor of *C. albicans*, was determined as reported by Alves-Silva *et al.* (2020)¹⁶.

Results and Discussion

Chemical composition of the essential oil

In the investigated sample, a total of 44 components were identified, constituting 95.3% of the oil (Table 1). Germacrene D, linalyl acetate and α -terpinyl acetate were the most abundant components (22.1, 21.0 and 17.3% of total oil, respectively). Also, high contents of 1,8-cineole, linalool and sclareol were observed (amounting to 6.7, 3.6 and 3.5%). The present findings are in accordance with published data. Ghizzoni *et al.*² analyzed the oils derived from herbs collected from different Sardinian areas that are characterized by 1,8-cineole, linalyl acetate and α -terpinyl acetate as main components in all samples. Peana *et al.*¹⁷ in a sample of cultivated *S. desoleana* found above mentioned components together with linalool and α -terpineol. Moretti *et al.*¹⁸ investigated the oils from different experimental stations in Sardinia. Another study reported the presence of sclareol (1.6%) in addition to the usual main components⁵. They found linalyl acetate (19.8%), α -terpinyl acetate (13.0%), linalool (7.9%) and 1,8-cineole (7.2%) as major components. However, in this work, the authors did not specify if the plants are spontaneous or cultivated⁵. Posadino *et al.*⁹ identified germacrene D as main component in hydrodistilled oil from cultivated plant followed by α -terpinyl acetate and sclareol. Recently, Rapposelli *et al.*¹⁹ showed the differences between wild and cultivated *S. desoleana* populations. The same compounds were found in all samples, but their relative concentrations varied quantitatively. With this intraspecific variability, it is difficult to identify the cultivated and spontaneous sage from the analysis of the chemical composition.

Antifungal activity and mechanism of action

The antifungal activity is presented in Table 2; the oil was more effective against *Cryptococcus neoformans* (MIC = 0.16 μ L/mL) and dermatophyte strains, particularly, *Trichophyton rubrum* (MIC = 0.32 μ L/mL). Antifungal activity of *S. desoleana* essential oil was previously reported against *Aspergillus* and dermatophyte strains⁵. Our sample showed a more preeminent antifungal activity against dermatophytes and *Aspergillus niger* than the oil previously analysed. This may be due to the different chemical profiles of the two oils, particularly in the amounts of germacrene D.

In our study, *Candida* species and *Cryptococcus neoformans* were evaluated for the first time.

Table 1 — Composition of *S. desoleana* essential oil.

R _I	R _I (Lit)	Compound	Identification	% Area
925	924	α-thujene	MS, R _I	0.2
932	932	α-pinene	MS, R _I , Inj	0.4
972	969	sabinene	MS, R _I , Inj	0.8
976	974	β-pinene	MS, R _I , Inj	0.9
990	988	myrcene	MS, R _I , Inj	0.8
1016	1014	α-terpinene	MS, R _I , Inj	0.1
1027	1024	limonene	MS, R _I , Inj	0.5
1029	1026	1,8-cineole	MS, R _I , Inj	6.7
1035	1032	cis-ocimene	MS, R _I	1.3
1046	1044	trans-ocimene	MS, R _I	0.4
1057	1054	γ-terpinene	MS, R _I , Inj	0.1
1088	1086	terpinolene	MS, R _I , Inj	0.2
1099	1095	linalool	MS, R _I , Inj	3.6
1165	1162	δ-terpineol	MS, R _I	0.2
1176	1174	terpinen-4-ol	MS, R _I , Inj	0.2
1189	1186	α-terpineol	MS, R _I , Inj	1.6
1255	1254	linalyl acetate	MS, R _I	21.0
1303	1299	terpinen-4-ol-acetate	MS, R _I	0.2
1316	1316	δ-terpinyl acetate	MS, R _I	0.1
1348	1346	α-terpinyl acetate	MS, R _I , Inj	17.3
1365	1359	neryl acetate	MS, R _I , Inj	0.4
1375	1374	α-copaene	MS, R _I , Inj	1.0
1384	1379	geranyl acetate	MS, R _I , Inj	1.1
1389	1387	β-cubebene	MS, R _I	0.4
1391	1389	β-elemene	MS, R _I	0.2
1417	1417	β-caryophyllene	MS, R _I , Inj	1.5
1427	1430	β-copaene	MS, R _I	0.1
1437	1439	aromadendrene	MS, R _I , Inj	0.6
1482	1484	germacrene D	MS, R _I	22.1
1486	1489	β-selinene	MS, R _I	0.4
1495	1500	bicyclogermacrene	MS, R _I	1.3
1503	1508	germacrene A	MS, R _I	0.4
1512	1513	γ-cadinene	MS, R _I	0.5
1522	1522	δ-cadinene	MS, R _I	0.6
1612	1628	1,10-di-epi-cubenol	MS, R _I	0.3
1639	1638	epi-α-cadinol	MS, R _I	1.0
1647	1649	β-eudesmol	MS, R _I , Inj	1.0
1650	1652	α-eudesmol	MS, R _I	0.5
1876	-	sclareol oxide	MS	0.6
1918	-	β-springene	MS	0.2
1985	1987	manool oxide	MS, R _I , Inj	0.4
2006	2009	13-epi-manool oxide	MS, R _I , Inj	0.2
2051	2056	manool	MS, R _I , Inj	0.5
2214	2222	sclareol	MS, R _I , Inj	3.5
Total identified				95.3
Monoterpene hydrocarbons				5.6
Oxygen containing monoterpenes				52.3
Sesquiterpene hydrocarbons				29.1
Oxygen containing sesquiterpenes				2.9

R_I, retention index determined on a HP-5 fused silica column relative to a series of n-alkanes (C8-C26); R_I (Lit), retention index reported from the literature (Adams, 2007); Inj, injection of authentic compound.

Table 2 — Antifungal activity (MIC and MLC) of *S. desoleana* essential oil for yeasts, dermatophyte and *Aspergillus* strains.

Strains	<i>S. desoleana</i>	
	MIC	MLC
<i>Candida albicans</i> ATCC 10231	10	>10
<i>Candida tropicalis</i> ATCC 13803	10	>10
<i>Candida krusei</i> H9	5	>10
<i>Candida guilliermondii</i> MAT23	5	>10
<i>Candida parapsilosis</i> ATCC 90018	10	>10
<i>Cryptococcus neoformans</i> CECT 1078	0.16	0.32
<i>T. mentagrophytes</i> FF7	0.64	1.25
<i>T. mentagrophytes</i> var. <i>interdigitale</i> CECT 2958	0.64	2.5
<i>Trichophyton rubrum</i> CECT 2794	0.32	0.64
<i>T. verrucosum</i> CECT 2992	1.25	1.25
<i>Microsporium canis</i> FF1	1.25	1.25
<i>M. gypseum</i> CECT 2908	1.25	1.25-2.5
<i>Epidermophyton floccosum</i> FF9	0.64	0.64
<i>Aspergillus niger</i> ATCC16404	1.25	>10
<i>A. fumigatus</i> ATCC 46645	2.5	>10
<i>A. flavus</i> F44	>10	>10

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

Table 3 — Influence of sub-inhibitory concentrations of *S. desoleana* essential oil on germ tubeformation of *Candida albicans* ATCC 10231.

Control ^(a)	MIC/128 ^(b)	MIC/64	MIC/32	MIC/16	MIC/8
100	65.2±0.5	28±4	7.3±0.8	0±0	0±0

^aUntreated samples including 1% DMSO are considered as control, with 100% filamentation; ^bAbsolute concentration in μL mL⁻¹. The results are expressed as mean ± standard deviation of a minimum of three independent experiments performed in duplicate.

Interestingly, the essential oil also decreases the germ tube formation on *C. albicans*, an important virulence factor responsible for disseminative candidiasis. The effect of sub-inhibitory concentrations of the essential oil on the inhibition of *C. albicans* germ tube formation is presented in Table 3. The oil was able to achieve about 40% of inhibition of filamentation at concentrations as low as 0.08 μL/mL (MIC/128) and more than 70% at 0.16 μL/mL (MIC/64). Strikingly, fluconazole, a conventional antifungal drug widely used in the clinic, failed to inhibit the germ tube formation even at concentrations much higher than its respective MIC. This is quite interesting, since filamentation (dimorphic transition from yeast to filamentous form) in *C. albicans* is essential for virulence²⁰ and it seems that filamentation inhibition *per se* is sufficient to treat disseminated candidosis²¹. Overall, these results justify and explain the traditional uses of this species as antiseptic.

Conflicts of Interest

No potential conflict of interest was reported by the authors.

Author Contributions

AP: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Software, Supervision, Writing – original draft, Writing - review & editing; DF: Resources, Investigation, Formal analysis; AM: Conceptualization, Methodology, Resources, Project administration, Funding acquisition, Supervision, Validation, Writing – review; SP: Resources, Supervision; DM: Resources, Investigation; LS: Resources, Investigation, Formal analysis, Writing - original draft; MJG: Resources, Investigation, Formal analysis; CC: Resources, Investigation, Formal analysis.

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