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Comparison of *Salvia officinalis L.* essential oil and antifungal agents against *candida* species

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Abstract

Background: Systemic fungal infections due to pathogenic yeasts are increasing in high-risk patients, and a need is emerging for novel antifungal agents with potent inhibitory activity toward a wide range of pathogenic fungi. In this study we investigated the composition and antifungal activity of the essential oil of *Salvia officinalis* (Lamiaceae) against standard species of *Candida* and compared the results with commercial antifungal agents.

Methods: The aerial parts of *Saliva officinalis* were collected in May 2011. The essential oil was extracted and analyzed by gas chromatography–mass spectrometry. The susceptibility profiles of different *Candida* species were determined by micro broth dilution assays with oil extracts and a panel of antifungal agents.

Results: The minimum inhibitory concentrations of essential oil extracts against *C. albicans, C. parapsilosis, C. krusei* (standard species), *C. albicans* and *C. glabrata* (isolated from patients) were 15.6, 3.9, 31.3, 31.3 and 1.9 µg/ml, respectively. Chemical analysis of the essential oil revealed the presence of 40 components that made up 99.58% of the total composition. Cineole, borneol, α-thujone, ledene, β-pinene, α-humulene and trans-caryophyllene were the major components of the oil.

Conclusion: The oil extract of *Salvia officinalis* showed good antifungal activity, and could serve as a natural alternative to synthetic fungicides for the control of some important fungal diseases.

Keywords: Antifungal activity, *Candida albicans*, Cineole, *Candida glabrata*

Background

Salvia (S) *officinalis* L., a member of the Lamiaceae family popularly known as salvia or sage, is an aromatic plant widely distributed in the world. Common sage, since ancient times, has been an ingredient in perfumes, a flavoring in a variety of food preparations, and a medicinal plant used in the healthy Mediterranean diet. Hence its name, *Salvia*, which derives from the Latin meaning "to heal" [1-3].

The increase in nosocomial systemicfungal infections due to pathogenic yeast has led researchers to seek novel antifungal agents with potent inhibitory activity toward a wide range of pathogenic fungi and low side effects for patients. Essential oil (EO) extracted from *S. officinalis* is used in the treatment of a large range of diseases such as respiratory and digestive syndromes, heart and blood circulation, metabolic and endocrine diseases, as well as for its many other therapeutic effects [4,5]. Many properties have been reported for this plant, including its antibacterial activity against gram-positive cocci and bacilli such as *Staphyloccocu saureus* and *Bacillus subtilis*, and against gram-negative bacilli such as *Escherichia coli*. The EO also has cytotoxic activity against Vero cells and antiviral activity against HIV, *Herpes simplex* virus 1 and vesicular stomatitis virus [6-8], anti-angiogenic and antitumor effects [9], and antioxidant activity due to osmarinic acid, carnosic acid and phenolic components [10-13]. Recent studies have identified diterpenoids, triterpenoids, flavonoids and phenolic glycosides isolated from the plant [14-16].

In this study, we investigated the composition and antifungal activity of *S. officinalis* EO extracted from the flowers and leaves against standard species of *Candida* (C.) *albicans* (a frequent pathogenic species), *Candida glabrata* (one of the most resistant fungi to routine antifungal agents [17,18]), *Candida krusei* and *Candida parapsilosis*. We compared this activity with polyene and azole antifungal agents in broth microdilution assays.

Methods

The aerial parts of *S. officinalis* L. were collected from the pharmacological plant garden of Isfahan University of Medical Sciencesin Isfahan, central Iran, in May 2011.

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Vouchers were deposited in the herbarium of the Faculty of Science and identified by one of the authors (ARN). The leaves and flowers were harvested and cleaned in a shaded, well-aired place for 15 days. Fifty grams of the dried plant material was cut into small pieces and place in 500 ml distilled water for 2.5 hours at 100 °C after boiling at 290 °C with a clevenger apparatus [19]. The oils were obtained with n-pentane as a collecting solvent, and were dried over anhydrous sodium sulfate (Fluka, Steinheim, Germany). The EO was extracted at a yield of 4% per 50 g dried plant material, and was stored in amber vials at 4°C until analysis.

A dilution of the EO in hexane (10 mg/ml) was analyzed by gas chromatography-FID (Agilent Technologies 7890A, Turin, Italy) with a 30 m×0.32 mm capillary column, a film thickness of 0.25 µm and injector temperature of 280°C with nitrogen as the carrier gas. Mass spectrophotometry was performed (Agilent 5975C) under the following conditions: 30 m capillary column, film thickness 0.25 µm, temperature program 60°C for 5 min, then heating to 210°C at a rate of 3°C/min, injector temperature 280°C, with helium as the carrier gas. The constituents were identified by comparison of their mass spectra fragmentation, retention indices, and standard materials with authentic compounds or with data from the literature [20].

As laboratory standard species, we used *C. albicans* ATCC 90028, *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 along with three species of each *C. albicans* and *C. glabrata* [17] isolated from patients and identified with the API system (bioMerieux, Marcy l'Etoile, France). The susceptibility patterns of the species were determined by microbroth dilution assay tested with *S. officinalis* EO and fluconazole, amphotericin B, ketoconazole (Sigma-Aldrich Chemie, Steinheim, Germany), itraconazole (Jenssen Pharmaceutical, Beerse, Belgium), posaconazole(Noxafil, Schering-Plough, Kenilworth, NJ, USA), caspofungin (Merck & Co., Whitehouse Station, NJ, USA) and voriconazole (Pfizer, Tadworth, UK), according to CLSI M27-A2 guidelines [21].

Stock solutions of antifungal agents were prepared in dimethyl sulfoxide or water. Briefly, 100 µL RPMI 1640 broth (RPMI, Sigma Chemical Co., St. Louis, MO, USA) buffered to PH 7.0 with 0.165 M morpholine propanesulfonic acid buffer (Sigma) was poured into each well of 96-well plates. In the first column of wells, 100 µL of each antifungal agent or EO was added. To dissolve the oil extract in RPMI, 5 µL Tween 80 was added and after pipetting 5 times, 100 µL of the solution was transferred to the second column. Ten serial two-fold dilutions of the EO and antifungal agents were prepared and evaluated for minimum inhibitory concentration (MIC). The density of the *Candida* spp. suspensions used as the inoculum was adjusted spectrophotometrically to 0.5 McFarland standard (equivalent to 1–5 × 10^6 C fu/ml) and diluted 1:1000 in RPMI 1640 medium (Sigma). Then 100 µL of the fungal suspension was added to each well except for negative controls. In each series, the positive control received no antifungal or EO extract, and one negative control with no fungal suspension was also used.

Antifungal activity was seen at 30 to 0.064 mg/ml for aqueous EO, at final concentrations from 8 to 0.016 µg/ml for amphotericin B, concentrations from 16 to 0.032 µg/ml for itraconazole, ketoconazole, voriconazole, posaconazole and caspofungin, and concentrations from 128 to 0.250 µg/ml for fluconazole. The plates were incubated at 35°C for 24 and 48 h. The MIC for amphotericin B and EO was defined as the lowest drug concentration that zone determined the point of complete inhibition (100%), and for itraconazole, fluconazole, voriconazole, ketoconazole, posaconazole and caspofungin the growth should be decreased by 80%, compared with the respective controls after 24 or 48 hours of visual growth. The results are reported as the mean values of the data recorded in three different experiments.

Results

The EO was effective against *Candida* spp. and inhibited the growth of all fungi tested in a dose-dependent manner, at a concentration comparable to that of some other antifungal agents. The MIC of the EO extract was 15.6 µg/ml against *C. albicans,* 3.9 µg/ml against *C. parapsilosis,* 31.3 µg/ml against *C. krusei* (standard species),31.3 µg/ml against *C. albicans,* and 1.9 µg/ml against *C. glabrata* (isolated from the patients). Because we compared the EO activity with that of antifungal agents, we report our results in µg/ml rather than mg/ml, as in similar studies. The MIC of the EO and other antifungal agents are shown in **Table 1**.

Chemical analysis of the EO revealed the presence of 40 components making up to 99.58% of the total material (**Table 2**). The major components were cineole (13.69%), borneol (13.77%), α-thujone (12.46%), ledene (11.05%), β-pinene (7.00%), α-humulene (6.92%), trans-caryophyllene (5.28%), β-thujone (4.56%), camphor (3.58%) and naphthalene (3.27%). Oxygen-containing monoterpenes including cineole, borneol, camphor, α-thujone and ledene predominated.

Discussion

The species of fungus used in this study were chosen primarily on the basis of their pathogenicity and susceptibility to antifungal agents. The standard species were used in this study because of the sufficient knowledge about the susceptibility pattern of these fungi and *C. albicans*, and *C. glabrata* are, the routine pathogenic fungi isolated from the patients. Resistance to antifungal agents such as fluconazole and itraconazole in some species of fungi involved in human infection including *C. glabrata* and *C. krusei* has reportedly increased in recent years [22-24]. At the same time, interest has grown in the possible use of natural medicinal plants and plant products as alternatives to inhibit fungal growth.

Our data show that the MIC for all species treated with EO was lower than in other reports, and the EO extracts exhibited substantial antifungal activity against *C. glabrata,*

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Table 1. Minimum inhibitory concentrations of *Salvia officinalis* L. essential oil and known antifungal agents after 24 hours.

Candida species	Essential oil (µg /ml)	Amphoericin B (µg/ml)	Itraconzole (µg/ml)	Fluconazole (µg/ml)	Voriconazole (µg/ml)	Ketoconazole (µg/ml)	Posaconazole (µg/ml)	Caspofungin (µg/ml)
C. albicans	31.3	0.38	0.004	1.5	0.023	0.016	0.125	0.094
C. glabrata	1.9	0.064	3.0	64	0.125	1.0	2.0	0.064
*C. albicans**	15.6	0.50	0.064	2.0	0.032	0.016	0.032	0.064
*C. parapsilosis**	3.9	0.25	0.032	1.0	0.032	0.032	0.032	0.064
*C. krusei**	31.3	0.5	0.5	16	0.25	0.5	0.250	0.032

* Standard species

Table 2. Chemical composition of the essential oil of Salvia officinalis aerial parts.

No	Compound	Percent of compound	Retention indices
1	Borneol	13.77	13.896
2	Cineole	13.69	8.717
3	Alpha.-Thujone	12.46	11.487
4	Ledene	11.05	31.239
5	Beta-Pinene	7.00	6.966
6	Alpha.-Humulene	6.92	25.803
7	Trans-Caryophyllene	5.28	24.413
8	Beta-Thujone	4.56	11.864
9	Alpha -Pinene	3.89	5.771
10	Camphor	3.58	12.946
11	Naphthalene	3.27	46.877
12	Camphene	2.86	6.165
13	Bicyclo	1.75	18.839
14	Limonene	0.94	8.586
15	Caryophyllene oxide	0.84	30.821
16	Beta.-Myrcene	0.69	7.310
17	Alpha Terpineol	0.64	14.823
18	Gamma.-Terpinene	0.63	9.616
19	Oxabicyclo	0.47	31.817
20	Cyclohexen	0.37	14.279
21	Alpha-Thujene	0.33	5.570
22	Dimethy	0.33	32.807
23	Alpha.-Terpinene	0.32	8.157
24	Alpha-Terpinolene	0.32	10.714
25	Linalool	0.32	11.224
26	Delta.-Cadinene	0.27	28.538
27	Sabinene	0.25	6.835
28	Bicyclo	0.25	9.919
29	Cyclohexadiene	0.24	12.751
30	Aromadendrene	0.22	25.162
31	H-Cycloprop	0.22	26.043
32	H-Cycloprop	0.22	30.615
33	cis-Ocimene	0.21	8.849
34	Benzene	0.19	8.425
35	Isoaromadendrene epoxide	0.18	32.664
36	Naphthalenemethanol	0.18	33.310
37	Bicyclo	0.17	11.126
38	Alpha.-Amorphene	0.17	26.690
39	Phenanthrene	0.15	42.288
40	Isoaromadendrene epoxide	0.14	34.077

which is highly resistantto itraconazole and fluconazole, two routine antifungal agents used in clinical practice, and posaconazole, a new antifungal agent [17,18].According to **Table 1**, the MIC of the *S. officinalis* EO extract was lower for *C. glabrata* than for other fungi. The EO obtained from anise seeds (*Pimpinellaanisu* mL.,Apiaceae) showed antimycotic activity against some pathogenic *Candida* species, but no activity against *C. glabrata* [25]. Earlier research showed that the antifungal activity of any agent depends on the species of fungus and on the plant species. *Salvia dominica* and *S. officinalis* inhibited the growth of *C. albicans*, but *Salvia spinosa* showed no activity against this yeast [26]. The EO of other *Salvia* species was previously shown to have antifungal activity against various *Candida* species [27,28]. In one report from Turkey, the MIC ranged from 3.12 to 25 mg/ml and as the authors reported " all the extracts exhibited a strong antifungal effect against the fungal cultures" [30]. Bioassays with the EO of *Salvia lachnocalyx* showed significant inhibition against fungi, with an MIC in the range of 5-10 mg/ml [31].

In the present study, the lower MIC for *S. officinalis* EO maybe related to regional variations in the composition of this species. Chemically, EO are primarily composed of mono- and sesquiterpenes and aromatic polypropanoids [30,31] with different amounts and types of oxygenated monoterpene components such as α-thujone, 1,8-cineol, camphor, borneol and bornyl acetate or sesquiterpene components, humulene, viridiflorol and manool [14,28,32]. The antifungal activities of sesquiterpenoid constituents were superior to those of monoterpenoid constituents. Among active sesquiterpenoids, T-murolol and α-cadinol possess the greatest activity against plant pathogenic fungi. Limonene and β-myrcene also showed weak antifungal activity [33]. The major components of *Salvia* species growing in Turkey are α-pinene, β-pinene, β-thujone, camphor, carvacrol, lynalyl acetate, sabinyl acetate and cineole [28]. The main differences between the compositions of the EO of sage from Turkey and the sage used in the present study were in the proportions of α-pinene, limonene, linalool and especially viridiflorol, humulene, manool, which were lower in our material.

Drinking sage (*S .officinalis*) tea could have a hepatotoxicity effect due to free radical formation of CCl_4 [34] and

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considering the toxicity of some components like thujone as reported [35]. However, there are some studies, which have not reported any level of hepatotoxicity [36]. Therefore, further studies are neededto make the findings more definitive.

Conclusions

Our results support the antifungal activityof the EO extract of S. *officinalis*L. against pathogenic *Candida* species. More investigations of the antifungal elements of EO and purification of these products will contribute to the development of new natural antifungal drugs for resistant strains of fungi, which could replace currently available synthetic agents.

List of abbreviations

S: Salvia
EO: essential oil
MIC: minimum inhibitory concentration

Competing interests

The authors report no competing interests related to this study.

Authors' contributions

PB conceived and designed the study, participated in the analysis and interpretation of the data and drafted the manuscript. ARN identified the plant species and performed gas chromatography-mass spectrometry analyses. MM extracted the essential oils. All authors read and approved the final manuscript.

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