ANTIFUNGAL ACTIVITY OF *Origanum syriacum* L. ESSENTIAL OILS AGAINST *Candida spp.*

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The infections caused by Candida species have been reported to cause serious life-threatening infections. Because the increased drug resistance among candida spp., new drug researches are intensively carried out. *Origanum syriacum* L. is a valuable medicinal plant in terms of its components. The aim of this study was to investigate components and the antifungal activity of *Origanum syriacum* L. essential oils on the growth of *Candida spp.* By GC-MS was highlighted that thymol (42,18 %), carvacrol (33,95 %), cymene (8,87 %) and γ-terpinene (8,21 %) are the main components. Antifungal activity of the essential oils of *Origanum syriacum* L. was evaluated by microdilution method. The most notable activity was obtained against *C. dubliniensis*, *C. albicans* and *C. parapsilosis*. MIC values were found to be 15.6 *C.* for *C. dubliniensis* and 31.2 µg/ml for *C. parapsilosis* and *C. albicans*. MIC values against *C. glabrata*, *C. kefyr*, *C. intermedia* were found to be 62.5, MIC values for *C. krusei* and *C. glabrata* were 125 µg/ml. The highest activity in terms of MBC values in the study was obtained against *C. intermedia* and *C. parapsilosis* (62.5 µg/ml). Our results are particularly promising to have significant activity against *C. dubliniensis*, *C. albicans* and *C. parapsilosis*. *Origanum syriacum* L. essential oils, which were also effective against non-albicans Candida species, may be a new hope for the treatment of candida infections.

Keywords: Candida, *Origanum syriacum* L., essential oils, antifungal, resistance

INTRODUCTION

Candida species are microorganisms found in the normal flora of many body parts such as human skin and mucosa. *Candida albicans* and Candida species other than *Candida albicans* have also been reported to cause serious life-threatening infections. Especially, in recent years, the increased drug resistance among candida spp. is striking. Therefore, new drug researches are intensively carried out (Spampinato and Leonardi, 2013).

Origanum syriacum L. is a valuable medicinal plant in terms of its components. It is possible to find many studies about Origanum genus. It has been reported to have major components of thymol and carvacrol in component analysis of *Origanum syriacum* L. It has been reported that these components have considerable pharmacological properties.

Candida species are the most common microorganisms found in the skin and mucous membranes of humans. It is found in the mouth and gastrointestinal tract of 30-50% of healthy people (Spampinato and Leonardi, 2013; Sanguinetti *et al*., 2015). Due to some predispose causes; Candida spp. can cause superficial, deep, acute and/or chronic infections in humans. Most candida infections are endogenous infections. Candida albicans is most frequently isolated from Candida infections. In recent years, the incidence of candidiasis caused by Candida species other than *C. albicans* has increased. Mortality and mortality are high in infections caused by candidiasis. In recent years, the increase in candida species other than *Candida albicans* and the use of antifungal drugs for prophylactic purposes have led to an increase in drug resistance. The increase in the frequency of Candida species other than *Candida albicans* has led to

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the development of resistance especially in azole derivative antifungals. It has also been shown that many Candida isolates develop amphotericin B resistance. Therefore, the search for new antifungal agents especially on natural products continues intensively (Sanguinetti *et al*., 2015; Golabek *et al*., 2015).

The aim of this study was to identify the components of *Origanum syriacum* L. essential oils and to investigate their antifungal activity against Candida spp.

MATERIALS AND METHODS

Clinical isolates and standard candida strains were used in the study. Eight strains were used from each of the clinical isolates used in the study. The strains used were:*C. albicans, C. tropicalis, C. kefyr, C. krusei, C. glabrata, C. dubliniensis, C. intermedia, C. parapsilosis*. Clinical isolates were Candida strains isolated from samples sent to routine mycology laboratory from various clinical specimens. Isolated strains were inoculated into Sabouraud-dextrose-agar media for identification and incubated at 37 °C for 48 hours. For typing, isolates were evaluated in terms of germ tube test, hif formation in corn flour aged with Tween 80, pseudo-hif formation, blastospor and chlamydospor formation properties. When needed, automated culture systems (Vitek-2, BioMerieux France) were utilized for typing.

Plant Materials

Origanum syriacum L. plants were harvested in Hatay province of Turkey during the full bloom period, when the amount of active substance was most intense and after being dried in the shade at room temperature.

Preparation of Plant Extracts

Plant material was weighted and placed in a round bottom flask with a volume of distilled water as extraction solvent; the herba-water mixture was refluxed about 2 h, during which the oil was collected in the side arm of the system. The installation was allowed to stand for about half hour to prevent the oil to reach room temperature. The oil was dried over anhydrous sodium sulphate and then stored in dark color glass bottles and keep to refrigerator (about 4 °C) until use for analysis.

GC/MS Analysis

Analysis of essential oil was performed using the Thermo Scientific Focus gas chromatograph equipped with a DSQ II single quadrupole mass spectrometer, Triplus autosampler and fused-silica capillary columnTR-5MS (5% phenyl-polysilphenylene-siloxane, 30 m×0.25 mm inner diameter, film thickness 0.25 µm). The injection volume was 2 µL. The samples were injected with a split ratio of 250:1 by using helium (99.99%) as carrier gas, at a flow rate of 1 mL/min; ionization energy was 70 eV. The transfer line temperature of the mass spectrometer was 220 °C, while the temperature of orifice injection was of 220 °C. The temperature of oven was programmed in the range 50–220 °C at a rate of 3 °C/min. Data acquisition was made in the scanning mode. Identification was done on full scan mode in the m/z range of 50–650 a.m.u.

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Determination of Minimal Fungicidal Concentration (MFC)

Antifungal activity of the essential oils of *Origanum syriacum* L. was evaluated by microdilution method. Clinical and standard isolates were grown in SDA (Saboraud's Dextrose Broth) medium at 37 °C for 48 hours. Final candida concentrations were adjusted to 10^8 cfu/mL with reference to the McFarland turbid meter.

Determination of Minimum Inhibitory Concentrations (MIC)

MIC values of *Origanum syriacum* L. essential oils were determined by the broth microdilution test. Firstly, all isolates were subcultured in Saboraud Dextrox Broth and incubated for 24 h at 37 °C. Then, two-fold serial dilutions of essential oils were made in Saboraud Dextrox Broth to achieve a concentration range from 0 to 500 µg/ml. (500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, 1.8, 0.9) The broth microdilution tests were performed according to the NCCLS guidelines (CLSI, 2008).

RESULTS

Seventeen essential oil components, representing 98,76 %, were detected in the essential oil of *Origanum syriacum* L. (Figure 1, Table 1). The major essential oil constituents were thymol (42,18 %), carvacrol (33,95 %), cymene (8,87 %) and γ-terpinene (8,21 %).

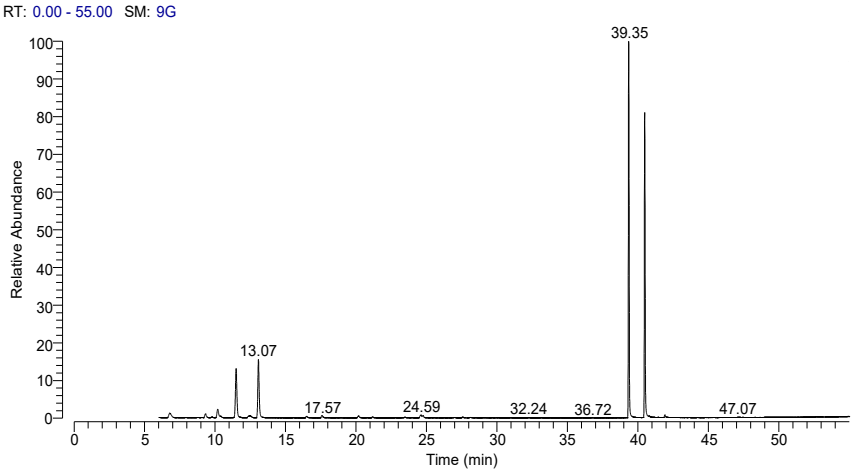

Figure 1. GC/MS chromatogram of *Origanum syriacum* L.

Table 1. Essential oil components of *Origanum syriacum* L.

RT	Compound Name	SI	RSI	Cas #	%
6,78	α-Pinene	942	969	80-56-8	1,02
9,31	β-Myrcene	956	985	123-35-3	0,64
9,76	α-Phellandrene	855	923	99-83-2	0,13
10,19	α-Terpinene	962	979	99-86-5	1,40
11,49	γ-Terpinene	988	992	99-85-4	8,21
12,45	Eucalyptol	876	889	470-82-6	0,61

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RT	Compound Name	SI	RSI	Cas #	%
13,07	Cymene	984	995	25155-15-1	8,87
16,47	3-Octanol	925	947	589-98-0	0,23
17,57	1-Octen-3-ol	955	982	3391-86-4	0,31
20,16	4-Thujanol	934	962	546-79-2	0,31
21,15	Linalool	913	930	78-70-6	0,15
24,58	trans-Caryophyllene	958	969	87-44-5	0,35
24,75	Terpinen-4-ol	930	954	562-74-3	0,20
27,55	α-terpineol	859	900	2438-12-2	0,12
28,12	Borneol	813	867	10385-78-1	0,08
39,35	Thymol	970	982	89-83-8	42,18
40,48	Carvacrol	966	966	499-75-2	33,95

The MIC and MBC results of *Origanum syriacum* L. were given in Table 2. In the study, considerable activities of *Origanum syriacum* L. essential oils were determined against all candida strains. The most notable activity was obtained against *C. dubliniensis*, *C. albicans* and *C. parapsilosis*. MIC values were found to be 15.6 *C.* for *C. dubliniensis* and 31.2 µg/ml for *C. parapsilosis* and *C. albicans* (Figure 2). MIC values against *C. glabrata*, *C. kefyr*, *C. intermedia* were found to be 62.5, MIC values for *C. krusei* and *C. glabrata* were 125 µg/ml. Table 2 also shows MFC values. The highest activity in terms of MFC values in the study was obtained against *C. intermedia* and *C. parapsilosis* (62.5 µg/ml) (Table 2).

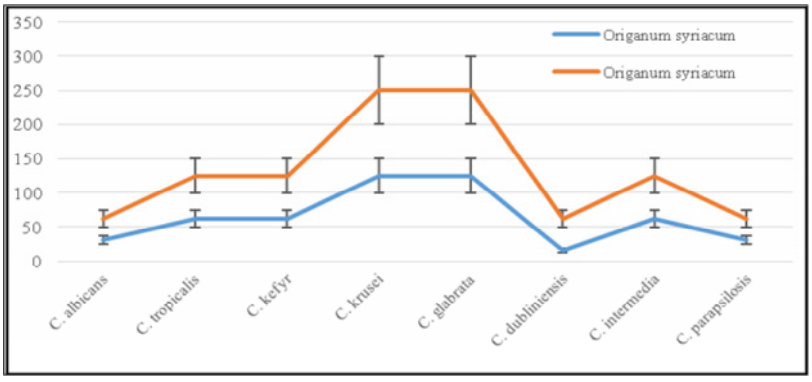

Figure 2. MIC and MFC values of *Origanum syriacum* L. essential oils against *Candida* species

Table 2. Comparison of MIC and MFC values of *Origanum syriacum* L. essential oils and fluconazole against *Candida* species

Candida species	*Origanum syriacum* L.		Fluconazole	
	MIC values µg/ml	MFC values µg/ml	MIC values µg/ml	MFC values µg/ml
C. albicans	31.6	62.5	1.8	3.9
C. tropicalis	62.5	125	3.9	3.9
C. kefyr	62.5	125	1.8	1.8
C. krusei	125	250	1.8	3.9

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C. glabrata	125	250	3.9	7.8
C. dubliniensis	15.6	125	3.9	3.9
C. intermedia	62.5	62.5	1.8	3.9
C. parapsilosis	31.2	62.5	1.8	3.9

DISCUSSION AND CONCLUSION

Recently, the prolongation of life span depending on the developments in medicine has led to an increase in the number of elderly and immunosuppressed patients (Spampinato and Leonardi, 2013; Sanguinetti *et al*., 2015; Sardi *et al*., 2013).

Candida infections have recently been isolated, especially in intensive care units and immunosuppressive patients. Due to its endogenous origin, *C. albicans* is the first line in nosocomial candida infections. But recently there has been a significant increase in the frequency of human infections caused by non-albicans candida species (Sardi *et al*., 2013).

It is known that antifungal treatment is more difficult to respond to non-albicans species such as *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. parapsilosis* and *C. lusitaniae*. Drug resistance is known to show significant increases in all candida species (Sanguinetti *et al*., 2015). For this reason, our results have a great prospect of investigating new active drug in increasing drug resistance.

Our results are particularly promising having significant activity against *C. dubliniensis*, *C. albicans* and *C. parapsilosis*. *Origanum syriacum* L. essential oils, which were also effective against non-albicans Candida species, may be a new hope for the treatment of candida infections.

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