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Original Article

In Vitro Anti-*Candida* Activity of *Zataria multiflora* Boiss

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Zataria multiflora Boiss known as Avishan Shirazi (in Iran) is one of the valuable Iranian medicinal plants. The aim of study was to evaluate anti-*Candida* activity of *Z. multiflora* against different species of *Candida in vitro*. Anti-*Candida* activity of the aqueous, ethanolic and methanolic maceration extract of the aerial parts of *Z. multiflora Boiss* was studied *in vitro*. Anti-*Candida* activity against *Candida* species was done using serial dilutions of extracts in Sabouraud's dextrose agar. Minimal inhibitory concentration (MIC) of the methanolic and ethanolic extracts was 70.7 and 127 mg l^{-1}, respectively. Aqueous extract showed no remarkable activity against *Candida* species. We conclude that methanolic extract of the aerial parts of *Z. multiflora* Boiss has more anti-*Candida* effect at 70.7 mg l^{-1} compared to ethanolic extract 127 mg l^{-1}. In addition, the isolates of *Candida parapsilosis* were more susceptible to methanolic extract than other tested species.

Keywords: anti-*Candida* – *Candida* – herbal medicine – *Zataria multiflora* Boiss

Introduction

Zataria multiflora Boiss (Lamiaceae) is a valuable medicinal plant grown extensively in Iran, Pakistan and Afghanistan (1). The chemical compositions of extracts have been extensively characterized in Iran (2–5) and Pakistan (6). The extract contains thymol, carvacrol (4,6), zatrinal, oleanolic acid, betulic acid, rosmarinic acid (5) and monoterpenoids, sesquiterpenoids, p-cymene, y-terpinene (3,4).

Aqueous and alcoholic extracts of *Z. multiflora* have been therapeutically used for relieving nociceptive pain (7,8), recurrent aphthous stomatitis (RAS) (9), and prevent growth of oral streptococci (10), *Plasmodium falciparum* (11) and *Trichomonas vaginalis* (12) as well as used as an insect repellent (6). Fataneh (13) has investigated anti-fungal properties of *Z. multiflora* extract *in vitr*o. In view of its potent antibacterial and anti-fungal activities, we hypothesized that *Z. multiflora* extracts may possess anti-*Candida* effects. We have tested the hypothesis *in vitro* by comparing the aqueous, ethanolic and methanolic extracts of *Z. multiflora* against 14 isolates of *Candida albicans, C. parapsilosis, C. tropicalis* and *C. glabrata.*

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Materials and Methods

Extraction

The plant was collected from Shiraz, Iran, and identified by Agricultural Research Centre, Ahwaz. The plant was identified in the Systematic Laboratory, Agricultural Sciences Centre, Ahwaz, Iran, where voucher specimens were deposited (ZM 1). Aliquots of 100 g of the dried powder of the plant were soaked in ethanol (1400 ml), methanol (1400 ml) and distilled water (2150 ml) for 24 h and then filtered with cloths. The extracts were concentrated to dryness in a *vacuo* at 53–55°C and yielded 11.39 g (aqueous extract), 15 g (ethanolic extract) and 13.3 g (methanolic extract).

Organisms

Fourteen isolates of *Candida* were studied including *C. albicans* ($n = 7$), *C. tropicalis* ($n = 3$), *C. parapsilosis* ($n = 2$) and *C. glabrata* ($n = 2$). All *Candida* species

were isolated from infected patients in the department of medical mycoparasitology, Jundishapour University of medical sciences, Ahwaz, Iran. All isolates were identified by CHROMagar Candida (CHROMagar Candida Company, Paris, France), germ-tube test, production of chlamydoconidia on Corn meal agar and growth at 45°C. Isolates were maintained on Sabouraud's dextrose agar (SDA) at 4°C. Organisms were subcultured on SDA and incubated at 37°C for 24 h. Several colonies of each *Candida* species were collected in 2 ml of sterile PBS to prepare a suspension. The suspension was adjusted to 70% transmittance (T) by a spectrophotometer at 530 nm. This should result in a suspension containing about 1×10^6 cfu per ml.

Test method

A serial dilution of each extract was prepared in SDA plates. Aqueous, ethanolic and methanolic extracts were diluted by the same solvent. The same solvent, at an appropriate concentration was also used as a negative control. A plate was considered as positive control without extracts and solvents. Aliquots of 20 µl of standardised suspension of different species of *Candida* were inoculated in to each plate. The plates were incubated at 30°C for 24–48 h. The lowest extract concentration where there was no visible growth was the minimal inhibitory concentration (MIC) when compared to control. All experiments were repeated three times and mean calculated.

Results

In the present study the anti-*Candida* activity of three extracts of *Z. multiflora* (aqueous, ethanolic and methanolic) was evaluated against 14 isolates of *Candida*. In the first stage, aqueous, ethanolic and methanolic extracts of *Z. multiflora* applied on one isolate of each *Candida* species. Aqueous extract of *Z. multiflora* showed no activity against *Candida* species. As a result this extract was removed from the next experiments. The ethanolic and methanolic extracts showed remarkable activities against *Candida* species. The MIC for both extract was between 50 and 150 mg l^{-1}. In the second stage, ethanolic and methanolic extracts were used for the detection of MIC.

Ethanolic Extract

Table 1 shows details of mean MICs of ethanolic extract against 14 isolates of *Candida*. As shown the lowest MIC was for 7 isolates of *C. albicans* (125 mg l^{-1}). Others MICs were respectively *C. glabrata* (126 mg l^{-1}), *C. parapsilosis* (125 mg l^{-1}) and *C. tropicalis* (131 mg l^{-1}). Totally, the MIC of ethanolic extract for 14 isolates of *Candida* was 127 mg l^{-1}. As shown both *C. albicans* and *C. parapsilosis* are more susceptible than other species.

Methanolic Extract

Table 1 shows the details of mean MICs of methanolic extract against tested *Candida*. As shown isolates of *C. parapsilosis* (64 mg l^{-1}) are more susceptible to methanolic extract of *Z. multiflora*, followed by *C. glabrata* (66 mg l^{-1}), *C. albicans* (76 mg l^{-1}) and *C. tropicalis* (76 mg l^{-1}). Totally, the MIC of methanolic extract for tested *Candida* was 70 mg l^{-1}. In the present study methanolic extract showed more activities than ethanolic extract against 14 isolates of *Candida*.

Discussion

Herbal and alternative medicines are popular in the general population worldwide. A great number of modern drugs are still derived from herbs (14). Iranian scientist, Avicenna (980–1037) and Razi (846–930) published several books on herbal medicine a few centuries ago and are still in use in different libraries in Europe (15). *Z. multiflora* grows wild in

Table 1. Minimal inhibitory concentration (MIC) of ethanolic (E) and methanolic (M) extracts of *Zataria multiflora* Boiss (Lamiaceae) required for inhibition of *Candida* species from 14 isolates from infected patients

Minimal inhibitory concentration (mg l^{-1})								
C. albicans (7)		*C. tropicalis* (3)		*C. glabrata* (2)		*C. parapsilosis* (2)		
E extract	M extract	E extract	M extract	E extract	M extract	E extract	M extract	
146	93	139	93	123	63	125	63	
130	73	126	66	130	70	125	66	
110	76	129	70					
116	70							
120	66							
125	86							
129	66							
876	530	394	229	253	133	250	129	Total
125.1	75.7	131.3	76.3	126.5	66.5	125	64.5	Mean
10.8	9.5	5.6	11.9	3.5	3.5	0.0	1.5	SD

Values in parenthesis refer to the number of isolates.
C. albicans (7): CA1, CA2, CA3, CA4, CA6, CA7, CA11; *C. tropicalis* (3): CT2, CT3, CT4; *C. glabrata* (2): CG 1, CG3; *C. parapsilosis* (2): CP1, CP2.

central and southern Iran. *Z. multiflora* is used in traditional herbal medicine for antiseptic, analgesic, and carminative properties (2,7,16). *Z. multiflora* was also used for treatment of 'Women disease' in Iranian folklore (17). The leaf powder of *Z. multiflora* is used as nutritional flavoring in Iran. It is important to investigate scientifically those plants, which have been used in traditional medicines as potential sources of novel antimicrobial compounds.

The presence of thymol, rosmarinic acid, and carvacrol in the different parts of the plant was observed (6). The present results indicate that methanolic extracts of the aerial part of *Z. multiflora* have marked activity against isolates of *Candida*. Probably, the anti-*Candida* activity of methanolic extract of *Z. multiflora* is due to both rosmarinic acid and thymol that extracted only into methanol (2). Probably, the anti-*Candida* activity of methanolic extract of *Z. multiflora* is due to above compounds. *Z. multiflora* is used in traditional herbal medicine for women disease (*Candidiasis vagina*). Fataneh (13) have shown that *Z. multiflora* has anti-fungal activity. They tested several isolates of dermatophytes and saprophytic fungi against *Z. multiflora* extract. Amanlou *et al*. (18) have shown that *Z. multiflora* has antierythema in denture stomatitis compared to miconazole gel, however, *Z. multiflora* gel did not reduce the colony count of the denture surface as efficiently as miconazole gel. The ethanolic extracts of aerial parts of *Z. multiflora* showed antinociceptive activity (19,20). Phytochemical screening supported the presence of flavonoids in *Z. multiflora*. Some flavonoids exert antinociceptive activity in mice (19). Ramazani *et al*. (8) reported six fractions of the extracts of aerial parts of *Z. multiflora* that have antinociceptive activity.

We conclude that *Z. multiflora* represents an untapped source of potentially useful anti-*Candida* and is worthy for future clinical study. In addition, measures must to be undertaken to preserve the traditional knowledge about medicinal plants.

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