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In-vitro inhibitory effect of ethanolic and methanolic extract of *Scrophularia striata* on *Candida spp.*

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Abstract

Candida species are the most common cause of opportunistic fungal infection worldwide. Due to increasing resistance of fungi against conventional drugs, as well as their side effects, alternative natural products have become a renewed interest. In the current study, the anti-Candida activities of *Scrophularia striata* extract were examined. In this experimental study, microdilution assay and well diffusion test were used to determine *in vitro* anti-fungal effects of ethanolic and methanolic extract of *Scrophularia striata* on four species of Candida including *Candida albicans ATCC 1167*, *ATCC 1677*, *tropicalis*, and *glabrata*. The results showed that 100 mg/ml methanolic extract has more anticandida activity than ethanolic extract. Diameter of inhibiting growth environment for ethanol extract was 37 mm, and MIC and MFC were 2.1 and 41.5 mm/ml. Results show that ethanolic and methanolic extract of *Scrophularia striata* have anti-fungal activities. Therefore results of this study suggest this extract is a promising anti-fungal agent, and future experiments will interest for performing *in vivo* study.

Keywords: *Scrophularia striata*, Candida, ehanolic extract, methanolic extract

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Introduction

In the past two decades, the prevalence of candidiasis has been increased. Candida species are able to create superficial and systemic infections (1). *Candida albicans* is an opportunistic pathogen, causing mycoses in immunocompromised patients as well as long-term antibiotic users (2-4). Also, other Candida species such as *C.glabrata*, *C.parapsilosis*, *C.tropicalis* and *C.krusei* are among the oral mucosal lesions suspected agents in AIDS patients (4-6). Medicinal plants may demonstrate a valuable, original source of recent antifungal drugs and have been shown to contain diverse biochemical and pharmacological actions (7, 8). Their antifungal activity has been showed against some Candida species (9-11).

One of the plants proposed to have immunomodulatory and anti-inflammatory effects is *Scrophularia striata* Boiss (Scrophulariaceae). *Scrophularia striata* (in the Scrophulariacea family) is vernacular to Iran. *Scrophularia striata* color is purple (12-14). Many Scrophularia species have been investigated and several compounds from different classes of secondary metabolites including iridoids, phenyl propanoids, phenolic acids, flavonoids, quercetin, isorhamnetin 3-Orutinoside and saponins by column chromatography have been isolated (15). These compounds usually are found in root, leaves, or buds of the plant (16, 17). *Scrophularia striata* traditionally has been used for infections, high blood pressure, and stomach disorders (18-20).

previous studies have suggested the inhibitory effect of *S.striata* extract on microorganisms (21, 22). In addition, anti-inflammatory, and immunomodulatory activity of some species of Scrophularia have been showed (23, 24).

In the present study, we investigated the *in vitro* inhibitory effects of *Scrophularia striata* ethanolic and

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metanolic extract on including *Candida albicans ATCC 1167, ATCC 1677*, *tropicalis*, and *glabrata*.

Materials and method

This is an experimental study conducted on *Candida albicans ATCC 1167*, *ATCC 1677*, *tropicalis*, and *glabrata*. *Scrophularia striata* was collected in 2013 May from Zagros mountain in Ilam, Iran and was detected by research center of natural resources in herbarium department in Isfahan.

All part of the plant were washed and dried. Then areal parts of plants such as stalk and leaves were powdered by electric blender. Themaceration method was used to make alcoholic extract. Then, 50 g was macerated with 100 mL of 80% ethanol and methanol on a rotary shaker for 48 and 72 hours, filtered, and then the solution. After, the extracts were taken and filtered by using a millipore filter paper. Then, the extracts were concentrated using a rotary evaporator at 40 °C under reduced pressure. Finally, the extracts were dried and stored at 4 °C till their usage in the different tests. The extracts were dissolved in dimethylsulfoxide (DMSO).

Herbal component can be dissolved in methanol, ethanol, percolation or maceration can be used to extract them. The advantage of alcohol extraction is that different alkaloids or alkaline salts can be suit, in addition to the water-soluble impurities such as polysaccharides, proteins are less extracted but its drawback is more fat-soluble impurities is extracted.

Antifungal activity was studied by the agar well diffusion method. Sabouraud dextrose agar (SDA) was used as the fungal medium. The effect of extract antimicrobial were assessed in the concentration include 3.14, 6.25, 12.5, 25, 50, and 100 (mg/ml) using agar well diffusion method and also by determination minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) using microdilution test method. The extracts were diluted in 1mL of dimethylsulphoxide (DMSO) at the concentrations of 3.14, 6.25, 12.5, 25, 50, and 100 (mg/ml) sabouraud dextrose agar. Petridishes (8 cm diameter) containing 20 ml of SDA were used for antifungal activity assay, performed on solid media by the well diffusion method sterile. Wells were prepared in the seeded agar petridishes. The study compound was introduced in the well (6 mm). The plate were incubated at 37 °C for 24- 48 h. Growth inhabitation of each fungal strains was counted as the percentage of prevent of radial grow relative to the control, fluconazol. A standard antifungal agent, fluconazol served as a positive control and DMSO was used as a negative control. To identify the minimum inhibitory concentration (MIC) microdilution method was used. In this experiment microplates of 96 cells were used in the first row wells 100 microliter of cultivation medium of SDA were put, then 100 microliter of the extract of the plant was added to the first well. Then 100 microliter of extract was added to the second wells and it was repeated until the stage in which 10 microliter of ferment suspension containing 1000 cells in each milliliter was added to the same well. In each row Alcohol with medium culture and ferment served as negative control and stril physiologic serum with culture medium and ferment served as positive control (24, 25).

Statistical analysis

The experiment was performed three times to minimize the error and the mean values are presented. Data were analyzed using ANOVA test in the $p < 0.05$ (SPSS Software, Chicago, USA)

Results

The growth inhibition value of ethanolic and methanolic extracts of *S.striata* on *Candida albicans ATCC 1167, ATCC 1677*, *tropicalis*, and *glabrata* in tables 1 and 2 are shown.

The strongest activity was seen against *C.glabrata* with a range of 41.3 mm mean inhibition zones and 15.62-21.87 mg/ml MIC values. The MIC and MFC of these extracts are currently being obtained in table 3.The methanolic extract of the tested plant has more anti-candida effects as compared to the ethanolic extract. Moreover, the results showed that 100 mg/ml methanolic extract has more anti-candida activity.

Discussion

Different studies have indicated that the many species of Scrophularia contains substances that have antimicrobial activities (26-29). Results obtained in pervious study show that *Scrophularia striata* extracts have selective antimicrobial activity on the basis of the cell-wall differences of bacterial and fungal microorganisms (30, 31). In recent years, tendency of using natural sources as alternative medicine has been raised (32).

Table 1 The antifungal activity of methanolic extract of *S. striata* on Candida spp*

Concentration Fungi	3. 12 mg/ml	6. 25 mg/ml	12. 5 mg/ml	25mg/ml	50mg/ml	100 mg/ml
***Candida albicans* (1167)**	3.66 ± 1.52	12.33 ± 1.50	19.8 3 ± 1.25	26.50 ± 1.80	37 ± 2	39.5 ± 0.5
***Candida albicans* (1677)**	4.66 ± 2.08	11.83 ± 1.60	22.16 ± 1.89	25.16 ± 1.60	28.8 ± 0.7	34.6 ± 4
Candida tropicalis	3.00 ± 0.30	12.00 ± 3.40	18.23 ± 23.00	24.50 ± 3.91	28.8 ± 2.0	34.6 ± 2.4
Candida glabrata	3.00 ± 2.00	13.86 ± 0.80	18.50 ± 0.50	28.00 ± 1.00	37.3 ± 2.0	41.3 ± 3.0

* Data present as mm (Mean ± SD)

Table 2 The antifungal activity of ethanolic extract of *S. striata* on Candida spp*

Concentration Fungi	3. 12 mg/ml	6. 25 mg/ml	12. 5 mg/ml	25mg/ml	50mg/ml	100mg/ml
Candida albicans (1167)	-	3.16 ± 0.28 mm	7.16 ± 0.28 mm	12.00 ± 0.50 mm	18.23 ± 0.76 mm	25.33 ± 4.33 mm
Candida albicans (1677)	-	4.83 ± 0.73 mm	10.66 ± 1.15 mm	13.20 ± 1.31 mm	29.9 ± 1.8 mm	26.8 ± 1.15 mm
Candida tropicalis	-	7.83 ± 1.04 mm	10.50 ± 0.80 mm	18.66 ± 1.60 mm	33.3 ± 1.5 mm	28.8 ± 3.7 mm
Candida glabrata	1.33 ± 1.52 mm	13.66 ± 3.51 mm	15.16 ± 2.56 mm	24.5 ± 3.77 mm	30.8 ± 1.0 mm	37.3 ± 2.0 mm

* Data present as mm (Mean ± SD)

Table 3 Determination of MIC and MFC of methanolic and ethanolic extract for Candida spp*

Extracts	MFC	MIC
Ethanolic	43/75 ± 0.63	21/87 ± 0.59
Methanolic	31.25 ± 0.71	15.62 ± 0.64

* Data present as mg/ml (Mean ± SD)

Plant-based drugs are promising agents for treatment of diseases. (33). Scrophularia as a member of Scrophulariaceae has been found to possess antibacterial, antiprotozoal, antitumor, anti-inflammatory, and diuretic activities and have been used in the treatment of mental, nervous and gastrointestinal conditions (34, 35). *S. striata*is traditionally used for treatment of infectious diseases. alchoholic extracts from aerial parts of *S. striata*have higher antimicrobial activity than that of aqueous extract (36). *S. striata* extract was shown to have antiseptic effects in treating infections caused by gram positive and negative bacteria, fungi and virus (37-39). One of the reason in treatment of infection due to the effects of flavonol and flavonoid compounds of the *S. striata* extract in combination with cell wall structures or extracellular proteins (40).

A few studies conducted on the essential oils of species of this genus and its family revealed a significant chemical diversity. However, some compounds such as anethole, anisaldehyde, eugenol, benzaldehyde, eugenol acetate are common in the *Scrophularia*. On the other hand, the presence of aromatic compounds in various genera of the *Scrophulariacea e*is one of the characteristics of this family (41, 42).Also they have been known to be rich in iridoid glycosides, mainly aucubin and catalpol. Iridoids represent a large group of cyclopentan-[c]-pyran monoterpenoids occurring as constituents of sympetalous plants including ornamental as well as wild ones (43, 44). In this study *invitro* inhibitory effect of ethanolic and methanolic extract of *Scrophularia striata* on Candida.spp have been studied. As it was expected, ethanolic and methanolic extract of *Scrophularia striata* had inhibitory effects on Candida and this effect was increasing by extract concentrations. The increasing public and economic implications caused by fungi means there is a stable fighting to produce safer food crops and to develop new antifungal agents (19). Plant extracts and essential oils are potential sources of novel antimicrobial compounds against bacteria and fungi pathogens. In traditional medicine *Scrophularia striata* has been used for wound disinfection. On the other hand, Scrophularia is an antipyretic and it used for treatment of kidney disorders and pulmmonary canceres (21). Plants have severe ability to synthesize phenolic products. These products are secondary metabolites and serve as plant defense mechanisms by microorganisms (12). Their activity could be due to their ability to complex with extracellular and soluble proteins (15). Thus, previous reports have demonstrated that: saponins, tannins, alkaloids, terpenes, carotenoids, and flavonoids possessed antifungal activity (18). Antimicrobial flavonoids have multiple cellular targets, Instead of one specific site of action. Alternatively, these common structural features may simply be necessary for flavonoids to gain presence to or uptake into the microbial cell. It may be that flavonoids are not killing microbial cells but singly inducing the formation of microbial aggregates and thereby reducing the number of CFUs in viable counts (41).

Our study is in agreement with several reports (41). Various investigations demonstrated antifungal effects of *Scrophularias triatat* extract. Based on Pirbalouti *et al*. *Scrophularia striata* water extract in different concentrations (10 to 15 mg) had anti-candida effects on Candida albicans (25). Bahraminejad *et al*. (45) showed that many Iranian plants like *Scrophularia striata* had antifungal role on *Fusarium Oxyparum* and Rhizoctonia (23). GhasemiPirbalouti *et al*. determined anti-candida activity of some of the Iranian medicinal plants. The extracts from different plant species studied showed antifungal activities, with the diameters of inhibition zone ranging from 7 to 46 mm. The most active of the concentration was high concentrations (50-55 µL) inhibiting the growth of yeast. Extracts of *Scrophularia striata* showed antifungal activity against *Candida albicans*. Among the plants tested essential oil the extracts of *Scrophularia striata* and *Ziziphus spina-christi* showed the best anti-*Candida* activity (22).

Bahmani *et al*. showed MIC of *Scrophularia deserti* ethanolic extract in dose of 480 mg/ml on *Saprolegnia parasitica* was 61% and for Formalin standard control was 57%, which it demonstrate antimicrobial effects of *Scrophularia deserti* (46). Other study showed anti-microbial effects of *Scrophularia striata* ethanolic extract on *E.coli*, in both agar diffusion and microdilution methods. Its ethanolic extract showed MIC and MBC as 90 mg/ml and100 mg/ml, respectively, but water extract had no anti-microbial activity (47). Moreover, *Scrophularia striatat* extract had anti-viral properties (48). Anti-inflammatory and antibiotic properties of *Scrophularia striatat* reported

in many studies (49). It is suggested that *Scrophularia striatat* extract could be used as an anti-inflammatory agent. Additionally, it could inhibit Helminth in gastro intestinal tract disorder patients (50). Many studies showed *Scrophularia striata* extract have inhibitory effect on various microorganisms and in some studies this extracts have better effects in comparison with antibiotics. However, research on the anti-fungal and anti-bacterial effects of *Scrophularia striata* are in the early stages, and most reports about this plant are based on anti-bacterial, anti-viral and anti-inflammatory traits. This explains the use of this plant in folk medicine for the treatment of various diseases whose symptoms might involve fungal infections. Future phytochemical research is needed to identify the active principles responsible for the antifungal effects of this medicinal plant.

Conclusion

In vitro antifungal activity of the methanolic and ethanolic extract of *Scrophularia striata* on fungi was tested. The methanolicand ethanolicextract of *Scrophularia striata* showed antifungal activity against *Candida albicans ATCC 1167*, *ATCC 1677*, *tropicalis*, and *glabrata*. Major investigations should be performed on a wider range of fungi in order to determine which component of the extract shows the most potent antifungal activity. The antifungal activity could also be a result of the synergism of its components.

Conflict of Interest

The authors declared no conflict of interest.

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