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Antifungal effects of *Allium ascalonicum*, *Marticaria chamomilla* and *Stachys lavandulifolia* extracts on *Candida albicans*

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ABSTRACT

Introduction: Due to increased risk for opportunistic fungal infections and increasing prevalence of hospital infections caused by pathogenic yeasts and fungus resistance to antifungal drugs, discovery of antifungal compounds with high efficiency is necessary. This study was aimed to evaluate and compare the antifungal activities of *Allium ascalonicum*, *Marticaria chamomilla* and *Stachys lavandulifolia* on *Candida albians*.

Methods: In this study the plants extracts were prepared with macerated method using ethanol 70%. Antifungal activities of the extracts were performed according to microbroth dilution method in 96 well microdilution plates. The amount of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) based on counting the number of fungal colonies (CFU) were evaluated for each of *Allium ascalonicum*, Chamomile and *Stachys lavandulifolia* extracts compared with the control group.

Results: MIC of *Allium ascalonicum*, *Marticaria chamomilla* and *Stachys lavandulifolia* were respectively 0.31, 3.75 and 15.13 mg/ml and also MIC50% of *Allium ascalonicum*, *Marticaria chamomilla* and *Stachys lavandulifolia* were respectively 0.93, 10.59 and 41.32 mg/ml and MIC 90% of them were respectively 8.65, 16.88 and 60.55 mg/ml and their MFC were respectively 20, 20 and 65 mg/ml.

Conclusion: The results indicate that all three extracts are effective, but *Allium ascalonicum* possesses the highest antifungal activity on *Candida albicans*. If clinical trials approve these findings, this plant may represent a new source of antifungal agent for control of *Candida albicans*.

Implication for health policy/practice/research/medical education:
The results indicate that *Allium ascalonicum* possesses high antifungal activity on *Candida albicans*. If clinical trials approve these findings, this plant may represent a new source of antifungal agent for control of *Candida albicans*.

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Introduction

Candidiasis is one of the most important and most common opportunistic fungal diseases in humans (1,2). *Candida albicans* is the most important agent in causing *Candidiasis*. Infection can be seen as acute, subacute or chronic in skin, nails, vaginal mucosa, bronchus, lung and gastrointestinal tract. Host response against infection is usually mild irritation and inflammation, then chronic form and acute purulent or granulomatous changes (3). Pravin in 2006 reported that 70% of human population always or occasionally use herbal medicines. WHO estimated that 80% of the population at risk of some diseases, use herbal medicines as first line treatment (4). Due to increased risk for opportunistic fungal infections and the increasing prevalence of hospital infections caused by pathogenic yeasts and fungus resistance to antifungal drugs, discovery of antifungal compounds with high efficiency and low toxicity is necessary. Plants present a

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great variety of compounds with therapeutic properties and might be considered as suitable alternative (4).

Synthetic drugs and chemicals used in fungal infections, especially in patients with underlying disease have many side effects (5). Researches on medicinal plants have shown promising results on different diseases (6-9). Some herbs have strong anti-oxidant activities and are rich in anti-microbial components that can be used to treat fungal infections and bacteria (10,11).

Allium genus have been shown to have antifungal activity which basically depends on the presence of sulfur compounds (12). The main compounds of scallion are composed of disulfide compounds, trisulfide, flavonoids (quercetin), saponins and sapogenine (13,14). The amount of flavonoids present in one ml of ethyl acetate extract of scallion is 156.87±5 µg or 38% of its extract (15).

Marticaria chamomilla is from Asteraceae family (16). The main components of *Marticaria chamomilla* are Chamazulen and α-Bisabolol with high antimicrobial properties against gram positive and gram negative bacteria (17).

Marticaria chamomilla and some components of this plant such as α- bisabolol, apigenin and luteolin have been shown to have antibacterial and antifungal activities (18). *Stachys lavandulifolia* has antioxidant activity and destroys free radicals (19).

This study was aimed to evaluate and compare the antifungal activities of *Allium ascalonicum*, *Marticaria chamomilla* and *Stachys lavandulifolia* on *Candida albians*.

Material and Methods

Extraction method

To prepare extracts, the plants, *Allium ascalonicum*, *Marticaria chamomilla* or *Stachys lavandulifolia* were purchased from a reputable grocery and after separation of brushwood with an electric mill, they were powdered. The resulting powder after passing through sieve No. 10 was poured in the flasks and mixed with 300 cc ethanol 70%. The solution was kept for 72 hours in a dry environment. After the solution was passed through the filter paper, it was transferred for extracting to percolation system. While the temperature was 38 °C on the system, the extract was transferred to the incubator and the alcohol was completely removed (20,21). When needed different concentrations of the extracts were prepared using distilled water and 0.5 ml of DMSO (Dimethyl Sulfoxide).

Preparation of Candida albicans suspensions

The standard fungal strain with PTCC5027 code used in this project was provided from Scientific Research Center of Iran. *Canida albicans* was cultured on dextrose agar medium at 35 °C. After 24 hours, some colonies were transferred to one ml of normal saline to prepared a solution with concentration of 0.5 McFarland 1.5×10^6 cfu/ml (neobar slides were used for colony counting). Then, the resulting solution was diluted to a ratio of 1:1000 and suspensions with concentrations of 0.5-2.5×10^3 cfu/ml was provided (22).

Different dilutions were prepared from *Allium ascalonicum*, *Marticaria chamomilla* and *Stachys lavandulifolia* extracts using Microbroth dilution method, and according to the method proposed by the National Committee for Clinical Laboratory Standards for yeasts (NCCLSM27-A). Thus, to the first well of Ninety-six well plates, two-fold dilution of the extract was poured, and subsequent dilutions were prepared with serial dilution (22).

Different Concentrations of the extracts were prepared as follows:

Allium ascalonicum: 0.31, 0.625, 0.93, 1.25, 1.87, 2.5, 3.75, 5, 7.5, 10, 12, 15 and 20 mg/ml,

Marticaria chamomilla: 2.5, 3.75, 5, 7.5, 10, 12,15 and 20 mg/ml,

Stachys lavandulifolia: 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 and 65 mg/ml.

100 microliters of each dilution of the extract was added separately to the socket of ninety-six well plates that contained 100 ml of Sabouraud broth liquid medium (Sabouraud broth). Then 100 ml of yeast cell suspension in volumes equivalent to 2500 cells per ml were inoculated into the all sockets, except the control plates. The plates were kept at 35 °C for 48 h in the incubator on the Shaker. After incubation time, 10 micro liters of the contents of each socket was cultured on Sabouraud dextrose agar medium and were placed for 48 hours at 35 °C. After this period, the amount of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) based on counting the number of fungal colonies (CFU) were evaluated for each of *Allium ascalonicum*, Chamomile and *Stachys lavandulifolia* extracts compared with the control group (23).

Results

Results of Allium ascalonicum extract

As can be seen in Figure 1, the extract has antifungal activity on *Candida albicans* and the effect is dose dependent.

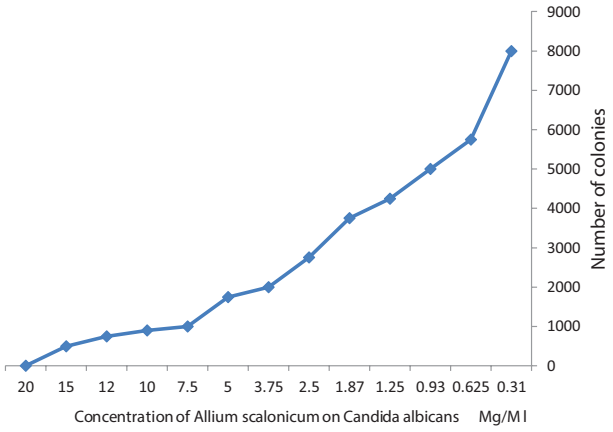

Figure 1. The number of colonies grown in culture medium, in different concentrations of *Allium ascalonicum* extract

MIC, MIC 50%, MIC 90% and MFC of *Allium ascalonicum* extract on *Candida albicans* were respectively 0.31, 0.93, 8.65 and 20 mg/ml (Figure 2).

Results of Marticaria chamomilla extract

The effect of the extract against *Candida albicans* was dependent on the dose of the extract. Hence, with increasing concentrations of *Marticaria chamomilla* extract the number of *Candida albicans* colonies reduced. In the concentration of 20 mg/ml, no colony was grown (Figure 3).

MIC, MIC 50%, MIC 90% and MFC of *Marticaria chamomilla* were respectively 3.75, 10.59, 16.88 and 20 mg/ml (Figure 4).

Results of Stachys lavandulifolia extract

The effect of *Stachys lavandulifolia* extract on the growth of *Candida albicans* was also examined. The extract the same as two other extracts had antifungal activity and in the concentration of 65 mg/ml, no colony could grow (Figure 5).

MIC, MIC 50%, MIC 90% and MFC of *Stachys lavandulifolia* on *Candida albicans* were respectively 15.13, 41.32, 60.55 and 65 mg/ml (Figure 6).

The MIC 50%, MIC 90% and MFC values (mg/ml) of three plants are compared in Table 1.

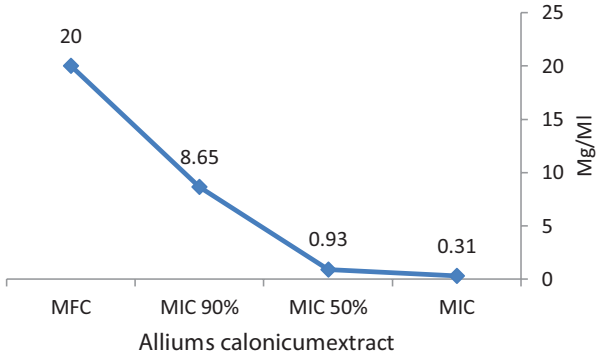

Figure 2. Comparison the MIC and MFC of *Allium scalonicum* extract on *Candida albicans*

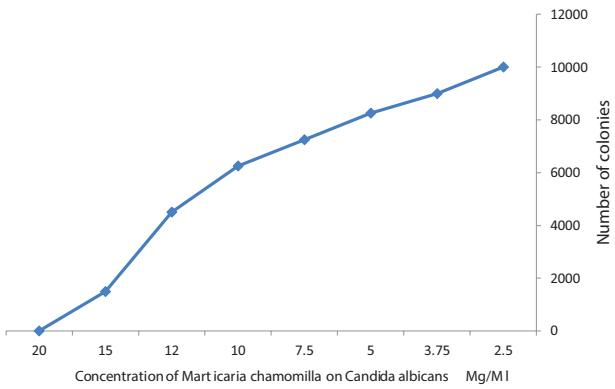

Figure 3. The number of colonies grown in culture medium, in different concentrations of *Marticaria chamomilla* extract

Comparison of inhibitory and fungicidal effects of plant extracts

The logistic regression test showed that there were significant differences between the inhibitory and fungicida effects of each of the plants extracts (P<0.05). *Allium ascalonicum* had the greatest inhibitory and fungicidal effects on *Candida albicans* then *Marticaria chamomilla*, and *Stachys lavandulifolia* had the least antifungal effect (Figure 7).

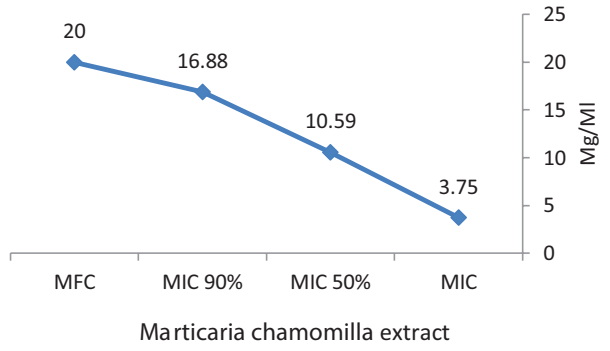

Figure 4. Comparison the MIC and MFC of *Marticaria chamomilla* extract on *Candida Albicans*

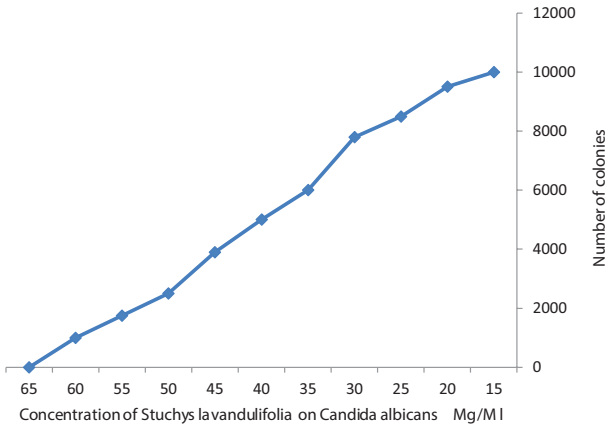

Figure 5. The number of colonies grown in culture medium, in different concentrations of *Stachys lavandulifolia* extract.

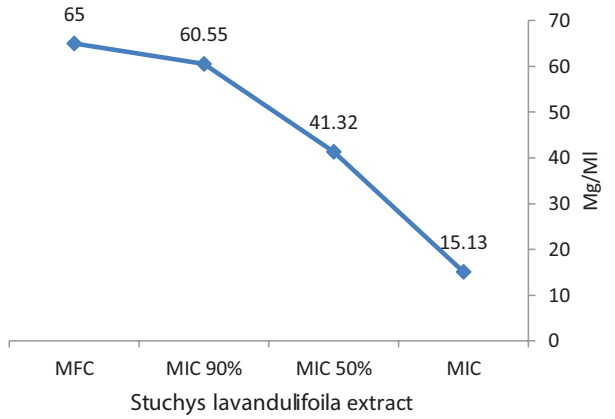

Figure 6. Comparison the MIC and MFC of *Stachys lavandulifolia* extract on *Candida Albicans*

Table 1. MIC 50%, MIC 90% and MFC values of the plants studied (mg/ml) and their 95% Confidence intervals

Plant	MIC 50%		MIC 90%		MFC	
	Value	95% confidence interval	Value	95% Confidence interval	Value	95% Confidence interval
Allium ascalonicum	0.93	0.15-1.92	8.65	7.19-10.31	20	19.5-20.95
Marticaria chamomilla	10.59	10.10-11.11	16.88	16.00-17.97	20	18.9-20.5
Stachys lavandulifolia	41.32	38.91-43.70	60.55	56.83-65.69	65	61.3-66.8

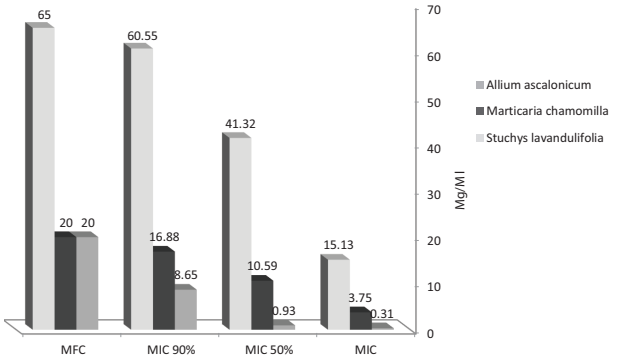

Figure 7. Comparison of MIC 50%, MIC 90%, and MFC *Allium ascalonicom*, *Marticaria chamomilla* and *Stuchys lavanulifolia extracts* on *Candida albicans*

Discussion

In this study the plants showed antifungal activity against *Candida albicans*. *Candidiasis* is the most common opportunistic fungal infection that can be seen on the skin, mucous membranes, respiratory tract and genitourinary system (3).

The results of this study are partially in agreement with previous ones which have proved the inhibitory effects of Allium genus especially *Allium ascalonicum* against bacteria, fungi, viruses and protozoa (24).

In a study Amin and colleagues evaluated and expressed anti-fungal and anti-bacterial effects of Allium ascalonicum, garlic and onion. Fungal species were more sensitive than bacteria. MIC values of dried *Allium ascalonicum* extracts for *Trichophyton mentagrophytes* was 0.62 mg/ml and for *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger* was 20 mg/ml (25).

In another study Yin and colleagues studied the antifungal effects of seven Allium species on three species of *Aspergillus*. In this study, the obtained MIC values of *Allium ascalonicum* extracts for *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus Niger* were respectively 0.474 mg/ml, 0.558 and 0.228 mg/ml (26).

Antifungal effect of aqueous and alcoholic extracts of *Allium hirtifolium* has also been tested. In this study, MIC values of *Allium hirtifolium* extract against the fungal species tested for the alcoholic extract were between 0.058 and 0.8 mg/ml and for aqueous extract between 0.26 and 3.84 mg/ml, and MFC of alcoholic and aqueous extracts were reported respectively between 0.1-12.8 mg/ml and 0.6-68.29 mg/ml (27).

It was shown by Zarei Mahmudabadi that the effect of *Allium ascalonicum* on saprophyte was more than its effect on *Candida albicans* (28).

In a study antifungal effect of onion and garlic on *Candida* albicans was evaluated. The MIC values of onion and garlic were respectively 4.522 mg/ml and 0.569 mg/ml, in which, the MIC values of *ketoconazole* for *Candida albicans* was 23.582 mg/ml (29).

In our study, MIC, MIC 50%, MIC 90% and MFC of *Allium ascalonicum* extract on *Candida albicans* were respectively 0.31, 0.93, 8.65, and 20 mg/ml, which they are consistent with the results of previous studies. These results may indicate that *Allium ascalonicum* has antifungal effect against the *Candida albicans* and may be a good candidate to be used in fungi infections.

Few studies have examined antifungal properties of *Marticaria chamomilla*, especially its effect on *Candida albicans.* Some studies have rejected the antifungal properties of this plant. For example Naiini and colleagues in 2009 evaluated the MIC and MFC of some plant extracts, including, *Marticaria chamomilla* against *Candida albicans*. In this study, the highest rate of used extracts, was 6.4 mg/ml. *Marticaria chamomilla* extract did not show antifungal effect in none of the concentrations (30).

In contrast, some studies have emphasized on antifungal activity of *Marticaria chamomilla*. Magro has mentioned, that dried *Roman chamomile* extract could inhibit the growth of *Aspergillus niger* in 920 mg/ml concentration (31).

In a research, by Abud latif in 2011, has been examined the pure and methanolic extracts of *Marticaria chamomilla* on the growth of *Candida albicans* and *Aspergillus niger*. In this study, MIC values of pure extract and methanol extracts of *Marticaria chamomilla* were obtained 0.002 mg/ml and 0.1 mg/ml, respectively (32).

In our study, MIC, MIC 50% , MIC 90% and MFC of *Marticaria chamomilla* were respectively 3.75, 10.59, 16.88, and 20 mg/ml.

Berry in 1995 has mentioned that α-bisabolol, spirother, camazolen and umbeliferone are some components with antifungal activities which all are present in *Marticaria chamomilla* (33).

The discrepancies in the results of different researchers can be due to differences in the types of used native plants

or differences in the types of extracts and compounds and/or differences in the used methods in the study. It seems that in this regard more studies are needed to evaluate the antifungal effects of *Marticaria chamomilla*.

Studies are also limited on the effects of antimicrobial and especially antifungal of *Stachys lavandulifolia*.

Dulger in Turkey studied the antifungal effects of methanolic extracts of four different types of *Stachys lavandulifolia* on several bacteria and yeasts. In this study the MIC and MFC of yeasts were obtained 0.032 mg/ml. It seems that due to equal amounts of MIC and MFC, *Stachys lavandulifolia* extract is a fungicidal plant (34). In another study by Skaltsa, the antifungal effect of *Stachys lavandulifolia* extract on *Candida albicans* was obtained between 0.3 and 1 mg/ml of extract (35). The results of this study indicate that *Stachys lavandulifolia* has a strong antifungal effect and plants of this family can inhibit fungal growth very well. In our study, MIC, MIC 50%, MIC 90% and MFC of *Stachys lavandulifolia* on *Candida albicans* were respectively 15.13, 41.32, 60.55 and 65 mg/ml.

Saidi *et al.* evaluated the effect of extracts of *S. Lavandulifolia* on *Candida albicans* using microbroth dilution method that the method used, is consistent with our study and results of both studies show that the extract had no effect on *Candida albicans* (17).

Conclusion

In the present study in order to be stopped Candida fungus growth, There were significant differences between the MIC, MIC 50% and MIC 90% three groups of plant extracts (*Allium ascalonicum*, *Marticaria chamomilla* and *Stachys lavandulifolia*). All three plants had the ability to inhibit the growth of *Candida albicans* but *Allium ascalonicum* extract had the greatest impact on controlling fungal. Also *Marticaria chamomilla* extract was in the next position, but the concentration required for *Marticaria chamomilla* extract was much higher than the other two plants.

In this study, the antifungal effect of *Allium ascalonicum, Marticaria chamomilla* and *Stachy lavandulifolia* extracts were determined on *Candida albicans* that as from increasing concentrations of plant extracts, growth of fungi was limited and since the chemical antifungal drugs are too expensive and also have some side effects and Harmful for patients, so if the herbal medicines produce, can be reduced side effects and drug resistance to chemical drugs.

Alliums ascalonicum extract had high effect on *Candida albicans,* so, this plant extract might be used to treat fungal infections. However, to better determine the impact of this plant to treat fungal infections, further researches should be done on clinical samples and directly effect of plant extract should be studied on *Candidiasis* patients.

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Authors' contributions

All the authors wrote the manuscript equally.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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