Evaluation of antifungal effect of \textit{Lavandula officinalis}, \textit{Salvia officinalis} L., \textit{Sumac}, \textit{Glycyrrhiza glabra}, and \textit{Althaea officinalis} extracts on \textit{Aspergillus niger}, \textit{Aspergillus fumigatus}, and \textit{Aspergillus flavus} species

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Using synthetic preservatives to control fungal disease has become important due to emergence of drug resistance and some side effects of the drugs’ remainder including carcinogenicity and teratogenicity. \textit{Lavandula}, \textit{Salvia officinalis} L., \textit{Sumac}, \textit{Glycyrrhiza glabra}, and \textit{Althaea officinalis} are some medicinal plants with proved therapeutic effects as anti-microbial, anti-viral, and anti-parasitic agents. The aim of the current study is to evaluate the effect of these plants on clinical species of \textit{Aspergillus niger}, \textit{Aspergillus fumigatus}, and \textit{Aspergillus flavus}. Plant extracts were prepared by maceration method. The extracts at concentrations of 500, 200, 100, 50, 25, and 12.5 mg ml\textsuperscript{-1} were prepared in dimethylsulfoxide. The effect of anti-fungal extracts was separately assessed using Broth macrodilithetion. Finally, the minimum inhibitory concentration (MIC) and minimum fungicidal concentrations (MFC) of extracts were determined. This study showed that \textit{Lavandula}, \textit{S. officinalis} L., \textit{Sumac}, \textit{G. glabra}, and \textit{A. officinalis} extracts have anti-fungal effects. The antifungal effect of \textit{Sumac}, \textit{G. glabra}, and \textit{Lavandula officinalis} was significantly different from that observed by \textit{S. officinalis} L. extract. Moreover, it was observed that \textit{A. flavus} and \textit{A. fumigatus} were the most sensitive and resistant fungal species to the antifungal effects of the extracts, respectively (p≤0.05). Further evaluation is necessary to elucidate the extent and mechanism of these changes.

\textbf{Key words:} Aspergillus, Lavandula, Salvia officinalis L., Sumac, Glycyrrhiza glabra, Althaea officinalis.

INTRODUCTION

\textit{Aspergillosis} consists of a large group of fungal diseases that are caused by different \textit{Aspergillus} species. The clinical signs and symptoms of the disease depend on the patient’s conditions because the pathogens of disease are ubiquitous and found in nearly everywhere (soil, plant debris, and indoor environment, especially in hospitals) (Soubani and Chandrasekar, 2002). The primary cases of \textit{Aspergillus} infection were reported in patients that suffered from pulmonary tuberculosis. The frequency of fungal diseases is increasing and the fungal diseases are becoming a significant cause of mortality and morbidity in immunocompromised patients.

Many immune system defects may increase the incidence risk of these opportunistic fungal infections, but the major predisposing factor is belonging to the group of immunocompromised population because of organ transplantation, cancer, and HIV/AIDS (Soubani and...
Chandrasekar, 2002; Mathew, 2005; Haynes et al., 1995). One of the most important fungal pathogens in these settings is Aspergillus spp. (Ajello and Hay, 1998; Anaisie and Bodey, 1989). In spite of availability of several antymycotic agents, treatment of immunocompromised patients is still limited due to a number of factors. The factors include low drug potency, poor solubility of drugs, emergence of resistant strains, and drug toxicity. This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections are a serious medical problem.

Therefore, it sounds essential to find new sources of antifungal agents. Plants are invaluable sources of pharmaceutical products that have drawn the attention of many scientists (Aliero et al., 2006; Capoor et al., 2005; Rangel, 2005). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena and Sharma, 1991; Ahmad and Beg, 2001). The World Health Organization (WHO) estimated that about 80% of the world’s population still believes in herbal drugs for their primary health care (WHO, 1993). The synthetic antimicrobial drugs are used indiscriminately for treatment of infectious diseases; thus drug resistance has developed in human beings as well as in plant (Davis, 1994; Loper et al., 1991; Service, 1995).

Furthermore, sometimes antibiotics cause adverse reaction like hypersensitivity, immunosuppression, and allergic reactions (Ahmad et al., 1998).

Therefore, there is a need to develop alternative antimicrobial drugs for treatment of infectious diseases from various sources, including Lavandula officinalis, Salvia, officinalis L., G. glabra, Althaea officinalis and Sumac (Clark, 1996; Cordell, 2000). These plants are cultivated throughout the world (Duke, 1986; Chiej, 1984) as well as in different parts of Iran. Indeed, it has been shown that these plants have anti-bacterial, fungistic, virustatic, astringent, and anti-hydrotic effects (Bors et al., 2004; Imandel and Adibnia, 2000; Fatima et al., 2009; Ayatollahi et al., 1996). These plants are native to Iran and the antifungal effects of these plants on Aspergillus spp. has not been studied so far. Considering this and also the side effects of antifungal drugs that has always been a health concern (Hosseinizadeh et al., 2009), the aim of the current study is to determine the antifungal effects of Salvia officinalis L., Sumac, Glycyrrhiza glabra and Althaea officinalis extracts on clinical species of Aspergillus niger, Aspergillus fumigatus and Aspergillus flavus isolated from patients under in vitro conditions.

### MATERIALS AND METHODS

#### Preparation of fungal species

The three Aspergillus spp. used to the assays were A. fumigatus, A. flavus, and A. niger, which were all obtained from the clinical specimens referred to the Mycology Department, Health Research Institute, Tehran University of Medical Sciences. The species were identified according to culture specificities and microscopic morphology. Stocks were maintained on Sabouraud’s Dextrose Agar (SDA) plates at 4°C prior to use for antifungal tests.

#### Preparation of plant extracts

The healthy leaves were dried in shade condition and to avoid decomposition of chemical constituents, dried leaves were powdered and stored in clean and dry airtight containers for further studies. The powder of leaves were macerated in 80% ethanol (MERCK, Germany) (10 g in 100 ml 80% ethanol) for 72 h (Fenner et al., 2005) and then filtered using Buckner funnel and filtered under weak vacuum. The ethanol extract was evaporated at room temperature. Plant extracts were filtered by syringe filters (0.2 μm) (Minisart, Sartorius Stedim Biotech GmbH, Germany). Then, 5 g of the dried plant extract was dissolved in 10 ml of dimethylsulfoxide (2%) (DMSO) (MERCK, Germany) to obtain a final concentration of 500 mg ml⁻¹. Then, the concentrations of 200, 100, 50, 25, and 12.5 mg ml⁻¹ were prepared (Sadish et al., 2010).

#### Preparation of inoculums

The microorganisms were activated on Saboraud Dextrose Broth (SDB) and then cultured on SDA for 3 days at 28°C to obtain adequate growth. Following the period of incubation, colonies were scraped with a sterile scalpel and macerated in 10 ml of sterile 0.05% tween 80 solution (in sterile distilled water) to prepare a suspension, and then fungal spores were washed and counted (Sadish et al., 2010).

#### Anti fungal activity of plant extracts

Then, 10⁶ colony forming units (CFU) ml⁻¹ of fungal spores (to obtain 0.5 McFarland turbidity standard) were counted. Triplicate tubes were used for each dilution. Finally, 200 μl of this fungal suspension was added onto the SDA plates and cultured (with streak method) using a sterile loop. The plates were incubated at 28°C and observed after 24 to 48 h. The control tubes were used including: tubes containing medium culture, tubes containing medium culture and the suspension (but a fungal suspension) (control for contamination), and tubes containing medium culture and different amounts of extracts, which were compared with regard to turbidity with the tubes with no fungus growth that contained the same amount of extract with fungal spores. The concentration of extract in the first tube that inhibits the growth of Aspergillus spp. was recorded as the minimum inhibitory concentration (MIC). The lowest concentration of the plant extracts that did not yield any colony on the SDA after subculturing and incubating at 28°C for 24 to 48 h for Aspergillus strains were recorded as the minimum fungicidal concentration (MFC) (Avijgan et al., 2006).

#### Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows version 12.0. Group comparisons were carried out using Mann-Whitney test. The p-value equal to or less than 0.05 was considered to be statistically significant.

#### RESULTS

The MIC and MFC of L. officinalis, S. officinalis L., Sumac,
Table 1. Average MIC (mg ml⁻¹) of Lavandula officinalis, Salvia officinalis L., Sumac, Althaea officinalis, and Glycyrrhiza glabra extracts on Aspergillus niger, Aspergillus fumigatus, and Aspergillus flavus in Sabouraud dextrose broth medium.

<table>
<thead>
<tr>
<th>Herbal extracts</th>
<th>Test fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aspergillus flavus (mg ml⁻¹)</td>
</tr>
<tr>
<td>Lavandula officinalis</td>
<td>16.67</td>
</tr>
<tr>
<td>Salvia officinalis L.</td>
<td>66.67</td>
</tr>
<tr>
<td>Sumac</td>
<td>41.67</td>
</tr>
<tr>
<td>Althaea officinalis</td>
<td>50</td>
</tr>
<tr>
<td>Glycyrrhiza glabra</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 2. Average MFC (mg ml⁻¹) of Lavandula officinalis, Salvia officinalis L., Sumac, Althaea officinalis, and Glycyrrhiza glabra extracts on Aspergillus niger, Aspergillus fumigatus, and Aspergillus flavus in Sabouraud dextrose agar medium.

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<th>Herbal extracts</th>
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<tr>
<td></td>
<td>Aspergillus flavus (mg ml⁻¹)</td>
</tr>
<tr>
<td>Lavandula officinalis</td>
<td>33.34</td>
</tr>
<tr>
<td>Salvia officinalis L.</td>
<td>83.34</td>
</tr>
<tr>
<td>Sumac</td>
<td>66.67</td>
</tr>
<tr>
<td>Althaea officinalis</td>
<td>50</td>
</tr>
<tr>
<td>Glycyrrhiza glabra</td>
<td>33.34</td>
</tr>
</tbody>
</table>

G. glabra, and A. officinalis extracts in SDB and SDA on the Aspergillus species are shown in Tables 1 and 2. The MIC of L. officinalis extract on Aspergillus spp. isolated from patients in SDB was in the range of 12.5 to 100 mg ml⁻¹, while the value was in range of 50 mg ml⁻¹ for S. officinalis L. extract, 50 to 100 mg ml⁻¹ for A. officinalis extract (except for A. niger, which was not effective), 25 to 50 mg ml⁻¹ for Sumac extract (it was not effective on A. niger), and 25 to 100 mg ml⁻¹ for G. glabra extract. The MFC of L. officinalis extract in SDA for the same strains was in range of 25 to 200 mg ml⁻¹, 50 to 200 mg ml⁻¹ for S. officinalis L. extract, 50 to 100 mg ml⁻¹ for sumac extract (it was not effective on A. niger), 50 to 100 mg ml⁻¹ for A. officinalis extract (the extract was not effective on A. niger), and 25 to 100 mg ml⁻¹ for G. glabra extract.

**DISCUSSION**

Aspergillus species are the most common causes of nosocomial infections in severely immunocompromised patients including those with bone malignancies, undergoing bone marrow transplantation, receiving corticosteroids (Anaissie and Bodey, 1989; Bodey et al., 1988) as well as those with fungal sinusitis (with mortality rate of 50 to 80%) (Parikh et al., 2004; Kordbacheh et al., 2004). The MIC and MFC of L. officinalis extract on A. niger, A. flavus, and A. fumigatus were in the range of 12.5-100 and 25 - 200 mg ml⁻¹, respectively. In a study by Ezatpour et al. (2009), the anti-Trichomonas vaginalis activity of Lavandula angustifolia essential oil under in vitro condition was examined and it was reported that T. vaginalis could remain alive for 48, 2, and 5 h in Trichomonas modified CPLM medium (TMCLPM) base, in the presence of metronidazole, and in TMCLPM, respectively. Also, the results indicated that the essential oils at concentration of 0.1, 0.01, and 0.001 were effective when applied at the beginning of inoculation (Ezatpur et al., 2009). The MIC and MFC of S. officinalis L. extract on fungal species listed were in range of 50 to 200 mg ml⁻¹. This value is consistent with the result reported by Atai et al. (2007). They evaluated the antifungal effects of Artemisia absinthium, Eucalyptus spp, Allium cepa, Cinnamomum zelanicum, Curcuma longa, S. officinalis L., Mentha piperita, and Calendula officinalis mouthwashes and obtained the MIC and MFC of S. officinalis L. extract at a dilution of 1/5 and 1/2, respectively (Atai et al., 2007). Although, integrated studies of antifungal effects of oak are not available, the data available on its effect on gram-positive and gram-negative bacteria, its antioxidant effects, as well as the long-term oral use indicate that it is not toxic (Davidson and Zivanovic, 2003). Thus, research on development of a food preservative from oak can be the subject of future studies. In the current study, the total MIC and MFC of Sumac extract for A. flavus and A. fumigatus were in range of 25 to 50 and 50 to 100 mg ml⁻¹, respectively. This is while the MIC and MFC values for A. niger were not achieved. There is a limited number of works on the antifungal effect of Sumac. These studies showed that Sumac has no effect on Candida albicans and Candida tropicalis (Sokman et al., 1999; Digraf et al., 2001). Therefore, further studies will be helpful in
clarifying this issue.

The MIC and MFC of G. glabra extract for the fungal species studied were in range of 25 to 100 mg ml⁻¹. In India, Fatima et al. (2009) studied the antifungal component *Officinale Glabrydin* (a component of *G. glabra*) on *Candida* fungus resistant to antibiotics. In this study, the MIC was obtained in range of 31.25 to 250 μg ml⁻¹ but the MFC was not reported (Fatima et al., 2009). The amount varies by the results of the study. The MIC and MFC levels of total extract of *A. officinalis* for the fungal strains were in the range of 50 to 100 and 50 to 100 mg ml⁻¹, respectively. In a study conducted by Ayatollahi et al. (1996) on the effect of methanol extract of *A. officinalis*, the MIC and MFC were in range of 10 to 15 and 12 to 17 mg ml⁻¹, respectively, nevertheless, the results obtained in the current study was different from those reported by Ayatollahi et al. (1996). In the current study, the antifungal effect of five different herbal extracts was examined on some clinically isolated fungal species. The MIC and MFC values were obtained in range of 25 to 200 mg ml⁻¹. The differences in results may be attributed to several factors, including testing methods, the fungal species isolated, types of extracts, extraction methods, and materials used for the experiment.

However, it is important to note that the results obtained are compatible with those of other studies on different plant extracts. As it was observed, *A. fumigatus* and *A. flavus* were the most resistant and sensitive species, respectively (p≤0.05). The MIC and MFC of *S. officinalis* L. and *G. glabra* extracts on the two species were the same.

**Conclusion**

The results of the study showed that *L. officinalis*, *S. officinalis* L. *officinalis*, *Sumac*, *A. officinalis*, and *G. glabra* extracts may have antifungal effects. The antifungal effect of *Sumac*, *G. glabra*, and *L. officinalis* extracts were higher than that obtained from *S. officinalis* L. extract (p≤0.05). The MIC of *Sumac* extract was 25 to 50 mg ml⁻¹, while the value was obtained to be 12.5 to 100 mg ml⁻¹ for *L. officinalis* extract. The MFC of *G. glabra* extract was in range of 25 to 100 mg ml⁻¹. Therefore, the antifungal effect of these three extracts was stronger than the others.

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**REFERENCES**


