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Full Length Research Paper

Evaluation of antifungal effect of *Lavandula officinalis*, *Salvia officinalis* L., *Sumac, Glycyrrhiza glabra*, and *Althaea officinalis* extracts on *Aspergillus niger*, *Aspergillus fumigatus*, and *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. *Lavandula*, *Salvia officinalis* L., *Sumac, Glycyrrhiza glabra*, and *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 *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus flavus*. Plant extracts were prepared by maceration method. The extracts at concentrations of 500, 200, 100, 50, 25, and 12.5 mg ml^{-1} were prepared in dimethylsulfoxide. The effect of anti-fungal extracts was separately assessed using Broth macrodilutitheon. Finally, the minimum inhibitory concentration (MIC) and minimum fungicidal concentrations (MFC) of extracts were determined. This study showed that *Lavandula*, *S. officinalis* L., *Sumac, G. glabra*, and *A. officinalis* extracts have anti-fungal effects. The antifungal effect of *Sumac*, *G. glabra*, and *Lavandula officinalis* was significantly different from that observed by *S. officinalis* L. extract. Moreover, it was observed that *A. flavus* and *A. fumigatus* were the most sensitive and resistant fungal species to the antifungal effects of the extracts, respectively ($p \leq 0.05$). Further evaluation is necessary to elucidate the extent and mechanism of these changes.

Key words: *Aspergillus, Lavandula, Salvia officinalis* L.*, Sumac, Glycyrrhiza glabra, Althaea officinalis*.

INTRODUCTION

Aspergillosis consists of a large group of fungal diseases that are caused by different *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 *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 immunocompramised population because of organ transplantation, cancer, and HIV/AIDS (Soubani and

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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; Anaissie and Bodey, 1989). In spite of availability of several antimycotic 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, fungistatic, 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 (Hosseinzadeh 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 Sabourad'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 (20%) (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, 10^6 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 *Aspergilus* 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.

Herbal extracts	Test fungi		
	***Aspergillus flavus* (mg ml^{-1})**	***Aspergillus fumigates* (mg ml^{-1})**	***Aspergillus niger* (mg ml^{-1})**
Lavandula officinalis	16.67	66.67	41.7
Salvia officinalis L.	66.67	133.34	-
Sumac	41.67	50	-
Althaea officinalis	50	83.34	66.67
Glycyrrhiza glabra	25	83.34	33.34

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.

Herbal extracts	Test Fungi		
	***Aspergillus flavus* (mg ml^{-1})**	***Aspergillus fumigates* (mg ml^{-1})**	***Aspergillus niger* (mg ml^{-1})**
Lavandula officinalis	33.34	133.34	83.34
Salvia officinalis L.	83.34	200	-
Sumac	66.67	83.34	-
Althaea officinalis	50	100	100
Glycyrrhiza glabra	33.34	83.34	41.67

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 12.5 to 100 mg ml^{-1}, while the value was in range of 50 to 200 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 include *A. flavus, A. niger*, and *A. fumigatus* are the most common causes of nosocomial infections in severely immunocompromised patients including those with blood 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 angyustifolia* 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 Trichomona modified CPLM medium (TMCPLM) base, in the presence of *metronidazole*, and in TMCPLM, 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; Digrak 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 *Oflicorice 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 µ 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 ($p \leq 0.05$). 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 ($p \leq 0.05$). 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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