Short Paper

Effects of *Zataria multiflora* and *Geranium pelargonium* essential oils on growth-inhibiting of some toxigenic fungi

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Summary

The effects of two Iranian medicinal plants including *Zataria multiflora* and *Geranium pelargonium* were evaluated on growth-inhibiting of some toxigenic fungi such as *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium verticillioides*. In this study, standard *Z. multiflora* and *G. pelargonium* essential oils (EOs) were diluted in 0.01% dimethyl sulfoxide. Different dilutions of *Z. multiflora* (500, 1000, 2000 and 4000 ppm) and *G. pelargonium* EOs (1000, 2000, 4000 and 8000 ppm) along with 0.1 ml of each fungal suspension were inoculated onto sabouraud glucose agar and incubated at 25°C for 7 days. *Zataria multiflora* (≥2000 ppm) and *G. pelargonium* (≥8000 ppm) EOs completely inhibited all the tested fungi. *Aspergillus* species were more susceptible than *F. verticillioides* to two EOs. The EOs considerably exhibited inhibitory effects against these important toxigenic fungi and their different concentrations demonstrated various degrees of growth inhibition. This study showed inhibitory effects of *Z. multiflora* and *G. pelargonium* EOs against some toxigenic fungi including *A. flavus*, *A. parasiticus*, *A. ochraceus* and *F. verticillioides*.

Key words: Antifungal activity, *Zataria multiflora*, *Geranium pelargonium*, Essential oil, Toxigenic fungi

Introduction

The herbal EOs have been known to show inhibition of proliferation or killing activity against a wide variety of micro-organisms including viruses, mycoplasma, chlamydia, bacteria, fungi, protozoans and harmful insects such as mites (Dorman and Deans, 2000; Isman and Machial, 2006). Numerous studies have documented the antifungal effects of plant EOs (Aligiannis *et al*., 2001; Elgayyar *et al*., 2001). Since the presence and growth of fungi in food- and feed-stuffs can cause spoilage and result in a reduction in quality and quantity, herbal EOs have been used against pre- and post-harvest fungi, particularly *Aspergillus* and *Fusarium* species (Juglal *et al*., 2002). Some *Aspergillus* species are responsible for many cases of food- and feed-stuffs contamination (Giorni *et al*., 2007). *Aspergillus flavus* and *A. parasiticus* are able to produce aflatoxins in food- and feed-stuffs, which have been known to be potent hepatocarcinogens in animals and humans. *Aspergillus ochraceus* produces ochratoxin A, which is a mutagen and animal carcinogen (Cary and Ehrlich, 2006). Also, toxigenic strains of *Fusarium* are able to produce fumonisins (Schollenberger *et al*., 2005). Therefore, the presence of toxigenic fungi and mycotoxins in foods and grains stored for long periods of time presents a potential hazard to human and animal health (WHO, 2006). Many investigators used herbal EOs such as cinnamon, peppermint, basil and thyme to protect grains against fungal infections, without affecting germination and plant growth. Considerable interest has developed during recent years on the preservation of foods and grains by the use of EOs to effectively retard fungal growth and subsequent mycotoxin production (Velluti *et al*., 2004). The herbal plants of *Z. multiflora* (Avishan-e-Shirazi in Persian and Sa'atar or Zaatar in the old Iranian medical books) and *G. pelargonium* grow wild in central and southern Iran (Tadjbakhsh, 2003). *Zataria multiflora* is used traditionally in food, especially in yoghurt flavouring. There are also commercial pharmaceuticals with formulae based on *Z. multiflora* EO. This oil has been used commonly in traditional folk remedies for its antiseptic, analgesic and carminative properties as well (Avicenna, 980-1037 AD; Tadjbakhsh, 2003). *Geranium pelargonium* EO with a wide spectrum of chemical compositions has shown antimicrobial activity, immuno-modulatory properties, leishmanicidal activity and interferon-like properties (Schelz *et al*., 2006). In addition, this oil was reported as being most active against some pathogenic fungi (Schelz *et al*., 2006) and to have antioxidative properties (Dorman and Deans, 2000). Many EO components are generally recognized as safe by the food and drug administration of the US and have been used as artificial flavourings and preservatives (Charlwood and Charlwood, 1991). Recently, there has been extensive research on the antimicrobial activity of EOs against food-borne pathogens (Elgayyar *et al*., 2001), seeking natural and safer means for food hygiene or preservation. This work was performed to determine the fungistatic effects of two Iranian herbal EOs, *Z. multiflora* and *G. pelargonium* on some important toxigenic fungi.

Materials and Methods

Fungal strains and preparation of the conidial suspension

Aspergillus flavus (ATCC 26768), *A. ochraceus* (ATCC 22947), *A. parasiticus* (ATCC 16869) and *F. verticillioides* (MRC 826) were used as test organisms and precultured onto potato dextrose agar [PDA] (Merck Co., Darmstadt, Germany) slant at 25°C for 10 days. Conidia were taken from the slants by the use of sterile distilled water containing 0.01% Tween 80. Mycelia were removed by filtration through gauze and the suspension was adjusted to a concentration of approximately 1×10^7 conidia/ml by means of a heamocytometer and light microscope (Khosravi *et al*., 2010).

Preparation of EOs

Standard *Z. multiflora* and *G. pelargonium* EOs were obtained from Barij Essence Pharmaceutical Company (Kashan, Iran). EOs were diluted in 0.01% dimethyl sulfoxide [DMSO] (Merck Co., Darmstadt, Germany). The following concentrations were tested; 500, 1000, 2000 and 4000 ppm for *Z. multiflora* and 1000, 2000, 4000 and 8000 ppm for *G. pelargonium*. Each concentration was mixed with 50 ml of sterilized semi-solidified sabouraud glucose agar [SGA] (Merck Co., Darmstadt, Germany) media and then poured into Petri dishes.

Antifungal activity of EOs

The solid media were punched out by a circular mould with a five mm inner diameter and four wells were made on each SGA medium containing different dilutions of EOs. The aliquot of 0.1 ml of each fungal suspension was inoculated in one well from each agar medium. Plates were incubated at 25°C for 7 days. Positive control plates containing only SGA media and fungal suspension, as well as negative control plates containing SGA media accompanied by EO and DMSO were prepared and incubated at the same conditions. Growth or absence of growth was monitored visually the second and fourth day after inoculation. Colony diameter (CD) was measured on the seventh day (CLSI, 2003). The average of the colony diameter was considered as the growth rate and an inhibitory diameter (ID) was calculated using the following formula, according to the Gosh and Haggblom (1985) method:

$$ID\ (\%) = [(\text{CD of control} - \text{CD of treatment}) / \text{CD of control}] \times 100$$

All experiments were repeated three times and mean calculated.

Statistical analysis

Statistical analyses were performed by

the use of SPSS for Windows version 10.0. Analysis of variance (ANOVA) and Student' t-tests were used to compare the means of the growth diameter and percent of growth inhibition of fungi treated with the EOs.

Results

The effects of two herbal EOs belonging to two families were presented in Table 1. The EOs of *Z. multiflora* and *G. pelargonium* showed inhibitory effects on four tested fungi including *A. flavus*, *A. parasiticus*, *A. ochraceus* and *F. verticillioides* at all concentrations. The higher EO concentration resulted in a higher inhibitory effect. *Zataria multiflora* EO had a more considerable inhibitory effect than other EOs. It completely inhibited four fungi at 2000 ppm, whereas *G. pelargonium* had the same effect at 8000 ppm. At 1000 ppm concentration, *Z. multiflora* EO significantly decreased the growth of *Aspergillus* species compared with the control, whereas it caused complete growth inhibition of *F. verticillioides* ($P<0.05$). Also, the *Aspergillus* species was considerably affected by the *G. pelargonium* EO at 4000 ppm concentration, whereas *F. verticillioides* was completely affected (100% reduction) ($P<0.05$). Therefore, *F. verticillioides* had more sensitivity than *Aspergillus* species against two EOs. The inhibitory effect of *G. pelargonium* against *A. ochraceus*, *A. flavus* and *A. parasiticus* (88.8, 82.2 and 79.2% reduction, respectively) was recorded at a concentration of 2000 ppm, whereas *Z. multiflora* EO completely inhibited the growth of four fungi (100% reduction) at the same concentration (Table 1).

Discussion

Spoilage and poisoning of food- and feed-stuffs by fungi is a major problem, especially in developing countries. In this study, the EOs of *Z. multiflora* and *G. pelargonium* showed the inhibitory effects on four tested fungi including *A. flavus*, *A. parasiticus*, *A. ochraceus* and *F. verticillioides* at all concentrations. *Z. multiflora* EO had a greater inhibitory effect than another essence. It completely inhibited four fungi at 2000 ppm, whereas *G. pelargonium* had the same effect at 8000 ppm. The antifungal activity of the EOs is related to the respective composition of the plant EO, the structural configuration of the constituent components and their functional groups and possible synergistic interactions between components (Dorman and Deans, 2000). It was demonstrated that the main components with phenolic structures in *Z. multiflora*, such as carvacrol and thymol, have higher activity against the tested microorganisms than geraniol as an active component of *G. pelargonium*, which is responsible for their antifungal activity (Maruyama *et al*., 2008). These compounds (carvacrol and thymol) are highly active, which is in agreement with published data (Lis-Balchin and Deans, 1997). *Zataria*

Table 1: The effect of different concentrations of essences on mean growth rate (GR) and percent of growth inhibition (PGI) of *Aspergillus flavus, A. parasiticus, A. ochraceus* and *Fusarium verticillioides*

Essential oils (ppm)	Fungus							
	A. flavus		*A. parasiticus*		*A. ochraceus*		*F. verticillioides*	
	GR (mm)	PGI (%)	GR (mm)	PGI (%)	GR (mm)	PGI (%)	GR (mm)	PGI (%)
Zataria multiflora								
0	20.2±0.7	0	17.8±1.6	0	16.1±1.4	0	41.5±2.2	0
500	3±0.3	85.1	4.6±0.8	74.2	3.6±0.8	77.6	5±1	88
1000	1±0.3	95	3.1±0.7	82.6	2.1±0.4	87	0	100
2000	0	100	0	100	0	100	0	100
4000	0	100	0	100	0	100	0	100
Geranium pelargonium								
0	20.2±0.7	0	17.8±1.6	0	16.1±1.4	0	41.5±2.2	0
1000	6.5±0.8	67.8	8.1±0.8	54.5	2.9±0.8	82	11.6±1.3	72
2000	3.6±0.8	82.2	3.7±1	79.2	1.8±0.4	88.8	7.5±1.2	81.9
4000	1.4±0.5	93	1.8±0.6	89.9	0.8±0.2	95	0	100
8000	0	100	0	100	0	100	0	100

multiflora EO at a concentration of 1000 ppm significantly decreased the growth of *Aspergillus* species in comparison with the control, whereas it resulted in complete growth inhibition of *F. verticillioides*. Also, the *Aspergillus* species was severely affected by *G. pelargonium* essential oil at 4000 ppm concentration, while *F. verticillioides* was completely affected (100% reduction). Therefore, *F. verticillioides* had a higher sensitivity than *Aspergillus* species against the two EOs. Some mutual properties of conidial and mycelia structure or metabolic capacity could be the reason for the *Aspergillus* resistance compared to *Fusarium*. The inhibitory effect of *G. pelargonium* against *A. ochraceus*, *A. flavus* and *A. parasiticus* (88.8, 82.2 and 79.2% reduction, respectively) was recorded at a concentration of 2000 ppm, whereas *Z. multiflora* EO completely inhibited the growth of four fungi (100% reduction) at the same concentration. These antifungal activities of *Z. multiflora* and *G. pelargonium* EOs were also demonstrated by Javidnia *et al.* (1999) on toxigenic fungi. Overall, these EOs exhibited considerable inhibitory effects against all fungi understudy and their different concentrations demonstrated various degrees of growth inhibition. In conclusion, our data confirmed that *Z. multiflora* and *G. pelargonium* EOs possessed *in vitro* antifungal activity against toxigenic species of *A. flavus*, *A. parasiticus*, *A. ochraceus* and *F. verticillioides*.

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