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Antifungal activity of the essential oil of Iranian medicinal plants

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Plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries. Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments as they contain antimicrobial properties. Antifungal activities of essential oil of four Iranian herbs including, *Thymus daenensis* var *daenensis* Celak, *Zataria multiflora* Boiss, *Thymbra spicata* var. *spicata* L. and *Bunium persicum* (Boiss.) K.-Pol. were investigated against of *Aspergillus niger* (PTCC 5298), *Aspergillus fumigates* (PTCC 5009), *Aspergillus flavus* (PTCC 5004) and *Aspergillus parasiticus* (PTCC 5018) by agar disc diffusion assay. Some of the essential oils showed relatively antifungal activity against the tested fungal. Of the herbs studied, the most active extracts were those obtained from essential oil of *T. daenensis* and *T. spicata*. The MIC values for active extract ranging between 64 and 256 µg/ml. The results obtained appeared to confirm the antifungal potential of the herbs investigated. The essential oils of *T. daenensis* and *T. spicata* could be used as natural antifungal against *A. niger*, *A. fumigates*, *A. flavus* and *A. parasiticus* in the food preservation and human health.

Key words: Iranian medicinal plants, *Aspergillus*, essential oil, antifungal activity.

INTRODUCTION

A very large number of fungi such as *Aspergillus* are responsible for producing mycotoxins in various foods and feeds (Abarc et al., 1994; Kedera et al., 1999; Lund et al., 1996; Lee et al., 2007). Fungus are also known to be responsible for the off-flavour formation and production of allergenic compounds, which often takes place before the growth of fungi is evident. *Aspergillus* is an extremely important genus concerning pathogenicity, mycotoxins, fundamental eukaryotic genetics and biotechnological exploration, several books have been dedicated to *Aspergillus* in general (Vanden et al., 1988; of *Aspergillus* in particular (Samson and Pitt, 1985, 1990, Bennett and Klich, 1992; Martinelli and Kinghorn, 1994;

Powell et al., 1994) and to the taxonomy and phylogeny (2000). *Aspergillus* species occur most frequently in soil, water, decaying vegetation and organic debris. *Aspergillus* is the most ubiquitous fungus with airborne conidia. It sporulates abundantly and the spores are simply dispersed into the environment by air. Because of this ubiquitous presence, people are probably constantly exposed to *Aspergillus* spores. Several species have been described as human pathogens (Girardin et al., 1994).

Aspergillus species have excellent growth characteristics and extracellular protein secretion capacities and have been used in industry for production of extracellular enzymes (Iwashita, 2002; Wang et al., 2003; Ward, 1989). Some of *Aspergillus* species produce mycotoxins, which are a serious problem as they have adverse effects on human health (Baird et al., 2006). *Aspergillus fumigates* is the organism most frequently

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isolated from human infections (Lin et al., 1995; Latge, 1999; Latge and Steinbach, 2008). *Aspergillus flavus* is the second most common etiologic agent of human aspergillosis (James et al., 2000).

The saprophytic species *A. fumigatus* is found worldwide and has an essential role in recycling carbon and nitrogen (Latge, 1999; Pitt, 1994). *A. fumigatus* is the most common mold pathogen of humans and causes both invasive disease in immunocompromised patients and allergic disease in patients with atopic immune systems (Denning et al., 2002; Barnes and Denning, 1993; Rinaldi, 1993; Kwon-Chung and Bennett, 1992; Patterson et al., 2000; Soubani and Chandrasekar, 1988). These diseases include allergic aspergillosis, aspergilloma (colonization of air spaces) and invasive pulmonary aspergillosis (Kwon-Chung and Bennett, 1992). *A. flavus* is the second leading cause of invasive and non-invasive aspergillosis (Hedayati et al., 2007). In addition, it is the main *Aspergillus* species infecting insects, and it is able to cause diseases in economically important crops, such as maize and peanuts, and to produce potent mycotoxins. *A. flavus* can infect corn, peanuts, cotton, and nut trees as well as other crops and growth on these agricultural commodities often leads to contamination with aflatoxin B1, a toxic and potent carcinogenic compound (Krishnan et al., 2009). *A. flavus*, the most common causal fungus, produces aflatoxins B1 and B2. It is close relative, *Aspergillus parasiticus*, produces aflatoxins B1, B2, G1 and G2 (Ehrlich et al., 2005; Yu et al., 2004). *Aspergillus niger* is a filamentous ascomycete fungus that is ubiquitous in the environment and has been implicated in opportunistic infections of humans (Perfect et al., 2001).

In the past decade, interest on the topic of antimicrobial plant extracts has been growing. Various spices and herb extracts have been used for the purpose of food preservation and appetizer promotion as well as medicinal purposes (Cowan, 1999; Zaika, 1988). In particular, extracts from many oriental spice plants and herbs such as sage, thyme, rosemary and mint have been known to possess antimicrobial effects (Nilsen and Rios, 2000; Shelef et al., 1980; Smith et al., 1998; Tassou et al., 2000; Yildirim et al., 2000). In this study, we examined the antifungal activity of herbs such as *Thymus daenensis* var *daenensis*, *Zataria multiflora*, *Thymbra spicata* var. *spicata* and *Bunium persicum*, which have been used as medicinal sources in Iran (Ghasemi Pirbalouti, 2009).

The objectives of this study were to examine the effect of different herb essential oils on some fungi by determination of the diameter of the zone of inhibition of fungi growth around the discs. Special interest was on the essential oils that were obtained from four herbs due to their better flavor.

MATERIALS AND METHODS

Herb sample preparation

Four Iranian traditional herbs used in this study were *Thymus daenensis* var *daenensis* Celak (Lamiaceae), *Zataria multiflora* Boiss (Lamiaceae), *Thymbra spicata* var. *spicata* L. (Lamiaceae) and *Bunium persicum* (Boiss) K.- Pol (Apiaceae). These herbs were chosen based on their reported biological activities. *T. daenensis* was collected from Bakhtiari Zagross Mountains, *T. spicata* was collected from Ilam, and other herbs were purchased from a local traditional Chaharmahal va Bakhtiari medicine store. The aerial parts of *T. daenensis*, *Z. multiflora* and *T. spicata* and fruits of *B. persicum* were air-dried, and then ground into fine powder using grinder and stored at room temperature.

Extract preparation

Dried plant materials were powdered (200 g) and subjected to hydro-distillation (in 2000 ml distilled water) for 4 h, using a Clevenger-type apparatus according to the method recommended in British pharmacopoeia (British Pharmacopoeia, 1988).

Fungal strains

Five fungal strains used in this study, including *A. niger* (PTCC 5298), *A. fumigatus* (PTCC 5009), *A. flavus* (PTCC 5004) and *A. parasiticus* (PTCC 5018) were obtained from the culture collection at Persian Institute of Industrial and Scientific Research (Persian Type Culture Collection). Cultures of fungal were grown on Sabouraud dextrose agar (Merck, Germany). Each microorganism was suspended in sterile saline and diluted at ca. 10^7 colony-forming unit (CFU/ml).

Antifungal test

The disc diffusion method of Jennette (1985) was used with some modification to determine the rate of growth inhibition of fungi by the examined plant essential oils. Sabouraud dextrose agar (Merck, Germany) was used to prepare the culture medium and autoclaved at 121 °C for 15 min. Plates (8 cm diameter) were prepared with 10 ml agar inoculated with 1 ml of each microbial suspension. The essential oils were dissolved in dimethyl sulfoxide (DMSO, 20 µl) before testing for antifungal activity. Sterile paper discs (6 mm in diameter) were impregnated with 60 µl of dilutions of known essential oils concentrations (8 to 256 µg/disc) and incubated at 37 °C for 48 h. A disc (6 mm diameter) of cycloheximide was used as a positive control. Microbial growth inhibition was determined as the diameter of the inhibition zones around the discs (mm). The growth inhibition diameter was the average of three measurements, taken at three different directions. All tests were performed in triplicate.

RESULTS

The growth inhibition value of essential oil on four fungal strains is shown in Table 1. The essential oils from the different plant species studied showed antifungal activities, with the diameters of inhibition zone ranging

Table 1. Antifungal effects of herb essential oils on the growth of four microbial strains.

Microbial strain	<i>Thymus daenensis</i>					
	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	128 µg/ml	256 µg/ml
<i>A. niger</i>	8 ^a	14	16	No growth	No growth	No growth
<i>A. fumigates</i>	9	12	16	No growth	No growth	No growth
<i>A. flavus</i>	-	11	15	No growth	No growth	No growth
<i>A. parasiticus</i>	-	9	12	No growth	No growth	No growth
	<i>Zataria multiflora</i>					
	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	128 µg/ml	256 µg/ml
<i>A. niger</i>	7	9	11	No growth	No growth	No growth
<i>A. fumigates</i>	9	11	13	22	No growth	No growth
<i>A. flavus</i>	-	11	17	24	No growth	No growth
<i>A. parasiticus</i>	-	-	15	No growth	No growth	No growth
	<i>Thymbra spicata</i>					
	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	128 µg/ml	256 µg/ml
<i>A. niger</i>	8	10	12	16	No growth	No growth
<i>A. fumigates</i>	10	11	13	18	No growth	No growth
<i>A. flavus</i>	8	9	10	12	No growth	No growth
<i>A. parasiticus</i>	7	9	11	23	No growth	No growth
	<i>Bunium persicum</i>					
	8 µg/ml	16 µg/ml	32 µg/ml	64 µg/ml	128 µg/ml	256 µg/ml
<i>A. niger</i>	-	-	-	-	-	13
<i>A. fumigates</i>	7	8	9	10	12	13
<i>A. flavus</i>	-	-	-	8	10	11
<i>A. parasiticus</i>	-	-	-	-	-	-
Cycloheximide	No growth	No growth	No growth	No growth	No growth	No growth

^a: Diameter of inhibition zone in mm; -: no inhibition.

from 7 to 24 mm. There were significant differences ($P \leq 0.01$) in the antifungal activities of plant extracts. Among the plants tested the essential oils of *T. daenensis* and *T. spicata* aerial parts (leaves and stem) showed the best antifungal activity (Table 1). These were followed by the antifungal essential oil of *Zataria multiflora* aerial parts (leaves and stem) and *B. persicum* fruits which exhibited strong inhibitory activities. The result showed that most of the essential oils could effectively inhibit the growth of *A. fumigates*. Among the plants that were tested, essential oil of *T. daenensis* and *T. spicata* could effectively inhibit the growth of *A. niger* (Table 1). The essential oils of *Z. multiflora* and *T. spicata* could effectively inhibit the growth of *A. flavus*. Interestingly, the essential oils of *T. spicata* showed promising inhibitory activity against *A. parasiticus*. Subsequent experiments were conducted to determine minimal inhibitory concentration (MIC) of all selected essential oils. The MIC values for active

essential oil ranging between 64 and 256 µg/ml (Table 1). The lowest MICs of *T. daenensis* against *A. flavus*, *A. fumigates*, *A. parasiticus* and *A. niger* were 64 µg/ml (Table 1).

DISCUSSION

The antifungal activities of the essential oils of the plants varied in relation to the test organisms. The most active was a 128 µg/ml concentration of the essential oils from *T. daenensis*, *Z. multiflora* and *T. spicata* that inhibited completely the growth of all yeast. In this study, the essential oils from *T. daenensis*, *Z. multiflora* and *T. spicata* exhibited inhibitory effect on fungal growth, suggesting that the studied plant essential oils are potentially a safe and natural source of antifungal agents. Brul and Coote (1999) have previously discussed the

mechanisms of how natural compounds in herbs exert their function. Many compounds are responsible for plant flavor, and humans use them to season the food, and serve as useful medicinal compounds. Some of the same herbs and spices. Among those antimicrobial compounds, phenolic compounds, terpenoids, and alkaloids are very important components in antimicrobial or antioxidant effects, and epidemiologists have observed that a diet rich in those compounds may result in a positive health effect (Fernandez et al., 1996; Houghton et al., 1994; Houghton and Paya, 1996; Ríos and Recio, 2005; Rojas et al., 1992; Scalbert, 1991). However, analyses of the active compounds, which inhibit the growth of tested fungi, were under the way in our laboratory. Many herb and spice extracts, for example *T. daenensis* and *T. spicata*, contained high levels of phenolics and exhibited antibacterial activity against food-borne pathogens (Ghasemi et al., 2010). Several researchers have demonstrated the antifungal and antibacterial activity exhibited by the extracts and essential oils of medicinal plants, but unfortunately, there are few data related to the antimicrobial activity of extracts obtained from different medicinal plants in Iranian medicinal plants. Previous studies (Rasooli et al., 2006) on the antimicrobial activity of the essential oils of some *Thymus* species showed that most of the species which possess large quantities of phenolic monoterpenes, have shown activity against viruses, bacteria, food-derived microbial strains and fungi.

The essential oil effect of *Z. multiflora* Boiss against growth, spore production and aflatoxin formation by *A. flavus* ATCC 15546 showed that at 200 ppm, the radial growth and sporulation reduced by 79.4 and 92.5%, respectively (Gandomi et al., 2009). Also, they suggested the potential substitution of the antifungal chemicals by this EO as a natural inhibitor to control the growth of molds in foods such as cheese. The other study the effects of essential oil different concentrations of *Z. multiflora* Boiss (EO: 0, 5, 15 and 30 μl 100 ml^{-1}) and nisin (N: 0, 0.25 and 0.5 μg ml^{-1}), temperatures (T: 25 and 8°C), and storage times (up to 21 days) on growth of *Salmonella typhimurium* and *Staphylococcus aureus* in a commercial barley soup were evaluated. The growth of *S. typhimurium* was significantly ($P < 0.05$) decreased by EO concentrations and their combinations with N concentrations at 8°C. For *S. aureus*, the viable count was significantly ($P < 0.05$) inhibited by EO and N concentrations and their combinations, incubated at both storage temperatures (Moosavy et al., 2008). All the essential oil showed varying degrees of antifungal activity on the microorganisms tested. Some of these plants were more effective than traditional antimicrobial to combat the pathogenic microorganisms studied. The essential oils of *T. spicata* and *T. daenensis* leaves and flowers had antifungal activities. The present study suggests that the

essential oil of these plants is a potential source of natural antifungal agents. After this screening experiment, further work should be performed to describe the antifungal activities in more detail as well as their activity *in-vivo*. In addition, phytochemical studies will be necessary to isolate the active constituents and evaluate the antifungal activities against a wide range of fungi population.

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