Antifungal Activity of Dried Extracts of Anise *(Pimpinella anisum* L.) and Star anise (*Illicium verum* Hook. f.) Against Dermatophyte and Saprophyte Fungi

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Receive: 6 Aug. 2008 **Acceptance:** 18 Feb. 2009

Abstract

Background: Medicinal plants synthesize a vast array of secondary metabolites that are important for human life. For medicinal purpose, antimicrobial activity of substances derived from plant extracts has been recognized for many years. *Pimpinella anisum* L. (*Apiaceae*) and *Illicium verum* Hook. f. (*Illiciaceae*) plant species, have been used for treatment of infectious diseases in Iranian traditional medicine.

Objective: In this study methanol extracts of *Pimpinella anisum* L. (*Apiaceae*) and Illicium *verum* Hook. f. (*Illiciaceae*), were tested for their potential antifungal activities.

Methods: Methanolic extracts were dried by freeze drying method, Minimum Inhibitory Concentration (MIC) was was determined according to agar dilution method and Minimum Fungicidal Concentration (MFC) was determined by incorporating various concentrations of extracts (2-256 mg/ml) in Sabouraud dextrose agar (SDA) in tubes against 4 dermatophyte and one saprophyte fungi.

Results: The extracts of anise seeds inhibited only dermatophyte species, while extracts of star anise fruits inhibited growth of all dermatophytes and saprophytes. MIC and MFC for each extracts were different and MFC was higher than MIC for all species.

Conclusion: As a result of this experiment, these plants can be candidate for further studies due to their antifungal potencies.

Keywords: Antifungal, *P. anisum*, *I. verum*, *Aspergillus niger*, Dermatophyte, Saprophyte

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[DOR: 20.1001.1.2717204.2009.8.29.24.5]

Introduction

Dermatophyte fungi are the main agents of skin diseases of man and remain a threat to public health in the tropics [1]. Although *Aspergillus* spp. are not considered to be a major cause of plant diseases, they are responsible for contamination of various plants and their products. They cause spoilage of agricultural products at different stages including pre-harvest, harvest, processing and handling [2]. Several species of *Aspergillus* genera are able to produce mycotoxin in foods [3]. Usage of medicinal plants by man have been known for centuries and therapeutic efficacy of several herbal species has been widely described [1, 4, 5].

Pimpinella anisum L. (*Apiaceae*) is an annual herb indigenous to the near east and widely cultivated in the Mediterranean regions as well as Mexico and Chile. It has been used as an aromatic herb and spice since Egyptian times [4]. *Illicium verum* Hook. f. (*Illiciaceae*) is the most frequently found among several species in the world. *I. verum* or commonly called star anise is non-poisonous and has been used in culinary preparations and also as medicine. It is distributed in North America, Atlantic and the tropical and subtropical zones of Asia [8].

The star anise and anise have both been widely used in Iranian traditional medicine for their antimicrobial effects [6]. The antimicrobial properties of these species have been reviewed by several researchers: anise [4, 7] and star anise [5].

Materials and methods

A) Plant extracts preparation

The plant materials (*P. anisum* seeds and *I. verum* fruits) used in this study were collected from herbal medicine traditional markets in Tehran, Iran. All plant materials were identified by the Herbarium of Institute of Medicinal Plants-ACECR, Tehran, Iran and authenticated by Dr. Amin Gh. of the Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences (TUMS).

The dried aniseeds and star anise fruits were first ground to powder, then 100 g powder of each were suspended in petroleum ether and kept at room temperature overnight for removal of all fatty substances. The supernatant was discarded and the residue was dried at room temperature. The residue was further suspended in 250ml of methanol in sterile conical flasks and kept at 4º C overnight. The methanolic phase were separated using separator funnel, subsequently filtered through Whatman filter paper No. 1 and the filtrate was dried in a freeze dryer. The dried extract was weighed and dissolved in 5% dimethyl sulfoxide (DMSO) in water using ultrasonic bath. The final concentration of DMSO was less than 2% (w/v).

B) Fungal inoculum preparation

Four of fungi, namely *Candida albicans PTCC* 5027, *Trichophyton mentagrophytes PTTC* 5054, *Microsporum canis PTTC* 5069 and *Aspergillus niger PTTC* 5011 were obtained from Persian Type Culture Collection, Iranian Research Organization for Science and Technology, Tehran, and *Epidermaphyton* floccosum was provided by Department of Mycology, Faculty of Health Science, Tehran University. All species were maintained by lyophilization form in small vials at 4ºC. The fungi were activated by 0.5 ml of sterile distilled water for half hour under sterile condition and were subculture on Sabouraud dextrose agar (SDA) in 9cm plate and incubated at 28ºC for 7 to 21 days (related to growth speed for each species). When fungi mycelium covered 80% of plate surface the spores were harvested

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aseptically using 5 ml of sterile distilled water. The spore suspensions were filtered through sterile fine texture cloth and adjusted to a concentration of 1-2 × 10^6 spores/ml in sterile distilled water, corresponding to absorbance of 1.0 for *A. niger* and 0.6 for other species at 450 nm wavelength.

C) Determination of Minimum Inhibitory Concentration (MIC)

MIC was determined according to agar dilution method [9, 10]. Various concentrations (20, 40, 80, 160, 320, 640, 1280, 2560 mg/ml) of each extracts were prepared in 10 cm experimental tubes containing SD broth. Each tube contains 9 ml of SDA and were sterilized by autoclaving. Upon cooling, 1 ml of each extract concentration were added to each tube, to make the final concentrations of 2, 4, 8, 16, 32, 64, 128, 256 mg/ml. The mixture of SDA and extracts were poured into plates aseptically in a laminar flow cabinet. Upon solidification of the agar medium, 2 µl of adjusted spore suspension were added to each plate by micropipette and incubated at 28° C. The SDA without any herbal extract served as control. The MIC was regarded as the lowest concentration of the extract that did not show any visible growth after 14 days of incubation (compared with control).

D) Determination of Minimum Fungicidal Concentration (MFC)

The *in vitro* fungicidal activities based on MFC were determined for each extracts as previously described [11, 12] with slight modifications. The MFC was determined by incorporating various concentrations of extracts (2-256 mg/ml) in SD broth in tubes. One milliliter adjusted spore suspension was added to each tube and incubated at 28°C for 3 days. The SD broth without incorporation of dried herbal extract and 1 ml of adjusted spore suspension served as positive control and SD broth alone served as negative control. The tubes which showed no visible growth after 3 days incubation were subculture on extract free SDA plates and incubated at 28°C for 7 days. The MFC was regarded as the lowest concentration of the extract that prevented the growth of any fungal colony on the solid medium.

Results

The *P. anisum* seed extracts showed MIC at 16 mg/ml concentration for 4 species but demonstrated no inhibitory effect on *A. niger* growth (Table 1). The MFC recorded for *P. anisum* seed extracts was different from 8 to 256 mg/ml but showed no any fungicidal effect on *A. niger* at the concentrations tested (Table 3).

The ethanol extracts of *I. verum* fruits showed MIC at 16 mg/ml concentration for *A. niger*, *C. albicans*, *M. canis* and 4 mg/ml for *E. fluccosum* and *T. mentagrophytes* (Table 2). The MFC recorded for *I.verum* was different from 8 to 256 mg/ml (Table 4).

Discussion

It is important to investigate scientifically plants that have been used in traditional medicines to determine potential sources of novel antimicrobial compounds [13].

The MIC and MFC of the *I. verum* fruit extracts were not similar, as shown in Table 2 and 4, where MIC for *A. niger*, *C. albicans* and *M. canis* were 16 mg/ml, while MFC for these 3 fungi were 256, 128 and 64 mg/ml respectively. The results showed that MFC was higher than MIC for fungi tested which were similar to the findings by Kosalec et al., 2005.

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Table 1: Minimum Inhibitory Concentration (MIC) of *P. anisum* dried extract on different fungi species

Fungi species	Dried extract concentration (mg/ml)				
	64	**32**	**16**	**8**	**control**
Aspergillus niger	-	-	-	-	-
Candida albicans	+	+	+	-	-
Microsporum canis	+	+	+	-	-
Epidermophyton fluccosum	+	+	+	-	-
Trichophyton mentagrophytes	+	+	+	-	-

+: Positive inhibition -: Negative inhibition

Table 2: Minimum Inhibitory Concentration (MIC) of *I. verum* dried extract on different fungi species

Fungi species	Dried extract concentration (mg/ml)						
	64	**32**	**16**	**8**	**4**	**2**	**control**
Aspergillus niger	+	+	+	-	-	-	-
Candida albicans	+	+	+	-	-	-	-
Microsporum canis	+	+	+	-	-	-	-
Epidermophyton fluccosum	+	+	+	+	+	-	-
Trichophyton mentagrophytes	+	+	+	+	+	-	-

+: Positive inhibition -: Negative inhibition

Table 3: Minimum Fungicidal Concentration (MFC) of *P. anisum* dried extract on different fungi species

Fungi species	Dried extract concentration (mg/ml)							
	256	**128**	**64**	**32**	**16**	**8**	**4**	**Control**
Aspergillus niger	-	-	-	-	-	-	-	-
Candida albicans	+	-	-	-	-	-	-	-
Microsporum canis	+	+	-	-	-	-	-	-
Epidermophyton fluccosum	+	+	+	+	+	+	-	-
Trichophyton mentagrophytes	+	+	+	+	-	-	-	-

+: Positive inhibition -: Negative inhibition

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Table 4: Minimum Fungicidal Concentration (MFC) of *I. verum* dried extract on different fungi species

Fungi species	Dried extract concentration(mg/ml)							
	256	**128**	**64**	**32**	**16**	**8**	**4**	**Control**
Aspergillus niger	+	-	-	-	-	-	-	-
Candida albicans	+	+	-	-	-	-	-	-
Microsporum canis	+	+	+	-	-	-	-	-
Epidermophyton fluccosum	+	+	+	+	+	+	-	-
Trichophyton mentagrophytes	+	+	+	+	-	-	-	-

+: Positive inhibition -: Negative inhibition

The MIC and MFC of the *P. anisum* seed extracts differed from those obtained for *I. verum*. Tables 2 and 4 showed that *P. anisum* seed extract had no inhibitory effect on *A. niger* growth and the MIC was found to be similar (16 mg/kg) for four fungi species.

The use of plant extracts in the treatment of diseases caused by various bacteria, viruses and fungi had been reported. Antifungal properties of plant extracts are widely recognized [1, 4, 8, 9].

The present study showed that *I. verum* fruit extracts and *P. anisum* seed extracts have high antifungal properties. The *I. verum* extract at a concentration of 4mg/ml inhibited the growth of *E. flucosum* and *T. mentagrophytes*. The extracts of both *I. verum* and *P. anisum* at 16 mg/ml controlled all dermatophyte fungi tested. The *I. verum* fruits extract inhibited growth of *A. niger*, one of the most important saprophytic fungus known to be associated with mycotoxin production in agricultural products and foods, at 16 mg/ml.

References

1. Natarajan V, Venugopal P & Menon T. Effect of *Azadirachta indica* (Neem) on the growth pattern of dermatophytes. *Indian J. of Medical Microbiol*. 2003; 21, 98 - 101.

2. Perrone G, Susca A, Cozzi G, Ehrlich K, Varga J, Frisvad JC et al. Biodiversity of *Aspergillus* species in some important agricultural products. *Studies in Mycology* 2007; 59: 53 - 66.

3. Palacios CH, Taniwaki MH, Hashimoto JM, Menezes HC. Growth of *Aspergillus ochraceus*, *A. carbonarius* and *A. niger* on culture media at different water activities and temperatures. *Brazilian J. of Microbiol*. 2005; 36: 24 - 8.

4. Kosalec I, Pepeljnjak S, & Kustrak D. Antifungal activity of fluid extract and essential oil from anise fruits (*Pimpinella anisum* L., *apiaceae*). *Acta Pharmaceutica* 2005; 55 (4): 377 - 85.

5. Sung og L, Park IK, Choi GJ, Lim HK, Jang KS, Cho KY et al. Fumigant activity of essential oils and components of *Illicium verum* and *Schizonepeta tenuifolia* against

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Botrytis cinerea and *Colletotrichum* gloeosporioides. *J. of Microbiol. Biotechnol.* 2007; 17 (9): 1568 -72.

6. Amin, G. Popular medicinal plants of Iran. Tehran-Iran: Tehran University of Medical Sciences. 2005.

7. Gulcın I, Oktay M, Kırecci E, Ku frevıog Lu OI. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food Chem*. 2003; 83: 371 - 82.

8. De M, De A, Sen P, Aanerjee BA. Antimicrobial actions of star anise *Illicium verum* Hook f. *Ars Pharm.* 2001; 42 (3-4): 209 - 20.

9. Benger S, Townsend P, Ashford RL, Lambert P. An *in vitro* study to determine the minimum inhibitory concentration of *Melaleuca alternifolia* against the dermatophyte *Trichophyton rubrum*. *The Foot* 2004; 14: 86 - 91.

10. Kuete V, Simo IK, Ngameni B, Bigoga JD, Watchueng J, Kapguep RN et al. Antimicrobial activity of the methanolic extract, fractions and four flavonoids from the twigs of *Dorstenia angusticornis* engl. (Moraceae). *J. of Ethnopharmacol*. 2007; 112 (2): 271 - 7.

11. LI RK, Ciblak MA, Nordoff N, Pasarell L, Warnock DW, Mcginnis MR. *In vitro* activities of voriconazole, itraconazole, and amphotericin B against *Blastomyces dermatitidis, Coccidioides immitis,* and *Histoplasma capsulatum*. *Antimicrob. Agents. Chemother*. 2000; 44 (6): 1734 - 6.

12. Espinel-Ingroff A, Fothergill A, Peter J, Rinaldi MG, Walsh TJ. Testing conditions for determination of minimum fungicidal concentrations of new and established antifungal agents for A*spergillus spp*.: NCCLS collaborative study. *J. of Clin. Microbiol*. 2002; 40 (9): 3204 - 8.

13. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. *J. of Appl. Microbiol.* 1999; 86: 985 - 90.

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