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Antifungal Effects of Various Extracts of *Ephedra major* Host on Common Fungal Pathogens

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Abstract: According to the fungal drug resistance and toxic adverse effect of antifungal drug, development of more effective and less toxic antifungal agents is the inevitable necessity of fungal treatment. Historically, plants have provided a source of inspiration for novel drug compounds, as plant-derived medicines have made large contributions to human health and well being. This study was undertaken to evaluate the *invitro* effects of *Ephedra major* Host on fungi agent that are common cause of infections. Four various Extracts from *Ephedra major* (collected from Alborz province, Iran) were assessed for antifungal activity against *Trichophyton mentagrophytes* (PTCC 5054), *Microsporum canis* (PTCC 5069), *Candida albicans* (ATCC 10231, PTCC 5027) and *Nocardia asteroids* (clinical isolated). The agar tubes containing known concentration of various extracts as well as extract free control, prepared and inoculated by microorganisms. The methanol, ethanol and acetone extracts showed broad spectrum antifungal activity although aqueous extract was not able to fully inhibit fungal growth even at highest concentration 125 mg/ml. It was concluded he ability of the arial part extracts of *E. major to* inhibit the growth of Fungi is an indication of its broad spectrum antifungal potential which may be employed in the management of fungal infections or food preservation.

Key words: *Ephedra major* Host · Herbal extract · Antifungal · Phytomedicine

INTRODUCTION

In recent decade worldwide, increase in fungal infections and antifungal drug resistance prevalence, putting public health at risk [1]. There is an increased awareness of the morbidity and mortality associated with fungal infections caused by resistant fungi in various groups of patients [2]. Because both fungi and humans are eukaryotes, thus fungal and human cells are similar at the molecular level. This makes it more difficult to find or design drugs that target fungi without affecting human cells [3]. As a consequence the majority of the clinically used anti-fungal suffer from various drawbacks in terms of toxicity, drug–drug interactions, lack of fungicidal efficacy, cost and emergence of resistant strains caused by the frequent use of some of them [4]. In spite of the recent introduction of new anti-fungal drugs, they are still limited in number there is a critical need for new anti-fungal agents to treat common fungal infections and life-threatening invasive infections [4]. Importance of medicinal plants has been continually increasing both for pharmaceutical industry and traditional users.

Plants are among the most important and common sources of potentially valuable new drugs [5]. Therefore, there is a need to investigate the biological properties of medicinal plants in order to develop new drugs.

Ephedra is a genus of gymnosperm shrubs, the only genus in the family Ephedraceae and order Ephedrales. These plants occur in dry climates over a wide area mainly in the Northern Hemisphere, across southern Europe, north Africa, southwest and central Asia, southwestern North America and, in the Southern Hemisphere, in South America south to Patagonia [6]. They are also called Joint-pine, Jointfir, or Mormon-tea. The Chinese name is mα huαng. Ephedra is also called Houm, soma and rish boz in Iran. *Ephedra major* is a shrub erect herb that growth widely in Iran, this family commonly known as Mahung is one of the oldest and most widely used drug in the orient. as reported *E. major* [7] have higher total alkaloid in compare to other 11 species that habitant Iran.

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Plants of the Ephedra genus, including *E. major* Host and others, have traditionally been used by indigenous people for a variety of medicinal purposes, including treatment of asthma, hay fever and the common cold [5, 8]. They have also been proposed as a candidate for the Soma plant of Indo-Iranian religion [9]. The alkaloids ephedrine and pseudoephedrine are active constituents of *E. major* and other members of the genus. These compounds are sympathomimetics with stimulant and decongestant qualities and are related chemically to the amphetamines. other phytochemical composition occurs in Ephedra such as Cyclopropyl-α- amino acid, Flavonoids, Kynorenates and etc [10].

MATERIALS AND METHODS

Plant Material: Wild plant *E. major* was collected during May–June 2010 from Alborz Province, Iran (N" 35.49' 29, "E 51.02' 10) and identified at Department of Biology, Islamic Azad University (IAU) Karaj branch.

Extraction Method: Arial plant parts were air dried and subsequently powdered using a mixer for the preparation of methanol, ethanol, acetone and aqueous extracts. Air-dried, powdered plant material (30 g) was macerated in 100% solvent (200cc) at the room temperature for 48 h on a rotary shaker. Aqueous extract obtained by 1 hours boiling without soaking. All extracts were filter through Buchner funnel with Whatman filter paper number one. The filtrates obtained extract was concentrated by rotary evaporator at 60°~ C under reduced pressure to final volume 20cc (1.5 g/cc).

Microorganism: The following microorganism used for the biological evaluation were, either purchased from Persian type culture (PTCC) or was clinical isolated kindly provided by Mycology Department of Science and Research Branch of IAU DR. S.Jamal Hashemi Hazaveh: *Microsporum canis* (PTCC 5069), *Candida albicans* (ATCC 10231,PTCC 5027), *Trichophyton mentagrophytes* (PTCC 5054) and *Nocardia asteroids* (clinical isolated).

Antifungal Activity Assessments: Antifungal bioassays were carried out by using agar tube dilution method (macro dilution). On the basis of company instruction the base media was made of sabouraud dextrose agar (SDA), in contrast to the instruction we added 80% volume of distilled water (DW) but later the remaining volume of DW was added along with extract.

Table 1: Final Extract concentration in culture media

Tube	*dilution*	*Extract concentration in 1ml of media*
1	1:2	125 mg
2	1:4	62.5 mg
3	1:8	31.25 mg
4	1:16	15.62 mg

At the temperature 25°C 1ml of extract mixed with 1ml DW to obtain 750 mg/ml extract concentration. on the basis of serial dilution method different concentration of extract were archived then 1ml diluted extract in various concentration were added to screw capped test tube containing 5ml media were autoclaved 121°C for 15 minute and allowed to cool to 50°C (before solidification). This mixture well shaken.the test tube that contain ethanol, methanol or acetone extracts were placed in water bath kept at temperature 50 for 30 minute for solvent evaporation after this stage tube left to solidify at room temperature on a slant. Culture media inoculated at least 12 hours after preparation to ensure complete solvent evaporation. Final extract concentrations in media containing extract are shown in Table 1.

The medium without the plant extracts and solvent and medium with solvent without plant extract served as control.

T. mentagrophytes and *M. canis* were stab inoculated in the culture media, supplemented with different concentrations of various plant extracts (`extract-included') or without the plant extract (controls) and cultures were incubated at 28°C for 14 days. *N. asteroids* and *C. albicans* streaked inoculums along the surface of slant medium (with extract and controls) before incubated at 37 for 1 week. Cultures were examined daily during incubation. All tests were repeated 3 times to ensure results accuracy.

RESULTS

Assay result of *Ephedra major* extracts against 3 standard fungi species and clinical isolated *N. asteroids* are listed in Table 1. Aqueous extract was not able to fully inhibited fungal growth, although culture media containing aqueous extract had less growth rate (compared to controls tube).*M. canis* growth completely inhibited even by lowest acetone extract concentration used in this survey.

Table 2: Minimum inhibitory concentration (MIC) of *Ephedra major* Extracts

	Extract			
Microorganism	Methanol	Ethanol	Acetone	Aqueous
Nocardia asteroids	31.25	62.5	31.25	U
Microsporum canis	31.25	31.25	<15.62	U
Trichophyton mentagrophytes	62.5	62.5	62.5	U
Candida albicans	125	62.5	125	U

U: undefined (fungi growth was not fully inhibited at the concentrations used in this study)

DISCUSSION

Ephedras; a known medical plant has long history of traditional healing therapies for centuries Zoroastrians of central Iran have collected Ephedra in them mountain and have use them as Haoma [8]. Ephedras employed as a bronchodilator and decongestant, ephedrine is used to relieve nasal congestion originating from allergic conditions, e.g. hay fever, or from bacterial or viral infection of the upper respiratory tract. It may be used as well to raise blood pressure. But, synthesized ephedrine also resulted in the discovery of an entire new class of drugs, (amphetamines) and is used in the production of methamphetamine [11].

Ephedra chemical compositions depend on species, harvesting time, Geographic Region of plant habitant and extraction technique accordingly different Ephedras exhibit variation in chemical and pharmacological properties [7].

Despite the long history of medical use of Ephedra in traditional medicine, enough research has not been taken on the medicinal properties of Ephedra. Few studies have been carried out to evaluate the antimicrobial properties of this plant. Most of which were performed on *E. intermedia* species.

Recent studies show several important species of Ephedra have antimicrobial activity [12-16]. As far as known to the writer, only one limited study of antimicrobial effect of *Ephedra major* Host extracts has ever been made so far [15].

According to test result showed in the Table 2. The ethanol extract of *E. major* exhibited antifungal activity against the test strains used. The important point is the most effective plant extracts against Candida albicans was ethanol extract, but in other fungi this extract was not more effective than acetone and methanol extract. Antifungal effects of acetone extracts was very strong against *Microsporum canis* and even at the lowest concentration of the extract, the fungus was not able to growth. *Candida albicans* exhibited very resistance to this extract So that only the first tube containing the highest concentration of extract inhibited fungi growth. Acetone seems to have more ability than other solvents to exploit active antimicrobial ingredients of this plant. Candida albicans showed weak sensitivity to methanol extract (such as acetone extracts) in comparison with ethanol extract. All fungi were able to growth at the concentrations of aqueous extract used in this study. Lower antifungal activity of aqueous extract may due following reason:

- Water has low ability to extract the active compound of plant
- Boiling plant powder lead to deterioration of active material of plant (boiling was not done for rest solvent)

Reasons of different response and resistance to various extracts of Ephedra major are probably for the following reason:

- Various solvent employed in extraction have different ability to dissolution of a definite chemical compositions
- Chemical composition of each extract is differ from the rest in case of chemical material or the quantity of each material and different sensitivity of microorganism to each of these materials causes different sensitivity pattern of antifungal test.

As mentioned above the only study on antimicrobial activity of *Ephedra major* conducted on inhibitory effect of extract and essential oil of *Ephedra major* Host (collected from Lorestan province, Iran) on growth and Aflatoxin production of *Aspergillus parasiticus*. Bagheri *et al.*[15] conducted this survey demonstrated that Methanol extract and essential oil (EO) of *Ephedra major* have antifungal activity on *A. prasiticous*. this antifungal activity attributed to the Flavonid, Heptadecan and Citronellol compound of plant extract that detected by GC-MASS. In addition the compound bis (2-ethyl) phthalate has been detected in EO of *Ephedra major* has been said that has antifungal activity [15]. Although we test the different fungus species for antifungal assessment but or result showed the Ephedra major extracts contained the antifungal active material.

In conclusion, according to the above mentioned study and tests results the *Ephedra major* extracts have

broad spectrum antifungal activity and these activities are varied among different Ephedra species and even in *E. major* species collected from different geographical region.

We recommend different species of Ephedra undertake to the phytochemical analyses to investigate new antimicrobial material. There is no proved best way (and solvent) for Ephedra extraction. Many physiological activities attributed to the Ephedra in traditional medicine that remain to be elucidated, scientific study of these allegations will help to better understanding of usage Ephedra in folk medicine.

Ephedra major is a resistance plant and can growth in extreme environmental condition screening more productive plant of these species and vast culturing are economically applicable.

The result of this study may form the basis for future investigation to isolate active compounds, elucidate the structure and their evaluation against wider rang of drug resistance fungal strains with the aim to fine new antifungal drug and food preservative agent.

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