Research Journal of Pharmacognosy (RJP) 1, 2014: 39-43
Received: Aug 2013
Accepted: Nov 2013

Original article

In vitro antifungal activity of four medicinal plants used in Iranian Traditional Medicine

S.J. Hashemi[1], J. Asgarpanah[2*], Z. Alaee[2], S. Sadeghian[2], H. Hasani[2], A. Azimi[2]

[1]*Department of Medical Mycology and Parasitology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.*
[2]*Department of Pharmacognosy, Pharmaceutical Sciences Branch, Islamic Azad University (IAU), Tehran, Iran.*

Abstract

Evaluating the *in vitro* antifungal activity of *Phlomis lanceolata, Rhynchocorys elephas, Otostegia persica* and *Eremurus persicus*, four species used in Iranian Traditional Medicine, has been performed on the clinical isolates of the pathogenic fungi *Aspergillus fumigatus, A. flavus, Trichophyton mentagrophytes, T. rubrum, T. verrucosum, Microsporum canis, M. gypseum and Epidermophyton floccosum* and the yeast *Candida albicans*. The susceptibility tests were done by agar disc diffusion method and the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of active extracts and sub-fractions were measured using the method of National Committee for Clinical Laboratory Standards (NCCLS). Among the investigated species, *P. lanceolata* sub-fractions were found to have fungicidal activity. The MIC and MFC was found to be considerable in petroleum ether, chloroform and ethyl acetate fractions (100 and 200 mg/mL) against the studied fungi and the yeast *Candida albicans*. The species appears to be a promising remedy for fungal based diseases, yet further studies are necessary.

Keywords: Antifungal activity, *Eremurus persicus, Otostegia persica, Phlomis lanceolata*, *Rhyncocorys elephas*

Introduction

Affordability and accessibility of the medicinal plants have made them an important part of many people's life all over the world. The medicinal plants selection is a conscious process, which has led to an enormous number of medicinal plants being consumed by many cultures in the world [1]. According to the World Health Organization (WHO), due to the poverty and lack of access to modern medicines, about 65-80% of the world's population in developing ountries rely on plants for their primary health care [2].

Regarding the lack of safe modern drugs, evaluation of active and effective plants for diseases such as diabetes and infectious conditions has been recommended by WHO [3].

It is estimated that close to 25% of the active compounds in currently prescribed synthetic drugs were first identified in natural sources especially plants [4].

Mycotic infections caused by pathogenic fungi are the most common cause of skin and systemic infections in developing countries.

**Corresponding author*: asgarpanah@iaups.ac.ir, Tel: +9821-22640051, Fax: +9821-22602059

Humid weather, over population and poor hygiene are the ideal conditions for these fungi growth [5]. In general, dermatophytes live in the dead, top layer of skin cells in moist areas of the body, such as toe web, the groin, and under the breasts. These fungal infections cause only a minor irritation. Other types of fungal infections could be more serious. They can penetrate into the cells and cause itching, swelling, blistering and scaling. In some cases, fungal infections can cause reactions elsewhere in the body. The dermatophytes, *Trichophyton, Epidermophyton* and *Microsporum canis* are commonly involved in such infections. However, their clinical differentiation is difficult. The clinical care is required by a physician or other healthcare professional in the treatment of these diseases [6].

Phlomis lanceolata Boiss. & Hohen., *Rhynchocorys elephas* (L.) Griseb., *Otostegia persica* (Burm.) Boiss. and *Eremurus persicus* (Jaub. & Spach) Boiss., are four medicinal plants which grow wild in Iran. The aerial parts of these plants have long been used in Iranian Traditional Medicine to treat skin disorders caused by fungi infections. The present investigation has been carried out to confirm the traditional use of these species as antifungal natural agents for treatment of mycotic infections.

Experimental

Plant materials

The flowering aerial parts of *P. lanceolata, E. persicus, O. persica* and *R. elephas* were collected from Ardabil (Northwest of Iran), Golpayegan (Center of Iran), Sistan-Baluchistan (South of Iran) and Rasht (North of Iran), respectively, in May 2011, and identified by Dr. Gholam Reza Amin. The voucher specimens (No. 823, 197, 475 and 178, respectively) were deposited in the Herbarium of the Department of Pharmacognosy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.

Extracts and sub-fractions preparation

The air dried flowering aerial parts of the collected plants (1 kg) were exhaustively extracted by percolation method with MeOH. The extracts were evaporated to yield the residue. 25 g of the extracts were kept separately in 4 clean vials, in a dark and cool place for further studies.

The remaining extracts were partitioned between water, petroleum ether, chloroform, ethyl acetate and MeOH. These sub-fractions were also kept separately in clean vials, in a dark and cool place for further studies.

Fungal strains

Trichophyton mentagrophytes, T. rubrum, T. verrucosum, Aspergillus fumigatus, A. flavus, Epidermophyton flucossum, Microsporum canis, M. gypseum and the yeast *Candida albicans* were the nine different clinical isolates of fungi and yeast taken for this study.

Antifungal assay

The selected isolates were grown on sabouraud dextrose agar (SDA). 21 days old culture of fungi was scraped with a sterile sculpture and macerated with sterile distilled water. The suspension was adjusted spectrophotometrically to an absorbance of 0.600 at 450 nm. For further studies, known quantity of this inoculum was used. Susceptibility testing was performed by agar well diffusion method described in the European Pharmacopoeia [7]. Minimum inhibitory concentration (MIC) was followed as per the National Committee for Clinical Laboratory Standards, USA.

For the agar diffusion method, 25±2 mL of sterile and melted sabouraud glucose agar (SGA) at 45–50 °C was inoculated with 1 mL of approximately $1–5\times10^6$ CFU/mL of inoculum of yeast or fungi in sterile physiological saline in Petri-dishes (9 cm). Inoculum density was measured with McFarland's standard solution of freshly prepared barium sulfate in sterile water; density of 0.01% $BaCl_2$ in 1% H_2SO_4 solution equals approximately 3×10^8 cells/mL [8]. After drying SGA at room temperature for a maximum of 30 minutes, holes of 6 mm in diameter were made with stainless steel cylinders and filled (80 µL) with different concentrations of the extracts or sub-fractions. One experiment was done in a Petri-dish for one fungal strain. Plates were then incubated at +4 °C for 1 h and at 25±2 °C for 48 h for the yeast and 10 days for fungi. After the

incubation period, inhibition zones were measured and expressed in mm.

For MIC value determination, samples (extracts and sub-fractions) were stocked in DMSO (<1%). The sample solution was further diluted (1:10) with RPMI1640 medium prior testing. Each sample was then diluted (1:2) and divided into 10 tubes. The nine strains were grown to 10^4 CFU/mL and then co-incubated with extracts or sub-fractions for 72 h at 28 °C. The anti-fungal agent, nystatin, was used as the positive control. For the conventional micro-dilution procedure, the growth in each sample well was compared with that of control with the aid of a reading mirror.

Each micro-dilution well was then given a "numerical score" as following: 4 meant no reduction in growth; 3 indicated a slight reduction in growth or approximately 75% compared to growth of control (drug-free medium); 2 implied a prominent reduction in growth or approximately 50% in comparison to the growth of the control; 1 was a slight growth or an approximately only 25% compared to control and 0 showed optically clear or absence of growth. The minimal inhibitory concentration (MIC) was then determined for each test sample [9].

Table 1. Inhibition zone diameter (mm) of *Phlomis lanceolata* extract and sub-fractions.

strains / Samples	*A. flavus*	*A. fumigatus*	*T. mentagrophytes*	*T. verrucosum*	*T. rubrum*	*E. floccosum*	*M. canis*	*M. gypseum*	*C. albicans*
MeOH extract									
100 mg/mL	-*	-	-	-	-	-	-	-	-
200 mg/mL	-	-	-	-	-	-	-	-	-
400 mg/mL	-	-	-	-	-	-	-	-	-
Pet fraction									
100 mg/mL	-	-	-	-	-	-	-	-	-
200 mg/mL	9.4	9.8	8.8	9.1	8.2	8.9	9.1	8.3	10.8
400 mg/mL	19.2	18.0	19.4	18.7	18.3	19.0	19.6	18.4	22.2
Chl fraction									
100 mg/mL	-	-	-	-	-	-	-	-	-
200 mg/mL	10.1	10.4	10.2	9.6	9.7	10.3	9.9	10.7	11.0
400 mg/mL	21.2	21.1	20.8	20.7	20.7	21.6	21.2	20.9	21.5
EthOAc fraction									
100 mg/mL	-	-	-	-	-	-	-	-	-
200 mg/mL	11.2	11.2	11.2	11.2	11.2	11.2	11.2	11.2	10.0
400 mg/mL	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	20.4
MeOH fraction									
100 mg/mL	-	-	-	-	-	-	-	-	-
200 mg/mL	-	-	-	-	-	-	-	-	-
400 mg/mL	-	-	-	-	-	-	-	-	-
Aqueous fraction									
100 mg/mL	-	-	-	-	-	-	-	-	-
200 mg/mL	-	-	-	-	-	-	-	-	-
400 mg/mL	-	-	-	-	-	-	-	-	-
nystatin 0.5 mg/mL	2.8	2.8	2.7	3.0	2.8	2.9	2.4	2.7	3.2

*: no inhibition

Results and Discussion

Occurrence of fungal diseases is a serious problem of the present world. This is because of the development of antifungal drugs resistance to the pathogens and side effects exhibited by the drugs used for fungal diseases. Hence there is a great demand for safer, alternative and effective chemotherapeutic agents. Use of medicinal herbs in the treatment of skin diseases including mycotic infections is an old practice in many parts of the world [10]. Plants contain a spectrum of secondary metabolites and their importance as antimicrobial or antifungal agents has been emphasized by several works [5].

The extracts and sub-fractions of four species, *P. lanceolata*, *E. persicus, O. persica and R. elephas* were investigated for antifungal activity against dermatophytic fungi and yeasts. As these plants were used widely as antifungal agents in Iranian Traditional

Table 2. MIC and MFC values of *Phlomis lanceolata* crude extract and sub-fractions (mg/mL).

strains / Samples	*A. flavus*	*A. fumigatus*	*T. mentagrophytes*	*T. verrucosum*	*T. rubrum*	*E. floccosum*	*M. canis*	*M. gypseum*	*C. albicans*
MeOH extract									
MIC	-*	-	-	-	-	-	-	-	-
MFC	-	-	-	-	-	-	-	-	-
Pet fraction									
MIC	1	0.5	1	1	2	2	0.5	0.5	1
MFC	4	2	2	2	4	4	2	1	2
Chl fraction									
MIC	1	2	1	1	1	1	0.5	1	2
MFC	2	4	4	4	4	2	2	2	4
EthOAc fraction									
MIC	0.5	1	0.5	0.5	0.5	1	0.5	0.5	1
MFC	2	4	1	1	2	2	1	1	2
MeOH fraction									
MIC	-	-	-	-	-	-	-	-	-
MFC	-	-	-	-	-	-	-	-	-
Aqueous fraction									
MIC	-	-	-	-	-	-	-	-	-
MFC	-	-	-	-	-	-	-	-	-
Nystatin									
MIC	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
MFC	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

*: not determined

Medicine to treat fungal infections based skin disorders, we were prompted to evaluate their antifungal activity against eight clinical isolates of fungal strains and the yeast *Candida albicans*. The results revealed that the extracts and sub-fractions of *E. persicus, O. persica* and *R. elephas* demonstrated no inhibitory activity on the growth of all the investigated clinical isolates of the yeast and fungal strains. *P. lanceolata* showed antifungal activity against all fungal strains (table 1). MIC and MFC of *P. lanceolata* extract and sub-fractions are presented in Table 2.

As shown in the table 1, ethyl acetate, chloroform and petroleum ether fractions showed good activity against the investigated fungi. *P. lanceolata* is one of the 17 endemic Iranian species of the genus *Phlomis* [11,12]. A number of *Phlomis* species have been used in Traditional Medicine as stimulant and tonic, and to treat pains, respiratory tract infections, diabetes and wounds [12,13]. Three phenylethanoid glycosides responsible for strong antibacterial activity have been isolated from this plant [14]; however, the present study showed that the polar sub-fractions of *P. lanceolata* including aqueous, methanol and the crude extract were inactive. The non-polar compounds such as triterpenoids present in the species could be responsible for these observations. It was overall concluded that among the four investigated species, *P. lanceolata* possessed biologically active constituent(s) that demonstrated antifungal activity which supports the traditional claims of the use of the plant in the management of fungal based skin disorders and has the potential to be introduced as an antifungal agent. Future studies such as isolation and identification of the responsible compound(s) is suggested and may help to design a new drug against pathogenic fungi and the yeast *Candida albicans*.

References

[1] Heinrich M, Barnes J, Gibbons S, Williamson EM. Fundamentals of pharmcognosy and *phytotherapy*. Oxford: Elsevier science Ltd, 2004.

[2] Calixto JB. Twenty-five years of research on medicinal plants in Latin America: A personal view. *J Ethnopharmacol.* 2005; 100: 131-134.

[3] Kim JD, Kang SM, Park MY, Jung TY, Choi HY, Ku SK. Ameliorative anti-diabetic activity of dangnyosoko, a chinese herbal medicine, in diabetic rats. *Biosci Biotechnol Biochem.* 2007 71: 1527-1534.

[4] Halerstein RA. Medicinal plants: Historical and cross-cultural usage patterns. *Ann Epidemiol*. 2005; 15: 686-699.

[5] Vaijayantimala J, Rajendra Prasad N, Pugalendi KV. Antifungal activity of oils. *Indian J Microbiol* 2001; 41: 325-328.

[6] Beentje HJ. Moringaceae. In Kenya Trees Shrub and Lianas. Nairobi: Majestic Printing Works Ltd, 1994.
[7] European Pharmacopoeia, 4th ed. Council of Europe, Strasbourg
[8] MacFaddin JF. *Biochemical tests for identification of medical bacteria.* Lippincott Williams & Wilkins, Philadelphia. 2000.
[9] Chuang PH, Lee CW, Chou JY, Murugan M, Shieh BJ, Chen HM. Anti-fungal activity of crude extracts and essential oil of *Moringa oleifera* Lam. *Biores Technol*. 2007; 98: 232–236.
[10] Irobi ON, Darambolo SO. Antifungal activity of crude extracts of *Mitracarpus villosus* (Rubiaceae). *J Ethnopharmacol.* 1993; 40: 137-140.
[11] Rechinger KH. *Flora Iranica.* Graz-Austria: Akademic Druck-u Verlagsanstalt. 1982.
[12] Mohajer M, Sarkhail P, Hajarolasvadi N, Zamani MJ, Khorasani R, Shafiee A et al. Antiinflammatory and analgesic effects of *Phlomis lanceolata* Boiss. and Hohen. extracts and examination of their components. *Int J Pharmacol.* 2006; 2: 50–54
[13] Sarkhail P, Amin G, Surmaghi MHS, Shafiee A. Composition of the volatile oils of *Phlomis lanceolata* Boiss. & Hohen., *Phlomis anisodonta* Boiss. and *Phlomis bruguieri* Desf. from Iran. *Flavour Fragra J*. 2005; 20: 327–329.
[14] Nazemiyeh H, Mukhlesur Rahman M, Gibbons S, Nahar L, Delazar A, Ghahramani MA et al. Assessment of the antibacterial activity of phenylethanoid glycosides from *Phlomis lanceolata* against multiple-drug-resistant strains of *Staphylococcus aureus*. *J Nat Med.* 2008; 62: 91-95.