

Full Length Research Paper

Anti fungal activity of alcoholic extract of *Peganum harmala* seeds

K. Diba^{1*}, M. Gerami Shoar², M. Shabatkhori³ and Z. Khorshivand¹

¹Department of Medical Parasitology and Mycology, School of Medicine, Urmia University of Medical Sciences, Uromia, Iran.

²Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

³Infectious Diseases Research Center, Department of Medical Parasitology and Mycology, Faculty of Medicine, Golestan University of Medical Sciences, Gorgan, Iran.

Accepted 8 June, 2011

In our study, it was aimed to determine antifungal activity of *Peganum harmala* on medically important yeasts and molds *in vitro*. Fungal suspensions of *Candida* spp and aspergillus spp isolated from the clinical specimens were treated with serial dilutions of *P. harmala* alcoholic extract in Sabouraud Broth. The titers included: 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, and incubated for 24 h at 30°C. Anti fungal activity of the extract was determined as MIC and MFC. For the determination of MFC, a small volume of above serial dilutions was cultured on SGA 4% medium. After 24 to 72 h of incubation at 30°C, the cultures were looked for fungal growth. The highest and lowest inhibitory effects of *P. harmala* extract were determined on *C. glabrata* (MIC: 0.312 mg/ml) and *C. albicans* (MIC: 1.25 mg/ml), respectively. The MFC for *Candida* species was differed from 0.62 to 2.5 mg/ml. The highest fungicidal effect was seen on *C. glabrata* and *C. tropicalis* (MFC: 0.62 and 0.125 mg/ml, respectively) and the lowest was for *C. albicans* (MFC: 2.5 mg/ml). The treatment of Aspergillus suspensions with Peganum extract showed a decreasing of growth and sporulation without definite inhibition. The alcoholic extract of *P. harmala* showed a fungicidal activity on opportunistic yeasts, *Candida* spp, and a decreasing sporulation for aspergillus most important species.

Key words: Growth inhibitory, *Peganum harmala*, fungi.

INTRODUCTION

Peganum harmala (Zygophyllaceae) is an intriguing herb with a long history of medicinal use. It is widely grow in the most parts of the world including North and South America, Mexico, Africa and Asia. *P. harmala* is widely distributed in the Southern part of Iran too. It is known as "espan" in Persian and is famous for its medical uses as analgesic and antiseptic in folk medicine. *P. harmala* has significant usages in industrial and medical aspects (Berrougui et al., 2006). It has been medically used by the people of ancient countries such as India, Iran, Egypt and Spain. Some people use it typically for enhancing limbs. People in the west Asia burn the seeds to make

smoke for keeping safe against voodoo (Rojhan, 2002). Levchenko (1987), Kang (1994) and Rashan (1989) carried out first studies on anti parasitic and anti viral activities of *P. harmala*. Also anti parasitic effect of the plant seeds have been recently reported by Iranian researchers (Mahdavi and Masoud, 2003; Nateghpour et al., 2006). Also anti tumor activity of *P. harmala* was shown by Lamchouri et al. (1999). Peganum seeds contain carbohydrates, lipids, proteins, minerals, amino acids and alcoholic compounds as well as saturated fatty acids.

On the other hand, during last decades, fungi have emerged as new life threatening agents, considering increased opportunistic infections in human and animals. In fact, many medical centers in the world are challenge with the event. Furthermore some of these organisms have earned a resistance to anti fungal drugs. Regarding the high frequency of fungal infections among the

*Corresponding author. E-mail: kambiz37diba@gmail.com. Tel: +98 441 367 4886, +98 912 446 4972 (Mobile). Fax: +98 441 278 0800-1.

immunocompromised patients, and also the desirable effects of the *P. harmala* on the growth of other micro organisms, such as bacteria and parasites, it is necessary to study the antifungal usage of *P. harmala* seeds which seem to be practical. Present research is one of the first studies in Iran which carried out on antifungal activities of *P. harmala in vitro*. The goal was to measure absolute minimum concentration of alcoholic extract of *P. harmala* for inhibiting and killing of most important fungi causing human infection.

MATERIALS AND METHODS

Collection and identification of the plant

About 2000 g of the plant seeds were prepared from traditional drug stores as the seeds of Espand (in Persian) and then confirmed as Iranian *P. harmala* by the Plants Systematic laboratory, UM School of Botany, Iran.

Preparation of alcoholic extract

Amount of 1000 g of *P. harmala* seeds was powdered in an electrical grinder and then added to 3200 ml of ethanol 96°, shaken for 2 h and incubated over night at room temperature to remove alcoholic solvent from the crude extract. Again, the same amount of solvent was added to the crude extract and the process was repeated five times. The solvent was separated completely by a vacuum filtering system. The final product was 110 g of a brown color extract with biting odor.

Micro organisms

Main subjects included 111 isolates of *Candida* species and *Aspergillus* species obtained from clinical specimens of patients in department of Medical Mycology, School of Public Health and Institute of Public Health Research. For the improvement of accuracy in the results MIC and minimum fungicidal concentration (MFC) tests, all of the 111 clinical isolates were used. *Candida* isolates were identified in the level of species using differential medium CHROM agar *Candida* included *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. tropicalis*, *C. kefyr* and *C. parapsilosis*. Identification of *Aspergillus* species by using morphological characteristics resulted three species of *A. fumigatus*, *A. flavus* and *A. niger*. Each fungal species was confirmed by Molecular Mycology laboratory of TUMS.

Preparation of fungal suspensions

Fungal suspensions including 10^5 *Candida* cells and 10^3 *Aspergillus* spores in aqueous Sabouraud medium were made. For avoiding spore propagations a little volume of Tween 80 were added into the suspensions. Standard Mc farland 0.5 containing barium chloride and sulphuric acid was used as turbidity control.

Serial dilution of *P. harmala* extract

Serial dilutions in tubes

Small volume (1 ml) of sabouraud broth in capped tubes was used as substrate. 1 ml of 10% solution of the crude extract in sterile

water was added into the substrate and mixed thoroughly. Then 1 ml of the solution was transferred into the next tube containing 1 ml substrate, after which it was mixed and transferred into the next tube with the same substrate. This was done sequentially till it got to the 6th tube, so that six decreasing titers of *P. harmala* alcoholic extract in substrate including: 1/20, 1/40, 1/80, 1/160, 1/320, 1/640 were made. Then 1 ml of each *Candida* suspension was inoculated into dilution tubes and incubated at 30°C for 24 h. Negative and positive controls were prepared by adding 1 ml of 1/20 diluted extract and *Candida* suspension, respectively, into the substrate.

Serial dilutions in agar media

Sabouraud glucose agar 4% (SGA 4%) was measured, mixed with distilled water, autoclaved and dispensed in medium size (8 cm diameter) plates, 10 ml in each after cooling up to 60 to 55°C. While it was melted, the alcoholic extract was added into media plates in a decreasing pattern to make titers: 1/10, 1/100, 1/200, 1/400, 1/800, respectively. After making solid agar media, 1 ml of *Aspergillus* suspensions was inoculated into the dilution plates and left at 30°C for 72 h.

MIC (minimum inhibitory concentration) determination

After incubation time at 30°C, the tubes and plates of reactions were investigated for fungal growth. It was performed for *Candida* isolates, comparing turbidity of test tubes with that of Mc Farland standard 0.5. Positive tubes were found without any turbidity of growth.

MFC (minimum fungicidal concentration) determination

All tubes without turbidity (negative for growth) in serial dilution test were used for the determination of MFC. A small volume from the negative tubes was transferred into plates of Sabouraud agar and the incubated at 30°C for 72 h. MFC of *peganum* extract on *Candida* isolates was determined finding the media plates without any growth of *Candida* colonies. For the *Aspergillus* isolates, the first dilution plate without fungal growth was considered as MFC point.

RESULTS

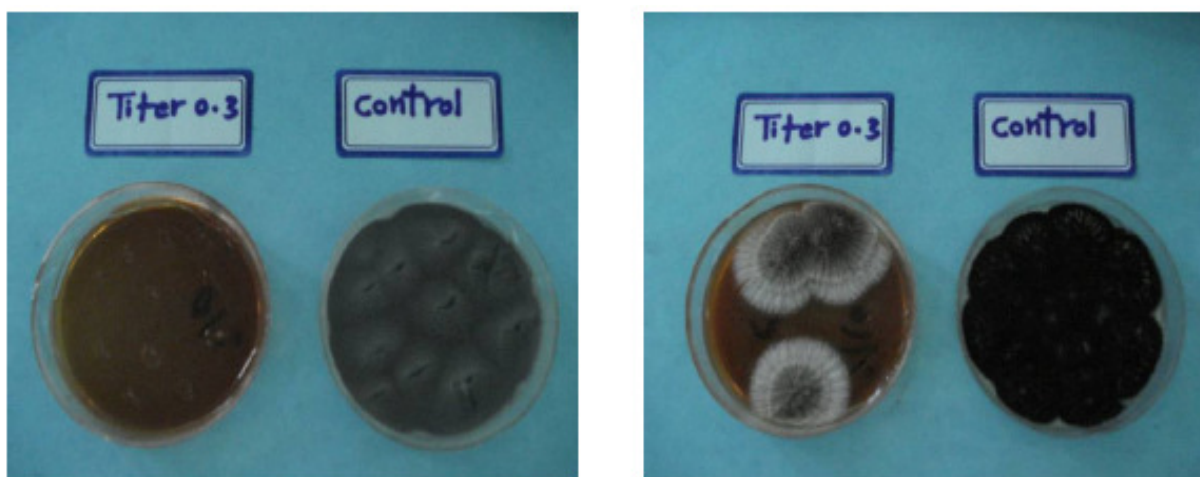
Totally 111 *Candida* isolates including *C. albicans*, *C. parapsilosis*, *C. keffir*, *C. glabrata* tested for MIC and MFC. Inhibitory test on 6 *Candida* species and 3 *Aspergillus* species resulted a range of MIC: 0.312 to 1.25 mg/ml. Alcoholic extract of *P. harmala* showed; MIC: 0.312 mg/ml on *C. glabrata* and MIC: 1.25 mg/ml on *C. albicans* as the highest and lowest inhibitory effects, respectively (Table 1). Moreover, minimum fungicidal concentration of the extract on *Candida* isolates was determined in a range of 0.625 to 2.5 mg/ml.

As our results, the highest fungicidal effect of the extract (MFC: 0.625 mg/ml) was found on *C. glabrata*, and the lowest on *C. albicans* (MFC: 2.5 mg/ml).

Our assay on *Aspergilli* showed different results in the level of species. The extract was effective on *A. niger* and *A. fumigatus* as growth inhibitory but for *A. flavus* the result was restricted to some changes in morphology

Table 1. MIC (minimum inhibition concentration) and MFC (minimum fungicidal concentration) in *Candida* species.

<i>Candida</i> species	No of isolates	MIC (mg/ml)	MFC (mg/ml)
<i>C. albicans</i>	102	1.25	2.5
<i>C. parapsilosis</i>	11	0.625	1.25
<i>C. Keiffir</i>	3	0.625	1.25
<i>C. glabrata</i>	2	0.312	0.625
<i>C. tropicalis</i>	2	0.312	1.25
<i>C. dubliensis</i>	1	0.625	1.25

**Figure 1.** Effect of PAE on *Aspergillus fumigatus* (left) and *A. niger* (right) in the dilution of 0.3.

characteristics, macroscopic features and sporulation rate, color and size of colonies (Figures 1 and 2). Microscopic variations were not included for *Aspergillus* species. Time effectiveness in MIC assay was shown in Table 2. It is clear that 24 h treatment for fungal suspension with *P. harmala* is much more effective than 2 and 6 h treatment.

DISCUSSION

P. harmala seeds have been considered from ancient time to date as a plant with drug usages regarding to some alkaloids compounds such as harmalin and harmalol. The compounds extracted from this plant have shown different medical characteristics such as anti inflammation effects of pitolin were reported by El-Saad El Rifaei (1980). He confirmed anti bacterial/anti fungal, anti fever and anti parasite effects on *P. harmala*. Most studies on medical effects of *P. harmala* are on parasite infections. Levchenko was one of the first people (Levchenko, 1987) who reported the medical effects of alkaloids of *P. harmala* on bovine Theileriosis et al. (1994) indicated protoscolocidal effects on *P. harmala* in hydatid cysts (Rashan, 1989). Some compounds of this

plant have anti parasite and anti microbial effects such as alkaloids that have drug usage on Babesia infected cattle (Fan et al., 1997). Anti bacteria and anti fungal activity of harmaline have reported by Abdel-Fattah et al, (1995). Most relevant researches are *in vivo* studies, but they are rare *in vitro* studies such as Shahverdi et al. on anti microbial effects of smoking of *P. harmala* seeds, also chemical effects of the plant on *Staphylococcus epidermidis* and *Cryptococcus neoformans* (Shahverdi et al., 2005).

The plant on *Staphylococcus epidermidis* and In this study anti fungal activity of *P. harmala* on 6 and 3 species of *Candida* and *Aspergillus*, (respectively) was evaluated *in vitro*. The inhibitory activity of alcoholic extract of *P. harmala* has been measured by the determination of MIC and MFC parameters. The lowest MIC was shown on *Candina albicans* the highest MIC on *C. glabrata*. Low activity of Peganum extract on *C. albicans* can be explained by the resistance of most of *C. albicans* clinical strains to anti fungal drugs. Shafiee and Gilani tested anti fungal activity of *P. harmala* seeds on *Microsporom*, *Trichophyton* and *Fonescae pedrosoei* with serial dilutions and they reported 0.125 mg/ml as lowest MIC. In the present study MIC differs in different species of *Candida*. MICs were decreased from 0.25 to 0.031 mg/

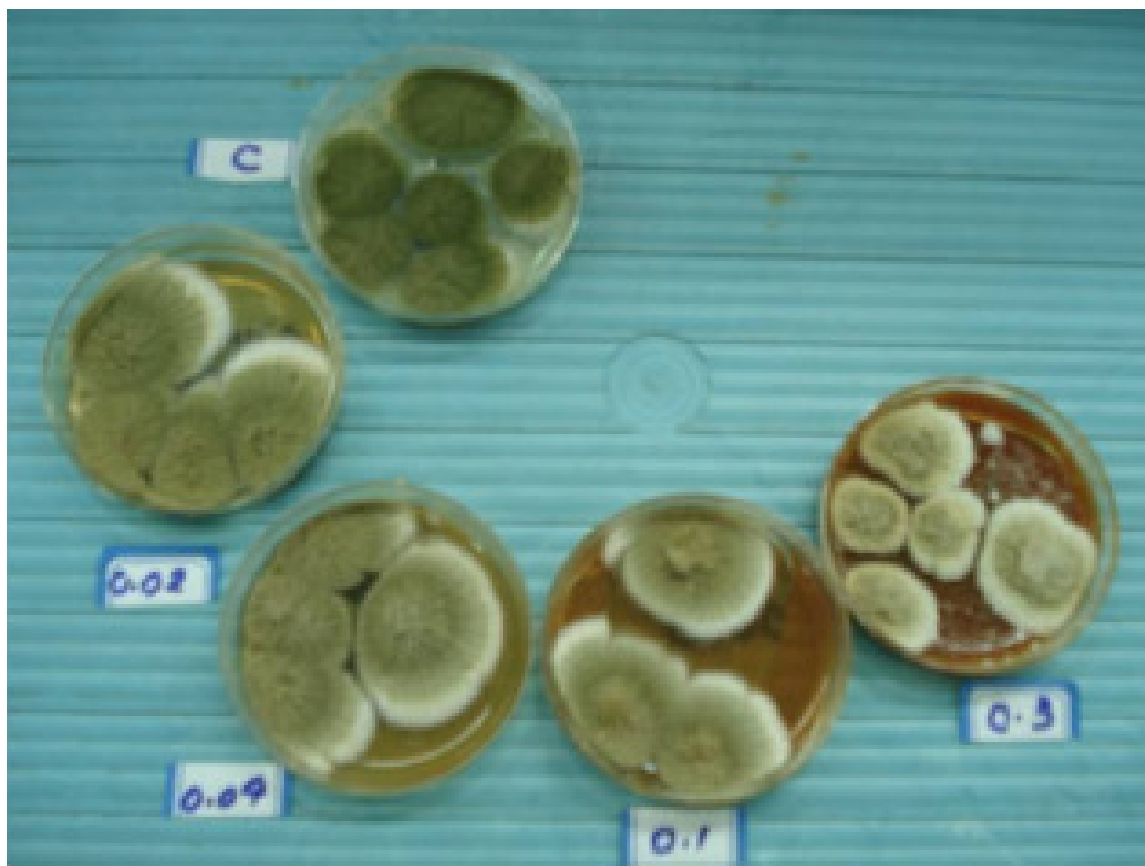


Figure 2. Modified Morphology of *A. flavus* in colony coloration and sporulation in the dilutions 0.3 to 0.02.

Table 2. Effect of incubation time on fungicidal activity of *P. harmala* extract.

Species	No. of isolates	Growth after 2 h	Growth after 6 h	Growth after 24 h
<i>C. albicans</i>	16	G	G	NG
<i>C. parapsilosis</i>	1	NG	NG	NG
<i>C. kefgyre</i>	1	NG	NG	NG
<i>C. glabrata</i>	2	G	NG	NG
<i>C. tropicalis</i>	1	NG	NG	NG

G: growth, NG: no growth.

ml on *Candida* species: *C. albicans*, to *C. tropicalis* and *C. glabrata*, respectively. By the consideration of dense and compact cell wall of yeasts comparing filamentous fungi cell walls, it seems to be reasonable that *Candida* spp. were resistant to *P. harmala* extract more than *Aspergillus* spp. Although *P. harmala* alcoholic extract inhibited mildly *A. niger* and *A. fumigatus* but it was not considerable. The alcoholic extract of Iranian *P. harmala* showed inhibitory and fungicidal activities on opportunistic pathogenic *Candida* spp, but it caused very low inhibition of growth and sporulation of tested *Aspergillus* spp.

ACKNOWLEDGEMENTS

We thank Dr. Jamal Hashemi for helping us to access the clinical isolates, also Leila Hosseinpur for assisting us in the lab. This project was supported by the deputy head of Research, Institute of Health Research, TUMS.

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