Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(6):140-142
(http://derpharmachemica.com/archive.html)

Evaluation of the antifungal effects of various extracts of *Amygdalus eburnea* on some fungal pathogens

Mehdi Rezaeifar[1], Seyyed Amin Ayatollahi Mousavi[2], Mitra Mehrabani[1] and Asghar Sepahvand[3,*]

[1]*Department of Pharmaceutics, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran*
[2]*Department of Medical Mycology and Parasitology, Kerman University of Medical Sciences, Kerman, Iran*
[3]*Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran*

ABSTRACT

The present study aims to evaluate the antifungal activity of Amygdalus eburnea Spach. (Rosaceae family) extracts against some fungi strains. Antifungal effects of A. eburnea were performed using Minimum Inhibitory Concentration (MIC) methods on Aspergillus flavus (ATTCC 15546) and Candida albican (ATCC 10321). Both aqueous and menthanolic extract of A. eburnea at all concentration demonstrated fungistatic activity against the tested fungi from week to potent with the MIC ranging from 5.33 to 9.33 mg/mL and 7.3 to 13.33 mg/mL, respectively. To conclude, the obtained findings demonstrated that A. eburnean extracts were found to be more active against some pathogenic fungi strains and thus provided the evidence for its traditional use value and it is suitable substitute for treatment of fungi infections.

Keywords: *Amygdalus eburnean*; MIC; *Aspergillus flavus* (ATTCC 15546) and *Candida albican*

INTRODUCTION

Nowadays, there has been an increasing incidence of fungal infections because of growth in immunocompromised people, such as HIV / AIDS patients [1, 2]. Fungal infections are usually related to *Candida*, *Aspergillus* and *Cryptococcus* species but those due to *Candida* species indicate the main opportunistic fungal infections worldwide, leading to high morbidity and mortality in the population [3, 4]. Current treatments of fungal infections are numerous, but, a few classes of antifungal drugs are currently available for treatment of infections in order to some limitations such as the high toxicity, the emergence of drug resistance, pharmacokinetic deficiencies, and/or insufficiencies in their antifungal activities [5, 6]. These reasons emphasize the urgent need for development of new effective treatment alternatives. Plant extracts and plant-derived compounds, due to having fewer side effects, low cost and high availability, are valuable sources that are commonly used to treat a wide range of disease conditions including infectious diseases [6-8]. One of these interesting plants is *Amygdalus eburnea* Spach. (called “Ghosk” in Persian) from family of Rosaceae as a type of almond which is naturally grown and distributed in Iran [9] In folk Iranian medicine *A. eburnea* has been used as laxative and anti-worm. Moreover, brew of dermal tissue are used for cough, respiratory distress and paregoric [9].

The present study aims to evaluate the antifungal effects of methanolic and aqueous extract of *A. eburnea* against some fungal pathogenic strains such as *Aspergillus flavus* and *Candida albican* to detect new sources of antifungal agents.

MATERIALS AND METHODS

Collection of plant materials

The shell root of *A. eburnean* was collected from rural regions of from Baft district, south east of Iran, in April 2013.

They were identified by a botanist of the Botany Department of Shahid Bahonar University, Kerman, Iran [10]. A voucher specimen of the plant materials was deposited at the Herbarium of Department of Pharmacognosy of School of Pharmacy, Kerman University of Medical Science, Iran (KF 1136).

Preparing of extracts

One hundred gram of powdered plant material was separately extracted by percolation method with methanol (80%) and water successively for 72 h. in room temperature. The extracts were passed through filter paper (Whatman No.3, Sigma, Germany) to remove plant debris. The extracts were finally concentrated in vacuum at 50°C using a rotary evaporator (Heidolph, Germany) and stored at -20°C, until testing [11, 12].

Antibacterial Activity

Microorganisms

Fungal pathogenic strains including *Aspergillus flavus* (ATTCC 15546) and *Candida albican* (ATCC 10321) were used for the experiment.

Cultivation of fungi

The fungi were grown on Sabouraud Dextrose Agar (SDA) slants at 28°C for 7 days. After complete growth the spores were collected using sterile ice cold doubled distilled water and homogenized for the antifungal study [13].

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the compound was performed according to the reference method described elsewhere [14, 15]. The extracts were dissolved in water together with 2% dimethyl sulfoxide (DMSO). The initial test concentration (0.5 mg/mL) was serially diluted twofold to obtain the extracts at the concentrations 0.5-16 mg/mL. Each well was inoculated with 5 µL of suspension containing 10^4 spore/mL of fungi, respectively. The plates were incubated for 24-72 h at 30°C. Ketoconazole and DMSO were also used as positive and negative control, respectively. Five µL of tested broth was placed on the sterile MHA plates and sealed in plastic bags to avoid contamination in the laboratory and at respective temperature. The MICs were calculated after an incubation time with no visible growth. The experiment was conducted in triplicate.

Statistical analysis

All the tests were performed in triplicate. Data analysis was carried out by using SPSS statistical package version 17.0 (SPSS Inc., Chicago, IL, USA). Differences between test and control groups were analyzed by *t*-test. In addition, $p<0.05$ was considered statistically significant [16, 17].

Table 1. Mean of minimum inhibitory concentration (MIC) of *Amygdalus eburnea* extracts against some pathogenic fungi strains

Tested sample (mg/ml)	Bacterial strain	
	Aspergillus flavus	*Candida albican*
Methanolic extract	9.33	5.33
Aqueous extract	13.33	7.33
Ketoconazol	0.5	0.5

RESULTS AND DISCUSSION

Table 1 shows the results of *in vitro* antifungal assay of *A. eburnean* extracts. Both aqueous and menthanolic extract of *A. eburnea* at all concentration demonstrated fungistatic activity against the tested fungi from week to potent with the MIC ranging from 5.33 to 9.33 mg/mL and 7.3 to 13.33 mg/mL, respectively. Methanolic extracts significantly ($p<0.05$) were much more effective than aqueous extract of *A. eburnea* once it exhibited lower MIC values for all the fungi. Among the tested fungi pathogens, *C. albicans* was the most sensitive to the extracts of *A. eburnean.* Moreover, ketoconazole as control drugs exhibited antifungal activities with the MIC 0.5 mg/ml tested fungi pathogens.

With the advent of industrial and synthetic antimicrobial agents in the middle of last century, lack of interest in plants as a natural and valuable source for antimicrobial drugs was caused [18]. Recently, with the emergence of some limitations in the use of these drugs, the situation has shifted and field of ethnobotanical research has been expanded [19]. The present study showed that *A. eburnean* extract was capable of inhibiting the growth of fungi strains that are as most common opportunist fungi. Approximately in phytochemical screening of the crude extract of all of plants there are some compounds such as terpenoids, phenols, flavonoids, fatty acids and sterols [19]. In several investigations biological especially antimicrobial activities of these components have been proven [20-24]. Thus, we can suggest that these components are responsible for the antifungal activity of *A. eburnean*; however their exact action mechanism is poorly understood. To conclude, the obtained findings demonstrated that *A. eburnean*

extracts were found to be more active against some pathogenic fungi strains and thus provided the evidence for its traditional use value and it is suitable substitute for treatment of fungi infections.

Declaration of Interest

The author declares that there is no conflict of interest in this study.

REFERENCES

[1] Watanabe S.. Japaneses *J Med Mycol* **1990**; 40: 151-155.
[2] Sepahvand A, Shams-Ghahfarokhi M, Allameh A, Razzaghi-Abyaneh M. *Jundishapur J Microbiol*. **2013**, 6(2), 186-192.
[3] Sepahvand A, Shams-Ghahfarokhi M, Allameh A, Jahanshiri Z, Jamali M, Razzaghi-Abyaneh M. *Folia Microbiol* (Praha). **2011**, 56(6), 527-34.
[4] De Pauw BE. *Clin Microbiol Infect* **2000**, 6, 23-28.
[5] Mahmoudvand H, Asadi A, Harandi MF, Sharififar F, Jahanbakhsh S, Dezaki ES. *Iran J Basic Med Sci*. **2014**, 17(12), 1001-6.
[6] Tavakoli Kareshk A, Keyhani A, Mahmoudvan d H, Tavakoli Oliaei R, Asadi A, Andishmand M, et al. *Iran J Parasitol*. **2015**, 10 (4), 625-631.
[7] Jahanbakhsh S, Azadpour M, Tavakoli Kareshk A, Keyhani A. Mahmoudvand H. *J Parasit Dis* **2015,** DOI 10.1007/s12639-015-0670-4.
[8] Saedi Dezaki E, Mahmoudvand H, Sharififar F, Fallahi S, Monzote L, Ezatkhah F. (**2015**). *Pharm Biol*. **2015**, 8, 1-7.
[9] Madani B, Rahemi M, Baninasab B, Mahmoodi M. *Horticulture Environment & Biotechnol* **2009**, 50(1), 63-67.
[10] Mahmoudvand H, Sharififar F, Rahmat MS, Tavakoli R, Dezaki ES, Jahanbakhsh S, Sharifi I. *J Vector Borne Dis*. **2014**, 51(4), 294-9.
[11] Mahmoudvand, H., Saedi Dezaki, E., Ezatpour, B., Sharifi, I., Kheirandish, F., & Rashidipour, M. (**2016**). *Planta Medica* **82**(4): 279-284.
[12] Ezatpour B, Saedi Dezaki E, Mahmoudvand H, Azadpour M, Ezzatkhah F. In vitro and in vivo antileishmanial effects of *Pistacia khinjuk* against *Leishmania tropica* and *Leishmania major*. *Evid Based Complement Alternat Med*. **2015,** 149707.
[13] Mahmoudvand H, Sepahvand A, Jahanbakhsh S, Ezatpour B, Ayatollahi Mousavi SA. *J Mycol Med*. **2014**, 24(4), 155-61.
[14] CLSI/Clinical and Laboratory Standards Institute, **2002**. Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi. Wayne. (Approved Standard M38-A).
[15] Mahmoudvand H, Ayatollahi Mousavi SA, Sepahvand A, Sharififar F, Ezatpour B, Gorohi F, Saedi Dezaki E, Jahanbakhsh S. *ISRN. Pharmacol* **2014**, 602436.
[16] Mahmoudvand H, Nadri S , Jahanbakhsh S Rezaeifar M. *Der Pharm Chem*, **2016**, 8 (6).
[17] Rezaeifar M, Mahmoudvand H, Amiria M. *Der Pharm Chem*, **2016**, 8 (5), 243-249.
[18] Cowan MM. *Clin Microbiol Rev*. **1999**, 12(4), 564–82.
[19] McCutcheon AR, Ellis SM, Hancock REW, Tower GNH. *J Ethnopharmacol* **1992**, 37: 213-223.
[20] Mahmoudvand H, Sharififar F, Saedi Dezaki E, Ezatpour B, Jahanbakhsh S. Fasihi Harandi M. *Iranian J Parasitol*. **2014**, 9(4), 26-34.
[21] Mahmoudvand H, Ezzatkhah F, Sharififar F, Sharifi I, Dezaki ES. *Korean Journal of Parasitology* **2015**, **53**, 21–27.
[22] Mahmoudvand H, Tavakoli R, Sharififar F, Minaie K, Ezatpour B, Jahanbakhsh S, Sharifi I. *Pharm Biol* **2014**, 4, 1–6.
[23] Abbaszadeh S, Sharifzadeh A, Shokri H, et al. *J Myco Med* **2014**, 24 (2), 51–56.
[24] Rezaeifar M, Behfarnezhad M, Moradi M, Mehrabani M,Mahmoudvand H. *Der Pharm Lett* **2016**; 8 (6).