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In vitro antidermatophytic effects of the methanolic extract of the Amygdalus eburnea

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Abstract : Dermatophytes are one of the main significant groups of fungi, which lead to globally human and animal's infections. The present study aims to determine the antidermatophyte activity of *A. eburnean* methanolic and aqueous extracts on *in vitro*. Minimum inhibitory concentration (MIC) of extracts against tested dermatophytes (*Trichophyton mentagrophytes* and *T. interdigitale*) was determined by broth macrodilution method, according to the protocol M38-A2 of the Clinical and Laboratory Standards Institute (CLSI) for filamentous fungi with some modifications. The results demonstrated that all the extracts had fungistatic activities with the MIC values 3.3 to 6.6 mg/ml. Methanolic extract of *A. eburnean* was much more effective than aqueous extract of plant once they showed lower MIC for tested dermatophyte strains. To conclude, the obtained findings demonstrated that *A. eburnean* extracts were found to be more active against some dermatophytic fungi strains and thus provided the evidence for its traditional use value and it is suitable substitute for treatment of fungi infections.
Keywords: Dermatophyte; MIC; Extract: *Trichophyton.*

Introduction

Dermatophytes are one of the main significant groups of fungi, which lead to globally human and animal's infections[1, 2]. Dermatophytes include the capacity to invade keratinized tissues, such as hair, skin and nails to produce dermatophytosis [3, 4]. Various types of the disease including tinea corporis, tinea pedis, capitis, barbae, cruris, manum and onychomycosis are present. Nowadays, treatments for these infections are still restricted to a few antifungal drugs. However, these drugs have limited in use due to having high toxicity and the emergence of drug resistance in their antifungal activities [5-7].

Historicalys, plant extracts and their 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 [8-10].

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 [11]. 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 [11]. The present study aims to determine the antidermatophyte activity of *A. eburnea* methanolic and aqueous extracts on *in vitro*.

Experimental

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[12]. 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.

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 [13-15].

Minimum inhibitory concentration (MIC) determination

The fungal strains used in this work are *Trichophyton mentagrophytes* (ATCC 9533), and *T. interdigitale* (ATCC 200099). The MIC of extracts against tested dermatophytes was determined by broth macrodilution method, according to the protocol M38-A2 of the Clinical and Laboratory Standards Institute (CLSI) for filamentous fungi with some modifications [12]. For the broth macrodilution method, 0.9 ml of the final conidia suspensions were mixed with 0.1 ml^{-1} of the different concentrations of extract (0.0625-16 mg/ml) in test tubes and incubated at 30°C for 7 days. The positive control tube contained 0.9 ml of conidial suspension and 0.1 ml of RPMI-1640, and the negative one contained 1 ml of RPMI-1640 only. The minimum concentrations at which no visible growth was observed were defined as the MIC, which were expressed in mg/ml[16-18].

Statistical analysis

Data analysis was done using SPSS statistical package, version 16.0 (SPSS Inc., Chicago, IL, USA). To assess the interaction of time and the experimental group, repeated measures analysis test was used. Differences were significant when the *p*-value was lower than 0.05 [19, 20].

Results and Discussion

Table 1 shows the results of *in vitro* antifungal activity of *A. eburnean* extracts against *T. mentagrophytes* and *T. interdigitale*. The results demonstrated that all the extracts had fungistatic activities with the MIC values 3.3 to 6.6 mg/ml. Methanolic extract of *A. eburnean* was much more effective than aqueous extract of plant once they showed lower MIC for tested dermatophyte strains. Among the tested dermatophytes, *T. interdigitale* was the most sensitive to the extracts of *A. eburnean*, while *mentagrophytes* was the less effective. Moreover, fluconazole as control drug revealed both fungistatic and fungicidal activities with the MIC value of 0.0184 to 0.0368 mg/ml against tested dermatophytes. Based on the statistical analysis, the difference in antidermatophytic effects between the extracts and the control drug was not statistically significant ($p>0.05$).

Table 1. Mean of minimum inhibitory concentration (MIC) of *A. eburnean* extracts against some pathogenic dermathopyte species.

Tested samples	MIC (mg/ml)	
	Trichophyton interdigitale	***Trichophyton mentagrophytes***
Methanolic extract	3.3	5.3
Aqueous extract	5.3	6.6
Fluconazole	0.0184	0.0364

Regarding antimicrobial effects of *A. eburnean,* previous study have reported that the extract from the A. eburnean were found to be more active against Gram-positive bacteria as an available and inexpensive herb possesses wound healing activity, and thus provided the evidence for its traditional use value and it is suitable substitute in healing of burn wounds[21]. Recently, Rezaeifar et al have demonstrated that *A. eburnean* extracts were found to be more active against some pathogenic fungi strains (*Aspergillus flavus* and *Candida albican*s) and thus provided the evidence for its traditional use value and it is suitable substitute for treatment of fungi infections [22].

According to the previous studies, in phytochemical screening of the crude extract of all of plants there are some compounds such as terpenoids, phenols, flavonoids, fatty acids and sterols [23-27]. Reviews have reported biological and antimicrobial activities of these components[28-33]. 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 dermatophytic 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.

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