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ANTIFUNGAL ACTIVITY OF *ASTRAGALUS ONOBRYCHIS* L. EXTRACTS

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Abstract

Ethanol and water extracts from aerial parts of Astragalus onobrychis were tested against 12 species of Candida, using the broth microdilution method, in order to determine the MIC values. The study showed a more potent activity on the ethanol extract with a MIC value of 2,57 mg/mL and a weaker activity on the water extract of 3,32 mg/mL on most of the Candida species studied. The results indicate that Astragalus onobrychis is a promising candidate for developing antifungal products.

Keywords*: Astragalus onobrychis, antifungal activity, broth micodilution, Candida.*

INTRODUCTION

Candida albicans, the most commonly isolated opportunistic human fungal pathogen, can cause skin and mucosal infections as well as life-threatening systemic infections (Wang et al., 2010). *Candida* infections, or candidiasis, are difficult to treat and create very serious challenge in medicine. Therefore, screening and testing various plants for potential antifungal activities is very important in order to develop antibiotics.

Although, *C. albicans* is the most commonly isolated yeast, other species are found with increasing frequency, including *C. parapsilosis* (Safdar, 2004). *C. parapsilosis* particularly affects critically ill neonates and surgical intensive care unit (ICU) patients, likely because of its association with parenteral nutrition and central lines (Kuhn et al., 2004).

In recent decades, many studies have been carried out on different plant species to discover compounds of possible interest for antifungal applications. Among these studies, several have focused on the biological and phytochemical properties of different species of *Astragalus*, the largest genus of the family Fabaceae and, with over 2500 species, probably the largest genus of flowering plants (Teyeb, 2012). Fabaceae is the third largest family of angiosperm plant with approximately 730 genera and over 19400 species worldwide, which includes the plants commonly known as legumes (Wojciechowski, 2004).

Astragalus is mainly distributed in cool to warm, arid and semiarid regions of the northern hemisphere, South America, and tropical East Africa; it is especially diverse in the south-western and Sino-Himalayan regions of Asia (ca. 1500–2000 spp.), in western North America (ca. 400–450 spp.), and the Andes of South America (ca. 100 spp.). Both the geographic center of diversity and the presumed origin of Astragalus in Eurasia – specifically in the steppes and mountains of south-western to south-central Asia and the Himalayan plateau. According to Ekuci and Ekim (2004) there are 142 species in Europe of which 50 are endemic. Several species of this genus are used in folk medicine due to their hepatoprotective, antioxidative biological activities and their antiviral properties. In Turkish folk medicine, the roots of *Astragalus* species are used for the treatment of leukemia and for the healing of wounds (Yesilada, 2005). Furthermore, *A. mongholicus* Bunge and *A. membranaceus* Bunge are among the most popular Chinese medicines and are used for a variety of diseases, including as an adjunctive in cancer chemotherapy. Some *Astragalus* products, such as gum tragacanth, are widely in use in the preparation of pharmaceuticals and as thickening agents in certain foods (Paul,

2007). Antibacterial activity has also been reported for some Astragalus species, such as *A. gymnolobus*Fisch and *A. brachystachys* DC. In addition, bioactive saponins have been extracted from *A. suberi* L. (Jassbi, 2002). Gođevac et al. (2008) investigated the antioxidant activity of methanol extract from the aerial part of A. *glycyphyllos* L. Another study showed that an ethanol extract of A. membranaceus roots may inhibit the growth of *Trypanosomacruzi* (Schinella, 2002).

Astragalus onobrychis has a wide distribution. It can be found in Europe, Asia and northern Africa. It is the first *Astragalus* species to be described by Linnaeus in his famous book, *Species plantarum* from 1753 (Ranjbar, 2007).

To the author's knowledge, there are no previous studies of antifungal properties of *A. onobrychis* on *Candida* species.

MATERIALS AND METHODS

Plant material

Fresh, young plants were collected in the spring, from the Botanic Garden of the University of Szeged.

Preparation of extracts

The plants were cleaned, washed and air dried under shade with occasional shifting and then powdered with a mechanical grinder and stored in an airtight container.

Aqueous solutions were prepared with 10 g of plant powder and 100 ml distilled water. The alcoholic solution was made with 100 ml of 95% ethanol. The solution was put in an orbital shaker for 24 hours and kept in a dark room at all times. The aqueous extract was filtered with a vacuum pump and concentrated using the lyophilization process. The alcoholic extract was concentrated using a rotavap. The dried substance was measured on a digital scale. The solution for testing was made by diluting the dried substance and making solutions to a known concentration.

The final concentration of the solutions, are as follows:

Astragalus onobrychis ethanol extract: 41,2, 20,6, 10,3, 5,15 and 2,57 mg/mL.

Astragalus onobrychis water extract: 26,6, 13,3, 6,65, 3,32 and 1,66 mg/mL.

Microorganisms

The *Candida* strains used are: *C. albicans, C. glabrata, C guilliermondii, C. inconspicua, C. krusei, C. lusitaniae, C. norvegica, C. parapsilosis, C. pulcherrima, C. zeylanoides, C. orthopsilosis, C. metapsilosis.*

Media

YEPD agar and broth (1% (w/v) yeast extract, 2% peptone, 2% glucose/dextrose, 2% agar in distilled water),

RPMI 1640 medium (Sigma –Aldrich , St. Louis, MO, USA; with L-glutamine , without sodium bicarbonate powered, buffered whit 0,165 mol 1-1 4-morpholinepropanesulfonic acid at pH=7,0).

Inoculum preparation

It was prepared a number of test tubes with YEPD agar medium in which the yeast strains were inoculated. The tubes were then incubated for 3 days at 37^{o}C.

Fungal Enumeration

Fungal populations were determined by plate counting. A modified version of the method described by Montville (2008) was used. In this procedure, five samples of different dilutions were individually surface plated onto one plate, in the form of "lanes" and then incubated at 37^{0}C for 24 h. Plates with colonies ranging between 30 and 100 were considered for colony counting to determine the fungal populations.The densities for each strain is shown in (Table 1).

Table 1. The densities measured for each strain

Fungal strains	CFU/ml
C. inconspicua	7,5 x 10^{8}
C. krusei	8,2 x 10^{8}
C. glabrata	7,6 x 10^{9}
C. pucherrima	9,6 x 10^{7}
C. methapsilosis	9,8 x 10^{9}
C. guilliermondi	9,2 x 10^{8}
C. albicans	8,2 x 10^{8}
C. norvegica	8,8 x 10^{8}
C. parapsilosis	7,8 x 10^{9}
C. kefyr	9,4 x 10^{7}
C. lusitaniae	9,8 x 10^{8}
C. zeylanoides	6,4 x 10^{8}

Antifungal assay:

In each well was put 1 ml of RPMI and inoculated with yeast. 100 µl of the stock solution of plant extract was prepared at the concentration of 41,2 mg/mL for the ethanol

extract and 26,6 mg/mL for the aqueous extract and was added into the first wells. Then, 100 µl of their serial dilutions was transferred into four consecutive wells. The last well containing 100 µl of nutrient broth without the compound and 100 µl of the inoculum from each strip was used as negative control. The final volume in each well was 200 µl. The plates were covered with a sterile plate sealer. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at 37^0C for 48 h. Microbial growth in each medium was determined at 24 hours and at 48 hours by reading the respective absorbance (A) at 620 nm using an ASYS Jupiter plate reader (Biochrom Ltd., Cambridge UK) and confirmed by plating 5 µl samples from clear wells on nutrient agar medium. The MIC was defined as the lowest concentration of the compounds to show no growth of microorganisms.

The tests were performed in triplicate. From the samples which showed activity, it was taken out 50 µl of solution and put in a Petri plate with YEPD agar medium and incubated at 37^0C.

Statistical analysis

Data were averages of three results ± Standard Deviations (SD) by using Microsoft Excel.

RESULTS AND DISCUSSIONS

The results obtained, show that *A. onobrychis* presents antifungal activity and inhibited the growth of most tested strains. As it can be observed from the tables below, a more intense inhibitory effect was noted in case of the ethanol extract and a weaker activity in the case of aqueous extract. As it can be seen from (Table 2), which shows the ethanol extract values, most of the 12 *Candida* species tested, had a MIC value of 2,57 mg/mL and a few were more resistant.

(Table 3) presents the aqueous extract values, which are higher, that means the aqueous extract presents a weaker activity. In the case of *C. lusitaniae, C. glabrata* and *C. metapsilosis*, the MIC values could not be determined. No significant change was found at the 48 hours.

Table 2: Ethanol extract

Species	MIC value mg/ml	Control
C. norvegica	2,57	62,5
C. inconspicua	2,57	70,1
C. zeylanoides	41,2	54,3
C. pulcherrima	2,57	55,6
C. guillermondi	41,2	64,3
C. albicans	2,57	41,5
C. krusei	41,2	45,3
C. lusitaniae	41,2	52,8
C. glabrata	2,57	67,3
C. parapsilosis	2,57	62,4
C. metapsilosis	2,57	54,3
C. orthopsilosis	2,57	54,1

The results are in accordance with the one obtained by Sulaiman (2012) on *A. atropilosulus* subsp. *abyssinicus* from Saudi Arabia on *Candida* sp. He tested leaf extracts and obtained a MIC value of 13 mg/mL with an ethanol extract and 30 mg/mL with an aqueous extract. Also, Mikaeili (2012) obtained a MIC value of 160 mg/mL on *Candida albicans*, with an aqueous extract from *Astragalus verus*.

Table 3: Aqueous extract

Species	MIC value mg/ml	Control
C. norvegica	3,32	54,2
C. inconspicua	26,6	65,2
C. zeylanoides	26,6	43,2
C. pulcherrima	26,6	44,7
C. guillermondi	3,32	46,2
C. albicans	26,6	57,3
C. krusei	26,6	53,2
C. lusitaniae	ND	63,2
C. glabrata	ND	61,6
C. parapsilosis	26,6	54,2
C. metapsilosis	ND	67,1
C. orthopsilosis	26,6	61,3

In the case of isolated constituents, tested separately, the MIC values are much lower, for example 31,25 µg/mL for soyasaponin I, isolated from *A. trimestris*, and tested on *C. albicans*, which shows that the isolated compounds have a much more intense antimicrobial properties (El-Hawiet, 2010).

Most *Astragalus* species exhibit a wide range of antimicrobial properties on very diverse microorganisms, like the ones tested by

Balachandar (2012). However, not all species do present antimicrobial properties, and one interesting example are the 13 *Astragalus* species from eastern Anatolia in Turkey, 4 of which are endemic, tested by Adigüzel (2009) on 40 different species of microorganisms. He found that at any concentration, "none of the extracts tested has inhibitory activity against any of the microorganisms tested" (Adigüzel, 2009).

CONCLUSIONS

The extracts prepared from aerial parts of *A. onobrychis*, with different solvents, exhibit a different antifungal effect. The ethanol extract presents a stronger antifungal activity than the aqueous extract. *A. onobrychis* extracts, inhibited almost all of the *Candida* species.

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