



## Article

Received 23 Aug 2011  
Accepted 14 Jan 2012  
Available online 20 Mar 2012

### Keywords:

*Astragalus verus*  
candidiasis  
chemical analysis  
guinea pig  
Kurdish ethnomedicine

ISSN 0102-695X  
<http://dx.doi.org/10.1590/S0102-695X2012005000035>

# Anti-candidal activity of *Astragalus verus* in the *in vitro* and *in vivo* guinea pig models of cutaneous and systemic candidiasis

Ali Mikaeili,<sup>1</sup> Isaac Karimi,<sup>\*2</sup> Tayebeh Shamspur,<sup>3</sup> Babak Gholamine,<sup>4</sup> Masoud Modaresi,<sup>5</sup> Ali Khanlari<sup>4</sup>

<sup>1</sup>Department of Medical Mycology, School of Medicine, Kermanshah University of Medical Sciences, Iran,

<sup>2</sup>Department of Biochemistry, Physiology and Pharmacology, College of Veterinary Medicine, Razi University, Kermanshah, Iran,

<sup>3</sup>Phytochemistry Group, Department of Chemistry, Shahid Bahonar University of Kerman, Iran,

<sup>4</sup>Department of Pharmacology, School of Pharmacy, Kermanshah University of Medical Sciences, Iran,

<sup>5</sup>Department of Pharmacognosy and Pharmaceutical Biotechnology, School of Pharmacy, Kermanshah University of Medical Sciences, Iran.

**Abstract:** This study was design to evaluate the anti-candidal activity of *Astragalus verus* Olivier, Fabaceae (Av). The GC/MS analysis of essential oils of Av showed that aqueous extract contains thymol while hexadecanoic acid, 1,2-benzenedicarboxylic acid, diisooctyl ester and phytol were found as major components of methanol and acetone extracts. The aqueous extract showed anti-candidal activity in the concentration 320 mg/mL using disc diffusion method and its minimum inhibitory concentration (MIC) was 160 mg/mL. To induce cutaneous candidiasis, the dorsum of immunocompromised guinea pigs was infected with *Candida albicans* and animals were divided into five groups (n=5 for each): NC, received a vehicle; PC, received topical ketoconazole 2% and three other groups which received topical 10, 20 and 40% aqueous extract of Av. On ninth day postinfection, skins were cultured and colony forming unite per gram (CFU/g) skin was recorded. Systemic candidiasis was obtained by intravenous inoculation of *C. albicans*, 4000 CFU/g body weight. Here, animals have been divided into five groups like cutaneous candidiasis but their medications have been delivered in drinking water for ten days before induction of infection. On second day postinfection, all internal tissues were taken for determining CFU/g tissue. The aqueous extract (40%) prevented heavy burden of *C. albicans* in tissues and skin in oral and topical application, respectively. The results indicate that Av represents a potential source of anti-candidal drug.

## Introduction

*Astragalus verus* Olivier (Av), an endemic Fabaceae, is a medicinal plant widely distributed in Northwest Persia and Asia Minor that highly used in kurdish ethnomedicine. The kurds used Av (black milkvetch or *Rasha Gavan* in kurdish linguistics) for treating various mucocutaneous diseases and its concrete juice of Av, tragacantha, has been used as emollient and demulcent in irritation of mucosa membranes while it being used as a vehicle for more active medicines in orthodox medicine (Chalechale & Karimi, 2009; Waring,

2009). According to these folklore usages of *Astragalus* and interviewing with shepherds, chiropractors and ethnic herbalists, we motivated to find the evidences beyond the ethnic application of Av for treatment of candidiasis in two experimental guinea pig models of cutaneous and systemic candidiasis.

Candidiasis is a condition that results from the overgrowth of a yeast-like fungus called *Candida albicans*. Candida cells are part of the normal flora of our bodies found in mouth, vagina, intestines, and other organs. When they grow unchecked, they can cause a number of health problems, including digestive disorders, fatigue, and vaginal yeast infections. There are a number

of conditions or medications that promote the overgrowth of yeast cells, either because they kill beneficial normal flora, or because they interfere with normal functions of immune and neuroendocrine systems. These medications include: antibiotics; chemotherapy, hormone replacement, corticosteroids, and oral contraceptives (Clemons & Stevens, 2000, 2001). Inappropriate diet, such as over-consumption of yeast products, sugar, or alcohol, also can promote yeast growth. Those people with immune system or endocrine gland disorders are more prone to candidiasis, such as patients with acquired immunodeficiency diseases, cancer, or diabetes mellitus (Clemons & Stevens, 2000, 2001).

Nowadays, the multiple drug resistance in different pathogenic microorganisms to commonly used antibiotics leads to search for new antimicrobial agents to combat infections and overcome problems of resistance and side effects of the currently available antimicrobial agents (Ali-Shtayeh et al., 1998; Primo et al., 2001; Karaman et al., 2003). As far as our literature survey could ascertain, neither the anti-candidal activities of the essential oils of Av nor the composition of the oils of Av have been studied before, although there was a report concerning the antidermatophytic activity of Av aqueous extract that we reported recently (Mikaeili et al., 2011). Hence, this study was aimed to find partial chemical composition of the essential oil and anti-candidal activity of different extracts of Av in the *in vitro* and in guinea pig models of cutaneous and systemic candidiasis.

## Material and Methods

### *Plant collection and authentication*

The aerial parts of *Astragalus verus* Olivier, Fabaceae, were collected from Khalichian village Mountains at Sanandaj, Iran in August 2010 and air dried in the shade. It was authenticated by Dr. Nastaran Jalilian, botanist in Research Herbarium of Jihad-Keshavarzi Organization, Kermanshah province of Iran. Aerial parts (15 kg) of dried Av were ground in an electric grinder to produce a fine powder.

### *Preparation of extracts*

#### Aqueous decoction

Aqueous decoctions were prepared by boiling 50 g Av powder in 500 mL sterile boiled distilled water with continuous shaking over a heater-stirrer at the 50 °C for 12 h. The flasks were then plugged and removed from heat and allowed to cool for 45 min. After cooling, the contents of the flasks were filtered with double filter paper and sterile filters to remove any impurities (Runyoro et al., 2006; Chaudhry & Tariq, 2008). The filtrate was dried

in rotary evaporator to obtain the aqueous extract (AE; 2.575 g; 5.150% w/w).

#### Methanol and acetone maceration

About 50 g of Av powder was extracted with 1.5 L of methanol by cold maceration for 24 h and filtered for three rounds. After cooling, the contents of the flasks were filtered with double filter paper and sterile filters to remove any impurities (Runyoro et al., 2006; Chaudhry & Tariq, 2008). The filtrate was dried in rotary evaporator to obtain the methanol extract (ME; 1.686 g; 3.372% w/w). The resulting powder was dissolved in 80 mL of methanol plus 40 mL of chloroform, a minimum ratio of two solvents. The similar methodology was applied to obtain acetone extract (AcE 2.569 g; 5.138% w/w). Here, the resulting powder was dissolved in 80 mL of acetone plus 25 mL of chloroform, a minimum ratio of two solvents. The sample extracts were kept in the refrigerator (4 °C) until the analyses were accomplished.

#### Preparation of essential oils

The essential oils were obtained from dried powdered of aqueous, methanol and acetone extracts of Av by steam distillation for 3 h, using a Clevenger apparatus.

#### *Essential oil chemical analysis*

Gas chromatography (GC) analysis of resulting essential oils of AE, ME and AcE extracts of Av was performed using a Hewlett-Packard chromatograph 5890 series equipped with FID detector and a HP-1 fused silica column (30 m×0.25 mm and film thickness 0.25 µm). GC/MS analysis was carried out on a Hewlett-Packard 5973 connected with a mass detector HP 6890 using a HP-1 column (30 m×0.25 mm and film thickness 0.25 µm). Oven temperature programming was 40-250 °C with an increase of 3 °C/min for both GC/FID and GC/MS. Injector and detector temperatures were 320 and 310 °C, respectively. The carrier gas was helium and flew at a rate of 1 mL/min. The mass spectrometer was operated at 70 eV with the mass range, 40-350 amu and scan time 1 s.

Identification was based on sample retention data and comparison with authentic standards, computer matching using NIST MS library. The identification was also confirmed by comparison of the retention indices with data in the literature (Adams, 1996; Shibamoto, 1987). The percentages of compounds were calculated by the area normalization method without considering response factors. The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes.

### Antimicrobial tests

#### *In vitro* antifungal assay: preparation of inocula

The fungal cultures of *C. albicans* isolates (ATCC 10231) were grown on Sabouraud dextrose agar (SDA; Merck, Germany) plates at 28±2 °C for two weeks. The conidia fungi were collected using sterile normal saline solution (NS) on the agar surface followed by gentle shaking. The suspension was vortexed and heavy particles were allowed to settle for 3-5 min. The inocula were prepared spectrophotometrically to give a final concentration of 1.0×10<sup>6</sup> CFU/mL in NS (NCCLS, 2009).

#### Antifungal assay using disc diffusion method

Testing of the plant extracts for antifungal activity was done by disc diffusion method described elsewhere (NCCLS, 2009). Briefly, the resulting inocula of *C. albicans* were swabbed in the amount of 1 mL/cm<sup>2</sup> over the surface of SDA plates. Then three filter discs uniformly loaded with given concentrations of the Av extract, corresponding solvents of Av extract as negative controls, and Ketoconazole (KTZ 20 mg/mL) as positive controls were placed with sterile tweezers in three equidistances onto the prepared plates, respectively. All the testing was done in triplicate.

The zones of inhibition on the growth of test strains were defined by the area where visible growth had been inhibited completely. All the measurements of zone of inhibition were carried out by one single examiner (A. Mikaeili).

#### Antifungal assay with determination of the minimum inhibitory concentration (MIC)

For quantitative assays five doses of each extract (20, 40, 80, 160, 320 mg/dL) were added to SDA (100 mL) at 45 °C, these being mixed rapidly and poured into four 9.5 cm<sup>2</sup> Petri dishes. After the agar had cooled down to room temperature 1.0×10<sup>7</sup> CFU/mL in NS were inoculated per each cm<sup>2</sup> of Petri dishes. The MIC was taken as the lowest concentration of plant extract to inhibit growth of the fungus after 24 h and 48 h (NCCLS, 2009). Each experiment was repeated two times. Then, each extract that showed the highest antifungal activity against *C. albicans* was used in guinea pig models of cutaneous and systemic candidiasis.

#### *In vivo* antifungal assay: preparation of inocula

In this study, *C. albicans* isolates were selected as the infecting agent. Candida was grown on SDA slants at 30 °C for one week. The conidia were scraped from

the plates in NS, centrifuged and washed twice in NS. The suspension was adjusted spectrophotometrically to a concentration of 1.0×10<sup>7</sup> conidia per mL (NCCLS, 2009). The working solution (WS) was prepared fresh in NS and 0.1 mL of WS used to inoculate the guinea pigs.

#### Cutaneous candidiasis

Male albino guinea pigs (mean body weight, 350 g) were used in the study of *in vivo* anti-candidal activity. Briefly, guinea pigs were acclimatized for seven days by feeding on a commercial solid diet (Dan-e-pars Co., Kermanshah, Iran). After acclimatization, guinea pigs were divided into five groups (n=5 in each group) on the basis of body weight: vehicle (the negative control animals received a vehicle containing 0.1 mL of normal saline solution plus 0.1 mL of distilled water; NC), the positive control animals received 0.1 mL of KTZ 2%; PC) and three other Av-treated, AE10%, AE20% and AE40%, which received 0.1 mL of 10%, 20% and 40% aqueous extract of Av, respectively. The procedures for induction of cutaneous candidiasis were according to the previously described methodology (Niwano et al., 1994; Maebashi et al., 1994). Briefly, guinea pigs were anesthetized by intramuscular administration of a combination anesthetic containing ketamine and xylazine and their dorsum were clipped and shaved in an area of 2.5 cm × 2.5 cm (6.25 cm<sup>2</sup>). The marked area was inoculated with a fungal suspension (0.1 mL, 2.0×10<sup>7</sup> cells) of *C. albicans* using a sterile pipette-tip and rubbed thoroughly. Prednisolone (Mitaka Pharmaceutical Co., Ltd., Tokyo, Japan) was subcutaneously administered four times (two days and immediately before and two and four days after the inoculation) at a dose of 30 mg/kg of body weight each time. In this part, formulations were topically applied to the infected area once a day for three consecutive days, starting on the fifth day postinfection.

#### Evaluation of cutaneous candidiasis

On the ninth day postinfection, all of the animals were deeply anesthetized by intramuscular administration of a combination anesthetic containing ketamine and xylazine and killed and the skin of the infected area was excised, minced with scissors, and homogenized in sterile physiological saline. A portion of the homogenate was cultured on a peptone yeast extract glucose agar (2% Bacto Peptone, 1% yeast extract, 2% glucose, 1.5% agar) plate for two days at 37 °C. Colonies grown on the agar plate were counted to calculate the number of CFU/g. An animal was considered fungus positive when more than one fungal colony was counted (assay limit, less than 20 CFU/g infected site) (Niwano et al., 1994; Maebashi et al., 1994).

### Systemic candidiasis

To evaluate prophylactic effects of Av against systemic candidiasis, systemic candidiasis in guinea pigs was induced by a slight modification of the procedure of Van Cutsem et al. (1992). Briefly, guinea pigs were acclimatized for seven days by feeding on a commercial solid diet (Dan-e-pars Co., Kermanshah, Iran). After acclimatization, guinea pigs were divided into five groups (n=5 in each group) on the basis of body weight: vehicle (distilled water here; NC), KTZ 2% (PC) and three other Av-treated, AE10%, AE20% and AE40%, which received 10, 20 and 40% aqueous extract of Av, respectively, in their drinking water *ad libitum* for ten days before induction of infection. Systemic candidiasis was obtained by intraperitoneal (*i.p.*) inoculation of *C. albicans*, 4000 CFU/g body weight. On the second day postinfection (day 12), after a 14- to 16-h fast, anesthesia was administered by an *i.p.* ketamine/xylazine injection and a blood sample was collected into a collection tube containing heparin via cardiac puncture and stored at -20 °C until analysis. After blood collection, the guinea pigs were decapitated and tissue specimens from the brain, lungs, heart, liver, spleen, eyes, stomach, intestines, kidneys, skeletal muscles, gonads (testes), lymph nodes, pancreas, thymus and adrenal glands of each animal were taken for qualitative and quantitative cultures. All the experiments were performed based on veterinary research ethics and this study was approved by the Iranian Society for the Prevention of Cruelty to Animals (ISPCA).

### Evaluation of systemic candidiasis

The visceral organs of the infected animals were removed, placed in glass vials containing 10 mL of sterile saline and subsequently homogenized. One gram of the homogenates was made in sterile normal saline

and cultured on Sabouraud glucose agar containing antibacterial antibiotics (20 IU penicillin and 40 µg/mL streptomycin). The plates were incubated at 37 °C for fourteen days, and the number of CFU per gram of tissue was determined (Khosravi et al., 2009).

### Statistical analyses

The results are expressed as the mean±SEM. All statistical analyses were performed using SPSS 16.0 statistical software. Student's t-test was performed to determine any significant difference between different extracts for *in vitro* antifungal assays. Comparison of means for *in vivo* antifungal assessment was carried out using one-way analysis of variance (ANOVA) and Tukey HSD test. *p*-value <0.05 was considered statistically significant.

### Results

#### Essential oil chemical analysis

Comprehensive GC/MS and GC/FID was used to analyze volatile components of Av top flowers in this study. The chemical constituents of EO are displayed in Table 1. The main components that isolated from EO of AV in the presence of different solvents that employed in this study were thymol in aqueous extract and hexadecanoic acid, 1,2-Benzenedicarboxylic acid, diisooctyl ester and phytol in both methanol and acetone extracts (Table 1).

#### In vitro antifungal assay

##### Antifungal assay using disc diffusion method

In the concentration 160 and 320 mg/mL, aqueous extract of Av has significant higher antifungal activity in

**Table 1.** Comparative chemical composition (%) of the essential oil of *Astragalus verus* (Black milkvetch) in the presence of different solvents.

| Extract   | Compound                                       | t <sub>R</sub> * | Percent | RI** |
|-----------|--|------------------|---------|------|
| aqueous†  | methanol                                       | 1.40             | –       | –    |
|           | thymol   | 25.47            | 98      | 1181 |
| methanol† | methanol                                       | 1.39             | –       | –    |
|           | <i>n</i> -hexadecanoic acid                    | 51.26            | 56      | 1799 |
|           | phytol   | 55.59            | 20      | 1899 |
|           | 1,2-benzenedicarboxylic acid, diisooctyl ester | 66.65            | 22      | 2157 |
| acetone   | acetone  | 1.58             | –       | –    |
|           | <i>n</i> -hexadecanoic acid                    | 51.32            | 58      | 1800 |
|           | phytol   | 55.61            | 10      | 1900 |
|           | 1,2-benzenedicarboxylic acid, diisooctyl ester | 66.69            | 22      | 2157 |

Note: \*t<sub>R</sub>: retention time (min); \*\*RI: retention indices in elution from HP-1 column. †the essential oils of aqueous and methanol extracts were prepared in the presence of methanol as solvent.

**Table 2.** *In vitro* antifungal activity of *Astragalus verus* extract on *Candida albicans* (determined by diameter of inhibition zones).

| Extract      | Dose (mg/mL) |            |            |             |             |
|--------------|--------------|------------|------------|-------------|-------------|
|              | 20           | 40         | 80         | 160         | 320         |
| methanol     | NI           | NI         | NI         | NI          | NI          |
| acetone      | NI           | NI         | NI         | NI          | 20.6±0.333* |
| aqueous      | NI           | NI         | NI         | 21.0±0.577* | 25.0±0.577* |
| ketoconazole | 29.4±0.444   | 30.0±0.235 | 29.4±0.293 | 30.3±0.440  | 30.1±0.309  |

Note: Values are mean±SEM (mm) of tri-plate test of the experiment; NI: no inhibition zone. \*Shows significant difference compared with KTZ.

comparison to acetone extract ( $p=0.000$ ). Both aqueous and acetone extracts have significant lesser antifungal activity in comparison to KTZ at the concentration 320 mg/mL ( $p=0.000$ ; Table 2). The methanol extract showed no antifungal activity at the maximum dose 320 mg/mL that studied in the present investigation (Table 2).

#### Antifungal assay with determination of MIC

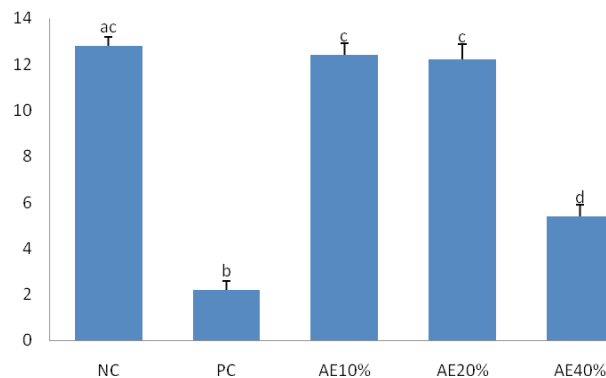
The aqueous extract showed MIC as 160 mg/mL while acetone extract showed MIC as 320 mg/mL for *C. albicans*.

#### Cutaneous candidiasis

The mean infection rate was higher in the skin of AE40% group ( $5.4±0.509×10^4$  CFU/g) compared to KTZ-treated group ( $2.2±0.374×10^4$  CFU/g;  $p=0.002$ ; see Figure 1). However, AE10% ( $12.4±0.509×10^4$  CFU/g) and AE20% ( $12.2±0.663×10^4$  CFU/g) showed similar results compared to NC group ( $12.8±0.374×10^4$  CFU/g;  $p=0.978$  and  $p=0.911$ , respectively; see Figure 1). The CFU/g in AE10% and AE20% groups was the same ( $p=0.998$ ; see Figure 1). The mean infection rate was lower in the skin of AE40% group compared to NC group ( $p=0.000$ ; see Figure 1).

#### Systemic candidiasis

The mean infection rate was higher in the kidneys, lungs, heart, liver and spleen than those of the other organs as shown in NC group (Table 3). The mean infection rates of different organs decreased in the presence of KTZ and different concentrations of aqueous extracts of Av. The KTZ-treated group (PC) showed lower mean infection rate in all studied organs compared with NC group (Table 3). However, the mean infection rates in all organs except thymus and eyes of AE40% group decreased significantly compared with NC group (Table 3). The AE10% and AE20% groups did not show significant difference in the mean infection rate of all organs in comparison to NC group (Table 3).



**Figure 1.** Contribution of aqueous extract of *Astragalus verus* to inhibition of *Candida albicans* in skin of guinea pigs. Note: NC: the negative control animals received a vehicle containing 0.1 mL of normal saline solution plus 0.1 mL of distilled water; PC: the positive control animals received 0.1 mL of ketoconazole 2%; and three other AE10%, AE20% and AE40% groups, which received 0.1 mL of 10, 20 and 40% aqueous extract of *Astragalus verus*, respectively. \*Values with various superscripts are significantly ( $p<0.05$ ) different in the Figure.

#### Discussion

This study shows that *Astragalus verus* Olivier, Fabaceae, can display anti-candidal activities both in the *in vitro* and in guinea pig models of cutaneous and systemic candidiasis. In this sense, the aqueous extract of Av showed more anti-candidal activity than acetone extract, however methanol extract did not show any anti-candidal activity *in vitro* as evaluated by disc diffusion method and determination of MIC.

As shown in Table 1, the chemical compositions of essential oils derived from both methanol and acetone extracts are approximately similar in both quality and quantity, however its direct *in vitro* anti-candidal activity of acetone extract must be related to other compounds that found in the whole acetone extract not in its essential oil. Three major compounds, hexadecanoic acid, phytol and 1,2-benzene dicarboxylic acid, diisooctyl ester, were identified by means of GC-MS analysis of the essential oils derived from acetone and methanol extracts of Av because the compounds participating in the mixtures in

**Table 3.** Comparison of mean infection rates of various tissues according to colony forming unit (CFU) of *Candida albicans* in fungal culture.

| Tissue×10 <sup>4</sup> | Group     |            |            |            |             |
|------------------------|-----------|------------|------------|------------|-------------|
|                        | NC        | PC         | AE10%      | AE20%      | AE40%       |
| Blood                  | 1.22±0.06 | 0.21±0.09* | 1.19±0.05  | 1.27±0.02  | 0.74±0.04*† |
| Urine                  | 1.49±0.03 | 0.51±0.05* | 1.48±0.01† | 1.54±0.03† | 0.94±0.03*† |
| Adrenal gds            | 1.40±0.04 | 0.21±0.09* | 1.43±0.04† | 1.42±0.03† | 0.72±0.04*† |
| Thymus                 | 0.21±0.09 | 0.00±0.00* | 0.27±0.12  | 0.31±0.08  | 0.06±0.06   |
| Pancreas               | 1.96±0.02 | 0.61±0.04* | 1.93±0.02† | 1.95±0.02† | 0.99±0.90*† |
| Lymph nds              | 0.81±0.03 | 0.12±0.07* | 0.72±0.04† | 0.78±0.06† | 0.63±0.05*† |
| Gonads                 | 1.69±0.03 | 0.49±0.05* | 1.72±0.04† | 1.74±0.03† | 0.78±0.04*† |
| Muscles                | 0.91±0.02 | 0.00±0.00* | 0.85±0.03† | 0.82±0.04† | 0.12±0.07*  |
| Kidneys                | 2.97±0.02 | 1.28±0.03* | 2.96±0.00† | 2.95±0.00† | 1.68±0.01*† |
| Gut                    | 2.32±0.00 | 0.71±0.03* | 2.30±0.00† | 2.30±0.00† | 1.13±0.04*† |
| Stomach                | 2.05±0.01 | 0.43±0.05* | 2.05±0.01† | 2.04±0.01† | 0.94±0.03*† |
| Eyes                   | 0.21±0.09 | 0.00±0.00* | 0.21±0.09  | 0.15±0.09  | 0.12±0.07   |
| Spleen                 | 2.33±0.01 | 0.61±0.04* | 2.32±0.00† | 2.32±0.01† | 1.01±0.03*† |
| Liver                  | 2.43±0.00 | 0.81±0.03* | 2.41±0.00† | 2.41±0.01† | 1.06±0.03*† |
| Heart                  | 2.54±0.00 | 0.92±0.02* | 2.57±0.05† | 2.52±0.00† | 1.21±0.02*† |
| Lung                   | 2.69±0.00 | 1.12±0.03* | 2.67±0.00† | 2.67±0.00† | 1.42±0.03*† |
| Brain                  | 0.69±0.02 | 0.00±0.00* | 0.68±0.06† | 0.63±0.05† | 0.12±0.07*  |

Note: \*Shows significant difference compared to NC group while †shows significant difference compared to PC or ketoconazole-treated group. Data were expressed as mean±SEM and *p*-value<0.05 was considered statistically significant.

percentages lower than 10% were not presented in Table I. The phytol content of methanol extract was twice the amount of acetone extract. Thymol was the major compound that found in the essential oil derived from aqueous extract of Av and consisted 98.0% of essential oil. Many fatty acids are known to have antibacterial and antifungal properties. For example, hexadecanoic acid and oleic acids are known to have potential antibacterial and antifungal activity (Dilika et al., 2000; McGraw et al., 2002; Seidel & Taylor, 2004; Bodoprost & Rosemeyer, 2007). The direct anti-candidal activity of acetone extract of Av might be the result of hexadecanoic acid and/or phytol action. It seems this antifungal activity is a dose dependent because at dose 1.0 mg/mL, n-hexadecanoic acid did not show any inhibitory action against *C. albicans* (Kabara et al., 1972). Phytol is a diterpene which is present in higher concentration in the methanol extract of Av. The phytol was observed to have dose-dependent antibacterial activities against *Staphylococcus aureus* (Inoue et al., 2005). 1,2-benzene dicarboxylic acid, diisooctyl ester also known as diisooctyl phthalate (DIOP) constitutes 22% of the essential oils derived from acetone and methanol extracts of Av. This constituent may therefore not be injurious to health and its antimicrobial activity has not reported yet. However its insecticidal, larvicidal and pesticidal activities have been reported previously (Hema et al., 2011).

To our knowledge, there is no similar study that investigated the tissue distribution of *C. albicans* following inducing an experimental systemic candidiasis in guinea pigs. In this study, the highest counts of *C. albicans* were recovered from the kidneys, followed by the lungs, heart, liver and spleen in NC group. These results are consistent with the distribution of *C. albicans* in the tissues of immunocompromised dogs on day 2 after challenge (Khosravi et al., 2009). However, the highest mean infection rate was reported in the lungs in immunocompromised dogs (Khosravi et al., 2009). The highest counts of *C. albicans* were recovered from the kidneys in the present investigation, probably are related to the prominent role of kidneys to excrete *C. albicans* throughout two days postinfection. As previously reported, the lowest counts of *C. albicans* were recovered from thymus and eyes (Khosravi et al., 2009). The PC group showed overt anti-candidal activity compared to the all groups that received aqueous extract. Ketoconazole 2% (PC) completely inhibited the growth of *C. albicans* in thymus, muscles, eyes and brain. Also, the minimum CFU/g skin was recorded in KTZ-treated group in the cutaneous candidiasis in the current investigation. In this form of candidiasis only the highest amount of aqueous extract of Av in AE40% group showed considerable anti-candidal effects when compared to NC group.

In this study, supplementation of aqueous extracts

of Av in drinking water prevented the heavy burden of *C. albicans* in different tissues of guinea pig. In this regard, the mean infection rates of all studied tissues except thymus and eyes ( $p > 0.05$ ) were considerably decreased for the AE40% group ( $p < 0.05$ ), while the AE10% and AE4% groups showed a moderate decrease when compared to the NC group ( $p > 0.05$ ). This means that the high amount of aqueous extract of Av in drinking water may exert an *in situ* anti-candidal activity or may prevent distribution or lodging of *C. albicans* in different tissues. Thymol was found as a main aromatic substance in an essential oil derived from aqueous extract of Av and the anti-candidal activity of aqueous extract of Av might be the result of thymol. Thymol is part of a naturally occurring class of compounds known as biocides, with strong antimicrobial attributes when used alone or with other biocides. The antibacterial, antioxidant and antifungal activities of thymol were the subject of many studies (Palaniappan & Holley, 2010; Ündeğer et al., 2009; Ahmad et al., 2010). Particularly, thymol has been successfully used *in vitro* against pathogenic fungi, including *Aspergillus*, *C. albicans* and moulds (Giordani et al., 2004; Shin & Kim, 2004). Thymol has been shown to be an effective fungicide, particularly against fluconazole clinical *C. albicans* isolates, including fluconazole-resistant and fluconazole-susceptible *C. albicans* strains, when combined with fluconazole or amphotericin B, although it shows weak antifungal activity when used alone (Guo et al., 2009; Ahmad et al., 2010).

The improvement of cutaneous candidiasis following topical application of aqueous extract of Av may be related to the boosting of immune systems. The immunostimulating activity of *Astragalus* species has been subject of several studies (Kong et al., 2004; Hu et al., 2006). Twenty-eight patients with systemic lupus erythematosus had significantly decreased natural killer cell activity when compared to normal controls. Preincubation of their peripheral blood mononuclear cells with *Astragalus* stimulated natural killer cell cytotoxicity in systemic lupus erythematosus patients and in healthy controls (Zhao, 1992). Beside its immunostimulating activity, other studies have shown the direct antimicrobial activity of different extracts and pure compounds that derived from *Astragalus* species. In mice infected with coxsackie B-3 virus, *Astragalus* inhibited viral replication in the myocardial tissue while improving abnormal myocardial electric activity (Lee et al., 2007). The antimicrobial and antifungal properties of several crude extracts from different parts of other *Astragalus* species have been reported (Bisignano et al., 1994; El Sebakhy et al., 1994; Pistelli et al., 2002). The aqueous extract of Av showed maximum antifungal activity in the present study. Antimycotic activity of aerial parts of *Astragalus verrucosus* was observed on more polar extracts as well (Pistelli et al., 2002). The aqueous extract of Av at dose

40% showed an acceptable efficacy in comparison to licensed compound KTZ and CFUs of skin and internal organs were significantly reduced and continued to decline in contrast to the progressive infection observed in the untreated animals throughout the observation period. Further studies are required to find the active compound in the aqueous extract of Av that demonstrates antifungal or possibly immune-modulating activities. In this sense, the active compounds including of pure saponins and astraverrucins I-VI derived from *Astragalus verrucosus*, antimicrobial isoflavans, astragaliquinone, 8-methoxyvestitol and 7-hydroxy-2',3',4'-trimethoxyisoflavan were isolated from roots of *Astragalus alexandrinus* and *A. trigonus* showed an appreciable antimicrobial activity (El Sebakhy et al., 1994; Pistelli et al., 2002).

In conclusion, we found aqueous extract of Av has antifungal activity *in vitro* and it exerted a significant anti-candidal effect against cutaneous and systemic candidiasis in guinea pig model. The detection of antifungal activity of Av gives the claims by traditional healers a significantly high credibility. However, in order to develop an *Astragalus*-based formulation for the treatment or control of candidiasis-related complications, more research will be needed to identify the whole active ingredients in Av and their mechanisms of action.

#### Acknowledgements

This project was financed by Department of Pharmacology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran.

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**\*Correspondence**

Isaac Karimi  
Department of Biochemistry, Physiology and Pharmacology,  
College of Veterinary Medicine, Razi University  
Kermanshah, Iran  
karimiisaac@razi.ac.ir  
Tel.: +98 831 8322599 8329540  
Fax.: +98 831 8320041.