IN VITRO ANTI-CANDIDA ACTIVITIES OF SALSOLA RIGIDA AND PROSOPIS FARCTA

Zahra Seifi¹, Fataemeh Seifi², Mohammad Java Mahdavi³, Raheleh Seifi⁴, Maral Gharaghani⁵, Ali Zarei Mahmoudabadi⁶

¹Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
²Counseling and Reproductive Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran.
³PhD student School of Natural Resources and Earth Sciences, University of Kashan, Iran.
⁴MSc of Horticulture
⁵Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
⁶Department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Health Research Institute, Infectious and Tropical Diseases Research Centre, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Correspondence author; E-mail: zarei40@hotmail.com. Tel.: +986133330074. Fax: +986133332036

ABSTRACT: Introduction: Traditional medicine is a potential source of new antifungal with worldwide. Salsola rigida is an Iranian traditional herbal medicine that it is useful for several diseases such as hypertension and leprosy. Prosopis farcta is another native species of arid and semi-arid regions of Iran. It is used for the treatment of stomach ulcers, abortion, dysentery, larynx rheumatism inflammation, cardiac pain, breath shortness and also has anti-inflammatory effect in folklore medicine. Objectives: The aim of the present study was to assess in vitro activities of ethanolic extracts of S. rigida and P. farcta against several clinical isolates of Candida species.

Materials and Methods: The aerial parts of S. rigida and the fruits of P. farcta were extracted using 85% ethanol for 72h at a room temperature. The extracts were filtered using Whatman filter papers and dried at ambient. A serial dilutions of both extracts from 3.12-400 mg/ml were prepared in dimethyl sulphoxide. In the present study agar well diffusion method was used for detection minimum inhibitory concentration (MIC) of several isolates of various Candida species. Results: It was found that the ethanolic extracts from the aerial parts of S. rigida and P. farcta were extracted using 85% ethanol for 72h at a room temperature. The extracts were filtered using Whatman filter papers and dried at ambient. A serial dilutions of both extracts from 3.12-400 mg/ml were prepared in dimethyl sulphoxide. In the present study agar well diffusion method was used for detection minimum inhibitory concentration (MIC) of several isolates of various Candida species. Results: It was found that the ethanolic extracts from the aerial parts of S. rigida and P. farcta were exhibited anti-Candida activity by ranges of inhibition zones and MIC by ranges of 25-400 mg/ml. The mean MICs for all tested Candida were 149.4 mg/ml and 120.6 mg/ml for S. rigida and P. farcta, respectively. Conclusion: Our result shows that both plants have antifungal activity potential and can be as a suitable source for the treatment of fungal disease. It is the worth of further investigations in order to identify the active compounds and their clinical applications for the treatment of candidiasis.

Keywords: Salsola Rigidia, Prosopis Farcta, Candida Albicans, Ethanol Extract.

INTRODUCTION

The number of immunosuppressive patients, patients undergoing chemotherapy and surgery, long stay in intensive care unit (ICU) and neonatal intensive care unit (NICU) wards were increased during last decades. In addition, the increasing use of antifungal drugs for the treatment of fungal disease and prophylaxes (especially in hospitalized patients), were increased resistance to antifungals. On the other hand, some Candida species such as Candida glabrata and C. krusei have an inherent resistance to some antifungals. As a results, the incidence of invasive fungal diseases were dramatically increased. Increasing of resistance to current antifungal drugs makes
it necessary to discover new antifungal drugs from traditional medicines. Herbal medicines have the potential sources of new antifungals, due to its minimal side effects, cost effective and availability. Salsola rigida (Alafe Shoor) belongs to the family of Chenopodiaceae. This plant is often shaped shrub and its height between 15 to 50 cm and the base is very wooden (Figure 1). The woody stem of plant includes branches with rough shoots and covered with short bristles (1). Some species of Salsola (S. aimbricata) are a traditional herbal medicine that these species have been useful for diseases such as hypertension, digestive disorders and leprosy (2). Plant exists in southwest of Tehran and is also abundant in the northwestern and southern Iran, Khorasan, Sistan-Baluchistan and Hormozgan provinces (3).

Prosopis farcta is endemic to arid and semi-arid regions of the America, southwestern Asia (from Kazakhstan to the Indian subcontinent and west to the Middle East) and Northern Africa (Algeria, Egypt and Tunisia) (4, 5). P. farcta is another native species of arid and semi-arid regions of Iran (South eastern). It belongs to the family of Leguminosae and subfamily Mimosoideae. P. farcta is a small, prickly shrub, 30-80 cm tall or “shrub-tree” 2-3 m or taller (Figure 1) (6). P. farcta is a traditional herbal medicine that it is useful for treatment of stomach ulcers, abortion, dysentery, cardiac pain and breath shortness, skin wound healing process and rheumatism inflammation of the larynx (4, 6-8). In addition plant has anti-diabetic, anti-spasmodic analgesic, anti-inflammatory properties and also decreases the cholesterol level in blood (3, 4, 9).

Figure 1: Salsola rigida (right) and Prosopis farcta (left) (10, 11)

Objectives
The aim of the present study was to evaluate in vitro activities of ethanolic extracts of two Iranian medicinal plants, S. rigida and P. farcta against several clinical species of Candida.

MATERIALS AND METHODS
Plant Materials and Extraction
The aerial parts of S. rigida were collected during its flowering stage in April 2012 from Kashan (in the province of Isfahan, Iran). The fruit of P. farcta was also collected during October and November 2012 from Kashan. Both plants were identified in the Lab School of Natural Resources and Earth Sciences, University of Kashan, Iran. The aerial parts of S. rigida and fruits of P. farcta were dried in shade and then powdered. Each powdered plant material (10g) was macerated with 100 mL of 85% ethanol for 72 h at a room temperature kept on a rotary shaker. The extracts were filtered using Whatman filter paper No. 1 (Whatman, UK) and dried at room temperature. Then extraction solvents were evaporated in room temperature.

Fungal Isolates and Suspension Preparation
In the present study several strains of Candida including C. glabrata, C. albicans, C. kefyr, C. humicola, C. tropicalis, C. dubliniensis, and Candida species were used. All strains were kept at the department of Medical Mycology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Iran. All strains re-identified using standard mycological methods, which including germ tube test, production chlamydoconidia on cornmeal agar (Difco, UK), ID 32C (bioMerieux SA, France) and CHROMagar Candida (CHROMagar Candida
Company, Paris, France) (12). An overnight culture of each of isolate on Sabouraud’s dextrose agar, SDA (Merck, Germany) was harvested in distilled water, washed with PBS and a suspension equal of 0.5 McFarland (106 cells/ml) was prepared in distilled water (13).

**Antifungal Assay**

A serial dilution of extract was prepared in Dimethyl Sulphoxide (DMSO) (Sigma, USA) from 25 to 400 mg/ml. In the present study agar well diffusion method was used for detection minimum inhibitory concentration, MIC (14). 50 µl of the fungal suspension were inoculated on the surface of the agar medium. Wells of 10 mm in diameter and about 7 mm apart were punctured in the plates using sterile glass tube. 100 µl of several dilutions of fresh extracts were loaded for each well. Bioactivities were determined after 24 h incubation at 37°C via measuring the diameter of inhibition zone diameter in mm. All experiments were made in triplicate and means were calculated.

**RESULTS AND DISCUSSION**

In this study the MIC of the ethanolic extracts of both plants S. rigid and P. farcta in the concentrations of 3.12-400 mg/ml evaluated against various Candida spp. (C. glabrata, C. albicans, C. kefyr, C. dubliniensis, C. humicola and C. tropicalis) using the agar well diffusion method. In this study the anti-Candida activity of extract ethanolic of S. rigida was evaluated against 20 isolates of species Candida that 6 samples of patients with candiduria and 14 samples were collected from patients with vulvovaginal candidiasis. It was found that the ethanolic extract from the aerial parts of S. rigida was exhibited anti-Candida activity with the MIC ranges of 25-400 mg/ml (Table 1). As shown C. humicola was the most sensitive tested organism to extract than other species. On the other hand the strains of C. glabrata recovered from vagina were more resistance to S. rigida than urine isolates. Totally, the mean MIC for all tested Candida was 149.4mg/ml.

### Table 1. In vitro activity of ethanolic extract of S. rigida against different isolates of Candida

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Sources</th>
<th>Range (mg/ml)</th>
<th>Mean (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans (no. 3)</td>
<td>Vagina</td>
<td>100-200</td>
<td>133.3</td>
</tr>
<tr>
<td>C. glabrata (no. 2)</td>
<td>Vagina</td>
<td>200-400</td>
<td>300</td>
</tr>
<tr>
<td>C. dubliniensis (no. 6)</td>
<td>Vagina</td>
<td>50-200</td>
<td>116.7</td>
</tr>
<tr>
<td>C. kefyr (no. 1)</td>
<td>Vagina</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>C. humicola (no. 1)</td>
<td>Vagina</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>C. tropicalis (no. 1)</td>
<td>Vagina</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>C. tropicalis (no. 5)</td>
<td>Urine</td>
<td>100-200</td>
<td>120</td>
</tr>
<tr>
<td>C. glabrata (no. 1)</td>
<td>Urine</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

The fruit extract of P. farcta were examined against 21 strains of Candida recovered from vulvovaginal candidiasis. All tested isolates were sensitive to P. farcta extract from 50-200 mg/ml, with 150 and 83.3 mg/ml as highest and lowest concentrations, respectively (Table 2). Totally, the mean MIC for all tested Candida was 120.6 mg/ml.

### Table 2: In vitro activity of ethanolic extract of P. farcta against vaginal Candida species

<table>
<thead>
<tr>
<th>Candida species</th>
<th>Range (mg/ml)</th>
<th>Mean (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans (no. 10)</td>
<td>100-200</td>
<td>150</td>
</tr>
<tr>
<td>C. dubliniensis (no. 5)</td>
<td>100-200</td>
<td>120</td>
</tr>
<tr>
<td>C. glabrata (no. 3)</td>
<td>50-100</td>
<td>83.3</td>
</tr>
<tr>
<td>C. kefyr (no. 2)</td>
<td>100-200</td>
<td>150</td>
</tr>
<tr>
<td>Candida species (no. 1)</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Several studies have shown that S. rigida and P. farcta are usually used in traditional medicine for the treatment of hypertension, digestive disorders, stomach ulcers, abortion, dysentery, cardiac pain, skin wound healing process and larynx rheumatism inflammation (2, 4, 6-8). However, there are a few report to evaluate the antifungal effect of these plants so far. Jawad et al., have shown that P. farcta exhibited acceptable activity against Candida species (15). In another study, the anti-Leishmania effect of P. farcta in in their vector,
Phlebotomus papatasi, after feed plant confirmed (16). Literatures shows that S. rigida was active against helminthes and used for the removal intestinal worms in animals (1). Our result shows that the species of Candida have different degree of sensitivity against ethanolic extract of S. rigida and P. farcta. The mean MICs for all tested Candida were 149.4 mg/ml and 120.6 mg/ml for S. rigida and P. farcta, respectively. These MICs shows that both plants have antifungal activity potential and can be as a suitable source for the treatment of fungal disease. However, more researches need to confirm and also non-toxicity plants compounds.

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