In vitro antifungal activity of Satureja Khuzestanica Jamzad against Cryptococcus neoformans

Majid Zarrin¹, Nasrin Amirrajab², Batool Sadeghi-Nejad³

ABSTRACT

Objective: Finding the antifungal activity of Satureja Khuzestanica Jamzad extract against Cryptococcus neoformans isolates.

Methodology: The dilution in agar method was used to test the plant extracts against C. neoformans. The minimum inhibitory concentration (MIC) was described as the lowest concentration capable to inhibit any visible fungal growth. All nine C. neoformans strains used in this study were environmental isolates.

Results: The MIC value of Satureja Khuzestanica against C. neoformans isolates observed in this study demonstrated that the extract of this plant have antifungal activity. The MIC of extract of Satureja Khuzestanica ranged from 62.5-2000 µg/ml⁻¹. The extract of this plant at concentration of 500 µg/ml⁻¹ inhibited 78% of C. neoformans isolates.

Conclusion: This study demonstrated that the extract of Satureja Khuzestanica has anticrptococcal activity. Our work open viewpoints of find more efficient medicines of herbal origin in the treatment of fungal mycoses.

KEY WORDS: Antifungal activity, Satureja Khuzestanica, Cryptococcus neoformans.

INTRODUCTION

Opportunistic mycoses are increasing as a result of the increase in number of immunocompromised patient. The condition is even more attentive with the present pandemic of AIDS. The frequently encountered fungal diseases in HIV patients are candidiasis and cryptococcosis. Cryptococcus neoformans is an opportunistic fungal pathogen that causes serious infection of the central nervous system in both immunocompromissed and it seems that immunocompetent patients.¹

The current drugs such as amphotericin B, miconazole and floconazole are toxic or can cause drug-drug interaction.² Moreover, drug resistant strains of C. neoformans are rising in the post-AIDS period.³

The antimicrobial properties of medicines from medicinal plants have been distinguished since ancient times.⁴ Plants characterize an excellent source of new antimicrobial molecules.⁵,⁶ This plant is widely grown in south of Iran. Satureja Khuzestanica has therapeutic value because of uses as an analgesic and antiseptic in folk medicine.⁷ The leaves base is attenuating and petioliiform. This plant traditionally is used for treating stomach and intestinal disorders. Recently, antibacterial,⁸,⁹ antiviral,¹⁰ antifungal¹¹,¹² effects of Satureja species have been reported from different parts of world. The highest antibacterial and antifungal actions of Satureja Khuzestanica has been seen against Staphylococcus aureus and Candida albicans.¹³
This encouraged us to assess Satureja Khuzestanica as source of possible chemotherapeutic drugs antifungal activity based on its ethnomedical apply. In this purpose we studied the antifungal activity of Satureja Khuzestanica Jamzad extract against C. neoformans isolates.

**METHODOLOGY**

**Plant materials:** Plant materials were collected from Andimeshk in Khuzestan province (Iran) in April 2006. The leaves of plants used in the experiment.

**Preparation of the extracts:** The leaves of Satureja Khuzestanica were air-dried at room temperature and powdered. About 10 g of powdered drug was extracted with add of 100ml of 80% ethanol (drug/solvent ratio=1:10 w/v) in a conical flask for maceration. The mixture was kept for 72 hour at room temperature shaking. The suspension was filtered with a Whatman filter paper #1 and the crude ethanol extracts were evaporated in room temperature. One gram of extract was dissolved in one ml 100% dimethyl sulfoxide (DMSO) and the final concentration of each extract adjusted to 1000mg/ml. Microorganisms: All nine C. neofoemans strains used in this study were environmental isolates from pigeon lofts, obtained at medical mycology laboratory, Ahvaz Jundishapur university of medical sciences. The stock cultures were maintained on Sabouraud dextrose agar medium (Merck) with chloramphenicol. Each isolate was subcultured twice in Sabouraud dextrose agar medium (Merck) with chloramphenicol. Each isolate was subcultured twice on Sabouraud dextrose agar to ensure purity and growth before experiment.

**Antifungal assay:** The dilution in agar method was used to test the plant extracts against C. neoformans. One thousands mg of the crude plant extracts were solubilized in 5 ml of DMSO and served as stock solution. This solution serially two-fold diluted in 0.85% sterile physiological saline ranging from 15.6-2000µg/ml. Of this solution, 100 µl were transferred into sterile Pertri dishes and 5 ml of liquefied Sabouraud dextrose agar at 45°C were added and mixed.

Fifty µl of spore suspension (10^6 CUF/ml) prepared in 0.85 saline was inoculated on agar plates and kept at 37°C for 48-72h. The minimum inhibitory concentration (MIC) was described as the lowest concentration capable to inhibit any visible fungal growth. Pertri dishes containing spore suspension with only DMSO diluted in the similar condition, which did not promote fungal growth, were used as negative control. The sensitivity of all C. neoformans strains to Amphotericin B (Bristol-Myers Squibb, Paris) was performed as positive control. The minimal fungicidal concentration (MFC) did not show any visible growth after incubation.

**RESULTS**

The MIC value of Satureja Khuzestanica against C. neoformans isolates observed in this study demonstrated that the extract of this plant have antifungal activity. The MIC of extract of Satureja Khuzestanica ranged from 62.5-2000 µg/ml.

The extract of this plant at concentration of 500 µg/ml inhibited 78% of C. neoformans isolates. The sensitivity of the same C. neoformans isolates to amphotericin B demonstrated a MIC of 2-4 µg/ml. The MFC of Satureja Khuzestanica extraction ranged from 125-4000 µg/ml. The extract of this plant at concentration of 1000 µg/ml was fungicidal for 44% of C. neoformans isolates. The MIC and MFC concentrations of plant extract against C. neoformans isolates showed in Table-I. The MIC and MFC ranges of plant extract against C. neoformans isolates showed in Table-II. Figure-1 shows the antifungal activity of the Satureja Khuzestanica in different concentrations.

**DISCUSSION**

This study demonstrated that the extract of Satureja Khuzestanica has anticryptococcal activity. Traditional uses of this plant suggest that it is favored by non toxicity to humans. Cryptococcosis is a worldwide fungal infection that has been distinguished by the following:

**Table-I: In vitro antifungal activity of Satureja Khuzestanica against 9 isolates of C. neoformans (µg/ml)**

<table>
<thead>
<tr>
<th>C. neoformans isolate</th>
<th>MIC (µg/ml)</th>
<th>MFC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2</td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>A9</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>F2</td>
<td>2000</td>
<td>4000</td>
</tr>
<tr>
<td>G3</td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>D</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>B</td>
<td>62.5</td>
<td>125</td>
</tr>
<tr>
<td>G1</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>E1</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>L4</td>
<td>2000</td>
<td>4000</td>
</tr>
</tbody>
</table>

**Table-II: Susceptibility pattern of C. neoformans to the extracts of Satureja Khuzestanica and amphotericin B (µg/ml)**

<table>
<thead>
<tr>
<th>C. neoformans</th>
<th>MIC (µg/ml)</th>
<th>MFC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B (MFC)</td>
<td>2-4</td>
<td>125-4000</td>
</tr>
</tbody>
</table>

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REFERENCES