

In vitro antifungal activity of *Satureja Khuzestanica* Jamzad against *Cryptococcus neoformans*

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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/ml¹. The extract of this plant at concentration of 500 µg/ml¹ inhibited 78% of *C. neoformans* isolates.

Conclusion: This study demonstrated that the extract of *Satureja Khuzestanica* has anticryptococcal 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*, *Crptococcus neoformans*.

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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 immunocompromised and it seems that immunocompetent patients.¹

The current drugs such as amphotricin 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 petioliform. This plant traditionally is used for treating stomach and intestinal disorders. Recently, antibacterial,^{8,9} antiviral,¹⁰ antifungal^{11,12} 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

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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⁻¹.

Microorganisms: All nine *C. neoformans* strains used in this study were environmental isolates from pigeon lofts,¹⁴ 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 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*.¹⁵ 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⁻¹. 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 (10⁶CUFml⁻¹) 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 Amphotricin 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⁻¹.

The extract of this plant at concentration of 500 µg/ml⁻¹ inhibited 78% of *C. neoformans* isolates. The sensitivity of the same *C. neoformans* isolates to amphotricin B demonstrated a MIC of 2-4 µg/ml⁻¹. The MFC of *Satureja khuzestanica* extraction ranged from 125-4000 µg/ml⁻¹. The extract of this plant at concentration of 1000 µg/ml⁻¹ 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.¹⁶ Cryptococcosis is a worldwide fungal infection that has been distinguished by the

Table-I: In vitro antifungal activity of *Satureja khuzestanica* against 9 isolates of *C. neoformans* (µg/ml⁻¹)

<i>C. neoformans</i> isolate	MIC	MFC
L2	250	500
A9	500	1000
F2	2000	4000
G3	250	500
D	500	1000
B	62.5	125
G1	500	1000
E1	500	1000
L4	2000	4000

Table-II: Susceptibility pattern of *C. neoformans* to the extracts of *Satureja khuzestanica* and amphotricin B (µg/ml⁻¹)

MIC	62.5-2000
MFC	125-4000
amphotricin B (MIC)	2-4

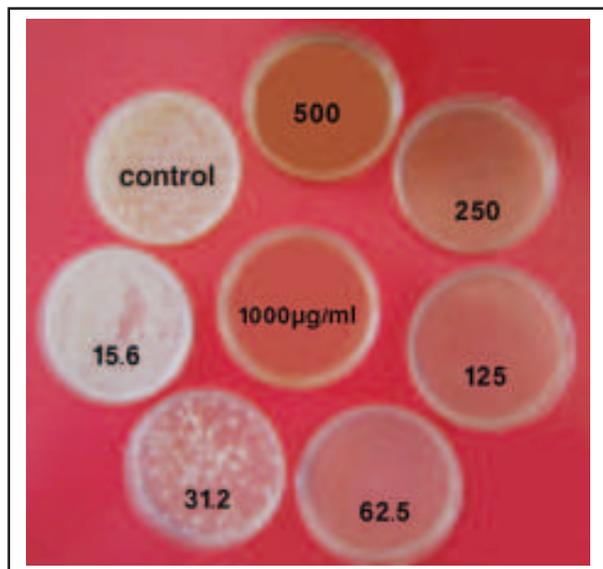


Fig-1: The antifungal activity of the *Satureja Khuzestanica* in different concentrations on isolate B of *C. neoformans* ($\mu\text{g}/\text{ml}^{-1}$).

progress of resistance among many strains following antifungal therapies.

The agar dilution method was used in our work for determination of the plant activity. Previous investigation has revealed that *Satureja Khuzestanica* has antifungal activity against other fungi.^{11,12} In our study 78% of *C. neoformans* isolates were inhibited at concentration of $500 \mu\text{g}/\text{ml}^{-1}$. The susceptibility tests of the *C. neoformans* isolates to *Satureja Khuzestanica* extract obtained in our study show MIC values ranging from 0.078 - 2.0 mgml^{-1} against *C. neoformans* isolates.

Satureja species have a variety of biologically active elements including essential oil, triterpenes, flavonoids and rosmarmic acid.¹⁷ The essential oil extracted from species of *Satureja* has been revealed to have fungicidal activities. Sahin et al, showed that *Satureja hortensis* inhibited the growth of isolates of *C. albicans* at the concentration of $300 \mu\text{g}/\text{ml}^{-1}$.⁸ A study demonstrated the antifungal activity of 4 species of *Satureja* against *C. albicans* and *Penicillium census*.⁹ Arvin showed the antifungal effect of the *Satureja Khuzestanica* against *C. albicans* and *Aspergillus niger*.¹⁸ The MIC for *C. albicans* was $100 \mu\text{g}/\text{ml}^{-1}$ and $200 \mu\text{g}/\text{ml}^{-1}$ for *A. niger*.

We conclude the *Satureja Khuzestanica* extract studied can be applied as a complementary treatment where an antifungal drug is frequently ineffective. Our work open further avenues to find more efficient medicines of herbal origin in the treatment of fungal mycoses.

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