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## Anti-fungal activity of *Glycyrrhiza glabra* (Licorice) against vaginal isolates of *Candida*

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### ABSTRACT

*Glycyrrhiza glabra* is a traditional medicinal herb and the root of plant contains several active constituents. Two major constituents are glycyrrhizin and glycyrrhetic acid that have a numerous pharmacological effects. The aim of study was to evaluate anti-*Candida* activity of *G. glabra* against vulvovaginal isolates of *Candida*. Minimal inhibitory concentration of the methanolic and ethanolic extracts was respectively 2% and <2%. Mean MICs of ethanolic extract diluted with DW, ethanol and DMSO were respectively 10 mg/ml, 625µg/ml and 312µg/ml. Our results indicate potential use of *G. glabra* root as anti-*Candida* agent isolated from vaginal candidiasis, deserving further investigation for clinical applications.

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### KEYWORDS

Herbal medicine;  
*Glycyrrhiza glabra*;  
*Candida*;  
Anti-*Candida*;  
Iran.

### INTRODUCTION

*Glycyrrhiza glabra* (Licorice root, Sweet licorice, Sweet wood) is a traditional medicinal herb grows in the various parts of the world, South-Eastern Europe, Italy, Spain, South-West Asia China, Russia, India and Iran<sup>[1]</sup>. Plant is growing in open fields close to running water. The roots of plant are brown, long and cylindrical and unearthed in the autumn of the fourth season. *Glycyrrhiza glabra* contains glycyrrhizin, glycyrrhetic acid, flavonoids (liquiritigenol), asparagine, iso-flavonoids, chalcones, triterpenoid glycosides (saponins), bitter principle (glycyrrmarin), volatile oil, coumarins, amino acids, amines (choline, betaine, asparagine), oestrogenic substances (including beta-sitosterol), glu-

cose and sucrose (5-15% sugars), starch, tannins (trace), gums, wax and antioxidants<sup>[2-5]</sup>.

In Greeks, the extract of herb is used for the treatment of gastric and peptic ulcers whereas in Asia and Europe, is used for psoriasis disease<sup>[1]</sup>. Glycyrrhizin and glycyrrhetic acid, major active constituents of herb have a numerous pharmacological effects, such as hepatoprotective activities<sup>[6]</sup>, memory improvement<sup>[7]</sup>, anti-inflammatory<sup>[8]</sup>, anti bacterial (Gram positive and negative)<sup>[1]</sup>, anti-viral<sup>[6]</sup> and anti-tumor<sup>[9]</sup>. *Glycyrrhiza glabra* is one of the most widely known medicinal plant in Iran, and records of its use include antiseptic, antimicrobial<sup>[10-11]</sup>.

*Candida* vulvovaginal is a common infection among women and approximately 75 % of all women will ex-

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perience an episode of disease at least once in their lifetimes and recurrent *Candida* vulvovaginal affect up to 5% women<sup>[12]</sup>. The majority of cases of *Candida* vaginitis are caused by *Candida albicans*. The vast majority of strains isolated from the recurrent vaginitis belong to *C. albicans*. In addition non *C. albicans* isolates have a natural greater resistance to the routine antifungal agents, i.e. various azole drugs<sup>[13]</sup>. The aim of the present study was to evaluate anti-*Candida* activity of *Glycyrrhiza glabra* against different isolates of *Candida* isolated from vulvovaginal candidiasis.

## MATERIALS AND METHODS

### Extraction

The dried roots of *Glycyrrhiza glabra* were obtained from grocery in Ahvaz, Iran and identified by agricultural research centre in Isfahan, where voucher specimens were deposited. Aliquots of 50 g of the dried powder of the plant were soaked in ethanol (200 ml), methanol (200 ml) and distilled water (200 ml) in room temperature for 72 h. Extracts were filtered with cotton wood filter paper and then concentrated to dryness in a *vacuo* at 53-55°.

### Tested organisms

Thirteen isolates of *Candida albicans*, one of *C. glabrata* and one *Candida* species isolated from vulvovaginal candidiasis were studied. The *Candida* isolates were identified by standard microbiology methods; CHROMagar Candida (CHROMagar Candida Company, Paris, France), germ-tube test, production of chlamydoconidia on Corn meal agar and growth at 45°C. All isolates were sub-cultured on slant of Sabouraud Dextrose Agar (Merck, Germany) (SDA) and maintained at 4°C until use.

### Assessment of *in vitro* activity

The anti-*Candida* bioactivity of the aqueous, ethanolic and methanolic extract of *Glycyrrhiza glabra* was evaluated against vulvovaginal isolates of *Candida* species. Isolates were sub-cultured on SDA and incubated at 37°C for overnight. A suspension of several colonies of each isolates was prepared in 2 ml of sterile PBS and adjusted to 70% transmittance (T) by a spectrophotometer at 530 nm. This should result in a sus-

pension containing about  $1 \times 10^6$  cfu/ml.

In the present study anti-*Candida* activity of *Glycyrrhiza glabra* was investigated by agar well diffusion method<sup>[14]</sup>. 25µl of yeast suspension was inoculated on the surface of the agar medium and spread using a sterile glass rod. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.2 ml of several dilutions of extracts was administered to fullness for each well. Plates were incubated at 37°C for 24 h and bioactivities were determined by measuring the diameter of inhibition zone diameter in mm. The lowest extract concentration where there was no visible growth around wells was the Minimum Inhibitory Concentration (MIC). All experiments were repeated three times and mean calculated.

## RESULTS AND DISCUSSION

In the present study the anti-*Candida* activity of *Glycyrrhiza glabra* (aqueous, ethanolic and methanolic) was evaluated against 15 vaginal isolates of *Candida*. In the first stage several dilutions (10-1%) of aqueous, ethanolic and methanolic extracts of *Glycyrrhiza glabra* applied on the isolates. Aqueous extract of *Glycyrrhiza glabra* did not show any activity against vaginal isolates. However ethanolic and methanolic extracts showed remarkable activities against tested isolates of *Candida*. Sato et al.<sup>[15]</sup> reported that hot water extracts of *Glycyrrhiza glabra* have no more activity against fungi than ethanol extracts. The Minimal inhibitory concentration (MIC) for methanolic and ethanolic extracts were respectively 2% and <2%. In the second stage, the serial dilutions of ethanolic extract (2-0.03125%) were used for the detection of MIC. The solvents for preparing dilutions were distilled water (DW), ethanol and DMSO. Mean MICs of ethanolic extract diluted with DW, ethanol and DMSO were respectively 10 mg/ml, 625µg/ml and 312µg/ml. The present results indicate that the ethanolic extracts of *Glycyrrhiza glabra* root have markedly activity against vaginal isolates of *Candida*.

*Glycyrrhiza glabra* is used in Iranian traditional herbal medicine for antiseptic, analgesic and carminative properties<sup>[10]</sup>. Several reports show that *Glycyrrhiza*

*glabra* have antibacterial activity<sup>[16]</sup>. In addition the antimycobacterial activity of root ethanolic extract of *Glycyrrhiza glabra* was detected against *Mycobacterium tuberculosis*<sup>[1]</sup>. They found that glabridin, chloroform: ethyl acetate fraction of *Glycyrrhiza glabra* is more active against strains of *Mycobacterium*. Several reports show that the antibacterial effect of *Glycyrrhiza glabra* attributed to flavonoids constituents<sup>[17]</sup>. Other studies have demonstrated therapeutic benefit for leishmania, malaria and viral infections<sup>[17]</sup>. Glycyrrhizin and glycyrrhizic acid two major constituents of *Glycyrrhiza glabra* have been shown to inhibit growth RNA and DNA viruses<sup>[17]</sup>. Chronic and recurrent vulvovaginal candidiasis have been increasing in recent years due to immunocompromised hosts, the increase of fungal resistance to antifungal drugs, antibiotic and corticosteroid therapy. As a result justify the search for new herbal drugs for new strategies.

We concluded that *Glycyrrhiza glabra* represent a remarkable anti-*Candida* bioactivity against *Candida* isolates from vulvovaginal candidiasis. In addition *Glycyrrhiza glabra* root extract has potential use for the treatment of vulvovaginal candidiasis.

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