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Research Article

IN VITRO SCREENING OF ANTI-*CANDIDA* ACTIVITY OF SAPONINS EXTRACTED FROM *GLYCYRRHIZA GLABRA* AND *QUILLAJA SAPONARIA*

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ABSTRACT

Objective: In recent years, the incidences of opportunistic fungal pathogens have increased and development of fungal resistance to antifungal drugs is a global concern. Therefore, it is important to identify new antifungal agents. Saponins are secondary metabolites that are found in various plant species and show antifungal activity. The aim of the study was to evaluate antifungal activity of saponin extracted from the *Glycyrrhiza glabra* against *Candida* species (*Candida albicans*, *Candida tropicalic* and *Candida glabrata*). Antifungal activity *Quillaja saponaria* total saponin (QST) was also evaluated.

Methods: The roots of the plant were dried, powdered and def-fatted with petroleum ether in a soxhlet apparatus. The air dried powder was successively extracted with methanol, n-butanol and diethyl ether. The antifungal activity of the saponins was carried out using well diffusion method and also the value of minimum inhibitory concentrations (MIC) was calculated. Clotrimazole was used as positive controls to determine the sensitivity of the species.

Results: According to the results, *C. albicans,* and *C. tropicalic* were sensitive to the saponins of *G. glabra*, and *Q. saponaria,* while saponin isolated from *G. glabra* just could inhibited the growth of *C. glabrata*.

Conclusion*: In vitro* studies have demonstrated that saponins extracted from *G. glabra*, and *Q. saponaria* can serve as potential candidates for the development of new antifungal agents.

Keywords: Saponin**,** *Glycyrrhiza glabra*, *Quillaja saponaria,* Anti-*Candida* activity

INTRODUCTION

The development of fungal resistance to many of the commonly used antibiotics provides further attempts to investigate for novel antifungal agents to combat infections and overcome the problems of resistance and side effects of the currently available antimicrobial agents. There are many approaches to search for new antimicrobial compounds from various kinds of sources such as soil, microorganisms, animals and plants [1- 3]. Plants are important sources of potentially useful constituents for the development of new therapeutic agents, because most of them are safe with little side effects [4]. Many plants synthesize secondary metabolites with powerful antimicrobial activities such as saponin. Saponins are composed of a sugar moiety usually containing glucose, xylose, glucuronic acid, galactose or rhamnose that is linked to a triterpene or steroid aglycone. Saponins have a lytic action on erythrocyte membranes, a property which has been used for their detection. These compounds have found many applications in food, pharmaceutical and cosmetics industries. They exhibit many pharmacological activities such as anti-inflammatory, hepatoprotective, anti-ulcer, antiviral, antifungal, antiprotozoal, antioxidant and antibacterial activities. Saponins also show anti-tumor effects against cancer cells [5-11]. *Glycyrrhiza glabra,* as herbal medicine has been used for treatment of chronic hepatitis, various types of ulcers, liver disease, psoriasis and shows antimicrobial and anti-inflammatory activity [12, 13, 14]. *Quillaja saponaria* is a tree native to the Andes region and the commercial saponins is extracted from this plant. *Q. saponaria* is a good source of triterpenoidal saponins. Different studies showed the saponin of *Q. saponaria* has antibacterial activity against *E.coli* [6, 9, 11, 15].

Opportunistic fungal pathogens such as *Candida, Cryptococcus* and *Aspergillus* are life-threatening to immunocompromised patients with AIDS, cancer and organ transplant. Despite advances in antifungal therapies, many problems remain for most current antifungal drugs [16, 17]. Therefore, the objective of this study was to investigate the antifungal activity of saponins extracted from *G. glabra*, and *Q. saponaria* against *Candida* species (*C. albicans*, *C. tropicalic* and *C. glabrata*).

MATERIAL AND METHODS

Candida species such as *C. albicans*, *C. tropicalic* and *C. glabrata* were isolated from clinical material collected from patients that referred to the School of ***Dentistry***, ***Ahvaz Jundishapour*** University of Medical Sciences, Ahvaz Iran. Sabouraud Dextrose agar (SDA) was purchased from Merck, Germany. QTS was obtained from Alfa Aesar, Germany. All of the solvents were of the analytical grade.

Plant Materials

The roots of *G. glabra* were collected from Ahvaz (Iran), and indentified in department of Pharmacognosy, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences. The roots of the plant were ground into powder and stored at room temperature (25ºC).

Extraction of Saponins

The powdered roots of *G. glabra* was defatted in a soxhelet apparatus with petroleum ether (boiling range 40-60 ºC) for removing lipids and phenolic compounds. The air-dried powder was extracted with methanol for 48 h. The solvent was removed under vaccum by rotary evaporator (Heidolph, Germany) and the resulting brown residue was suspended in water, then centrifuged at 2500 rpm for 45 min, and the supernatant was separated and extracted with water saturated n-butanol. Butanol phase concentrated in rotary evaporator at 80ºC and the dry residue was dissolved in the

least methanol quantity (30 ml), and then precipitated by addition of diethyl ether. Finally, total saponin of the plant (GTS) was freeze-dried (Operon, Korea) and stored at room temperature [18, 19].

Antifungal Activity

The microorganisms were cultured on Sabouraud Dextrose agar (SDA) and incubated at 37°C for 24 h. Inoculums containing 10^8CFU/ml according to the McFarland turbidometry was spread on Sabouraud Dextrose agar medium. For determination of the antifungal activity, well diffusion method was used. Wells were made on the media by using cork borer. Each plate was inoculated with 50 µl of the fungal suspension. The dried saponins were dissolved in DMSO 50% and various serial dilutions of the saponins were prepared (200, 100, 50 and 25 mg/ml). Then, 100 µL of each serial dilution transferred to the wells and incubated at 37°C for 24 h. Clotrimazole (4mg/ml) was used as positive control against *Candida* species. After the incubation period, the diameter of inhibition zone to each well was measured in mm. The Minimumal Inhibitory Concentration (MIC) was determined as the lowest concentration of the saponins that inhibited growth after 24 h of incubation [20, 21]. All experiments were done in three replicates.

RESULTS AND DISCUSSION

The yield of the total saponin extract of *G. glabra* was 0.8% w/w. The results of zone of inhibition (mm) at different concentrations of GTS and QTS are shown in Table (1). According to the results, GTS at concentrations of 20 and 10 mg ml^{-1} showed antifungal activity against all microorganisms that were tested and at concentration 5 mg ml^{-1} only showed activity against *C. glabrata* (Figure 1). The highest inhibition zone of 22±2.82 mm for GTS was observed against of *C. glabrata.*

QST inhibited the growth of *C. albicans* and *C. tropicalic* at concentrations 20 and 10 mg ml^{-1}, while *C. glabrata* was resistant to QST. Both saponins at concentration 2.5 mg ml^{-1} did not inhibit the growth of any of the microorganisms under study. According to the results in Table 1, anti-*Candida* activity was enhanced with the increase of the saponin concentration.

The results (Table 1) indicated a significant antifungal effect GTS *against C. glabrata.* Also, the lowest MIC value of 5 mg ml^{-1} (Table 2) in the presence of GTS was observed against *C. glabrata*. The MIC values of GTS and QTS against *C. albicans* and *C. tropicalic* was 10 mg ml^{-1} (Table 2).

Table 1: The zone of inhibition (mm) of GTS and QTS against *Candida* sp. at different concentrations (Mean ±SD)

Microorganisms	Concentrations GTS (mg ml^{-1})				Concentrations QTS (mg ml^{-1})				Clotrimazole (mg ml^{-1})
	20	10	5	2.5	20	10	5	2.5	2
C. albicans	15.33±4.61	11.66±3.51	0±0.0	0±0.0	16.5±2.12	14±2.82	0±0.0	0±0.0	17
C. glabrata	22±2.82	13.5±2.12	9.5±3.53	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	17
C. tropicalic	13.38±3.21	9±3.60	0±0.0	0±0.0	16±1.41	11±1.41	0±0.0	0±0.0	17

Table 2: Minimumal inhibitory concentration (MIC) of GTS and QTS (mg ml^{-1})

Microorganisms	GTS	QTS	Clotrimazol
C. albicans	10	10	0.078
C. glabrata	5	NI*	0.078
C. tropicalic	10	10	0.078

***NI: No inhibition.**

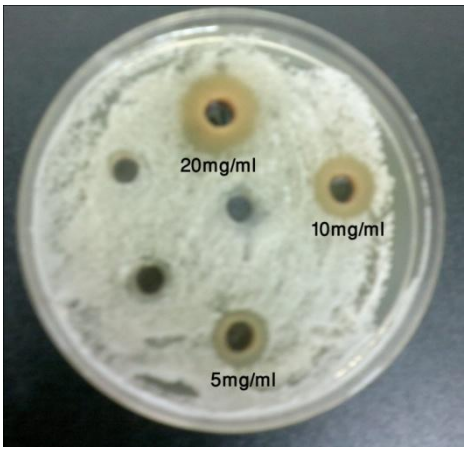

Figure 1: Inhibition zone of the GTS against *C. Glabrata* with MIC= 5 mg ml^{-1}

In the past few decades, a worldwide increase in the incidence of fungal infections has been reported. The used antifungal agents have various disadvantages as a result of toxicity, cost and their frequent use has led to the emergence of resistant strains. Therefore, there is a need to search for new agents with greater antifungal activity. Plants have been shown to be potential sources for new antimicrobial agents [22]. Saponins are secondary metabolites that are present in a wide range of plant species. It is believed that the interaction with steroids of the fungal membrane is the mechanism of antifungal activity of the saponins [23, 24]. The antifungal properties of saponins have been evaluated by a number of investigators.

Soetan *et al.* in 2006 investigated the antifungal activity of saponins extracted from *Sorghum* against *C.albicans.* Their results showed no significant inhibitory effect. They demonstrated that the ineffectiveness of the saponins on *C.albicans* may be as a result of the protective effect of the microbial coats that saponin could not be able to penetrate the cell membranes of the microorganisms [5]. Unlike the results of Soetan *et al.,* our findings imply that GTS and QTS have remarkable antifungal activity against *C.albicans* in comparison with saponins of *Sorghum.*

Maatalah *et al.* in 2012 reported that saponin extracts of *Anabasis articulata* was active against *C.albicans* and inhibition zone at concentrations of 5, 2.5, 1 and 0.5 mg/ml was 13, 10.8, 9.3 and 8.8 mm, respectively [25]. While, our finding showed that GTS, and QTS at concentrations 5 and 2.5 mg/ml could not affect the growth of *C.albicans.* It seems that saponin extracted from *A. articulate* may be more effective than GTS and QTS on *C.albicans.* Sanng *et al.* in 2005 evaluated the antifungal activity of eight steroid saponins from *Tribulus terrestris* (TTS-8, TTS-9, TTS-10, TTS-11, TTS-12, TTS-13, TTS-14 and TTS-15. TTS-12 and TTS-15) against *Candida sp.* They used the final concentrations of saponins in the range of 128.0 to 0.25 µg/ml. According to their results, TTS-12 and TTS-15 had significant antifungal activities against *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. neoforman* and *C. krusei*. Particularly, TTS-12 and TTS-15 inhibited the growth of *C. albicans*, and the MIC value was determined to be 4.4 and 9.4 µg/ml, respectively [26]. In comparison with our findings, it appears that the sapaonin of *Tribulus terrestris* is more effective than GTS and QTS against *C. albicans.*

kannabiran *et al.* in 2009 evaluated the antifungal activity of saponin isolated from *Solanum xanthocarpum* and *Centella asiatica* against *Aspergillus niger* and *A. fumigates*. According to their results, *A. fumigatus* was more susceptible than *A. niger* [27]. Consequently, the saponins of *Solanum xanthocarpum* and *Centella asiatica* can be considered as new antifungal agents for treatment of fungal infections. It is suggested that the potent antifungal activity of saponin isolated from *G. glabra* or *Q.saponaria* may be enhanced in combination with saponin of *Anabasis articulate, Tribulus terrestris, Solanum xanthocarpum* or *Centella asiatica.* This combination may effectively disrupt the fungal membrane and inhibit their growth. More studies are needed to prove their exact mechanism of action.

The results of our study showed that both GTS and QTS can be regarded as new sources of natural antifungal agents. However, further studies are needed to determine their chemical structure and to confirm their broad spectrum of antifungal activity against pathogenic microorganisms as well as saponins isolated from these plants should be further studied in animal models in order to evaluate their *in vivo* efficacy and toxicity.

CONCLUSION

It is concluded that GTS and QTS show *in vitro* antifungal activity against *C. albicans,* and *C. tropicalic.* It should be noted that *C. glabrata* was sensitive to the GTS*,* whereas it was resistant to the QTS. The results of the investigation suggest that these saponins are suitable candidates for further pharmacological evaluation.

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