ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# *In-vitro* Evaluation of Antibacterial Activity of *Glycyrrhiza glabra* and *Acanthopyllum squarrusom* Total Saponins.

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**ABSTRACT**

In recent years, with increasing prevalence of antibiotic resistant bacteria, there is an urgency to develop new antimicrobial compounds from various sources such as medicinal plants. Saponins are secondary metabolites that are found in various plant species. The aim of this study was to evaluate antibacterial activity of saponins extracted from the *Glycyrrhiza glabra* and *Acanthopyllum squarrusom* against *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus mirabilis*. The results were compared to data of *Quillaja saponaria* as a commercial saponin. The roots of the plants 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 antibacterial activity of the saponins was determined using well diffusion method and also the value of minimum inhibitory concentrations (MIC) was evaluated by 96-well microtiter plates. Ampicillin and gentamicin were used as positive controls to determine the sensitivity of the strains. According to the results, the best growth inhibitory activity was observed with saponin of *G. glabra*, while saponin fraction of *A. squarrusom* and *Q. saponaria* showed less activity against bacteria. The zone inhibition of the saponin extract from *G. glabra* was 40.00±0.00 mm for *E. coli*, 35.00±0.00 for *S. aureus,* 34.00±0.05 for *P. aeruginosa* and 30.00±0.00 for *P.mirabilis.* The value of MIC of saponin of *G. glabra* against *E. coli, S. aureus* and *P. aeruginosa* was 3.12 mg/ml, and against *P.mirabilis* was 25 mg/ml, whereas saponins of *A. squarrusom* and *Q. saponaria* were found comparatively less effective against bacterial pathogens. The results of the study indicated that saponin of *G. glabra* has a broad spectrum of antimicrobial activity and can be used as a natural antimicrobial agent.

**Keywords:** Saponin**,** *Glycyrrhiza glabra*, *Acanthopyllum squarrusom, Quillaja saponaria,* antibacterial activity

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ISSN: 0975-8585

## INTRODUCTION

Resistance to antimicrobial agents has increased and caused significant morbidity and mortality worldwide. Bacteria have the genetic ability to transmit and acquire resistance to drugs, which are employed as therapeutic agents. In addition, increasing the cost of drug regimens has contributed to the high frequency of opportunistic and chronic infection in developing countries. To control these infections, there is a necessary search for new agents with greater antibacterial activity and less toxicity [1- 4]. Plants have been shown to be potential sources for new antimicrobial agents. 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 pharmaceutical and cosmetics industry. They show many pharmacological activities such as anti-inflammatory, hepatoprotective, anti-ulcer, and antimicrobial activities. Saponins also show anti-tumor effects against cancer cells [5-10]. *Glycyrrhiza glabra,* as herbal medicine has been used for treatment of chronic hepatitis, various types of ulcers, liver disease, psoriasis and shows antimicrobial activity [11, 12]. *Acanthophyllum squarrosum* is growing wild in different locations of Iran. The roots of the plant have saponins that are used as soaps and detergents [13]. *Quillaja saponaria* is a tree native to the Andes region and the commercial saponins is extracted from this plant. Different study showed the saponin of *Q. saponaria* has antibacterial activity against *E.coli* [6, 9, 14].

Antibacterial resistance in bacteria is an international concern. Therefore, the purpose of the study was to determine the antibacterial activity of saponins extracted from *Glycyrrhiza glabra*, *Acanthopyllum squarrusom* and *Quillaja saponaria.*

## MATERIALS AND METHODS

Nutrient agar and nutrient broth were obtained from Merck, Germany and QUELAB, Canada, respectively. P-iodonitrotetrazolium violet (p-INT) was purchased from Hiemedia, India and *Quillaja saponaria* were obtained from Alfa Aesar, Germany. The freeze-dried sealed glass ampoule of *E. coli* (ATCC No. 25922), *Staphylococcus aureus* (ATCC No. 25923), *Pseudomonas aeruginosa* (ATCC No. 27853) and *Proteus mirabilis* (ATCC No. 12453) were procured from Iranian Research Organization for Science and Technology, Tehran, Iran. Ampicillin and gentamicin were kindly donated by Farabi and Daroupakhsh Pharmaceutical Co., respectively. 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. *A. squarrusom* roots were collected from Tabas, south Khorassan, Iran, and indentified in herbarium, Mashhad University of Medical Sciences. The roots of these plants were ground into powder and stored at room temperature (25ºC) until use.

### Extraction of Saponins

The powdered roots of *G. glabra* and *A. squarrusom* were 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, centrifuged at 2500 rpm for 45 min, and the supernatant was separated and was 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 precipitated by addition of diethyl ether. Finally, total saponins were freeze-dried (Operon, Korea) and stored at room temperature [5, 15, 16].

### Antimicrobial Activity

Microorganisms were grown in nutrient broth at 37°C for 24 h. Final cell concentrations were $10^8$cfu/ml according to the McFarland turbidometry. 100µl of the inoculum was added to each plate

containing nutrient agar. For determination of antibacterial activity, well diffusion method was utilized. Wells were made on the media by using cork borer. The dried saponins were dissolved in distilled water to final concentration 400mg/ml. Each well was filled with 50 µl of saponin. The cultured plates were incubated at 37ºC for 24 h. After the incubation period, the diameter of inhibition zone to each well was measured in mm [17, 18]. The test was performed three times.

**MIC Determination**

Sterile 96-well (12×8) microtitre plates were used for the assay of MIC. 100 µl of the inocula were added to all wells (B-H) except well (A) in the first column where 200 µl of the inoculums were piptted. 50 µl of the sample was added to the first well and mixed thoroughly with the inoculums in well A before transfer 100 µl of the resultant mixture to well B. The same procedure was repeated for inocula mixture in well B-H, therefore, creating a serial dilution so that a concentration of 200, 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/ml of the test materials is obtained. Gentamicin used as standard antibacterial agents *E. coli, S. aureus*, *P. aeruginosa*, whereas ampicillin was employed against *P. mirabilis*. The microplates were incubated at 37°C for 24 h. A solution of 50 p-INT (0.2 mg/ml) was then added to each well and plates were incubated for an additional 1 to 2 h. The MIC was determined as the lowest sample concentration at which no violet color developed. Samples from clear wells were subcultured by plotaing on to nutrient agar for determining the effect of bacteriostatic or bactericide saponins [10, 19].

## RESULTS AND DISCUSSION

The yield of the total saponin extract of *G. glabra* and *A. squarrusom* were 0.8% w/w and 2.7% w/w, respectively. The results of zone of inhibition (mm) saponins of *G. glabra*, *A. squarrusom and Q. saponaria* are shown in Table [1]. According to the results, the strongest activity was observed by *G. glabra* against four pathogens .The highest inhibition zone of 40.00±0.00 mm the saponin of *G. glabra* was observed against of *E. coli* [Figure 1]. In comparison with the saponin of *G. glabra,* saponin of *A. squarrusom and Q. saponaria* showed the less growth inhibition zone. The results of minimal inhibitory concentrations (MIC) are presented in Table [2]. According to the results, the lowest MIC of saponin extracted from *G. glabra* was 3.12 mg/ml against *E. coli, S .aureus and P. aeruginosa*, whereas this saponin was found less effective against *P.mirabilis* (MIC = 25 mg/ml).

The lowest MIC values of 25 mg/ml in the presence of saponin of *A. squarrusom* was observed against *E. coli,* while saponin had less effect on the *S .aureus* and *P.mirabilis* (MIC=200 mg/ml). *P. aeruginosa* was resistant to the saponin of this plant. Saponin of *Q. saponaria* revealed the highest MIC (200 mg/ml) against all of microorganisms that were tested. On the other hand, this saponin exhibited weak antimicrobial activity.

**Table 1: Inhibition zone diameter of the saponin of *G. glabra, A. squarrusom* and *Q. saponaria* (mm)**

| Microorganisms | *G. glabra* | *A. squarrusom* | *Q. saponaria* |
|---|---|---|---|
| *E. coli* | 40.00±0.00 | 11.00±0.17 | 10.60±0.11 |
| *S.aureus* | 35.00±0.00 | 10.00±0.00 | 10.30±0.05 |
| *P. aeruginosa* | 34.00±0.05 | 10.00±0.00 | 10.60±0.11 |
| *P.mirabilis* | 30.00±0.00 | 10.00±0.00 | 10.00±0.00 |

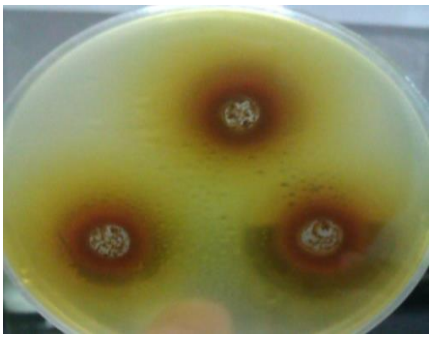

**Figure 1: Inhibition zone of the saponin of *G. glabra* against *E. coli***

ISSN: 0975-8585

**Table 2: Minimum inhibitory concentration (MIC) of the *G. glabra, A. squarrusom and Q. saponaria saponin* (mg/ml)**

| Microorganisms | *G. glabra* | *A. squarrusom* | *Q. saponaria* | Gentamicin | Ampicillin |
|---|---|---|---|---|---|
| *E. coli* | 3.12 | 25 | 200 | 0.025 | - |
| *S.aureus* | 3.12 | 200 | 200 | 0.00312 | - |
| *P. aeruginosa* | 3.12 | R | 200 | 0.025 | - |
| *P.mirabilis* | 25 | 200 | 200 | - | 0.1 |

Gram negative bacteria was reported to be more resistance to antibiotics treatment, therefore, it is important to identify effective agents that have broad spectrum of inhibition against gram negative bacteria [20].

The antimicrobial properties of saponins have been investigated by a number of researchers worldwide. Soetan *et al.* in 2006 investigated the antimicrobial activity of saponins extract of *Sorghum Bicolor.* The results showed that the saponins had inhibitory effect on gram positive organism but had no effect on gram negative bacteria [5]. Khanna *et al.* in 2008 evaluated the antimicrobial activity of saponin fractions of the leaves of *Gymnema sylvestre* and *Eclipta prostrate.* They reported the MIC of saponin fraction of *G. sylvestre* and *E. prostrate* against bacterial strains were in the range of 600-1200 mg/l and 1000-1200 mg/l, respectively. They suggested that saponin of *G. sylvestre* and *E. prostrate* can be used as a potential antibacterial agent [20]. Maatalah *et al.* in 2012 indicated that saponin extracts of *Anabasis articulata* was active against *E.coli, S. aureus, K. pneumonia*, *B. subtilis* and *P.aeruginosa* with MIC values ranging from 0.5 to 1 mg/ml [21].

Based on the results of this study we will further investigate the antibacterial activity of saponins as a source of anitibiotic *in vivo* against human pathogens. The results suggest that different way should be used for extracting of saponin to achieve the highest antibacterial activity and further studies are needed to indentify and evaluation chemical nature of saponin.

## CONCLUSION

Saponin of *G. glabra* showed great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes.

## ACKNOWLEDGEMENT

The authors are grateful to Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran for providing financial support and also gratefully thank Farabi and Daroupakhsh Pharmaceutical Co., for donating of antibiotics.

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