Antibacterial activity of Glycyrrhiza glabra roots against certain gram-positive and gram-negative bacterial strains

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Received: September 19, 2013; Revised received: November 10, 2013; Accepted: November 20, 2013

Abstract: The present study aimed to evaluate the antibacterial potency of grinded crude material (root of Glycyrrhiza glabra) against some gram-positive and gram-negative bacterial strains. Two solvents (methanol and acetone) were used to extract the phytochemicals from the test material. Four different concentrations (100%, 75%, 50% and 25%) of methanolic and acetic extract were used to investigate the inhibiting properties against Salmonella typhi, Escherichia coli, Vibrio cholerae, Staphylococcus aureus, Bacillus cereus and Bacillus subtilis strains. Among methanol and acetone extracts, later exhibited low antibacterial activity. The 100% (w/v) concentration of both extracts showed maximum inhibition against B. subtilis followed by E. coli, S. aureus, B. cereus, S. typhi and V. Cholerae. Maximum activity in acetic extract was obtained against B. cereus followed by S. typhi, E. coli, V. cholerae and S. aureus and minimum in B. subtilis. A reverse pattern of inhibition activity was found in both extracts (methanolic and acetic) against B. subtilis. Maximum activity was found in methanolic extract against B. subtilis (18.6 mm) but it was only 14.3 mm against this strain in acetic extract. The antibacterial activity of the crude samples corresponded to that of concentration. Hence there was positive correlation of antibacterial activity with the test material.

Keywords: Antibacterial activity, Glycyrrhiza glabra, Gram-positive bacteria, Gram-negative bacteria

INTRODUCTION

Today, the increasing failure of chemotherapeutic and antibiotics resistance exhibited by microorganisms have been a major problem and this leads to the screening of medicinal plants for their potential for antimicrobial activity (Laxmi et al., 2011). In general antimicrobial nature of the drug plants is due to the secondary metabolite in the form of generation of new chemical component some of them are highly effective against certain pathogenic and they can be exploited for their industrial applications. As the G. glabra has been used since ancient times as a folk medicine and still has its more significance in indigenous system of medicine. Its root contains commercially important chemical called licorice. The chemicals components responsible for antioxidant and antibacterial activity present in Glycyrrhiza glabra root have been reported such as Glycyrrhizin, Glycyrrhizinic acid etc (Tang and Eisenbrand., 1992); glabridin, glabrene, glabrol, licoflavonol, glycyrol, licoricone, formononetin, phaseollinisoflavan, hispaglabridin AandB, 3-hydroxyglabrol, 3’-methoxyglabridin (Kinoshita et al., 1976; Mitscher et al., 1978, 1980; Saitoh et al., 1978; Fukai et al., 1996, 2002a,b, 2003; Glenn et al., 2005); glabranin isomer, narinigen, lupiwightenone (Biondi et al., 2003, 2005). Therefore, it is aimed to turn our investigations to natural products from G. glabra roots for antibacterial potential so as to develop new drug molecule. The bacterial strains used in the present study are such as B. subtilis, B. cereus, E. coli, S. typhi, S. aureus, V. cholerae. B. subtilis is a gram-positive and rod shaped bacterium, which has the ability to form a tough, protective endospore, allowing the organisms to tolerate extreme environmental conditions. B. cereus is an endemic, soil dwelling, gram-positive, rod shaped beta haemolytic bacterium. It is the cause of “Fried Rice Syndrome” as the bacteria is classically contracted from fried rice dishes that have been kept at room temperature for hours (Glenn et al., 2005). E. coli is a gram-negative, rod shaped bacterium found in lower intestine of warm blooded organisms (endotherms). Virulent strains of E. coli can cause gastroenteritis, urinary tract infections and neonatal meningitis. S. aureus is a facultative anaerobic gram-positive coccoc bacterium also known as “golden staph”. S. aureus is frequently found in human respiratory tract and on skin. It is the common cause of skin infections (e.g. boils) and food poisoning. V. cholerae is gram-negative comma shaped bacterium. V. cholerae secretes cholera toxin, a protein that cause profuse,
water diarrhoea during the course of infection. Sweetness of *G.* glabra root is mainly due to presence of glycyrrhizin component. Glycyrrhiza *glabra* L. has been used since long in indigenous medicine system either alone or with the combination of other drug plant materials. The antimicrobial activity of *G.* glabra against *Mycobacterium tuberculosis* has been established (Gupta et al., 2008).

Licorice extracts have been used for more than 60 years in Japan to treat chronic hepatitis and also have therapeutic benefits against other viruses, including human immunodeficiency virus (HIV), cytomegalovirus (CMV) and Herpes simplex. Fukai et al. (2002a, b) reported the Anti-Helicobacter pylori and antibacterial activities of flavonoids from licorice extracts. The present study was undertaken to evaluate the antibacterial activity of *G.* glabra against gram-positive and gram-negative bacterial strains.

**MATERIALS AND METHODS**

**Plant material:** The roots of *G.* glabra were procured locally from Hansa Pharmacy, located in Prem Nagar Ashram, Haridwar. The roots were washed with sterilized distilled water, shed hot air dried and then grinded into coarse powder under sterilized condition. The grinded powder was packed in sterilized poly bags than kept at room temperature in research laboratory.

**Test organisms/bacterial strains:** Pure cultures of *B. subtilis* (MTCC 6728), *S. typhi* (MTCC 3216), *S. aureus* (MTCC 7443), *V. cholerae* (MTCC 9304), *B. cereus* (MTCC 441) were obtained from microbial type culture collection centre of institute of microbial technology (IMTECH) Chandigarh, India and *E. coli* from SGPGI Lucknow. All the organisms were subcultured on nutrient agar medium (NAM), *V. cholerae* was subcultured on Luria Bertani Agar (LBA) and incubated at 37±1°C for 24 hours and preserved at low temperature.

**Preparation of root extract:** The solvents used for the extraction procedure were acetone and methanol of analytical grades. These two solvents already have been used by Meena et al. (2002). Further ethyl alcohol and other solvents also have been used by Meena et al., 2010. After weighing 125gm of grinded root powder it was extracted using 500ml of the individual solvent (25% w/v) for 24 hrs in soxhlet extractor and was then subjected to filtration through filter paper (Whatman No.1). The solvent was allowed to evaporate under controlled temperature to get the volume of 125ml. The final extract was so further dried to obtain 1.0 ml of extract solution represented 1.0gm of powdered material. The obtained extract was 100% concentrated and was used as the stock solution which was further diluted to prepare 75%, 50% and 25% concentration of the extract. Methanol and acetone (100%) were used as control.

**Antibacterial activity:** The antibacterial activity of *G.* glabra was tested by Well-agar diffusion method. About 100µl of standardized microbial stock suspension (1x10^5 cfu/ml) of 24 hrs old cultures of test organism was thoroughly mixed with melted nutrient agar medium (NAM) and poured into sterile petriplates. Five wells of 6mm diameter were made in agar medium using sterile borer and filled with 100µl of each of the extract concentration. All the solvents served as negative control. Zones of inhibition obtained around well was measured after an incubation period of 24 hours at 37°C was used as positive control.

**Phytochemical analysis** by high performance liquid chromatography (HPLC): HPLC has ability to separate and identify the compounds present in any specific sample that can be dissolved in a liquid in trace concentrations as low as parts per trillion. Due to this versatility this is being used in pharmaceutical industry. Therefore, in the present investigation, phytochemical analysis of *G.* glabra of methanolic and acetonic extract was investigated by HPLC. It is the advanced form of Column chromatography that pumps sample mixture/analytes in a solvent at high pressure through column with chromatographic packing material. The column of HPLC used was made up of stainless steel.

**RESULTS AND DISCUSSION**

In the present study, maximum effective inhibition in methanolic extract at 100% was found against *B. subtilis* (18.6 mm) followed by *E. coli* (18.3 mm), *S. aureus*, *B. cereus* (17.6 mm) and *S. typhi* (16.3 mm), whereby the minimum inhibition zone was recorded against *V. cholerae*. Acetonic extract did not show same trend of antimicrobial activity of bacterial strains as were found in methanolic extract. Maximum effective inhibition in 100% acetic drug extract was recorded against *B. cereus* (16.3 mm) followed by *S. typhi* (16.0 mm), in *E. coli* (15.3 mm), in *V. cholerae* and *S. aureus* (15.0 mm), whereas the minimum inhibition zone was (14.3) recorded against *B. subtilis*. In general declined effective pattern of inhibition zone with dilution was recorded in all dilutions of both extract (Table 1). Makky et al. (2012) studied the phytochemicals and their role in antimicrobial activity of six drug plants including *G.* glabra against antibiotic resistance bacterial strain (ARB) isolated from pharmaceutical product and from hospital. The methanolic extract of *G.* glabra showed antibacterial activity against both gram-positive and gram-negative antibiotic resistant bacterial strains (ARB) isolated from pharmaceutical product i.e. *Alcaligenes xylosoxidans* (UNO99) and *Staphylococcus xylosus* (ASP13D). Gupta
Fig. 1. Methanolic extract of G. glabra. A-Chlorogenic acid; B-Caffeic acid; C-Rutin; D-Mycricitin; E-Quercetin; F-Kaempferol; STD-standard.

Fig. 2. Acetonic extract of G. glabra. A-Chlorogenic acid; B-Caffeic acid; C-Rutin; D-Mycricitin; E-Quercetin; F-Kaempferol; STD-standard.
et al. (2008) have also found the antibacterial activity of G. glabra against Mycobacterium tuberculosis. Trend of variant behaviour of different crude extract against all test bacterial strains may be due to the presence or absence as well as quantitative variations of chemicals responsible for bioefficacy. Drug efficacy loss in term of reduction of inhibition zone in mm in last dilution i.e 25% drug concentration in comparison of 100% concentration in methanolic extract was recorded i.e 4.0 mm against S. typhi, 7.0 mm against E. coli, 7.0 mm against V. cholerae, 5.6 mm against S. aureus, 5.0 mm against B. cereus and 6.0 mm against B. subtilis. In acetonic extract total decreased value is 5.7 mm against S. typhi, 5.3 mm against E. coli, 8.7 mm against V. cholerae, 5.7 mm against S. aureus, 5.3 mm against B. cereus and 4.7 mm against B. subtilis was recorded. Total loss of drug potency at 25% concentration in comparison of 100% of each specific crude drug extract against specific bacterial strains may be either due to maximum dilution or biologically active compounds which were present in very small traces and were reduced surely responsible for the much loss of the bioefficacy. But the potency loss ratio was quiet different against test strains among different crude extract. A definite ratio of reduction of extract potency in term of reduction of inhibition zone at last concentration could not be observed. In methanolic extracts, maximum loss

Table 1. Antibacterial activity of methanolic and acetonic extract of G. glabra (Mean±SD of three replicates).

| Test organisms       | Extract concentration (%) | Effective inhibition zone (mm) |
|----------------------|---------------------------|-------------------------------|
|                      | Methanol                  | Acetone                       |
| Control (methanol/acetone) | Absolute (100%)          | --                            |
| S. typhi             | 100                       | 16.3±0.57                     |
|                      | 75                        | 14.3±0.57                     |
|                      | 50                        | 13.0±1                        |
|                      | 25                        | 12.3±0.57                     |
| E. coli              | 100                       | 18.3±1.15                     |
|                      | 75                        | 15.0±0                        |
|                      | 50                        | 13.6±0.57                     |
|                      | 25                        | 11.3±0.57                     |
| V. cholerae          | 100                       | 15.3±1.15                     |
|                      | 75                        | 12.3±0.57                     |
|                      | 50                        | 10.6±1.15                     |
|                      | 25                        | 8.3±0.57                      |
| S. aureus            | 100                       | 17.6±1.15                     |
|                      | 75                        | 15.3±0.57                     |
|                      | 50                        | 13.6±0.57                     |
|                      | 25                        | 12.0±0                        |
| B. cereus            | 100                       | 17.6±1.52                     |
|                      | 75                        | 14.3±0.57                     |
|                      | 50                        | 13.6±0.57                     |
|                      | 25                        | 12.6±0.57                     |
| B. subtilis          | 100                       | 18.6±0.57                     |
|                      | 75                        | 16.3±0.57                     |
|                      | 50                        | 14.0±0                        |
|                      | 25                        | 12.6±0.57                     |

Well size in each case: 6 mm; ** Well size in each case: 6 mm; significantly different at 1% level of ANOVA, (—): No activity.

Table 2. Phytochemical analysis of G. glabra.

| Extract type | Quantification on percent dry weight basis of different compounds |
|--------------|---------------------------------------------------------------|
|              | Chlorogenic acid | Caffeic acid | Rutin | Myricitin | Quercetin | K aemperol |
| Methanol     | 0.014008          | 0.062226     | 0.05312 | 0.016671 | 0.003467 | 0.007102 |
| Acetone      | ND                | 0.034316     | 0.087007 | ND        | 0.006814 | 0.013876 |

(ND: Not detected)
of drug potency was found in E. coli and V. cholerae (7 mm) followed by B. subtilis (6 mm) and lowest value was found against B. cereus. In acetone extract maximum reduction of drug plant was found against V. Cholerae i.e 8.7 mm followed by S. aureus 5.7 mm and minimum against B. subtilis i.e., 4.9 mm.

Results of phytochemical analysis based on HPLC revealed that both methanolic and acetone extracts showed variation in terms of absence or presence of certain compounds as well as in their quantification on percent dry weight basis of different compounds. Two compounds i.e. chlorogenic acid and myricitin were not found in acetone crude extract of G. Glabra (Figs.1, 2).

In general lowered value bioefficacy was recorded in acetone extract (Table 1) may be due to the absence of certain effective biological components as it is evident by Table 2 in which chlorogenic acid and myricitin were absent and possibility cannot be ignored about involvement of these two in the bioefficacy. Chlorogenic acid compound also possesses inhibitory effect on tumor formation in mice showed anti cancerous property (Mou-Tuan Huang et al., 1988). Fukai et al. (2002 a) reported certain flavonoids for licorice such as glabridin, glabrene, licochalcone A, licoisoflavone B which showed antibacterial activity against drug resistant H. pylori. Fukai et al. (2002 b) further reported antibacterial activity of flavonoid against methicillin resistant strain of S. aureus.

Caffeic acid outperformed the other antioxidants reducing aflatoxin production by more than 95%. These studies are the first to show that the oxidative stress that would otherwise trigger or enhance Aspergillus flavus aflatoxin production can be stymied by caffeic acid. This opens the door to using natural fungicide methods by supplementing trees with antioxidants.

Chlorogenic acid is reported to be a chemical sensitizer responsible for human respiratory allergy to certain types of plant material (Freedman et al., 1964). Quercitin seems to exert antibacterial activity against almost all the strains of bacteria known to cause respiratory, gastrointestinal, skin and urinary disorders (Rigano et al., 2007). Quercitin appeared active against different viruses (Kaul et al., 1985) including HIV (Mahmood et al., 1996), probably due to inhibition of reverse transcriptase (Nakane and Ono 1990).

**Conclusion**

It was concluded that both methanolic (acetonic) extracts of G. glabra had potential in vitro antibacterial activity against all the studied gram-positive and gram-negative bacterial strains. However, the study needs evaluation of various compounds (chlorogenic acid, caffeic acid, quercitin, myricitin, kaempferol, rutin) detected in the ethanolic and methanolic extracts for the antibacterial activity. As the extracts obtained are of plant origin, therefore it would be safer than modern medicines in terms of side-effects for their use in antibacterial activity of various microbes.

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