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# Antioxidant and antibacterial activity of root extracts of Licorice (*Glycyrrhiza glabra*)

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**ABSTRACT**

*This study has been initiated to reduce burden of Extended-spectrum beta-lactamase (ESBL)-producing Gram negative pathogens using Licorice (Glycyrrhiza glabra) root extracts as natural source of therapeutics molecules. In present investigation, phytochemical analysis of aqueous and methanol extract of Glycyrrhiza glabra was compared as an antioxidant and as antibacterial against ESBL gene containing Escherichia coli. Ascorbic acid for antioxidant activity and cefotaxime for antibacterial potential was taken as standard. The 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and ferric reducing antioxidant power (FRAP) assays indicated that aqueous and methanol extracts have antioxidant activity comparable to ascorbic acid. Methanol extract shows better antibacterial activity than aqueous extract against E. coli. Both extracts do not have significant antibacterial activity when used alone but can be able to enhance efficacy of cefotaxime on synergistic application. Its high antioxidant potential may help immunity to eradicate these infections.*

***Keywords*** **:** ESBL; *glabra;* antioxidant; phytochemical; antibacterial; root extract

## INTRODUCTION

Gram negative pathogens which can produce Extended-spectrum beta-lactamase (ESBL) have been developed into serious distress over the last few decades. It is considered that ESBLs will definitely be able to create significant therapeutic problems in the future. Literature showed that Extended-spectrum beta lactamases can hydrolyze broader range of beta lactam antibiotics that contain an oxyimino-group such as oxyimino-cephalosporins (e.g., ceftazidime, ceftriaxone, cefotaxime) as well as oxyimino-monobactam (aztreonam) (Gupta, 2007; Livermore *et al.,* 2007) than the simple parent â-lactamases from which they are derived. It is third generation cephalosporins that causes mutations in TEM-1, TEM-2 and SHV-I enzymes that leads to generate ESBLs (Nathisuwan *et al.,* 2001; Ayyagari and Bhargava, 2001). Thus, developing ESBLs is leading cause of resistance to cephalosporins.

In last four decades a variable and rising incidence and prevalence of ESBLs has been reported ESBL-producing *Klebsiella spp*. and *E. coli* worldwide i.e. Germany (Knothe *et al.,* 1983), France (Sirot *et al.,* 1987; Philippon *et al.,* 1989), England (Du Bois *et al.,* 1995) USA (Saurina *et al.,* 2000; Mathai *et al.,* 2001; Winokur *et al.,* 2001), Canada (Cordero *et al.,* 2004), Spain (Romero *et al.*, 2007), Taiwan (Kuo *et al.*, 2007), Turkey (Hoþoðlu *et al.*, 2007), Algeria (Messai *et al.,* 2008), China (Xiong *et al.*, 2002) etc. Researchers have recorded presence of ESBL positive *E coli* from Hospital intensive care units (Shakya *et al*., 2017; Singh *et al*., 2016), farm livestock (Dahms *et al.,* 2015) and from house hold latrines' door handle (Erb *et al.,* 2018). It is recommended that the ESBL infected patients should be avoided to visit public settings to prevent spread among other.

Antibiotic resistance of bacterial pathogens is one of the most worldwide maltreatment to public

health care. To prevent selection and dissemination of resistance, the use of traditional antibiotics must be limited and alternative effective therapies must be sought (Wood *et al.,* 1996). To overcome this problem about 80% world populations depends on Plants or agricultural products. However, due to environmental pollution there are high level toxic residues in the agricultural products which may harm the human health (Premathilake *et al*., (2018). Thus, there is a high need of study and scientific evaluation of traditional knowledge of plants based medicines or recipes/preparations.

The licorice plant (*Glycyrrhiza glabra* Family *Leguminoceae* or *Fabaceae*) has been used in folk medicine since time immemorial. Many of the claims for the effectiveness of licorice extracts have been shown by modern science to be credible, a root component (Glycyrrhizin) being generally regarded as the major biologically-active principle (Nitalikar *et al.,* 2010). Glycyrrhizin is widely used in pharmaceutical and confectionery industries (Fenwick *et al.,* 1990).

Despite ESBL production antibiotic resistant bacteria can damage mitochondria and then damaged mitochondria release large amount of free radicals. These free radicals are able to suppress the immune system. Free radical stress leads to tissue damage and may eventually lead to death of patient. Several studies are ongoing worldwide to find natural antioxidants of plant origin. *Glycyrrhiza glabra* has also been studied for same. Further, we initiated this study to evaluate specific antioxidants and antibacterial activity against gram negative pathogens especially, *Escherichia coli.*

## MATERIALS AND METHODS

### Collection of samples

The roots of *Glycyrrhiza glabra* were purchased from local market of Agra, India. The same were cross-identified by their vernacular names and later validated at the Department of Botany, School of Life Sciences, Dr. Bhim Rao Ambedkar University, Agra. Voucher specimens (accession number Bot.0001/2012/0010) were deposited for future reference in the herbarium of same department.

### Processing of samples

The withered roots (300 g) of *Glycyrrhiza glabra* (Family: Fabaceae) were washed vigorously with tap water. After that, roots were placed in shade to dry for 10-15 days. All dried material was chopped into small fragments. They were then reduced into a fine powder with a kitchen grinder. The powder could then pass through a sieve of pore size 0.5 mm. The part left in sieve was grinded again and again till we get all material in coarse powder.

### Preparation of methanol extract

Powdered samples (30 gm) were extracted at 65$^{o}$C with methanol (400 ml) for 72 h (25 cycles) using Soxhlet apparatus to make methnol extracts. The thimble was carefully filled in with keeping at least 1 cm gap between the sample and the top of the thimble. Weight of the filled thimble was 30.2 g. Finally, the extract was filtered and concentrated in oven at 40$^{o}$C ± 5°C under atmospheric pressure, to obtain semisolid paste, after drying; they were weighed in order to know the amount of extract of plant sample and percentage yield. The same procedure of extract preparation was repeated with the remaining powder for two times more.

### Preparation of aqueous extract

The aqueous extract of *G. glabra* was obtained by using a hot water extraction method. The dried powdered sample (30 gm) was mixed in 300ml distilled water at 70°C in a marked flat bottom flask. The distilled water was evaporated during this incubation. A known quantity (in volume) of distilled water was added repeatedly after evaporation up to the mark. This step was repeated till 5L of water was utilized for resulting extract. Then it was filtered using Whatman filter paper (No. 1) and concentrated in oven at 45$^{o}$C under atmospheric pressure to give a semisolid paste. The % yield of plant extract was calculated. Both extracts were stored at 4$^{o}$C till further use.

### Phytochemical analysis

The presence of phytochemicals was screened. Alkaloids, saponins, tannins (5% ferric chloride), terpenoids (2, 4-dintrophenyl hydrazine) and steroids (Liebermann-Burchard test) were estimated according to the methods described by Edeoga *et al.* (2005) and Roy *et al.,* (2011).

**Detection of Alkaloids**

Protocol was adopted from Edeoga *et al.(*2005); Roy *et al. (*2011).

**Dragendorff's test**

A few drops of Dragendorff's reagent (0.4 g of bismuth subnitrate in 10 ml HCl 12 N; mixed with 5 g of potassium iodide in 50 ml distilled water) was added to 1ml solution filtrate obtained from 0.5 g of the extract and stirred with 5 ml of 1% aqueous HCl on steam bath. Orange precipitation indicated the presence of alkaloids.

**Wagner's test**

A fraction of the extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml distilled water). The formation of reddish brown coloured precipitate indicates presence of alkaloid.

**Detection of Steroids**

Protocol was adopted from Edeoga *et al.(* 2005); Roy *et al. (*2011).

**Liebermann-Burchard test**

Acetic anhydride (2 ml) was added to 0.5 g methanol extracts in 2 ml of $H_2SO_4$. The change in color from violet to blue or green indicated the presence of steroids.

**Detection of Terpenoids**

Protocol was adopted from Edeoga *et al.(*2005); Roy *et al.(*2011).

**Salkowski test**

5 ml of extract were mixed in 2 ml of chloroform and layered over 3 ml of concentrated $H_2SO_4$. A reddish-brown color of the interface demonstrated the presence of terpenoids.

**Detection of Tannins**

Protocol was adopted from Edeoga *et al. (*2005); Roy *et al. (*2011).

About 0.5 g of the dried powdered sample was boiled in 20 ml of water and then filtered. A few drops of 0.1% ferric chloride was added to the filtrate and observed for brownish green or a blue-black colouration which suggested the presence of tannins.

**Detection of Saponins**

Protocol was adopted from Edeoga *et al.(* 2005); Roy *et al. (*2011).

**Frothing test**

The frothing test was used to check the presence of saponins. 2 gm of the extract was mixed in 20 ml of distilled water, boiled in a water bath, and filtered. 10 ml of the filtrate was taken aside, and an additional 5 ml of distilled water added and shaken vigorously to generate a stable, persistent froth. Froth formation indicated the presence of saponins.

**Detection of Flavonoid**

Protocol was adopted from Edeoga *et al.(* 2005); Roy *et al. (*2011).

**Alkaline reagent test**

Crude extract was mixed with 2 ml of 2% NaOH solution. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid indicates presence of flavonoids.

**Detection of Carbohydrates**

Protocol was adopted from Edeoga *et al.(* 2005); Roy *et al. (*2011).

**Molish Test**

In 2 ml of extract solution 3 drops of á-napthol solution (0.5 gm á-napthol in 100ml ethanol) was added, then 2 ml of conc $H_2SO_4$ poured along the side of the test-tube. Reddish purple ring at the interface indicates carbohydrates.

**Detection of Protein**

Protocol was adopted from Edeoga *et al.(* 2005); Roy *et al. (*2011).

**Ninhydrin Test**

Crude extract was boiled with 2 ml of 0.2% Ninhydrin solution, development of violet color indicated the presence of proteins.

**HPLC analysis**

The methanol & aqueous extracts were prepared for High Performance Liquid Chromatography (HPLC) by dissolving completely dried samples in HPLC grade methanol at 10 mg/ml

concentration. All samples were filtered through 0.45µm (Millipore, Bedford, MA) filter. Standard (1.2 mg/5ml), was prepared in HPLC grade methanol. Analysis was performed with waters HPLC system (Waters, Miliford, MA ), equipped with 515 binary gradient pumps, 717 plus injector, 2996 PDA detector and Empower software (version 3.0). Extracts were separated on RP-18 column (4.0 X 250mm, 5µm, merck). The mobile phase consisted of acetonitrile – 2% acetic acid (40:60) delivered at a flow rate of 0.8 ml/min. The column temperature was maintained at 30° C. The UV chromatograms were recorded at 254 nm. 10 µL of the methanol & aqueous extract was injected.

**Antioxidant assays**

In this phase, after testing the phytochemicals, in order to check the other medicinal value of *G. glabra*, the antioxidant property of the candidate plant was analysed. Based on the results of phase I, the root of *G. glabra* was taken for further study and the antioxidants were analyzed in them. The methodology adopted for analyzing these parameters is given below.

**1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay**

The effect of aqueous extract on DPPH radicals was estimated according to the method of Molyneux (2004) and Roy *et. al.*(2011), with minor modifications. The aqueous extract and methanol was lyophilised and dilutions from 1.4 mg/ml to 3.0 mg/ml prepared in ethanol and methanol respectively. Again this solution was diluted to make working solution by adding 100 µl of extract with 1.9ml ethanol or Methanol respectively. Then, One millilitre (300 mM) of DPPH solution was mixed with 1.0 ml of working extract solution. The reaction mixture was vortex-mixed thoroughly and incubated at room temperature in the dark for 20 min. Reduction in the absorbance of the mixture was measured at 517 nm using ascorbic acid as a control. Scavenging of DPPH radicals by the extract was calculated. The half maximal inhibitory concentration ($IC_{50}$) values denoted the concentration of sample required to scavenge 50% of DPPH free radicals.

Reduction in the absorbance of the mixture was measured as mentioned above for aqueous extract using ascorbic acid as a control. To cover a wide range of concentration doubling dilution of aqueous ascorbic acid solution (10mg/ml) was prepared.

The reactions were repeated three times for each dilution, and then take the mean value for % of inhibition DPPH scavenging activity.

**Ferric reducing antioxidant power (FRAP) assay**

Method of Ferreira *et al.* (2007) and Roy *et al.* (2011) was adopted with modification to estimate reducing power in root. Briefly, crude extract was mixed with phosphate buffer (0.2 M, pH 6.6) to make working solution of different dilutions (1.4mg/ml to 3.0 mg/ml). Then working solutions (2.5 ml) was added with 1% potassium ferricyanide (2.5 ml). After 30 minute incubation at 50°C, 10% trichloroacetic acid (2.5 ml) was added and the mixture was centrifuged at 2000 × g for 10 min. Then, supernatant (1.5 ml) was mixed with distilled water (1.5 ml) and a freshly prepared 0.1% $FeCl_3$ solution (0.3 ml). After 10 minute incubation, absorbance were read at 700 nm using a PC based UV/visible light spectrophotometer, 2201 (Systronics Inc., India). The iron (III) reducing activity determination was performed in triplicate.

**Evaluation of antibacterial activity of plant root extract**

ESBL positivity of *E coli* was defined based on interpretation of results of Kirby-Bauer disc diffusion (1966) and recommendation by Clinical and Laboratory Standard Institute (CLSI) guidelines, 2012. The disc diffusion method adopted by Kirby-Bauer was applied to test the antibacterial activity of plant root extracts. TEM SHV and CTXM positive strain of *Escherichia coli* was grown on different culture media to performing antibacterial assay and the maintenance of strains. Nutrient broth (NB), Nutrient agar media (NA) and Muller Hinton agar media (MHA) were used in the study. Strains were activated by inoculating a loopful culture in the nutrient broth (30 ml) incubated for 4 hours to maintain McFarland standard turbidity ($10^8$cells/ml). Then 0.1ml of inoculums was inoculated on MHA & NA and spread uniformly using sterile cotton swab. Simultaneously, various dilution (20mg/ml, 10mg/ml, 5mg/ml and 2.5 mg/ml) of root extracts were

obtained using doubling dilution of 20mg root extracts rehydrated in distilled water. Of these 40 µl was introduced on the filter paper disc (6mm) and allowed to dry. Thus, disc had 0.8 mg, 0.4 mg, 0.2 mg and 0.1mg dose of extract. The dried impregnated discs were placed on both media. Dimethyl sulfoxide (DMSO) was used as a negative control where as cefotaxime (Taxime, 1gm, Manufactured by Alkem India pvt Ltd, reconstituted in 5ml distilled water to make a stock of 200mg/ml) was used as a positive control. Plates were incubated at $37^0$C for 24 hours. Antimicrobial activity was expressed as the mean diameter of zone of inhibition (mm) around the disc as measured with vernier calliper. For positive control antibiotic disc cefotaxime was placed on similarly prepared plates with the same culture. The assay was done in triplicates and plates were incubated for 24 hours at 37°C for antibacterial activity. The mean of three readings of diameter of zone of inhibition were presented. The *E coli* strain was also positive for TEM, SHV and CTX-M genes as per results of specific PCR.

**MIC/MBC Estimation**

Macro Broth dilution method (Clinical and Laboratory Standard Institute (CLSI) guidelines, 2012) was used to determine the MIC/MBC of both extracts. Serially diluted extracts (3.125, 6.25, 12.5, 25 & 50 mg/ml) was impregnated in 5 test tubes separately. Thereafter, 200 µl of fresh broth culture was inoculated in each and left to grown at $37^o$C for 24-36 hrs. The Minimum Inhibitory Concentration (MIC) value was noted from the tube with no visible growth but lowest concentration of extract. All the test tube without visible growth was then streaked over Nutrient agar plate. Test tube concentration belong to a non visible growth on Nutrient agar plate after incubation for 24-36 hrs at $37^o$C was considered to be having Minimum Bactericidal Concentration (MBC).

**Statistical analysis**

All data were expressed as mean ± standard deviation of three independent replicates. The results were statistically analyzed by analysis of variance (ANOVA) and significant differences among means from triplicate analyses at ($P < 0.05$) were determined by Bonferroni multiple comparisons using the GraphPad Prism (ver 5.0).

## RESULTS

**Phytochemical screening**

The yield obtained from aqueous extract (71.42%) was higher than methanol extract (65.09%) of *G. glabra*. Phytochmical analysis of both extracts of *G. glabra* showed presence of terpenoids, sugars, saponins, tannins, and flavonoids (Table1).

These peaks were at different retention time (rt). The contents of the *G. glabra* were investigated using standard curve for validated HPLC method. The analytical results showed that the amount of glycirhizin (rt 5.9 min) in the methanol and aqueous extract was 0.1991 mg/ml and 0.1676 mg/ml, respectively.

On comparing the chromatograms, it was observed that few peaks were exclusive to aqueous extract and few were exclusive to methanol extract.

**Identification and quantification of *glycirhizin* in both extracts using HPLC**

The HPLC chromatogram of the methanol extract (Figure 1) and aqueous extract (Figure 2) has shown 10 and 9 well defined chromatographic peaks, respectively.

**Screening of antioxidant activity**

**DPPH assay (Free Radical scavenging activity)**

The dose-response curves of DPPH radical scavenging activity of the methanol & aqueous extract of roots of *G. glabra* were compared with those of ascorbic acid (Figure 3 & 4). The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecules and radical progress which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in colour from purple to yellow. *Glycyrrhiza glabra* exhibited a comparable anti oxidant activity with that of standard ascorbic acid at varying concentration tested. There was a dose dependant increase in the percentage antioxidant. The scavenging activity of control (ascorbic acid) was comparable to methanol and aqueous extracts. The obtained $IC_{50}$ values of methanol and aqueous extract of roots were 1.372 ± 0.36 mg/ml and 1.338 ± 0.34 mg/ml, respectively and for ascorbic acid 1.482 ± 0.11 mg/ml.

**Table 1: The presences of Phytochemicals in root of *Glycyrrhiza glabra***

| Phytochemicals | Methanol extract | Aqueous extract |
|---|---|---|
| Terpenoids | + | + |
| Tannins | + | + |
| Alkaloids-<br>Dragendorff's reagent<br>Wagner's reagent | — | — |
| Flavonoids | + | + |
| Saponins | + | + |
| Carbohydrates | + | + |
| Proteins | - | - |

Note- (+) Present, (-) Absent

**Table 2a: Evaluation of antibacterial activity of aqueous extract and methanol extract of *Glycyrrhiza glabra* against *E coli* strain**

| S.No | Extract | 20 mg/ml | 10 mg/ml | 5 mg/ml | 2.5 mg/ml |
|---|---|---|---|---|---|
| 1 | *Aqueous Extract* | 15 mm | 14.5 mm | 13 mm | 10 mm |
| 2 | *Methanol extract* | 20 mm | 12.9 mm | 10.6 mm | 7.15 mm |

**Table 2b: Evaluation of cefotaxime susceptibility against *E coli* strain as per CLSI 2012 recommendation**

| S.No | *Medicine* | 40 µg/ml | 30 µg/ml | 20 µg/ml | 10 µg/ml |
|---|---|---|---|---|---|
| 1 | *Cefotaxime* | 19.75 mm | 16.25 mm | 17.00 mm | 16.25 mm |

**Note:** Zone of inhibition e"22 mm for 30µg/ml dose of cefotaxime is suggestive of resistance strain (CLSI 2012 Guidelines).

**Table 3: MIC and MBC of methanol and aqueous root extract of *Glycyrrhiza glabra***

| | | ***E. coli*** |
|---|---|---|
| **methanol extract** | MIC (mg/ml) | 12.5 |
| | MBC (mg/ml) | 12.5 |
| **aqueous extract** | MIC (mg/ml) | 6.25 |
| | MBC (mg/ml) | 6.25 |

MIC & MBC of cefotaxime against *E. coli* were 4ì g/ml and 13ì g/ml, respectively.

**Note:** MIC>2ìg/ml for cefotaxime against *E. coli* is suggestive of ESBL production.

**Table 4: Evaluation of synergistic and alone antibacterial activity of glycirhhizin and cefotaxime against *E coli* strain**

| S. No. | Concentration(µg/ml) | Zone of Inhibition(mm) | | |
|---|---|---|---|---|
| | Glycirhhizin | Cefotaxime | Glycirhhizin + Cefotaxime | |
| 1 | 10 | 8.2 | 16 | 16.2 |
| 2 | 20 | 12.2 | 17 | 16.8 |
| 3 | 30 | 14.5 | 16.3 | 17.4 |
| 4 | 40 | 14.5 | 18.6 | 18.2 |

**FRAP (Ferric Reducing Antioxidant Power) assay**

In vitro ferric reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide ($Fe^{3+}$) to form potassium ferrocyanide ($Fe^{2+}$), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. FRAP activity of the methanol & aqueous extracts and standards increases with the increase in amount of standard and sample concentrations (Fig 5).

**Screening and evaluation of antibacterial activity**

The antibacterial properties of the *Glycyrrhiza glabra* roots were checked by Disc diffusion method. Methanol and aqueous extracts were significantly active against *E. coli* (Table 2a) as they showed Zone of inhibition (in mm) in comparison to control. Cefotaxime was used as positive control (Table 2b). In the present study the MIC/MBC values were recorded same (Table 3).

The major compound Glycirhhizin of *G. glabra* was used to check synergistic antibacterial activity with cefotaxime (Table 4). The half dose of cefotaxime and glycirhhizin was applied in synergistic experiments of disc diffusion.

**DISCUSSION**

The major side effect and also the resistance to conventional medicines has increases the interests of using the natural plant extracts as antioxidant and antimicrobial source against oxidation and microbial growth to protect human health. These reasons as well as the emergence of novel microbial infections and oxidation damages are behind the recent rise in work to isolate an antioxidant & antimicrobial drugs from plants (Marchese and Shito, 2001) or other natural sources. Indigenous evidences indicated a well-known role of the plants in providing better health and nutritional security at an inexpensive price to human beings and animals due to their antioxidants and other nutrients (Momin *et al.,* 2018). In present study we addressed the antioxidants and antibacterial effects of *G. glabra* root purchased from the local market. The root of *Glycyrrhiza glabra* was selected on the basis of literature about their traditional use in folk lore medicine in India and other countries.

The day by day appearance of multi-drug resistance in microbes due to ESBLs has decreased the choice of safe drugs. Recently, to suggest novel therapeutic molecule researchers explored phytochemicals. Phytochemicals are ubiquitously present in plants, and when plants are consumed as foods or medicine (decoction, tonic, syrup, etc), these phytochemicals contribute to the intake of natural antioxidants in humans as well as animals. *G. glabra* extracts have antioxidant activity that have been proved by FRAP and DPPH assays. Ascorbic acid, a well known reference of antioxidant capacity was used to determine the plant root extracts antioxidant power. The aqueous and methanol both extracts have higher antioxidant capability that is comparable to ascorbic acid. Both extracts have shown significant FRAP & DPPH activity ($P<0.05$). However mehanolic extract of *Glycyrrhiza glabra* showed better antioxidant activity than aqueous extract.

Evidences suggest that phytochemical compounds (primary and secondary antioxidants) with reducing power are electron donor and can

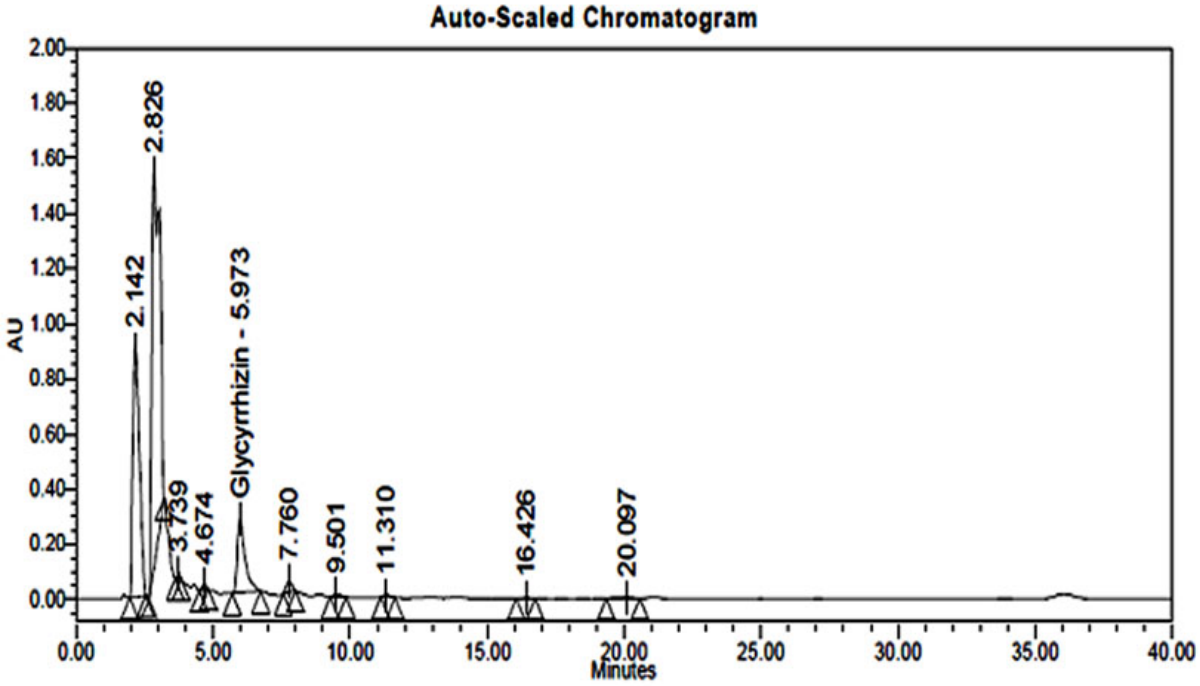


**Figure 1: High performance liquid chromatographic pattern of *G glabra* Methanol extract.**

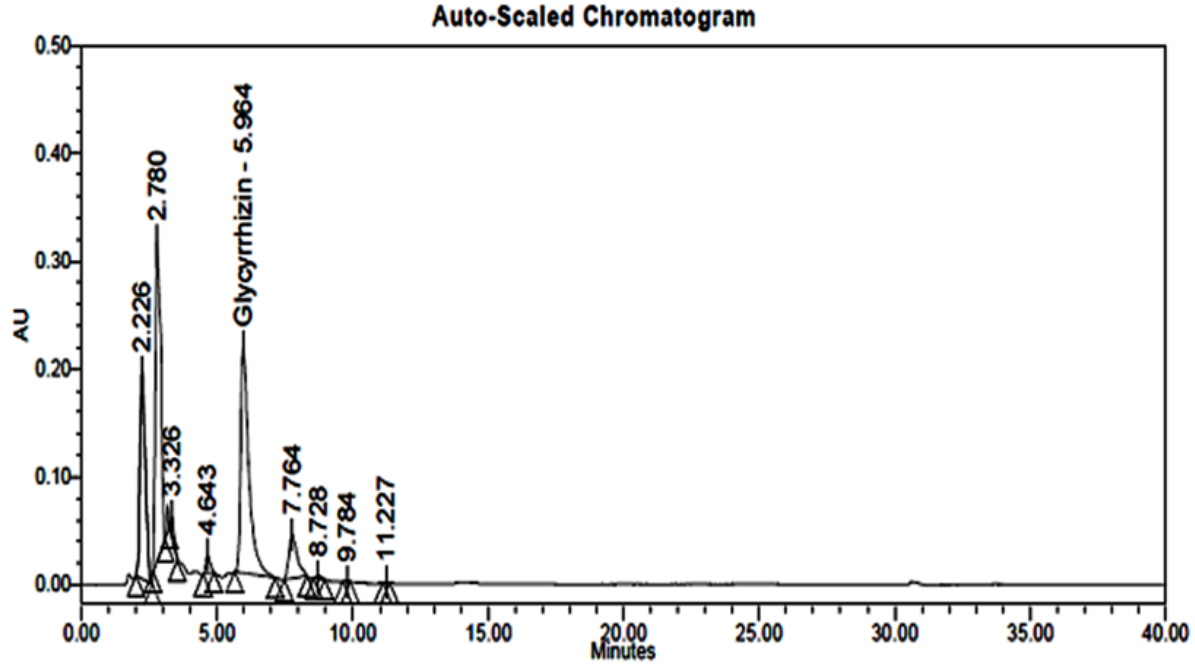


**Figure 2: High performance liquid chromatographic pattern of *G glabra* aqueous extract.**

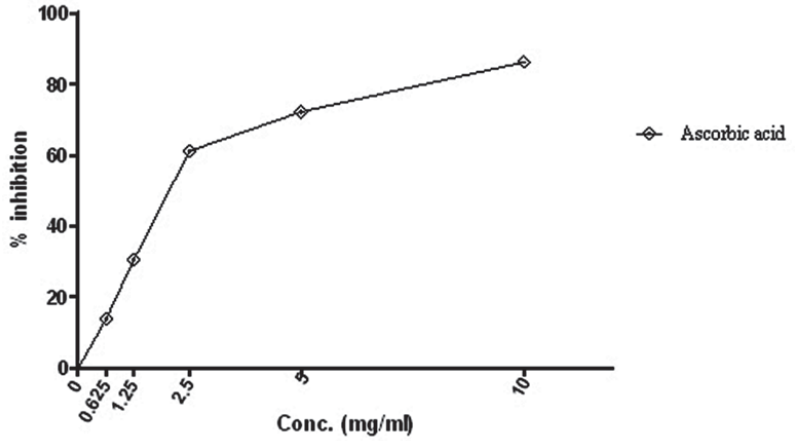


**Figure 3: Percent inhibition of standard (Ascorbic acid) at various concentrations (mg/ml) in DPPH assay.**

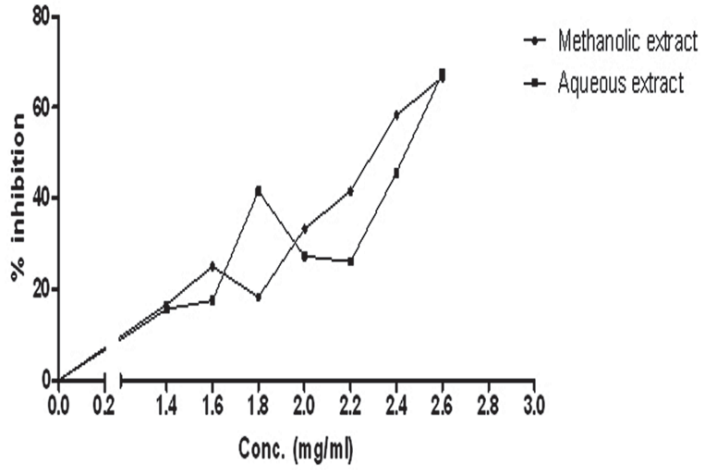


**Figure 4: Percent inhibition of aqueous and methanol extract of *G. glabra* at various concentrations (mg/ml) in DPPH assay**

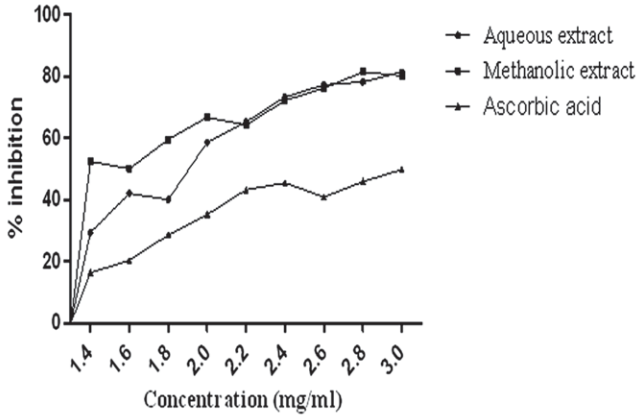


**Figure 5: The percent inhibition of methanol and aqueous extract of *G. glabra* and also of ascorbic acid at various concentrations (mg/ml) in ferric reducing power assay**

reduce free radicals (Chanda and Dave, 2009). In biological systems, phenolic compounds are major group of secondary metabolite that could be an important part of plant defense with supplying an antioxidant activity (Wuyts *et al.,* 2006) to plant. *Glycyrrhiza glabra* have various group of phytochemical compounds viz terpenoids, tannins, flavonoids, and saponins (Table 1). Free radical scavenging property may be one of the mechanism by which this plant is effective as a traditional medicine. It is well documented previously that most of tannins, terpinoid, & saponins are phenolic compounds and responsible for antioxidant properties of many plants. So this activity may be due to the presence of phenolic compounds tannins, terpenoids, & saponins in these extracts. These results are also in conformity with the findings of several workers viz. Roy *et al.* (2011).

An attempt has been made to identify the antibacterial activity of the methanol & aqueous extract of *Glycyrrhiza glabra* against the cefotaxime resistance *E. coli.* These initial findings show inhibitory effects against resistance *E coli* with high dose but that is also equivalent to cefotaxime. Still because of higher antioxidant potential we suggest *G glabra* could be useful in developing medicine against resistance pathogen. The antioxidant molecule improves immunity to fight against pathogens (Puertollano *et al*., 2011; Brambilla *et al*., 2008).

Furthermore, HPLC analysis indicates that 10 and 9 peaks in methanol and aqueous extracts, respectively, including glycyrrhizin. The amount of glycyrrhizin was more in methanol extract than aqueous extract. On comparing retention time, 8 peaks were seems to be common in both extract while one was present exclusively in aqueous and 2 in methanol extracts. These compounds responsible for these peaks might be responsible for variation in antimicrobial activities between two extracts. The synergistic antibacterial potential of glycyrrhizin with cefotaxime indicates that the *G glabra* could be an important supplement to enhance the antibacterial effect of conventional medicine in drug resistances cases (Table 4).

This study on *G. glabra* root determined the therapeutic potential of this plant that can be used to discover bioactive natural products that may serve as lead for development of new pharmaceuticals that the address hitherto unmet the therapeutic needs. This plant have established edible role in Ayurveda hence *in vivo* clinical testing can be perform to confirm these *in vitro* results.

## CONCLUSIONS

*Glycyrrhiza glabra* is a significant choice to inhibit the infections caused by ESBL positive Gram negative pathogens, by presence of potent novel therapeutic and antioxidant molecules. The *G glabra* extract is also useful to enhance the antibacterial activity on synergistic uses.

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