# In-vitro Antibacterial Activity of Licorice Root on Food-Borne Pathogens

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**ABSTRACT**

The antimicrobial activity of *Glycyrrhiza glabra* showed that *Klebsiella pneuminiae* ATCC 31488 and *Klebsiella aerogenes* ATCC 9621 were resistant for all the extarcts. Aqueous extract of *Glycyrrhiza glabra* did not showed any antimicrobial effect on any of the test organism as well as on bacterial cultures isolated from food samples also. *Enterococcus faecalis* ATCC 29212 showed highest sensitivity against chloroform extract of the spice. Growth of *Salmonella sp*, *Staphylococcus aureus* and *E. coli* isolated from food samples was inhibited by all the extracts except aqueous one indicating its use for inhibiting growth of food microbes.

**INTRODUCTION**

*Glycyrrhiza glabra*, also known as licorice and sweet wood, is native to the Mediterranean and certain areas of Asia. Historically, the dried rhizome and root of this plant were employed medicinally by the Egyptian, Chinese, Greek, Indian, and Roman civilizations as an expectorant and carminative. In modern medicine, licorice extracts are

often used as a flavoring agent to mask bitter taste in preparations, and as an expectorant in cough and cold preparations. A number of components have been isolated from licorice, including a water-soluble, biologically active complex that accounts for 40-50 percent of total dry material weight. This complex is composed of triterpene saponins, flavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, and various other substances (Obolentseva *et al*., 1999).

Shirazi M.H. *et al*. (2007) had studied the in vitro inhibitory effects of *G. glabra* extract against the growth of *Salmonella typhi, S. paratyphi B, Shigella sonnei, S. flexneri* and enterotoxigenic *E. coli* was investigated using well and disc diffusion method. *Salmonella paratyphi* B showed no susceptibility to liquorice with concentrations lower than 7.5%, however all tested bacterial strains exhibited susceptibility to high concentration of liquorice. The antibacterial activities of the alcohol, ethyl acetate, acetone and chloroform extracts of 5

plant species were studied by Ates and Erdoúrul (2003). The extracts of *Glycyrrhiza glabra Linn* (liquorice) (root), was tested in vitro against 13 bacterial species and strains by the agar diffusion method. The extracts of *Glycyrrhiza glabra Linn* roots

showed various antibacterial activities (7-11 mm/20 µl inhibition zone) against the microorganisms tested. The alcohol extracts did not inhibit *B. subtilis* var. *niger*, *B. brevis*, *E. faecalis*, *L. monocytogenes*, *P. aeruginosa* and *Y. enterocolitica*. The ethyl acetate extracts did not inhibit *B. subtilis* or *Y. enterocolitica*, and the acetone extracts did not inhibit *E. faecalis*, *L. monocytogenes*, *P. aeruginosa* or *Y. enterocolitica*. The chloroform extracts showed no inhibition effect against *P. aeruginosa* or *Y. enterocolitica*. Alonso J. had studies the glycyrrhizin is habitually used as a vehicle in orally administered products, where it inhibits the growth of some bacteria, as well as dental plaque formation. In regards to its antibacterial action, *in vitro* studies have demonstrated inhibitory effects for licorice aqueous and ethanolic extracts on *Staphylococcus aureus* and *Streptococcus pyogenes* cultures, the first one showing the strongest inhibition with 10-15mm halo diameters. It exhibited antimicrobial activity against both Gram-positive and Gram-negative bacteria by Gupta *et al*. (2008).

## MATERIALS AND METHODS

### Herbal Materials

The Roots *Glycyrrhiza glabra* are used as herbal material for present study. These materials were collected from local area and place of work was Department of Microbiology, School of life science, Singhania University.

### Bacterial Culture

#### *Bacterial strains collected from culture banks*

Ten bacterial strains (6 Gram neagtive and 4 Gram positive) and 2 fungal strains for the present work were collected from American type culture collection (ATCC), in the form of lyophilized culture. The cultures used were *Escherichia coli* ATCC11840, *Escherichia coli* ATCC 12632, *Pseudomonas flourescens* ATCC 13525, *Klebsiella pneumoniae* ATCC 9621, *Klebsiella aerogenes* ATCC 31488, *Proteus mirabilis* ATCC 29245, *Streptococcus lactis* ATCC 8043, *Staphylococcus aureus* ATCC 12600, *Bacillus cereus* ATCC 12826, *Enterococcus faecalis* ATCC 29212, *Aspergillus niger* ATCC 26603 and *Rhizopus oryzae* ATCC 8993.These strains

used as standard test microorganism.

***Bacterial strains isolated from food samples***

Food sample collected from University canteen and road side was used for isolation. From each sample 25 g was aseptically weighed and macerated and 225 ml of sterile distilled water was added and shake for 1hr in incubator shaker at $37^0$C. Serial dilution was carried out using sterile distilled water as diluents. From each dilution 50 µl was spreaded using the spread plate methods. Enriched sample were then streaked on nutrient agar media. Isolated colonies were subcultured on independent plates for further morphological and biochemical screening of microorganisms. The following Bacteria are isolated and identified from food samples. These strains used as isolated test microorganism.

1. *Staphylococcus aureus* 2. *Escherichia coli* 3. *Bacillus cereus*4. *Pseudomonas aeruginosa* 5. *Salmonella sp*

**Preparation of herbal plants**

All samples were washed in 50 µg/mL hypochlorite solution, sliced and air-dried at 50°C in a hot air oven. The final moisture content determined by gravimetrically method was 5-8% (dry basis). Dried samples were ground to powder using a mechanical grinder, and kept separately in air tight containers in dry condition until use.

**Preparation of extracts**

Because of the limitation of solubility of fine particles of the spices, the highest preparable concentration was 500 µg/ml.

**Hot Water Extraction**

30 g of the sample were batch extracted by dissolving it in 100 ml boiled distilled water, and shaken for 30 minutes at 100°C in a shaking water bath. Crude extracts were centrifuged at 10000×g for 30 minutes and then vacuum filtered using a 47 mm glass microfibre filter to obtain the clear extracts. The extracts were filtered again using 0.45 µm filter to obtain the sterile extracts. The hot water extracts were kept in sterile glass bottle and stored frozen at -40°C

**Solvent Extraction**

Thirty Gram of shade dried, powder of plant materials were filled separately in the

thimble and extracted successively with 150 ml of ethanol, methanol, chloroform and ethyl acetate separately using a Soxhlet extractor for 4-8 h. Extract was concentrated using vaccum rotary evaporator and frozen at -80°C before freeze drying. After complete solvent evaporation, solvent extract was weighed and preserved at $4^{o}$C in airtight bottles until further use. Stock solutions of solvent extracts were prepared by diluting the dried extracts with 10% dimethyl sulphoxide (DMSO) solution to obtain a final concentration of 500 µg/ml

## Determination of the Antimicrobial Activity

### Preparation of the Inoculums

For the preparation of the inocula, test organisms were taken from the stock culture and were grown separately in 50 ml Trypticase Soy Broth and incubated at 37°C for 24 hours on an orbital shaker at 200 rpm. Each test organism suspension was subsequently streaked out on Trypticase Soy Agar and incubated at 37°C for 24 hours. A single colony was transferred to Trypticase Soy Agar slants and incubated at 37 °C for 24 hours. These stock cultures were kept at 4°C. For use in experiments a loop of each test organism was transferred in 50 ml Trypticase Soy Broth, and incubated separately at 37°C for 18-20 hours. This culture was used for the antibacterial assays.

### Determination of Zone of Inhibition

The antimicrobial assay was performed by agar well diffusion method for extracts. The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum (1 x $10^8$ cfu/ml) and poured into the Petri plate (Hi-media). For agar well diffusion method, wells were prepared in the plates with the help of a cork-borer (0.85 cm). 25 µl, 50 µl, 75 µl and 100 µl of the test compound were introduced into separate wells along with controls. The plates were incubated overnight at $37^{0}$C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented. Media are made up to the final concentration is used in the usual methods of microbiological assay, but with addition of 1.5 percent of agar. During preparation of the plates strict asepsis is unnecessary; normal cleanliness and the use of oven dried apparatus have so far avoided all trouble from contaminating organisms and it has also been found unnecessary

to sterilize either test or standard solution.

**Preservative agent**

The standard preservative used in the present study was benzoic acid (1%) 20 µl quantity.

## RESULT AND DISCUSSION

**Antimicrobial activity of *Glycyrrhiza glabra***

This study was performed to observe the antimicrobial potential of *Glycyrrhiza glabra* against standard test microorganisms (Fig 4.18) and food borne microorganisms (Fig 4.19). Antimicrobial activity was studied in terms of Zone of Inhibition, Minimum Inhibitory Concentration and Minimum Lethal Concentration.

**Determination of Zone of Inhibition of *Glycyrrhiza glabra***

Antimicrobial activity of *Glycyrrhiza glabra* was expressed by Zone of Inhibition against the test microorganisms and the food isolated microorganisms. Table 1 and Table 2 shows Zone of inhibition of different extracts of *Glycyrrhiza glabra* against all microorganisms used. The extract was dissolved in sterilized 10% DMSO at concentration of 400 µg/ml. Extracts were used in different concentrations of 25 µl, 50 µl,
75 µl and 100 µl against all the microorganisms.

***Antimicrobial activity of aqueous extract of Glycyrrhiza glabra***

The aqueous extract of *Glycyrrhiza glabra* showed no antimicrobial activity against any of the test organisms used. Even in the case of isolates from food samples this extract showed no antimicrobial activity (Table 1 and Table 2).

***Antimicrobial activity of methanolic extract of Glycyrrhiza glabra***

The findings shows maximum zone of inhibition of methanolic extract of *Glycyrrhiza glabra* against *S. aureus* ATCC12600 and *E. coli* ATCC 11840 (21 mm and 20 mm respectively). Both the strains of *Klebsiella* were found to be resistant against methanolic extract of *Glycyrrhiza glabra*. The extract also showed antifungal activity against both *Aspergillus* and *Candida* (17 mm and 18 mm) (Table 1).

Good antimicrobial potential of methanolic extract of *Glycyrrhiza glabra* was observed against all the isolated microbes. Maximum zone of inhibition was 25 mm against *S. aureus* and minimum zone of inhibition was 17 mm against *E. coli* (Table 2).

***Antimicrobial activity of ethanolic extract of Glycyrrhiza glabra***

Ethanolic extract of *Glycyrrhiza glabra* showed less antimicrobial activity against test microorganisms. Maximum zone of inhibition observed was 23 mm against *Streptococcus lactis* ATCC 8043 . The extract showed no antimicrobial activity against *Klebsiella pneumoniae* ATCC 31488, *Klebsiella aerogenes* ATCC 9621, *Pseudomonas flourescens ATCC 13525*, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 12826 (Table 1). Findings against both fungal cultures showed 19 mm and 16 mm zone of inhibition when treated with ethanolic extract of *Glycyrrhiza glabra* (Table 1). The ethanolic extract also had good antimicrobial potential against *S. aureus* and *Salmonella sp.* (21 mm zone of inhibition). *Pseudomonas aeruginosa* showed no zone of inhibition against this extract (Table 2).

***Antimicrobial activity of chloroform extract of Glycyrrhiza glabra***

The observations showed that chloroform extract of *Glycyrrhiza glabra* has a good ability to suppress the growth of *Enterooccus faecalis* ATCC 29212 with zone of inhibition 29 mm. The extract showed no zone of inhibition against *Pseudomonas flourescens ATCC 13525*, *Proteus mirabilis* ATCC 29245, *Klebsiella aerogenes* ATCC 9621 and *Klebsiella pneumoniae* ATCC 31488 (Table 1). Zone of inhibition of 19 mm and 13 mm was observed against *Aspergillus niger ATCC 26603* and *Rhizopus oryzae ATCC 8993* (Table 1).

The results indicated that isolated *B. cereus* and *Pseudomonas aeruginosa* are resistant for this extract. Maximum zone of inhibition observed against isolated microbes was 20 mm against *S. aureus* and minimum zone of inhibition was 17 mm against *E. coli* (Table 2).

***Antimicrobial activity of ethyl acetate extract of Glycyrrhiza glabra***

Both the species of *Klebsiella* and *Bacillus cereus* ATCC 12826 were resistant for ethyl acetate extract of *Glycyrrhiza glabra*. This extract had a good antifungal potential showing zone of inhibition of 20 mm and 17 mm for *Aspergillus niger ATCC 26603* and *Rhizopus oryzae ATCC 8993* (Table 1).

The ethyl acetate extract of *Glycyrrhiza glabra* served as an effective antimicrobial agent against all the isolated microbes with zone of inhibition lying between 25 mm to 12 mm against *S. aureus* and *E. coli* respectively (Table 2).

The observations showed that ethyl acetate extract of *Glycyrrhiza glabra* had maximum zone of inhibition against *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC

12600 and *E. coli* ATCC 12632, i.e., 22 mm, 19 mm and 19 mm respectively (Table 1).

**Table 1.Antimicrobial activity of *Glycyrrhiza glabra* showing Zone of Inhibition against microorganisms**

| S.No | Solvent | Conc (µl) | *E. coli* ATCC 11840 | *E. coli* ATCC 12632 | *P. flourescens* ATCC 13525 | *K. pneumonia* ATCC 31488 | *K. aerogenes* ATCC 9621 | *P. mirabilis* ATCC 29245 | *S. lactis* ATCC 8043 | *S. aureus* ATCC 12600 | *B. cereus* ATCC 12826 | *E. faecalis* ATCC 29212 | *A. niger* ATCC 26603 | *R. oryzae* ATCC 8993 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Aqueous | 25 | - | - | - | - | - | - | - | - | - | - | - | - |
| | | 50 | - | - | - | - | - | - | - | - | - | - | - | - |
| | | 75 | - | - | - | - | - | - | - | - | - | - | - | - |
| | | 100 | - | - | - | - | - | - | - | - | - | - | - | - |
| 2. | Methanol | 25 | 12 | 5 | 8 | - | - | 9 | 5 | 11 | 10 | 11 | 11 | 13 |
| | | 50 | 13 | 9 | 9 | - | - | 12 | 9 | 14 | 12 | 15 | 13 | 15 |
| | | 75 | 17 | 11 | 11 | - | - | 15 | 13 | 19 | 14 | 17 | 15 | 16 |
| | | 100 | 20 | 15 | 14 | - | - | 16 | 15 | 21 | 14 | 19 | 17 | 18 |
| 3. | Ethanol | 25 | 9 | 11 | - | - | - | 10 | 12 | 6 | - | - | 13 | 9 |
| | | 50 | 13 | 15 | - | - | - | 14 | 16 | 9 | - | - | 15 | 12 |
| | | 75 | 14 | 17 | - | - | - | 16 | 19 | 12 | - | - | 17 | 14 |
| | | 100 | 14 | 19 | - | - | - | 18 | 23 | 14 | - | - | 19 | 16 |
| 4. | rofor m | 25 | 10 | 9 | - | - | - | - | 11 | 8 | 7 | 20 | 12 | 19 |
| | | 50 | 15 | 12 | - | - | - | - | 13 | 13 | 9 | 22 | 14 | 12 |
| | | 75 | 18 | 15 | - | - | - | - | 15 | 15 | 11 | 25 | 16 | 13 |
| | | 100 | 19 | 19 | - | - | - | - | 17 | 19 | 14 | 29 | 19 | 13 |
| 5. | Ethyl acetate | 25 | 11 | 13 | 8 | - | - | 9 | 10 | 11 | - | 13 | 12 | 11 |
| | | 50 | 12 | 15 | 10 | - | - | 11 | 14 | 13 | - | 17 | 14 | 13 |
| | | 75 | 15 | 17 | 12 | - | - | 16 | 15 | 14 | - | 20 | 18 | 14 |
| | | 100 | 17 | 19 | 14 | - | - | 17 | 17 | 19 | - | 22 | 20 | 14 |
| 6. | 10 % DMSO | 20 | - | - | - | - | - | - | - | - | - | - | - | - |
| 7. | 1 % Benzoic acid | 20 | 14 | 14 | 13 | 12 | 12 | 13 | 14 | 15 | 16 | 15 | 12 | 12 |

**Table 2. Antimicrobial activity of *Glycyrrhiza glabra* showing Zone of Inhibition against microorganisms isolated from food samples**

| S.No | Solvent | Conc (µl) | Bacteria isolated from Rice | | | Bacteria isolated from Burger | | |
|---|---|---|---|---|---|---|---|---|
| | | | *herichia coli* | *Bacillus cereus* | *hylococcus aureus* | *herichia coli* | *seudomonas aeruginosa* | *Salmonella sp* |
| 1. | Aqueous | 25 | - | - | - | - | - | - |
| | | 50 | - | - | - | - | - | - |
| | | 75 | - | - | - | - | - | - |
| | | 100 | - | - | - | - | - | - |
| 2. | Methanol | 25 | 10 | 12 | 16 | 9 | 12 | 12 |
| | | 50 | 14 | 16 | 18 | 12 | 15 | 14 |
| | | 75 | 17 | 20 | 22 | 15 | 18 | 16 |
| | | 100 | 19 | 22 | 25 | 17 | 22 | 20 |
| 3. | Ethanol | 25 | 11 | 5 | 10 | 11 | - | 13 |
| | | 50 | 13 | 8 | 15 | 14 | - | 16 |
| | | 75 | 15 | 10 | 17 | 16 | - | 19 |
| | | 100 | 18 | 12 | 21 | 19 | - | 21 |
| 4. | Chloroform | 25 | 9 | - | 9 | 7 | - | 11 |
| | | 50 | 13 | - | 12 | 10 | - | 14 |
| | | 75 | 16 | - | 18 | 15 | - | 17 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **100** | **17** | **-** | **20** | **19** | **-** | **19** |
| **5.** | **Ethyl acetate** | **25** | **9** | **11** | **11** | **8** | **10** | **5** |
| | | **50** | **10** | **13** | **15** | **10** | **15** | **11** |
| | | **75** | **12** | **16** | **18** | **11** | **16** | **12** |
| | | **100** | **14** | **18** | **20** | **12** | **17** | **16** |
| **6.** | **10% DMSO** | **20** | **-** | **-** | **-** | **-** | **-** | **-** |
| **7.** | **1% Benzoic acid** | **20** | **21** | **19** | **19** | **22** | **18** | **18** |

The results of this study showed that the aqueous extract of *Glycyrrhiza glabra* was not efficient antibacterial as well as antifungal agent and thus did not showed inhibition against any of the test microorganisms. Even microbes isolated from food samples were resistant for this extract.

*Klebsiella pneumoniae* ATCC 31488 and *Klebsiella aerogenes* ATCC 9621 are not sensitive towards any extract of *Glycyrrhiza glabra* (Irani *et al*., 2010). Methanolic extract of *Glycyrrhiza glabra* showed excellent antibacterial and antifungal potential, inhibiting in the order of *S. aureus* ATCC 12600 > *Escherichia coli* ATCC 11840 > *Enterococus faecalis* ATCC 29212 > *Rhizopus oryzae* ATCC 8993 > *Aspergillus niger* ATCC 26603 > *Proteus mirabilis* ATCC29245 > *Streptococcus lactis* ATCC 8043 > *Pseudomonas flourescens* ATCC 13525 > *Bacillus cereus* ATCC 12826. Results for antimicrobial activity against microorganisms isolated from food samples also indicate strong antimicrobial potential of methanolic extract of *Glycyrrhiza glabra* against *Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa* and *Salmonella sp.*

Evidences from study performed by Sultana *et al*. (2010), also shows highest antimicrobial activity of the methanolic extract of *Glycyrrhiza glabra* against *Staphylococcus aureus.* They also showed that *G. glabra* exhibited antimicrobial activity against *Escherichia coli, Bacillus subtilis* and *Salmonella sp.*

In comparison to methanolic extract the ethanolic extract of *Glycyrrhiza glabra* was not so much effective against microorganisms. It showed highest inhibition for *Streptococcus lactis* ATCC 8043. Inhibition against *Escherichia coli* ATCC 11840, *E. coli* ATCC 12632, *Proteus mirabilis* ATCC29245 and *Staphylococcus aureus* ATCC 12600 was also observed (Patil *et al*., 2009). This extract also showed sensitivity against microbes isolated from food samples like *E. coli, Bacillus cereus, S. aureus* and *Salmonella sp.* (Nitalikar *et al*., 2010). *Pseudomonas flourescens* ATCC 13525, *Bacillus cereus* ATCC 12826 and *Enterococcus faecalis* ATCC 29212 were found to be resistant for this extract (Ates *et al*., 2003). *Pseudomonas aeruginosa,* isolated from food samples was resistant for this extract (Irani *et al*., 2010). It was also found that this extract also had antifungal potential (Tharkar *et al*., 2010).

The growth of *Enterococcus faecalis* ATCC 29212 was highly suppressed by the chloroform extract of *Glycyrrhiza glabra.* Microbes isolated from food samples also showed good inhibition against this extract. No inhibition was found for *Pseudomonas flourescens* ATCC 13525 and *Proteus mirabilis* ATCC 29245. Even *Pseudomonas* aeruginosa isolated from food samples showed its resistance for chloroform extract of *Glycyrrhiza glabra.* Ates *et al*. (2003) also confirms our study. Studies performed by Nitalikar *et al*. (2010) also showed the antimicrobial potential of chloroform extract of *Glycyrrhiza glabra* against *Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli.* Our findings also support this result. Both the fungal strains tested showed sensitivity for the chloroform extract of *Glycyrrhiza glabra* indicating its antifungal potential.

*Glycyrrhiza glabra* had an excellent antimicrobial potential in its ethyl acetate extract form against standard microorganisms as well as bacterial strains isolated from food samples also. It inhibited all the microorganisms except *Bacillus cereus* ATCC 12826, *Klebsiella pneumoniae* ATCC 31488 and *Klebsiella aerogenes* ATCC 9621. Ates *et al*. (2003) also showed the resistance of *Bacillus subtilis* for chloroform extract of *Glycyrrhiza glabra.* Our study supports the results of the study performed by Patil *et al*. (2009) that the ethyl acetate extract of *Glycyrrhiza glabra* inhibits the growth of *S. aureus, Pseudomonas aeruginosa* and *E. coli.*

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