

Antioxidant and Antibacterial Activity of Organic Extracts of Roots of *Glycyrrhiza Glabra Linn*

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Abstract: Extracts of root of *Glycyrrhiza glabra linn* were prepared by using *n*-hexan, toluene, tetrahydrofuran, ethylacetate, and ethanol and their antioxidant and antimicrobial activities were measured. Secondary metabolites like flavonoids, alkaloids, terpenoids, glycosides, and steroids were measured and it was found that highly polar solvents like ethyl alcohol, ethyl acetate and THF extract more metabolites as compared to less polar solvents. In vitro antioxidant activity of organic extracts of *glycyrrhiza glabra* root was measured by using DPPH scavenging radical. Ethanolic extract gave highest antioxidant activity of 92% followed by ethyl acetate 89%, THF 87%, toluene 45% and hexane 23% respectively. The organic extracts of the plant root was further tested for its antibacterial activity against two bacteria viz. *Staphylococcus aureus* and *Escherichia coli*. Ethanolic extract and THF extracts have showed mild antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* respectively whereas *n*-hexane extract failed to inhibit the growth of bacteria. The results have shown that organic extract of *Glycyrrhiza glabra linn* is rich source of secondary metabolites, a strong antioxidant and possess mild antibacterial activity.

Keywords: *Glycyrrhiza Glabra Linn*, Organic Extracts, Antioxidant, Antibacterials

1. Introduction

From the time when life has originated on this planet, plants continue to play a curative and a medicinal role in protecting human health against various diseases and deterioration [1, 2]. Plants are an essential source of new structures leading to medications in all major disease areas. The starting materials for about one-half of the medicines we use today come from natural sources [3]. In this context, Pakistan being an Asian country is a good storehouse of herbal plants that are widely used in the preparations of herbal medicines.

Glycyrrhiza glabra linn is hardly an herb or under shrub of pea family named leguminosae, having habitat of subtropical and warm temperate regions. It is more than four or five feet, leaflets are elliptical, leaves multifoliate, imparipinnate,

flowers in axillary spikes, papilionaceous, lavender to violet in color, pods are trampled, seeds are rein formed, white to purplish flower clusters, an widespread root system which consist of main tap root [4], approximately 1.5 cm long and further divides into 3-5 subsidiary roots, about 1.25 cm long from which the horizontal, numerous runners and woody stolon's arise. The main tap root which is harvested for medicinal use, is soft, fibrous, and has a bright yellow interior with a characteristic odour and sweet taste [5].

Glycyrrhiza glabra linn, also known as sweet wood, is present in central and south west Asia, mediterranean basin of Africa, south Europe and certain areas of India [6]. Historically, the dried root of this plant were used medicinally by Chinese, Greek, Indian, Egyptian, and roman

civilization as a carminative and expectorant [7]. *Glycyrrhiza glabra* linn is used as a demulcent, anti-tussive, anti-bacterial, anti-oxidant, estrogenic, anti-ulcer, anti-diabetic, anti-cancer, drug delivery agent, anti-thrombin, anti-malarial, hepatoprotective, tyrosinase inhibitor, immune stimulating [8], laxative, sedative properties, sweetner, diuretic, anti-pyretic [9], anti-skin pigmentation [10], anti-hyperglycemic [11], anti-inflammatory, anti-tuberculosis [12], hair growth promotor [13], anti-acne [14].

The main constituent of the *Glycyrrhiza glabra* linn is glycyrrhizin, a triterpenoid saponins which is 50-80 times sweeter than sugar. Glycyrrhizin is present in root 10-15% of its total constituents. Both glycyrrhizin and glycyrrhetic acid exist in 18 α and 18 β stereoisomers and responsible for curing many ailments like ulcer, diabetes, cancer and microbial invasion [15]. Secondary metabolites glycyrrhizin, glabrin A & B, glycyrrhetol, glabrolide, isoglabrolide, isoflavones, coumarins, triterpenes, sterols, liquitin, isoliquitin, flavones, chalcones (licochalcone A and licochalcone B) isoflavonoids such as glabridin, glabrene, glabrol, hispaglabridin A, hispaglabridin B; 40-methyl glabridin, and 3-hydroxyglabrol are responsible for the antimicrobial and antioxidant activity of the *Glycyrrhiza glabra* linn [16, 17 and 18].

Medicinal plants contains varying amount of bioactive/metabolites contents and their extraction depends upon the solvent and method of extraction. In this study program five solvent; ethanol, THF, ethyl acetate, toluene and *n*-hexane; with varying polarity were selected and extraction of bioactive metabolites was carried out and then their evaluation for antioxidant with DPPH and antimicrobial activity with *Staphylococcus aureus* and *Escherichia coli* was also carried out.

2. Materials and Method

2.1. Collection of Plant Material

Authenticated sample of *glycyrrhiza glabra* linn root was collected from herbal medical store at Lahore. It was cut into small pieces and then shade dried for 10 days and ground, sieved and stored in a dessicator.

2.2. Chemicals

All the materials and reagents used for the study were AR grade taken from PCSIR Labs complex, Lahore and University of Engineering and Technology Lahore and used as such without further purification.

2.3. Preparation of the Extract

Hundred grams of finely grinded root of *Glycyrrhiza glabra* linn was poured into five flasks and extracted against various solvents like; ethanol, ethyl acetate, tetrahydrofuran, toluene and *n*-hexane of different polarity and heated at 60-80°C for 6 hours with stirring. It was allowed to cool and then filtered. The resulting filtrates were stored in glass bottles [19].

2.4. Phytochemical Screening

The plants are considered as biosynthetic store of variety of compounds like flavonoids, glycosides, tannins, alkaloids, saponins, and volatile oils, etc. These compounds are classified as secondary metabolites and capable of curing many diseases. These metabolites were analysed in these extracts by following the already reported procedures [20, 21].

2.5. Antioxidant Activity of Extracts of *Glycyrrhiza Glabra* Linn

Antioxidant activity of the various organic solvent extracts of the *Glycyrrhiza glabra* linn was determined by the scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by using UV-Vis double beam spectrophotometer model Hitachi-U 2000 Japan (P). This assay was performed by following the method reported by Epton et al., 2000 [22]. The samples (20, 40, 60, 80, 100 μ l each) of different solvent extracts were mixed with 3 ml of DPPH (0.004% methanolic sol.). The absorbance of the resulting solution and the blank (with only DPPH and no sample) was recorded at 517 nm after an incubation of 30 minutes at room temperature against BHT as a positive control. The scavenging activity on the DPPH radical was expressed as % inhibition using the following formula:

$$\% \text{ Inhibition} = [(A_B - A_S) / A_B] \times 100$$

Where as A_B was the absorbance shown by control reaction (containing all reagents except the extract of the plant material).

Anti- bacterial activity of organic extracts of *Glycyrrhiza glabra* linn.

2.5.1. Test Bacteria

The bacteria used for the determination of antibacterial activity was *staphylococcus aureus* (Gram positive) and *Escherichia coli* (Gram negative). These strains were obtained from a micro genetics laboratory of Lahore and after that standard confirmatory tests were performed for these bacterial strains.

2.5.2. Bacterial Culture and Preparation of Media

In vitro antibacterial activity was performed by using Mueller Hinton Agar. Two Fresh suspensions of sterilized water, pure and isolated strains of bacteria (*Staphylococcus aureus* and *Escherichia coli*) were prepared and then mixed with the sterilized Nutrient Agar separately for evaluation of antibacterial activity. Its temperature was maintained at 45°C and poured into Petri dishes and allowed to solidify.

2.5.3. Anti-Bacterial Activity Using Disc Diffusion Method

Anti-bacterial activity was studied using disc diffusion method. Fresh culture suspension of test bacteria gram positive and gram negative was uniformly spread in separate petri dishes. Concentration of cultures was used 10 mg/ml. The diameter of the disc of filter paper was used 6 mm soaked with extract and put on the surface of inoculated

media agar plates. Incubation temperature was 37°C for 24 hour under optimum conditions. Strains of bacteria used were *Staphylococcus aureus* and *Escherichia coli*. Zone of inhibition was measured against different extracts of *glycyrrhiza glabra linn* including diameter of disc in mm. zone of the inhibition for the standards used in preparing extracts were also measured and compared [23].

3. Results and Discussion

Phytochemical screening of *Glycyrrhiza glabra linn* root organic extracts showed the presence of wide range of secondary metabolites, alkaloids, flavonoids, glycosides, terpenoids, tannins, triterpenoids, saponins, fats mainly in the ethanolic, ethyl acetate and THF extracts. Tetrahydrofuran extract showed presence of all above mentioned constituents may be due to better penetration and affinity while *n*-hexane extract has least extraction of metabolites (Table 1). As the *n*-hexane has least polarity so extraction of non polar molecules will be high but as the polarity increases the extraction of polar metabolites increases. Furthermore it may have less solubility for polar compounds. Similarly, Ethanol showed negative results for the fat contents, this may also due to polarity and its solubility behavior. Extraction of metabolites

depends upon the parameters like solid liquid ratio, particle size, time, temperature and solvent. In this experiment solvents with different polarities were selected to find out the best suitable solvent for the extraction of maximum metabolites. It was found that high polarity solvent extract more metabolites. It may be due to better penetration of solvent into the plant matrix, better solubility and more affinity toward the metabolites [24].

Table 1. Qualitative Phytochemical results of different extracts of *Glycyrrhiza glabra linn*.

phytochemicals	Ethanol	Ethyl acetate	THF	Toluene	Hexane
Flavonoids	+	+	+	-	+
Alkaloids	+	-	+	-	-
Saponins	+	-	+	+	-
Glycosides	+	+	+	-	-
Triterpenoids	+	+	+	-	-
Tannins	+	+	+	-	-
Steroids	+	+	+	+	-
Fats	-	-	+	+	+

(+) sign means constituent is present and (-) sign show absence of that constituent.

Antioxidant activity of all five extracts of *Glycyrrhiza glabra linn* was summarized in fig. 1 given below:

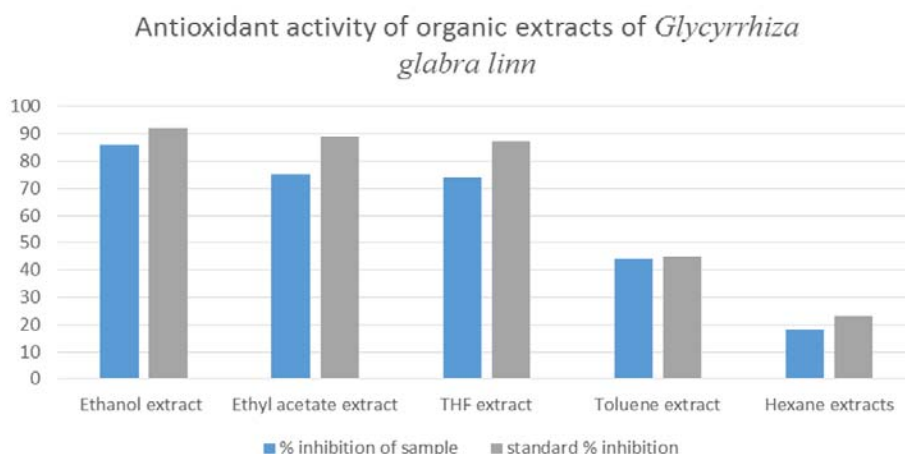


Figure 1. Antioxidant activity of organic extracts of *glycyrrhiza galabra linn* roots.

In this graph standard (BHT) % inhibition and sample (organic extracts of *Glycyrrhiza glabra linn*) % inhibitions were compared. Standard which is butylated hydroxy toluene shown more antioxidant activity as compared to *Glycyrrhiza glabra linn* organic extracts but values were close enough and *Glycyrrhiza glabra linn* considered as strong antioxidant. The highest 92% antioxidant activity was found in ethanolic extract followed by ethyl acetate 89%, THF 87%, toluene 45% and hexane 23% respectively. This trend can be seen that when the polarity of solvent (hexan > toluene > THF > ethyl acetate > ethanol) increases, its antioxidant increases. This may be due to better extraction of phenolics from the *glycyrrhiza galabra* roots. As extraction depends upon solvent polarity and solubility of the target molecules. Furthermore, solvent with low density and high diffusivity allow to easily diffuse into the pores of plant material to

leach out the bioactive constituents [24]. High polarity solvents have better affinity toward extraction of polar molecules. Gernerally, if extract has more phenolic and polyphenolic compounds it gave higher antioxidant activity [25, 26 and 27].

Antibacterial activities of extracts were evaluated by measuring the zone of inhibition (Fig. 2). Zone of inhibition of the extracts are THF extract is greater against gram negative bacteria (*Escherichia coli*) and zone of inhibition against gram positive bacteria (*Staphylococcus aures*) were 10 mm, 6 mm, 5 mm, 1.2 mm and 1 mm for ethyl alcohol, ethyl acetate, THF, toluene and *n*-hexan repectively. While zone of inhibition again gram negative bacteria (*Echerichia coli*) were 6 mm, 3.5 mm, 9 mm, 2 mm and 3 mm for ethyl alchol, ethyl acetate, tetrahydrofuran, touluene and *n*-hexan extracts respectively.

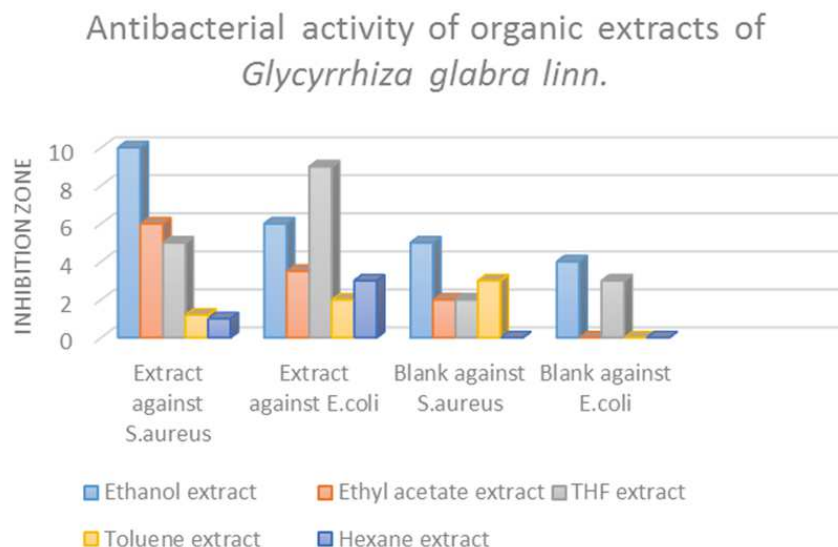


Figure 2. Antimicrobial activity of organic extracts of *glycyrrhiza galabra* linn roots.

It can be seen from the fig. 2 that ethyl alcohol extract gave maximum inhibition (10 mm) against gram positive bacteria (*Staphylococcus aureus*) while tetrahydrofuran gave maximum inhibition (9 mm) against gram negative bacteria (*Escherichia coli*). Hexane and toluene extract show negligible antibacterial activity as compared to ethyl alcohol, ethyl acetate, and THF. The antibacterial activity of phytochemical constituents is possibly linked to the presence of alkaloids, flavonoids, tannins, phenols, saponins and several other secondary metabolites that serve as defence mechanism against the invasion by many micro-organisms, insects and other herbivores [27, 28 and 29]. Natural product has their own importance and each component play a primordial important role in the plant. Such as flavonoids are hydroxylated phenolic substances, known to be synthesized by plant in response to microbial action [29, 30 and 31], saponins are active due to causing leakage of proteins and certain enzymes [32, 33] and tannin bind to proline rich proteins and interfere with the protein synthesis [34].

4. Conclusion

Organic extracts of root of *Glycyrrhiza glabra* linn were prepared by using ethanol, n-hexane, ethylacetate, tetrahydrofuran, toluene, and their constituents were determined qualitatively. Furthermore their antioxidant and antimicrobial activities were performed. Secondary metabolites like flavonoids, alkaloids, saponins, glycosides, terpenoids, tannins, steroids and fat were measured and it was found that highly polar solvents like ethyl alcohol, ethyl acetate and THF extract more metabolites as compared to less polar solvents. These solvents have better diffusibility and solubility as compared to less polar solvent for secondary metabolites. Antioxidant activity of these extracts was measured and it was found that ethanolic extract gave highest antioxidant activity of 92% followed by ethyl acetate 89%, THF 87%, toluene 45% and hexane 23% respectively. The

antioxidant activity depends upon the secondary metabolites particularly phenolics, flavonoids, tannins, saponins etc. As high polar solvent has better affinity toward polar secondary metabolites, so they gave better activity as compared to less polar solvent extracts. Similarly, antimicrobial efficacy of the organic extracts tested against two bacteria viz. *Staphylococcus aureus* and *Escherichia coli* and it was found that extracts with high polar solvent gave better efficiency as compared to less polar solvent extracts.

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