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Study of Biochemical Analysis and Antibacterial Activity of *Glycyrrhiza Glabra* Root Extracts

- Dr. K. Prabu*
- P. Kanagalakshmi**

Abstract

Phytochemical analysis, biochemical analysis, *in vitro* antioxidant activity, *in vitro anti* inflammatory activity, *in vitro* antiarthritic activity and antibacterial activity of root extracts of *Glycyrrhiza glabra* were studied. The aqueous and ethanolic root extracts were tested for the presence of phytochemicals. The anti-inflammatory activity of *Glycyrrhiza glabra* at 250, 500,750,1000 mcg/ml were studied using human red blood cell membrane (HRBC) stabilization method. The anti-arthritic activity of *Glycyrrhiza glabra* at the same concentration was analysed using bovine serum albumin denaturation (BSA) method. Diclofenac, at concentration of 1000 mcg/ml, was assessed as standard anti-inflammatory and anti-arthritic drug. Antibacterial activity was evaluated using Agar well diffusion method. Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids. The extracts of *Glycyrrhiza glabra* had shown significant amount of ascorbic acid and catalase. The aqueous and ethanolic extracts of *Glycyrrhiza glabra* results showed significant protection of HRBC and significant inhibition in BSA denaturation and which are comparable to standard drugs diclofenac. Aqueous and ethanolic extracts of *Glycyrrhiza glabra* exhibited significant antibacterial activity. All the analysis was made with the use of standard procedure. These results indicate the Anti-inflammatory, Antiarthritic and antibacterial potential of the *Glycyrrhiza glabra* root extracts and can be used as a promising herb in treating the clinical ailment.

Keywords: Phytochemical, Antioxidant activity, Anti-inflammatory activity, Antiarthritic activity, Anti bacterial activity, *Glycyrrhiza glabra.*

Introduction

The world is abundant with natural and medicinal plants. Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacology. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological actions on the human body [1]. Phytochemical, natural compound occur in plants such as medicinal plants, vegetables and fruits that work with nutrients and fibers to act against diseases or more specifically to protect against diseases.

*Assistant Professor, Department of Biochemistry, Indo-American College, Thiruvannamalai District, Cheyyar 604 407. Email: kprabu.cas@gmail.com Mobile: +9894387994
**Department of Biochemistry, Indo-American College, Thiruvannamalai District, Cheyyar 604 407.

Glycyrrhiza glabra, family Leguminoseae, is a plant which grows in India and other countries of the world. Its roots possess some nutritive value and medicinal properties. They are widely used as a cold beverage, in preparing some pharmaceutical preparations such as haematinic pills and to disguise the bitter taste of other remedies [2]. It is a very sweet, moist, soothing herb that detoxifies and protects the liver and is also a powerful anti-inflammatory, being used in conditions as varied as arthritis and mouth ulcers. Phytochemical analysis of *Glycyrrhiza glabra* root extract showed that it contains saponin, triterpenes (glycyrrhizin, glycyrrhetinic acid and liquirtic acid), flavonoids (liquirtin, isoflavonoids and formononetin) and other constituents such as coumarins, sugars, amino acids, tannins, starch, choline, phytosterols and bitter principles[3, 4].Aim of the study was to optimize extraction methods in order to maximize the recovery of secondary metabolites in the crude extracts of *Glycyrrhiza glabra*.

In the present study, an attempt has been made to identify the active ingredients present in the root powder extracts (Different solvents) of *Glycyrrhiza glabra* plant and was subjected to phytochemicals screening, Antioxidant activity (Enzymatic and Non-enzymatic), *In-vitro* anti-inflammatory activity on human red blood cell membrane, *In-vitro* anti-arthritic activity on Inhibition of protein denaturation and Antibacterial activity by well diffusion method.

Materials And Methods

Collection of *Glycyrrhiza Glabra* Root

The plant of *Glycyrrhiza glabra* root was collected from Chetpet, in Thiruvannamalai district. The Plant was recognized based on the plant anatomy. The plant was washed thoroughly in tap water and the stem was removed from the plant. The root parts of the plant were air dried in the shade for six days. The plant samples were grounded into uniform powder using milling machine. The powder was used for the further studies.

Preparation of Extracts

Aqueous Extract

Aqueous extract of the sample was prepared by soaking 10g of dried powder in 100ml of distilled water for 24 hours. The extract was filtered using the No: 1 Whatmann filter paper and it was used for further studies.

Ethanol Extract

Ethanol extract of the sample was prepared by soaking 10 grams of dried powder in 100ml of Ethanol for 24 hours. The extract was filtered using the Whatmann No.1 filter paper and it was used for further studies.

Phytochemical Screening was carried out using standard procedures of Harborne, quantitative analysis of Glucose by ortho-Toluidine Method, Estimation of Protein by Lowry's Method, Estimation of tannins by Tyler and Herbalgram & Harborne, antioxidant analysis of Estimation of Ascorbic acid by Roe & Ruther, Determination of Catalase by Sinha method. Determination of total phenol contents by Singleton & Rossi, *In-vitro* anti-inflammatory activity of *Glycyrrhiza glabra root* (GGR) ethanol Extract on human red blood cell (HRBC) stabilization method , *In-vitro* Anti-arthritic activity of *Glycyrrhiza glabra* root (GGR) extract on Inhibition of (protein) bovine serum albumin (BSA) denaturation method, antibacterial activity by Agar well diffusion method.

Results and Discussion

The results confirm the presence of constituents which are known to exhibit medicinal as well as physiological activities. The phytochemical characteristics of the root extract of *Glycyrrhiza glabra* investigation reveal the presence of medicinally active constituents like tannins, Alkaloid, terpenoids, steroids and saponins in the leaves of *Glycyrrhiza glabra*. While Flavnois, Phlobatannins, Glycosides were absent in aqueous extract, the alkaloids contained in plants are used in medicine as anesthetic agents [15]. The presence of saponins in plants have been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs [16] . The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the root of the plants studied. The presences of Phytochemical compounds have also been confirmed to have antimicrobial activity [17]. Hence it could be inferred that the plant extracts could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection.

The qualitative phytochemical screening of *Glycyrrhiza glabra* investigated were summarized in Table - 1. The results of analysis showed that *Glycyrrhiza glabra* contains most of the phytochemical

in root extract. Ethanol and aqueous extracts of root gave similar results for alkaloids, carbohydrates, saponins and proteins. *Glycyrrhiza glabra* root extract contains alkaloids, carbohydrates, Glycosides, proteins, Saponins, Tannins, Terpenoids, Anthraquinone and Polyphenols as phytochemical constituents as shown in the present study on ethanol and aqueous solvent extracts. Preliminary Phytochemical investigations revealed the presence of alkaloids. The therapeutic properties of *Glycyrrhiza glabra* are due to alkaloids documented to possess antibacterial action.

Polyphenols possess several antioxidant mechanisms including scavenging or quenching free radicals, chelating metal ions, and inhibiting enzymatic systems responsible for free radical generation. The potent antioxidant activity of Polyphenols may provide the best protection against elevated oxidative stress. The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported the antibacterial properties of tannins were also reported.

Quantitative analysis was carried out in aqueous, and Ethanolic extracts prepared from root powders of *Glycyrrhiza glabra* and the results were presented in Table – 2 & figure-1. The glucose content of *Glycyrrhiza glabra* root extract was higher in ethanol extract (250mg %) compared to aqueous extract (220mg %). Similarly the protein content of *Glycyrrhiza glabra* root extract was higher in Ethanolic extract (845mg %) compared to aqueous extract (115mg %).

The Total Phenolic content of *Glycyrrhiza glabra* extract was higher in Ethanol extract (263mg %) when compared to aqueous extract (140mg %). The Tannin content of *Glycyrrhiza glabra* leaf extract was higher in Aqueous extract (70mg %) when compared to ethanol extract (15mg %). Quantitative Results indicate that *Glycyrrhiza glabra* are rich in protein, and antioxidant components such as ascorbic acid, catalase, etc. In view of the current research focusing on exploiting plants as sources of antioxidants, potential exists for exploring the antioxidant properties in food and biologic systems. Several of the plant-derived phenolic compounds, such as flavonoids, may be successful target antioxidants to treat oxidative stress.

The results of *in-vitro* anti-inflammatory activity of *Glycyrrhiza glabra* on human red blood cell membrane were given in figure-2. *In-vitro* anti-inflammatory activity of *Glycyrrhiza glabra* was performed by using human red blood cell membrane stabilization method. *Glycyrrhiza glabra* showed significant anti-inflammatory activity in a concentration dependent manner. *Glycyrrhiza glabra* at concentration of 250 and 500 mcg/ml showed 15%, 40% 60% and 75% protection of HRBC in hypotonic solution respectably. All the results were compared with standard diclofenac at 1000 mcg/ml which showed 65% protection of HRBC in hypotonic solution respectably.

The results of *in-vitro* Antiarthritic activity of *Glycyrrhiza glabra* on Inhibition of protein denaturation method were presented in figure-3. *In-vitro* anti-arthritic activity of *Glycyrrhiza glabra* was performed by using Inhibition of protein denaturation method. *Glycyrrhiza glabra* showed significant anti-arthritic activity in a concentration dependent manner. *Glycyrrhiza glabra* at concentration of 250, 500, 750, 1000 mcg/ml showed 20%, 40%, 60%, 80% inhibition of protein denaturation respectively. All the results were compared with standard Diclofenac at 1000 mcg/ml which showed 72 % inhibition of protein denaturation respectably. Antibacterial activity of Aqueous, Ethanolic root extracts of *Glycyrrhiza glabra* against (Microorganism) Bacteria of *Escherichia coli, Klebsiella pnemoniae, Proteus vulgaricus, Proteus mirabilis, Pseudomonas, Staphylococcus aureus, Salmonella typhi* and the results shown in figure 4.

Table 1: Phytochemical Screening Of Aqueous And Ethanolic Extract Of *Glycyrrhiza Glabra*

S. No	Test / Root Extract	Aqueous	Ethanol
1.	**Test for Alkaloids**		
	a) Mayer's test	+	+
	b) Wagner's test	+	+
	c) Dragendorff's test	+	+
2.	**Test for flavonoids**		
	a) Shinoda's test	+	+
	b) Alkaline reagent test	+	+
3.	**Test for carbohydrates**		
	a) Benedict's test	+	+
	b) Molisch's test	+	+
4.	**Test for glycosides**		
	a) Borntrager's test	+	+
	b) Keller – Killani test	-	+
5.	**Test for Proteins**	+	+
	b) Biuret test	+	+
6	**Test for saponins**		
	a) Froth test	+	+
	b) Lead acetate test	+	+

7	**Test for Tannins**		
	a) Ferric chloride test	+	+
	b) Lead acetate test	+	+
8	**Test for Terpenoids**		
	a) Salkowski test	+	+
9	**Test for Anthraquinones**		
	a) Ammonia test	+	+

- → indicates the absence of phytochemicals,
+ → indicates the presence of phytochemicals.

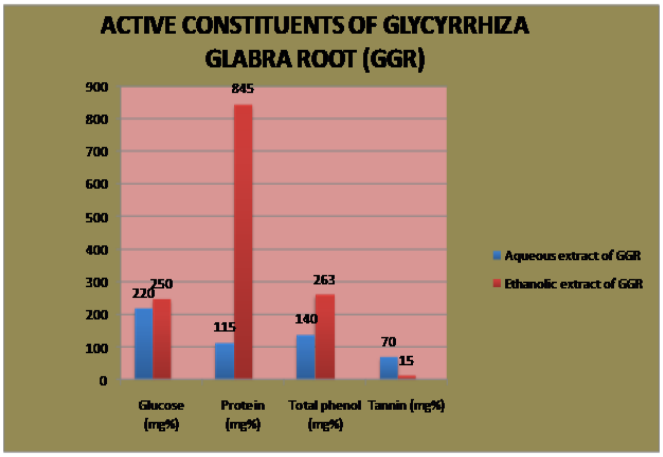

Figure 1: Active Constituents of Glycyrrhiza Glabra Root

Table 2: Non- Enzymatic-Antioxidant and Enzymatic-Antioxidant components of Glycyrrhiza glabra

S.No	Antioxidant Components	Aqueous extract (Mg %)	Ethanol extract (Mg %)
1	Vitamin C	12.5	20.8
2	Catalase	480	640

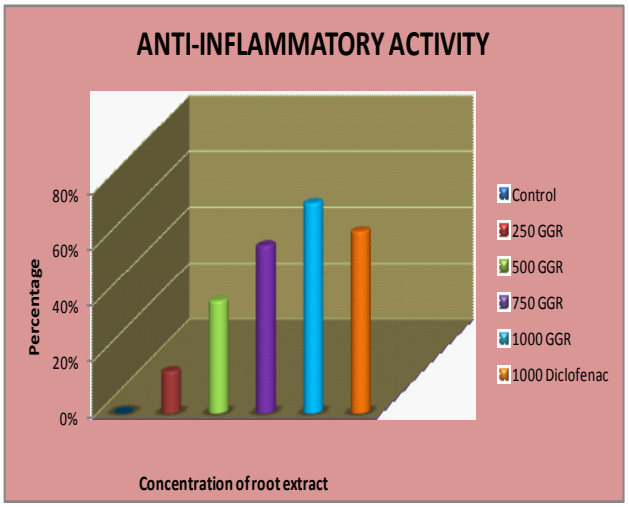

Figure 2: Anti Inflammatory Activity

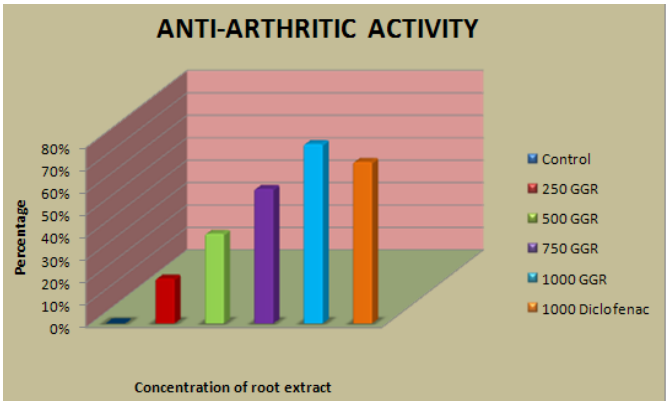

Figure 3: Anti-Arthritic Activity

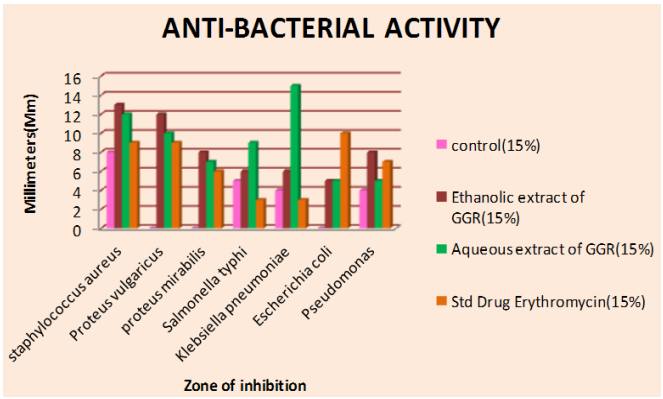

Figure 4: Anti-bacterial activity

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