

PRELIMINARY PHYTOCHEMICAL SCREENING AND *IN VITRO* FREE RADICAL SCAVENGING ACTIVITY OF ROOT EXTRACTS OF *GLYCYRRHIZA GLABRA L*

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ABSTRACT

Objective: Obesity is an international health problem caused by various environmental and genetic factors. Herbal supplements have proved to be active against obesity. *Glycyrrhiza glabra* belongs to the family of Fabaceae. *G. glabra* was used traditionally to treat various diseases. Hence, the current study was planned to investigate the phytochemicals and antioxidants present in *G. glabra*.

Methods: The preliminary qualitative, quantitative phytochemicals, and antioxidants were investigated from the root extracts of *G. glabra*.

Results: The preliminary qualitative analysis of various extracts of *G. glabra* showed the presence of phenols, flavonoids, tannins, saponins, and quinones. Quantitative analysis of *G. glabra* showed elevated amounts of phenols, flavonoids, and tannins in ethanolic extracts. Aqueous, ethanolic, hydro-alcoholic extracts of *G. glabra* showed 211±2.64, 281.66±1.57, 263±14.2 mg of phenols, 152.66±3.05, 187.33±1.52, 183.33±3.05 mg of flavonoids, 21.33±0.57, 41±3.46, 34.3±1.15 mg of Tannins, and 42.33±2.05, 63.33±1.52, 36.33±1.57 mg of terpenoids. The antioxidant properties of the ethanolic extract of *G. glabra* were evaluated with the 2,2-diphenyl-1-picryl hydrazyl (DPPH), nitric oxide radical (NO), superoxide radical (SO), hydrogen peroxide radical (H₂O₂), and hydroxyl radical (HO) scavenging activity. The inhibitory concentration 50% value of *G. glabra* is 120 µg/ml for DPPH, 208 µg/ml for (NO), 196 µg/ml for (SO), 148 µg/ml for (H₂O₂), and 252 µg/ml for (OH). *In vitro* method suggests that *G. glabra* is more powerful against nitric oxide and hydroxyl radicals.

Conclusion: This preliminary study shows that *G. glabra* is a promising herb with many phytochemicals and antioxidants and it can be further investigated for its anti-obesity potential.

Keywords: *Glycyrrhiza glabra L*, Phytochemicals, Antioxidant and obesity.

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INTRODUCTION

Obesity can be described as an imbalance between energy intake and expenditure such that excess energy is stored in fat cells, which enlarge or increase in number [1]. There are many different treatments that are used to control obesity including diet, exercise, behavior modification, and medication. Among these treatments, diet and exercises have helped to manage obesity [2,3]. Obesity induces oxidative stress, which may be the cause of obesity-related chronic diseases, such as diabetes, hypertension, cardiovascular disease, stroke, asthma, musculoskeletal problems, kidney diseases and certain cancers such as endometrium, ovaries, breast and colon [4,5], gallbladder disease, arthritis, respiratory disease, pregnancy complication, menstrual difficulties and psychological problems [6].

Obesity is a medical condition involving an excess accumulation of body fat. The prevalence of obesity has increased steadily over the last five decades not only in adults but also among children and adolescents [7]. Obesity is also an extremely costly health problem which accounts for 2-6% of total healthcare costs in developed countries [8]. According to the WHO standards, a body mass index (BMI) of 25.0 kg/m² or higher is categorized as overweight; the BMI 30.0 kg/m² or more as obese.

Plants play an important role in traditional medicine systems. Numerous preclinical and clinical studies with various herbal medicines reported significant improvement in controlling bodyweight, without any noticeable adverse effects [9]. Various herbal supplements have proved to be active against obesity such as *Allium sativum*, *Citrus aurantium L*, *Cyperus rotundus L*, *Nelumbo nucifera*, *Piper nigrum L*, *Piper longum*, and *Vitis vinifera L*. By literature survey, it is proved that *Glycyrrhiza glabra* Linn was used traditionally to treat various diseases recently [10]. Hence, this study was selected to evaluate the anti-obesity efficacy of *G. glabra*.

Licorice (*G. glabra L*) belongs to the family Papilionaceae/Fabaceae. It is a traditional medicinal plant that grows in various parts of the world [11]. Phytochemical analysis of *G. glabra* root extract showed that it contains flavonoids, carbohydrates, saponins, phenols, tannins, terpenoids, and quinones. In the traditional system of medicine, the roots and rhizomes of the *G. glabra* have been employed clinically for their anti-inflammatory, antiulcer, expectorant, antimicrobial, antioxiolytic, antiallergic, antibacterial, antioxidant, and anti-arthritis activity [12,13]. This study was performed to analyze the qualitative, quantitative phytochemicals, and antioxidant investigation of various extracts of *G. glabra L* was determined.

METHODS**Collections of plant materials**

The roots of *G. glabra L* which were collected from the local market and was authenticated by Professor P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai.

Extraction

The different extracts of dried root powder of *G. glabra* were prepared using various solvents such as hexane, ethyl acetate, ethanolic, hydroalcohol, and aqueous using soxhlet apparatus. The extracts were subjected to rotary evaporator to remove the solvents and then the extract was used for the further analysis.

Phytochemical screening

The phytochemical screening of the root extracts of *G. glabra L* was performed using the standard protocol.

Qualitative analysis

Chemical tests were carried out using standard procedures to identify the constituents as described by Sofowara, Evans, and Harbone [14-16].

Quantitative analysis*Determination of total phenols*

Total phenols were determined by Folin-ciocalteu reagent by the method [17].

Procedure

About 5 ml of Folin-ciocalteu reagent and 4 ml of aqueous sodium carbonate were added to 0.5 ml of each extract. After 15 minutes incubation at room temperature, the absorbance was read at 765 nm. The standard curve was plotted using chlorogenic acid. Total phenols were expressed in terms of chlorogenic equivalents (mg/g).

Determination of tannin-phenolics

Tannin - phenolics was determined by the method [18].

Procedure

Tannins were precipitated by adding either cinchonine sulfate, caffeine, or lead acetate solutions. The concentrations of the reagent required for completely precipitating the tannins were determined earlier. The precipitate containing tannin was dissolved in a known volume of 10% methanol, and the concentration of tannins was estimated by the Folin-ciocalteu methods. The concentration of tannins was expressed in terms of chlorogenic acid equivalents (mg/g).

Determination of total flavonoids

Total flavonoids were determined using the colorimetric method [19].

Procedure

About 0.5 ml of each extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. It was kept at room temperature for 30 minutes and the absorbance of the reaction mixture was measured at 415 nm. The standard calibration curve was plotted using catechin.

The values were expressed as mg/g.

Determination of terpenoids

Terpenoids were estimated according to the method [20].

Procedure

About 0.2 ml of each extract prepared in ethanol (mg/ml) was evaporated by keeping it in boiling water bath and to the residue; 0.3 ml of vanilin/glacial acetic acid (W/V) was added. 1 ml of perchloric acid was added and incubated at 60°C for 45 minutes. Tubes were cooled in ice and to the mixture, 5 ml of glacial acetic acid was added and the color intensity was measured at 548 nm. The standard curve was plotted using ursolic acid.

The values were expressed as mg/g.

Assessment of scavenging activity*Determination of free radical scavenging assay*

DPPH (2, 2-diphenylpicrylhydrazyl) scavenging activity was determined by the method [21].

Procedure

The ability to scavenge the stable free radical DPPH is measured by a decrease in the absorbance at 517 nm. To an ethanolic solution of DPPH (0.05 mM), an equal volume of extract dissolved in water was added to a final volume of 1 ml. An equal volume of alcohol was added to the control. After 20 minutes, absorbance was recorded at 517 nm in a UV-visible double beam spectrophotometer. The scavenging activity of the plant extract was calculated using the formula.

$$\% \text{ inhibition} = [(Abs_{\text{control}} - Abs_{\text{sample}}) / Abs_{\text{control}}] \times 100$$

Where,

Abs_{control} = Absorbance of control

Abs_{sample} = Absorbance of sample

Determination of nitric oxide scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically by the method [22].

Procedure

Sodium nitroprusside (5 mM) in PBS was mixed with different concentrations of the extract dissolved in methanol and incubated at 25°C for 150 minutes. The samples from the above were treated with Griess reagent (1% sulfanilamide, 2% phosphoric acid, 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with naphthyl ethylenediamine hydrochloride was measured at 546 nm and compared with that of standard solutions treated in the same way.

$$\% \text{ inhibition} = [(Abs_{\text{control}} - Abs_{\text{sample}}) / Abs_{\text{control}}] \times 100$$

Where,

Abs_{control} = Absorbance of control

Abs_{sample} = Absorbance of sample

Determination of superoxide anion scavenging activity

Superoxide anion scavenging activity was carried out by employing NBT reduction assay [23].

Procedure

A reaction mixture containing 0.4 µl of sodium pyrophosphate, 25 µl of phenazine methosulfate, 25 µl of nitroblue tetrazolium, and 100 µl of NADH was mixed with various concentrations of extracts and incubated for 90 s at 30°C. Purple colored chromogen formed was measured spectrophotometrically at 560 nm. The scavenging activity of the plant extract was calculated using the formula.

$$\% \text{ inhibition} = [(Abs_{\text{control}} - Abs_{\text{sample}}) / Abs_{\text{control}}] \times 100$$

Where,

Abs_{control} = Absorbance of control

Abs_{sample} = Absorbance of sample

Determination of hydroxyl radical scavenging activity

The scavenging activity of hydroxyl radical was measured by the method [24].

Procedure

The reaction mixture contained 0.8 ml of phosphate buffer solution (50 mmol/L, pH 7.4), 0.2 ml of sample at different concentrations (100-500 µg/ml), 0.2 ml of EDTA (1.04 mmol/L), 0.2 ml of FeCl₃ (1 mmol/L), and 0.2 ml of 2-deoxyribose (60 mmol/L). The mixture was kept in a water bath at 37°C and the reaction was started by adding 0.2 ml of ascorbic acid (2 mmol/L) and 0.2 ml of H₂O₂ (10 mmol/L). After incubation at 37°C for 1 hr, 2 ml of cold thiobarbituric acid (10 g/L) was added to the reaction mixture followed by 2 ml of HCl (25 %). The mixture was heated at 100°C for 15 minutes and then cooled with water. Ascorbic acid was used as a standard. The absorbance of solution was measured at 532 nm with a spectrophotometer. The hydroxyl radical scavenging capacity was evaluated with the inhibition percentage of 2-deoxyribose oxidation of hydroxyl radicals. The scavenging percentage was calculated according to the following formula

$$\% \text{ inhibition} = [(Abs_{\text{control}} - Abs_{\text{sample}}) / Abs_{\text{control}}] \times 100$$

Where,

$Abs_{control}$ = Absorbance of control

Abs_{sample} = Absorbance of sample

Determination of hydrogen peroxide radical scavenging activity

Hydrogen peroxide radical scavenging activity was assayed by the method [25].

Procedure

The reaction mixture containing 500 μ l of phosphate buffer and 400 μ l of 2 mM hydrogen peroxide was mixed with various concentrations of the extract and incubated at room temperature for 5 minutes. Then, 2 ml of dichromate reagent was added and the decrease in color intensity was measured at 570 nm. 2 ml of dichromate acetic acid reagent alone served as the blank, whereas the reaction mixture without compound served as control.

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

Where,

$Abs_{control}$ = Absorbance of control

Abs_{sample} = Absorbance of sample

RESULTS AND DISCUSSION

Phytochemicals screening and determination of phenols, flavonoids, tannins, and terpenoids

Qualitative analysis was carried out in the root extracts of *G. glabra* and this extracts showed the presence of phytochemical constituents, and the results are summarized in Table 1. The phytochemical screening of various extracts of *G. glabra* showed the presence of carbohydrates, flavonoids, saponins, tannins, phlobatannins, terpenoids, glycosides, phenols, alkaloids, quinones, phytosterol, and proteins. It was observed that the preliminary phytochemicals are present in higher amounts in ethanol, hydroalcohol and aqueous extract followed by hexane and ethyl acetate extracts. Hence, further studies were carried out using the aqueous, ethanol and hydro-alcohol extracts.

Table 2 represents the quantitative analysis of the root extracts of *G. glabra*. The above results showed the presence of total phenols, flavonoids, tannins, and terpenoids. Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities [26]. The aqueous, ethanol, and hydro-alcohol extracts showed the presence of the total phenols and were estimated to be 211 \pm 2.64, 281.66 \pm 1.57, 263 \pm 1.42 expressed as chlorogenic acid equivalents/g of extracts of the *G. glabra*.

Secondary chemicals are widely used [27]. It was found that flavonoids help to reduce the blood lipids and glucose in humans. Flavonoid constitute a wide range of substances that play an important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules such as carbohydrates, proteins, lipids and DNA [28]. It belongs to the group of polyphenolic compounds, which are classified as flavones, flavonols, isoflavones, and chalcones. The flavones possess a variety of pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Flavonoids are hydroxylated phenolic substances known to be synthesized by plant in response to microbial infection and the ability to modify the body reactions like allergens [29]. The aqueous, ethanol, hydro-alcohol extracts showed the presence of the flavonoids and were estimated to be 152.66 \pm 3.05, 187.33 \pm 1.52, 183.33 \pm 3.05 of Quercetin equivalents/g.

Most of the true tannins are high molecular weight compounds. These compounds are complex polyphenols, which are produced by polymerization of simple polyphenols. These are polyphenolic

Table 1: The qualitative analysis of various extract of *Glycyrrhiza glabra*

Compounds	Hexane	Ethyl acetate	Ethanol	Hydro alcohol	Aqueous
Carbohydrates	-	+	++	++	+++
Saponins	-	-	+	++	+++
Coumarins	-	-	-	-	-
Tannins	-	+	+++	++	+++
Phlobatannins	-	+	++	+	+
Terpenoids	-	++	+++	+++	+
Glycosides	-	+	+++	++	++
Alkaloids	-	++	++	++	++
Phenols	-	+	+++	+++	+++
Quinones	-	-	+++	++	-
Proteins	-	+	++	++	++
Flavonoids	-	+	+++	++	++
Phytosterol	++	+	+	+	-

+ = Present; ++ = Moderately present; +++ = Present in higher amount; - = Absent

Table 2: Quantitative analysis of *Glycyrrhiza glabra*

S.N	Contents	Aqueous	Ethanol	Hydro alcohol
1	Phenols (mg/g)	211 \pm 2.64	281.66 \pm 1.57	263 \pm 1.42
2	Flavonoids (mg/g)	152.66 \pm 3.05	187.33 \pm 1.52	183.33 \pm 3.05
3	Tannins (mg/g)	21.33 \pm 0.57	41 \pm 3.46	34.3 \pm 1.15
4	Terpenoids (mg/g)	42.33 \pm 2.0	63.33 \pm 1.52	36.33 \pm 1.57

compounds divided into two main groups, namely hydrolyzed and condensed tannins. Tannins and flavonoids have been shown to have numerous health protective benefits, which includes lowering of blood lipids [30]. Thus, this plant has been used to lower lipid content. Tannins bind to proline-rich protein and interfere with protein synthesis. They are also known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Tannins are a major group of compounds that act as a primary antioxidant or free radicals scavengers and it plays an important role in promoting wound healing process [31]. The aqueous, ethanol, hydro-alcohol extracts showed the presence of the tannins and were estimated to be 21.33 \pm 0.57, 41 \pm 3.46, 34.3 \pm 1.15 of chlorogenic acid equivalents/g, respectively.

Terpenoids are a large and diverse class of naturally occurring organic chemicals found in all classes of living organisms. Plant terpenoids are used extensively for their aromatic qualities and play a role in traditional herbal remedies. They are currently under investigation by numerous groups for antitumor, antibiotic, anticancer, antineoplastic, antibacterial, anti-inflammatory, and other therapeutic properties [32]. Terpenoids are the most widespread, chemically interesting groups of secondary metabolites with over 30,000 known compounds [33,34]. Many terpenes have biological activities and are used for the treatment of human diseases. The aqueous, ethanol, hydro-alcohol extracts showed the presence of the terpenoids and were estimated to be 42.33 \pm 2.05, 63.33 \pm 1.52, 36.33 \pm 1.57 of Ursolic acid equivalents/g, respectively. It was observed the ethanolic extract showed high amounts of phenols, flavonoids, tannins, and terpenoids. Hence, further studies were carried out using the ethanolic extract of *G. glabra*.

In vitro antioxidant activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule DPPH radical is usually used as a substrate to evaluate antioxidant activity of antioxidants. It involves reactions of specific antioxidant with stable free radical 2, 2-diphenyl-1-picryl-hyrazyl (DPPH). As a result, there is a reduction of DPPH concentration by the antioxidant, which decreases the optical absorbance of DPPH, and it is detected at 517 nm [35]. The DPPH radical scavenging effects of *G. glabra* are presented in Table 3 and Fig. 1. The

inhibitory concentration (IC₅₀) value of DPPH scavenging activity of the ethanolic extract was found to be 120 (µg/ml) While the IC₅₀ value for ascorbic acid was 84 (µg/ml), respectively. The maximum scavenging activity was found to be at 50% of methanolic extract of *P. nigrum* Linn. and IC₅₀ value is found to be 189µg/ml as reported [36].

Nitric oxide radical scavenging procedure is based on the sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrate ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitric oxide [22]. The nitric oxide scavenging effects of *G. glabra* are shown in Table 3 and Fig. 2. The IC₅₀ value of nitric oxide scavenging activity of ethanolic extract was found to be 208 (µg/ml) and the IC₅₀ value for ascorbic acid was found to be 192 (µg/ml), respectively. The maximum scavenging activity was found to be 50% of ethanolic extract of *Centella asiatica* and IC₅₀ value was found to be 200 µg/ml [37].

Table 3: IC₅₀ value of *Glycyrrhiza glabra*

S.N	Free radicals	IC ₅₀ values of ethanolic extract (µg/ml)	
		<i>Glycyrrhiza glabra</i>	Ascorbic acid
1	DPPH	120±2.64	84±3.21
2	Nitric oxide	208±1.15	192±4.35
3	Super oxide	196±3.51	152±2.08
4	Hydrogen peroxide	148±1.52	96±2.08
5	Hydroxyl radical	252±1.52	172±2.51

DPPH: 2,2-diphenyl-1-picryl hydrazyl, IC₅₀: Inhibitory concentration

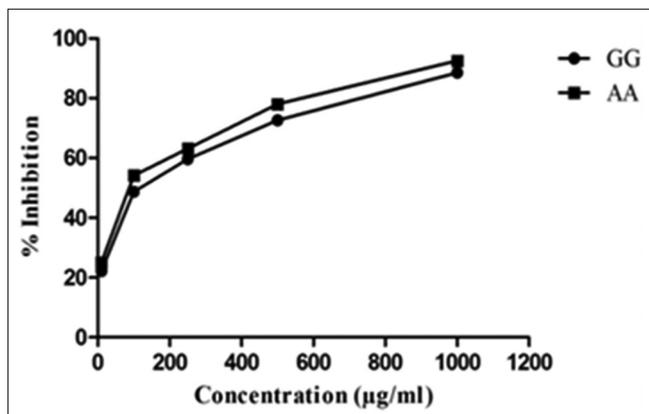


Fig. 1: 2,2-diphenyl-1-picryl hydrazyl radical scavenging activity of ethanolic extract of *Glycyrrhiza glabra*

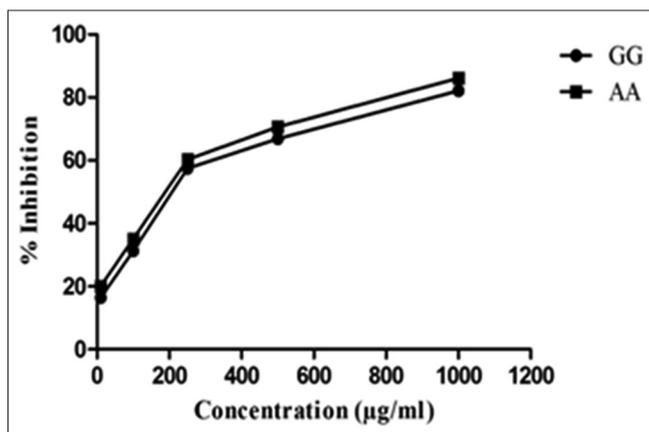


Fig. 2: Nitric oxide radical scavenging activity of ethanolic extract of *Glycyrrhiza glabra*

Super oxide known to be very harmful to cellular components as a precursor of the more reactive oxygen species is contributed to tissue damage and various diseases. In a biological system, its toxic role can be eliminated by super oxide dismutase. The radicals may also play an important role during the peroxidation of unsaturated fatty acids and other potential susceptible substances [38]. The super oxide scavenging activities of *G. glabra* are shown in Table 3 and Fig. 3. The IC₅₀ value of super oxide scavenging activity of the extract was found to be 196 (µg/ml) and the IC₅₀ value for ascorbic acid was 152 (µg/ml), respectively. The maximum scavenging activity was found to be 50% of methanolic extract of *Hibiscus taiwanensis* and the IC₅₀ value was found to be 200 µg/ml [39].

Hydrogen peroxide is a weak oxidizing agent and it is not very reactive, but can cross biological membranes. Because of the possible involvement of hydrogen peroxide in the generation of hydroxyl radicals, hydrogen peroxide becomes very important radical has a more prominent role to initiate cytotoxicity than its chemical reactivity. Thus, removing hydrogen peroxide is very important for the protection of living systems [40]. The hydrogen peroxide scavenging radical effects of *G. glabra* is shown in Table 3 and Fig. 4. The IC₅₀ value of hydrogen peroxide scavenging activity of the extract was found to be 148 (µg/ml) and the IC₅₀ value for ascorbic acid was 96 (µg/ml), respectively. The maximum scavenging activity was found to be 50% of methanolic extract of *Torilis leptophylla* (L) and IC₅₀ value was found to be 130 µg/ml [41].

Hydroxyl radical is the more reactive chemical species known. The hydroxyl radical induces some oxidative damage to biomolecules such as protein, DNA and almost any biological molecule it touches, as food additive to increase the shelf life of foods by preventing lipid

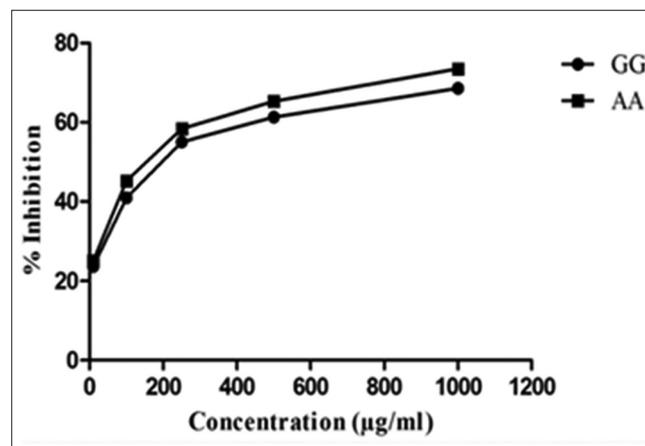


Fig. 3: Super oxide radical scavenging activity of ethanolic extract of *Glycyrrhiza glabra*

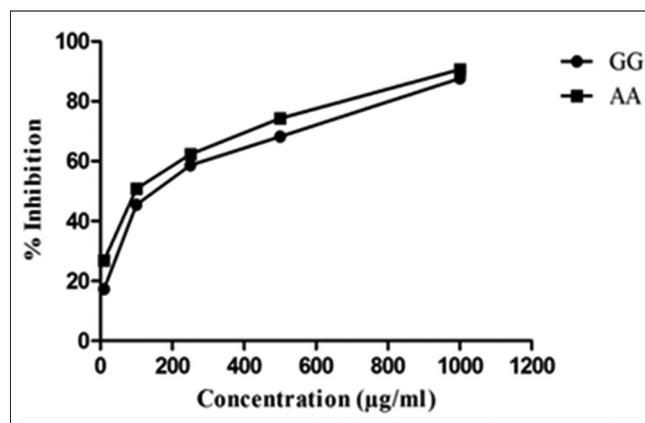


Fig. 4: Hydrogen peroxide scavenging activity of ethanolic extract of *Glycyrrhiza glabra*

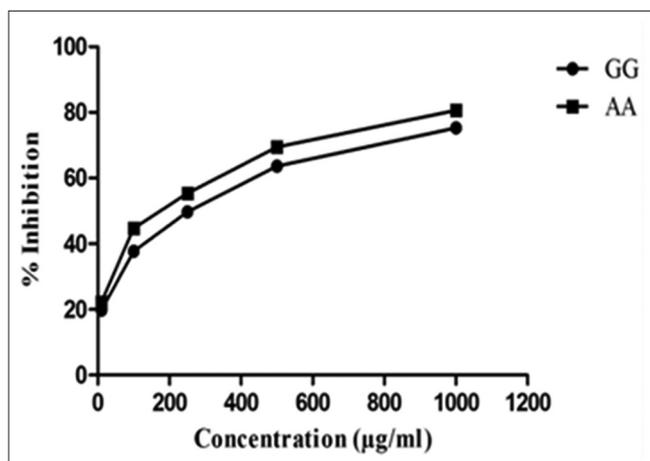


Fig. 5: Hydroxyl radical scavenging activity of ethanolic extract of *Glycyrrhiza glabra*

peroxidation [42]. Hydroxyl radical scavenging activities of *G. glabra* are shown in Table 3 and Fig. 5. The IC_{50} value of hydroxyl radical scavenging activities of the extract was found to be 252 ($\mu\text{g/ml}$) and the IC_{50} value for ascorbic acid was 172 ($\mu\text{g/ml}$), respectively. The maximum scavenging activity was found to be 50% of methanolic extract of *Flemingia strobilifera* (L) and IC_{50} value was found to be 337 $\mu\text{g/ml}$ [43].

CONCLUSION

The phytochemical evaluation can be used for further assessment of secondary metabolites. This investigation will help in further extraction, analysis of compounds from various parts of *G. glabra*. This study reveals that all the extracts have a significant amount of phytochemicals, especially tannins and flavonoids which contribute to potential health benefits and also acts as a therapeutic agent for obesity. The extracts of the root of *G. glabra* show the presence of many bioactive secondary metabolites and the ethanolic extract of *G. glabra* possessed antioxidant potential. It was able to scavenge hydroxyl and nitric oxide radicals very efficiently.

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