ISSN 2582-788X

RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF
APPLIED CHEMICAL AND BIOLOGICAL SCIENCES

DETERMINATION OF ANTI-DIABETIC AND ANTI-OXIDANT ACTIVITY OF *Glycyrrhiza glabra* ROOT EXTRACTS AND ITS PHYTOCHEMICAL SCREENING

Kavita Tiwari* and Hina Alim

Department of Life Sciences, University of Mumbai, Mumbai, India

ABSTRACT

Glycyrrhiza glabra is used as Ayurveda medicine for many decades due to its great and broad medicinal activity. The present study aimed at carrying out phytochemical analysis and evaluates its in vitro antidiabetic, and antioxidant activities of traditional medicinal plant *Glycyrrhiza glabra* which was collected from the nearby area of Mumbai, Maharashtra, India. An in vitro antidiabetic activity was evaluated by alpha-amylase methods and in vitro antioxidant activity by DPPH (2, 2-diphenyl-1- picrylhydrazyl) assay. The Aqueous extracts of *Glycyrrhiza glabra* root showed significant inhibition of alpha-amylase as compared to methanolic extract at various concentrations, whereas methanolic extract showed significant scavenging activity as compared to Aqueous extract at various concentrations. The phytochemical analysis revealed that *Glycyrrhiza glabra* root showed presence of secondary metabolites flavonoids, Saponin, glycosides, terpenoids etc. in different extracts which might be responsible for significant in vitro antidiabetic and antioxidant activities. Identification and isolation of active substances and secondary metabolites from such medicinal plants and their in vivo efficacy evaluation will help in further validation of pharmacological activities.

Keywords: Phytochemical, Anti-diabetic, Antioxidant, In-vitro assays

INTRODUCTION

Ayurveda is a curative and health maintaining science to overcome the deficiencies through a medicinal plant for example *Piper nigrum, Piper Longum. G. glabra* which are found majorly in Middle East and rest of Asia but the use of herbal medicine in developed countries has expanded sharply in the latter half of twentieth century. Plants and their secondary metabolite constituents are traditionally used for curing many diseases and have a long history of use in modern medicine such as atropine, morphine, quinine, vincristine, codiene, digoxine, etc. The pharmacological treatment of disease began long ago with the use of medicinal herbs [1] that grows in various parts of the world. Many medicinal plants are a rich source of a variety of phytochemicals like saponin, triterpenoid, flavonoids, phenolic compounds, alkaloids, which possess many bioactive actions via different mechanisms [2-3]. Due to deformities in carbohydrate, fat, and protein breakdown in diabetes is a failing action of insulin on

target tissue [4]. The main advantages of medicinal plants are, they are economical, readily available and has no side effects. Oxidative stress is produced during normal metabolic process in the body as well as induced by a variety of environmental factor and chemicals. Oxidative stress has been shown to have a significant effect in the causation of diabetes as well as diabetes related complications in human beings [5]. Oxidative stress in diabetes has been shown to co-exist with a reduction in the antioxidant status [6]. Oxidative stress leads to imbalance between oxygen species and antioxidant defense. High level of free radicals in the body can cause many degenerative diseases such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer [7-8]. The damage prevention of free radical associated with diabetes is helped by antioxidant [9]. Phytochemicals like flavonoids, phenolic compounds, phenolic acids, etc., are known to have strong antioxidant action. These kinds of phytochemicals are found in many medicinal plants [10- 11]. The use of modern medicine is largely preferred but leads to many side effects. In the traditional system of medicine, the roots and rhizomes of *G. glabra* (family: Legumenacea/ Fabaceae) have been employed clinically for centuries for their anti-inflammatory, anti-tumor, antiulcer, expectorant, antimicrobial, anti-oxidant and anxiolytic activities [12-13]. *Glycyrrhiza glabra* root extract have been exploited for more than 60 years to treat chronic hepatitis, anti-tussive, mild laxative and anti-aging activities and also have therapeutic benefit against other viruses [14-15]. The most commonly found secondary metabolites (phytochemicals) of the *G. glabra* plant are Saponin, tannins, flavonoids, alkaloids, and terpenoids. The presence of secondary metabolites mostly specifies the uses of plant for traditional medicines [16]. Therefore this study aims at identifying the medicinal potential of *G. glabra* for curing diabetes and oxidative stress without any side effects.

MATERIALS AND METHODS

All the plant materials were collected from the surroundings of Mumbai, Maharashtra, India and identified and authenticated by Botanist. A specimen of plant material was deposited in the Department of Life Sciences, University of Mumbai, India. Collected plant materials were washed with tap water and dried. The material was grounded, and was pulverised an air-tight container for further use.

Preparation of extracts

The root powder of *G. glabra* was used to prepare different solvent extracts (Aqueous and methanol) by Soxhlet extraction method. The extracts were obtained by dissolving 10 grams of the powdered root in 100 ml of each solvent for 24 hours. The solvent was then filtered through Whatman filter paper no.1, and the solvent was evaporated in a rotary evaporator [17] to get extract. The obtained test extracts in sufficient quantities was prepared and stored at -20 ^{o}C which was used to evaluate in-vitro anti-oxidant and anti-diabetic activities.

Phytochemical screening

Total phenolic content

Qualitative phytochemical screening was performed for test extract as per standard procedures [18] with little modification. Gallic acid used as standard for total phenolic content assay. Aboutt 250 µl of extract solution was mixed with 2 ml Folin-Ciocalteu (FC) reagent (diluted 1:10), to it 1 ml solution (75 g/l) of sodium carbonate was added. The mixture was allowed to stand for 1 hour. The observation was made at 760 nm in a UV-vis spectrophotometer

(Shimadzu). Tests were performed in triplicate. Total phenolic content (TPC) was expressed as milligrams of gallic acid equivalents in milligram per gram (mg GAE/g) of dried extract [19].

Determination of total flavonoid content

A preliminary test for flavonoids was performed using the lead acetate, sodium hydroxide reagent, and the results were positive. Thus, the quantitative determination of total flavonoid content was attempted. Quercetin was used as standard to plot a calibration curve. An aliquot of 0.5 mL of test solution was mixed with 0.1 mL of 10% aluminum nitrate, 0.1 mL of potassium acetate, and 4.3 mL of 80% ethanol. The reagents were mixed thoroughly and were incubated for 40 min at room temperature. The observation of absorbance was measured at 415 nm [20]. Yellow coloration of the mixture indicated the presence of flavonoids. The results were expressed as quercetin equivalents in milligram per gram (mg QE/g) of dried extract.

Determination of Carbohydrates

Small quantity of test extract was dissolved in 4ml of double distilled water and then filtered. To the filtrate was added molisch's reagent and further addition of Fehling's reagent to detect the presence of carbohydrates. The presence of brick red colour confirmed the presence of reducing sugar [21].

Determination of Proteins

About 2ml of 10% sodium hydroxide solution was added to 2ml of filtrate solution in a test tube and heated for 10 minutes. To the reaction mixture added a drop of 7% copper sulphate solution. The presence of purplish violet colour indicates presence of proteins [21].

In vitro anti-diabetic activity

Alpha-amylase inhibition was evaluated by Dinitro Salicylic acid (DNSA) method Bernfeld (1955) with little modification. The control solution was prepared by adding all reagents except test or standard. About 100 µl extracts and standard solution (Acarbose 1 mg/ml) were prepared in phosphate buffer solution. The mixture was added with 200 µl α-amylase (from malt) solution and 1 ml starch solution. The solvent was mixed properly and put the reaction mixture in an incubator for 20 minutes. After incubation 1 ml of DNSA reagent was added into the reaction mixture, and the test-tubes were immediately placed in boiled water for 5 min. The test tubes were cooled at room temperature and added with 6 ml distilled water. The observation of reaction mixture was taken at 540 nm in UV-vis spectrophotometer.

% Inhibition = [(A control –A sample)/ A control] ×100

Where;

A control = Absorbance of the control, A sample = Absorbance in the presence of the extract.

In vitro antioxidant activity

In-vitro anti-oxidant activity of *Glycyrrhiza glabra* was estimated by DPPH (1,1-Diphenyl-2-picrylhydrazyl)

Table 1. Total phenolic and flavonoid contents of *G. glabra* root extracts (mean ± SEM, n =3)

Extract	Total phenolic content (mg GAE/g of dry material)	Total flavonoid content (mg QE/g of dry material)
Methanolic extract	137.8 ± 6.7	108.03 ± 2.09
Aqueous extract	132.1 ± 3.04	92.78 ± 4.15

free radical scavenging assay. The DPPH free radical scavenging assay was carried out according to Senguttuvan et al., (2014) with some modifications where ascorbic acid was used as standard. In 1 ml of reaction mixture, different concentration (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, 250µg/ml) 100 ml of test sample were taken. To the reaction mixture 100 ml of methanol was added, followed by addition of 1 ml DPPH solution. The reaction mixture was incubated at room temperature for 30 minutes. Only DPPH was used as negative control. The absorbance was taken at 517 nm using UV-vis spectrophotometer (Shimadzu). The assay was performed in triplicates. The following equation shows the radical scavenging activity of the sample, which is stated as the inhibition percentage.

% Inhibition = [(A control –A sample) / A control] ×100

Where;

A control = Absorbance of the control, A sample = Absorbance in the presence of the extract.

Statistical analysis

All numerical data are expressed as mean ± Standard deviation (SD). Selected data have been analyzed by one-way ANOVA, followed by the Duncan Multiple Range Test (DMRT) to compare the differences in means with SPSS 23.0 statistical software, significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening

Total phenolic content

The root extract of *G. glabra* showed presence of phytochemical properties. The presence of light brick red color and purple colour confirms the presence of carbohydrate and protein content in root extract respectivel. The total flavonoid content and total phenolic content were determined from the calibration curves of quercetin (0.9991) and gallic acid (0.9998), respectively. The total phenolic and total flavonoid contents of different root extracts are presented in Table 1. The results showed that methanol extract possessed the highest phenolic (137.8 ± 6.7) mg GAE/g of dry material and flavonoid components (108.03 ± 2.09) mg QE/g of dry material, as compared to Aqueous extract which is (132.1 ± 3.04) mg GAE/g of dry material and flavonoid components (92.78 ± 4.15) mg QE/g of dry material.

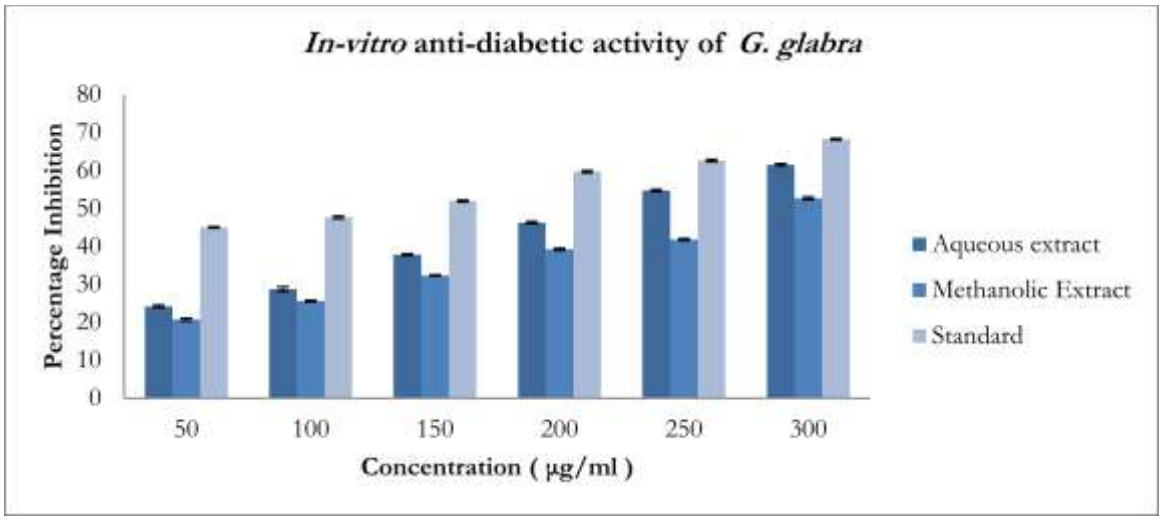

Fig 1. Percent inhibition of alpha-amylase by various extracts of *G. glabra* and acarbose. Values with different superscript were differ significantly at respective concentration ($p<0.05$)

In vitro anti-diabetic activity

The per cent inhibition of alpha-amylase by various test extracts of *G. glabra* is depicted in Figure 1. The methanol extract and aqueous extract of *G. glabra* produced significantly ($p<0.05$) higher inhibition up to 61.49 ± 0.35% and 52.66 ± 0.37% of alpha-amylase at 300 µg/ml concentration respectively. The per cent inhibition of standard acarbose is found to be 68.22 ± 0.24% at 300 µg/ml concentration. The IC50 of the methanol extract was (294.42 ± 0.43) µg•mL−1, IC50 of aqueous extract was of (224.96 ± 0.27) µg•mL−1 and of standard was (114.96± 0.36) µg•mL−1 which twice the concentration of methanolic extract. However, the inhibition by both these extracts was found comparable to inhibition by acarbose which indicates well anti-diabetic activity of *G. glabra* extracts. An alpha-amylase is responsible for the conversion of starch molecules into simple sugar molecules which absorb in the body and raises blood glucose level [22].

In vitro antioxidant activity

The per cent inhibition of DPPH (2, 2-diphenyl-1-picrylhydrazyl) by various root extracts of *G. glabra* is presented in figure 2. The methanol extract and aqueous extract of *G. glabra* produced significantly ($p<0.05$) higher inhibition up to 62.26 ± 0.56% and 53.31 ± 0.23% respectively at 250 µg/ml The per cent inhibition of standard ascorbic acid was found to be 71.46 ± 0.49% at 250 µg/ml. As the positive control, ascorbic acid showed high scavenging activity with IC50 of (3.3 ± 0.01) µg•mL−1 followed by methanolic extract with IC50 of and aqueous extract IC50 of (17.8 ± 0.38) µg•mL−1. The root extract of *G. glabra* showed a concentration-response relationship in DPPH scavenging activity. An increase in concentration is synonymous with an increase in scavenging capacity. It is presumed that the antioxidant activity in plants is mainly due to the presence of phenolic and flavonoid content. As an important phytochemical bearing very reactive hydroxyl group, phenolics have attributed antioxidant action [9-10].

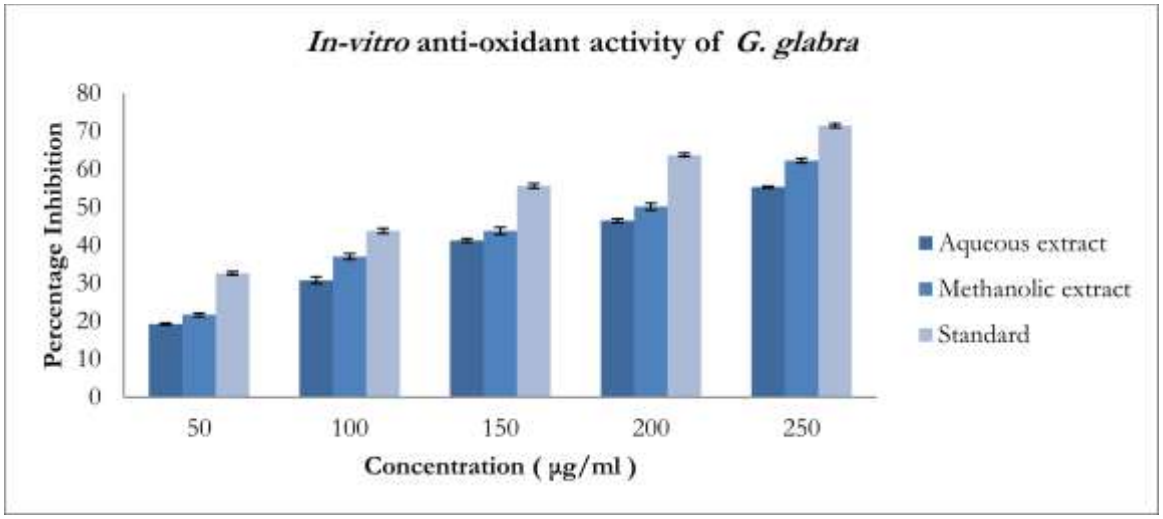

Fig 2. Percent inhibition of DPPH by various extracts of *G. glabra* and ascorbic acid. Values with different superscript were differ significantly at respective concentration ($p<0.05$)

CONCLUSION

Methanolic extracts and aqueous extract of *G. glabra* root have shown good in vitro anti-diabetic activity and anti-oxidant activity. The methanolic extracts comparatively showed better results with respect to aqueous extract. It may be an important drug for prevention of bacterial infection and scavenging of hydroxyl radicals, which are generated during carcinogenesis, respiratory disease. Further, isolation and identification of active principles from *G. glabra* other parts may be helpful to explore pharmacological activity.

ACKNOWLEDGEMENT

The authors acknowledge Mrs. Prerna Malik and Ms. Komal; University of Mumbai and Mr. Brijesh Shukla for their support in research activities.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

SOURCE/S OF FUNDING

No source of funding

REFERENCES

1. Schulz V., Hänsel R. and Tyler V.E., (2001). A Physician's Guide to Herbal Medicine. Ratio. Phytoth., **(4th edt.)**, Berlin, Springer- Verlag, 153.

2. Harborne, J. B. (1973). "Phytochemical methods", London. Chapman and Hall, ltd.: 49-188.

3. Bhatt PR, Pandya KB, Patel UD, Modi CM, Patel HB, & Javia BB (2019). Antidiabetic, antioxidant and anti-inflammatory activity of medicinal plants collected from nearby area of Junagadh, Gujarat. *Annals of Phytomedicine*; **8(2)**: 75-84.

4. Ghosh S, Suryawanshi SA (2001). Effect of vinca rosea extracts in treatment of alloxan diabetes in male albino rats. *Indian Journal of Experimental Biology*; **39**: 748-75.

5. Molyneux. (2004). The use of the stable free radical Diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin *Journal of Science and Technology*; **26(2):** 211-219.

6. Sabu MC & Kuttan R (2004). Antidiabetic activity of Aegle Marmelos and its relationship with its antioxidant properties. *Indian Journal of Physiology and Pharmacology*; **48(1)**: 81–88.

7. Nagappa AN, Thakurdesai PA, Venkatrao N and Singh N (2003). Antidiabetic activity of terminalia catappa Linn fruits. *Journal of Ethnopharmacology*; **88**: 45-50.

8. Kavitha S, Rameshkannan MV, and Mani P (2018). Analysis of antioxidant and antidiabetic activity of piper nigrum leaf extract by in vitro assay. *Journal of Pharmacy and Biological Sciences*; **13(2)**: 53-56.

9. Bidchol AM, Wilfred A, Abhijna P and Harish R (2011). Free radical scavenging activity of Aqueous and Ethanolic extract of Brassica oleracea L. Var. Italica. *Food Bioprocessing and Technology*; **4(7)**:1137-1143.

10. Bhatt PR, Patel UD, Modi CM, Pandya KB, and Patel HB (2019a). Thin layer chromatography and in vitro free radical scavenging activity of few medicinal plants from the surroundings of Junagadh, Gujarat, *Indian Annals of Phytomedicine*; **8(1)**:45-55.

11. Khushwah P, Vir DK, Kayande N, Patidar R (2014). Phytochemical Screening & Evaluation of antimicrobial Activity of *Glycyrrhiza glabra* Linn. *PharmaTutor Magazine*; **2(5)**: 144-147.

12. Bhakuni SD. Drugs from plants. Science Reporter. CSIR. New Delhi, 1990.

13. Nassiri-Asl M. and Hosseinzadeh H., (2008). Review of pharmacological effects of Glycyrrhiza sp. and its bioactive compounds. *Phytotherapy Resources*; **22(1)**: 709–724.

14. Nassiri-Asl M., Saroukhani S. and Zamansoltani F (2007). Anticonvulsant effects of aqueous extract of *Glycyrrhiza glabra* root in PTZ-induced seizure in mice, *International Journal of Pharmacology*; **3(2)**: 432–434.

15. Senguttuvan J, Paulsamy S, and Karthika K (2014). Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. *Asian Pacific ournal of Tropical Biomedicine*; **4(1)**:S359-S367.

16. Manjeet Singh. (2012) Comparative phytochemical and antioxidant study of aqueous extract of *Glycyrrhiza glabra* (Mulethi) &Piper Longum (Long pepper). *International journal of drug research and technology*; **2(2)**: 203-207.

17. Sarker SD, Latif Z and Gray AI (2006). Natural product isolation. 2nd Edn. Humana Press, New Jersey.

18. Harborne AJ (1998). Phytochemical methods-A guide to modern techniques of plant analysis. **3rd edn**. Springer science, Netherland

19. Encarnacão S, de Mello-Sampayoa C, Graça NAG, Catarinob L, Moreira da Silvaa IB, Limaa BS, and Duarte Silvaa OM (2016). Total phenolic content, antioxidant activity and pre-clinical safety evaluation of an Anacardium occidentale stem bark Portuguese hypoglycaemic traditional herbal preparation. *Industrial Crop and Products*; **82**:171-178.

20. Asok kumar K, Umamaheswari M, Baharudeen A (2010). Antioxidant and hepatoprotective activities of the fractions of Ficus microcarpa using in vitro and ex vivo models [J]. *Functional Plant Science and Biotechnology*; **4(1)**: 17-27.

21. Varsha S, Agrawal RC, & Sonam P (2013). Phytochemical screening and determination of anti-bacterial and anti-oxidant potential of *Glycyrrhiza glabra* root extracts. *Journal of environmental Research and Development*; **7(4A)**: 1552.

22. Gopinath BS, Anbu P, Md Arshad MK, Lakshmipriya T, Hong Voon C, Hashim U and Chinni SV (2017). Biotechnological processes in microbial amylase production. *Biomedical Research*; Int.:1-9.