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Nutritional and therapeutic potential of *Glycyrrhiza glabra* L. roots

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Abstract: This study was aimed to estimate the antioxidant, anti-diabetic, anti-amnesic, anti-biofilm, thrombolytic, hemolytic, and cytotoxic effects of *G. glabra* root extracts for nutritive and medicinal purpose. Antioxidant, alpha amylase, antiglycation, anti-amnesic, cytotoxic, thrombolytic and antibiofilm assays were performed. In the current research, *G. glabra* root extracts showed significant antioxidant (80.96 mg GAE/100 g TPC, 38.96 mg CE/100 g TFC, 46% DPPH scavenging), antidiabetic (75% antiglycation, 38.7% α -amylase inhibition), anti-amnesic (5.5%), hemolytic (19.46%), thrombolytic (35.4%) and antibiofilm (72% *P. multocida*, 51.57% *S. aureus* inhibition) activities. *G. glabra* root extracts have remarkable activity to fight against oxidative stress, hyperglycemia, blood coagulation, bacterial growth, DNA damage and memory enhancing ability that ensure its uses in food and pharmaceutical industries.

Keywords: cytotoxic; food additives; *Glycyrrhiza glabra*; root extracts; therapeutic

1. Introduction

Glycyrrhiza glabra L. is among one of the active valuable plant used worldwide because of its healthcare and nutritional values. Indian, Roman, Greek, Chinese and Egyptian civilizations used its dried roots in soups, drinks and teas (Sharma and Agrawal, 2013). It is enriched with biologically active components such as starch, pectin, gum, mucilage, terpenes, saponins, flavonoids, bitters, asparagine, sex hormones (estrogen and progesterone), tannin, resins and sterols that represent 40-50% dry weight of *G. glabra* (Parvaiz *et al.*, 2014). Main component of *G. glabra* is glycyrrhizin that gained importance due to use as artificial sweetening agent in jam, jellies, candies and tobacco products. Plant has been studied by many researchers for its biochemical properties like antidiabetic, antioxidant, antineurotic, anticoagulation, antimutagenic, antihemorrhoid, anti-inflammatory, anti-hepato toxic and antihyperlipidemic. The most important and frequent use of *G. glabra* roots is to cure respiratory disorders such as cough, bronchitis and sour throat (Damle, 2014; Kaur *et al.*, 2013). Although medicinal potential and nutritional efficacies of *G. glabra* has been broadly assessed but investigation on some vital biochemical parameters of its root part are missing. The current research aims to reveal the selected bioactive potential of *G. glabra* roots to eradicate hyperglycemia, reactive oxygen species, psychomotor abnormalities, cytotoxicity, blood clot and bacterial infections.

2. Materials and Methods

2.1. Plant material and extracts preparation

G. glabra roots (liquorice, mulethi) were purchased from Akbari market, Lahore, Pakistan. The sample material was authenticated by Department of Botany, University of Agriculture, Faisalabad. Methanolic extract of sample was partitioned into different solvents which are ethanol, *n*-butanol, *n*-hexane, chloroform, ethyl acetate and aqueous (Mehmood *et al.*, 2012).

2.2. Anti-oxidant profile

Antioxidant profile was assessed by measuring total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical scavenging potential. TPC of the sample extracts were determined by Folin-Ciocalteu colorimetric method (Chaharedhi *et al.*, 2009). Aluminum chloride colorimetric assay was used to determine TFC (Siddique *et al.*, 2010). The extracts antioxidant activity was assessed on the basis of their free radical scavenging potential against stable radical 2, 2-diphenyl 1-picrylhydrazyl (DPPH) (Souri *et al.*, 2008).

2.3. Antidiabetic potential

Antidiabetic profile was estimated by glycation and alpha amylase inhibition. For glycation inhibition test sample extracts were dissolved with bovine serum albumin, glucose, phosphate buffer of pH 7.4 and incubated for 11 days at 37°C (Mastuda *et al.*, 2003). Alpha amylase inhibition assay was performed by incubating samples solutions at 25°C for 90 minutes, along with phosphate buffer (pH 6.9), porcine pancreatic α -amylase (Sigma Aldrich). Now starch solution is added and incubated again for another 30 minutes at 25°C. With the addition of 3, 5 dinitro-salicylic acid reagent solutions were heated in boiling water for few minutes. Percent inhibition was calculated by taking absorbance at 540 nm (Apostolidis *et al.*, 2006).

2.4. Anti-amnesic activity

Acetylcholinesterase effect was analyzed by the procedure described previously (Rahman *et al.*, 2005). 2.8 mL phosphate buffer, 100 μ L 5, 5-dithio-bis-2-nitrobenzoic acid stock solution and 30 μ L acetylcholinesterase enzyme (Sigma-Aldrich Germany, source: *Electrophorus electricus*; electric eel) and 30 μ L fractions were mixed. After incubation for 15 minutes at 25°C, 30 μ L substrate stock solution was added and absorbance at 412 nm was recorded.

2.5. Cytotoxic potential

2.5.1. DNA damage protection assay

For DNA damage protection assay, reaction mixture (supercoiled pBR322 plasmid DNA and extracts) was incubated, following the addition of Fenton reagent and analyzed by agarose gel electrophoresis. The positive control contained Fenton reagent and plasmid DNA whereas plasmid DNA was used as negative control. The results were analyzed by trans-illuminator (Ruma *et al.*, 2013).

2.5.2. Hemolytic activity

This assay was performed according to described procedure (Powell *et al.*, 2000). Red blood cells (RBCs) suspension were mixed with fractions, incubated for 30 minutes at 37°C, centrifuged at 1500 rpm for 5 minutes and supernatant was diluted with phosphate buffer saline. The absorbance was determined at 576 nm.

2.6. Thrombolytic activity

Thrombolytic activity was carried out by taking micro-centrifuge tubes. These were weighed empty, added with blood samples and incubated. Serum was discarded and tubes with clot were again weighed. Clots were added with plant extracts and incubated. After incubation, tubes were again weighed see the difference in weight after clot disruption (Hossain *et al.*, 2012).

2.7. Biofilm inhibition assay

Antibiofilm activity was investigated by using two bacterial strains namely *Staphylococcus aureus* and *Pasteurella multocida*. Plant extracts, nutrient broth and microbial strains were incubated for 24 hr. at 37°C in microtiter plates. Then plates were washed with sodium phosphate buffer saline pH 7.4 and staining was done by crystal violet dye. Absorbance was taken at 630 nm (Dheepa *et al.*, 2011).

2.8. Statistical analysis

The results were expressed as mean \pm standard deviation (S.D) and analyzed by SPSS software (version 22) with level of significance set at $p < 0.05$.

3. Results and Discussion

3.1. Anti-oxidant activity

TPC, TFC and DPPH assay investigations of all fractions showed significant ($p < 0.05$) results. Chloroform fraction showed highest phenolic contents (80.96 ± 0.019 mg GAE). TPC results for other fractions in descending order were as <methanol<ethanol<ethyl acetate<*n*-butanol <aqueous<*n*-hexane. In current research,

TPC contents were in range of 3.14-80.96 mg GAE which are in accordance to earlier reported results. An earlier study reported the TPC in range of 75.7 to 185.7 μg GAE for aqueous and ethanolic extracts of *G. glabra* aerial parts, respectively (Tohma and Gulcin, 2010). Our results are comparatively similar to these earlier findings, although variations may be due to different solvents used and units of measurement used.

Maximum flavonoid contents were shown by chloroform extract (38.96 ± 0.017 mg CE). TFC values for other fractions were found to be in ascending order as: aqueous >*n*-butanol>ethyl acetate>methanol> ethanol>*n*-hexane. Flavonoids possess the antioxidant activities that are important for various diseases produced by free radicals. In present study *G. glabra* fractions exhibited TFC in range of 5.13-38.96 mg CE. Contrary to present results an earlier study described the 2.6-5.1 μg quercetin flavonoid contents (Tohma and Gulcin, 2010). Variation among results is due to use of diverse TFC units.

Optimum DPPH inhibition (46%) was shown by *n*-butanol. Whereas other fractions showed the following DPPH inhibition indescending order: *n*-hexane <aqueous<ethyl acetate<ethanol <chloroform<methanol (Table 1). *G. glabra* root extracts showed DPPH scavenging activity in range of 5.08-46%. Current results are in accordance with the previous investigation that reported antioxidant activity of *G. glabra* roots in range of 42.9-57.5 % (Tohma and Gulcin, 2010). It is concluded from the antioxidant profile that *G. glabra* roots have potential to eliminate free radicals to be used in food industries for preservation of food and to avoid the food spoilage and rancidity. It also protects the biological systems to minimize the detrimental effects of oxidative stress.

3.2. Antidiabetic potential

Range of antiglycation values was 35-75%; $p < 0.05$ and trend in descending order was: chloroform <ethyl acetate<*n*-hexane <aqueous <methanol<ethanol<*n*-butanol (Table 1). Restriction of protein glycosylation process is a vital therapeutic option to reduce diabetic complications. *G. glabra* extracts exhibit up to 75% antiglycation potential. Another study reported the 69% glycation inhibition from *G. glabra* roots owing to the presence of triterpenoid saponin and glycyrrhizin that reduced level of advanced glycation end products (Tupe *et al.*, 2017).

Significant ($p < 0.05$) alpha amylase inhibition ranged from 7.2 to 38.7%. Inhibition percentage for all fractions in descending order was as: *n*-hexane <chloroform<ethyl acetate <*n*-butanol <ethanol <methanol <aqueous (Table 1). About 7.2-38.7% inhibition potential shown by *G. glabra* root extracts in current research was supported by earlier study that stated amylase inhibition range from 24.12 to 51.07% (Rodda *et al.*, 2014). Findings of these results suggested that *G. glabra* root extracts are valuable alternatives sweetening agent used in food products to avoid diabetic complications.

Table 1. Antioxidant and antidiabetic profile.

Fractions/ Extracts	Antioxidant profile			Antidiabetic profile	
	TPC	TFC	DPPH	Antiglycation	Alpha amylase
Methanol	38.96 ± 0.003	12.40 ± 0.015	5.08	37	11
Ethanol	34.96 ± 0.011	15.77 ± 0.002	9.8	35.5	15.5
<i>n</i> -butanol	13.87 ± 0.010	10.18 ± 0.002	46	35	17.6
<i>n</i> -hexane	3.14 ± 0.011	19.63 ± 0.011	24	65	38.7
Chloroform	80.96 ± 0.019	38.96 ± 0.017	8.6	75	35
E. acetate	23.32 ± 0.008	10.68 ± 0.010	10.6	72	20.9
Aqueous	13.32 ± 0.009	5.13 ± 0.022	12	51	7.2
Control	-	-	66.23	61	45

Data expressed as (mean \pm SD) or percentage inhibition. All the extracts showed statistically significant results $p < 0.05$.

TPC: total phenolic contents expressed mg gallic acid equivalents /100 g dry weight; TFC: total flavonoid contents expressed as mg catechin equivalents/100 g dry weight; DPPH: 2, 2-diphenyl 1-picrylhydrazyl. Control: BHT; butylated hydroxy toluene (antioxidant), metformin (antiglycation), glucobay (alpha amylase inhibition).

3.3. Anti-amnesic activity

Maximum acetylcholinesterase (AChE) inhibitory potential (5.5%) was shown by ethanol fraction. Whereas other extracts showed inhibition in ascending order as: ethyl acetate >aqueous> methanol >*n*-butanol >chloroform >*n*-hexane (Table 2). AChE inhibition range shown by *G. glabra* was 3.12-5.5%. An earlier investigation (Young *et al.*, 2013) perceived that glabridin containing aqueous extract suppressed the neuron cell

death by inhibiting the AChE activity up to 11.1%. As AChE enzyme is involved in the termination of impulse transmission by rapid hydrolysis of the neurotransmitter acetylcholine. Therefore, current study demonstrated the impressive ability of *G. glabra* root extracts to regulate hormonal imbalance involved in neurodegenerative disorders.

3.4. Cytotoxic potential

3.4.1. DNA damage protection assay

Chemical constituents of *G. glabra* organic extracts have the potential to protect the DNA. As shown in Figure 1, *n*-butanol, *n*-hexane and aqueous fractions have no ability to protect DNA from Fenton-induced damaged besides other fractions (methanol, ethanol, ethyl acetate, chloroform) which protected DNA from distortion (Figure 1). Cell life cycle is affected by damage to nucleic acids, which have been linked to cell cycle regulation and repair pathways. Pretreatment of plasmid DNA with *G. glabra* root extracts and fractions secured DNA with similar efficacies, as four fractions (methanol, ethanol, ethyl acetate, chloroform) saved DNA from oxidative stress. Similar effect was attributed by glycyrrhetic acid component of *Glycyrrhiza uralensis* that exhibit 20-80% protection to DNA (Chen *et al.*, 1994). It is concluded that *G. glabra* root extracts provide encouraging results against DNA alterations associated with positive health effects.

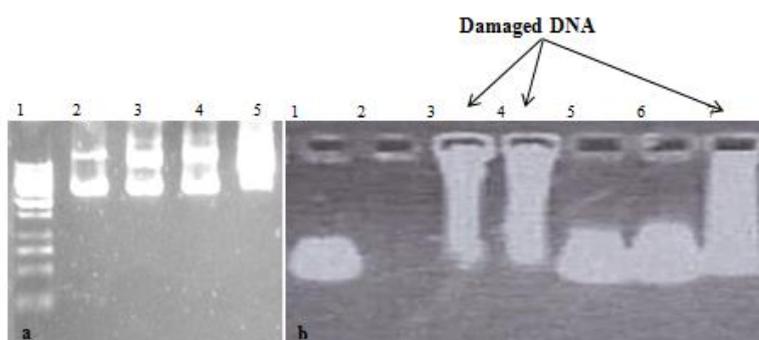


Figure 1 (a-b). Agarose gelelectrophoresis of DNA-damage protection assay.

a: Lane 1: ladder; Lane 2: positive control; Lane 3: negative control (FeSO_4); Lane 4 and 5: negative control (H_2O_2), b: Lane 1-7: plasmid DNA incubated with methanol, ethanol, *n*-butanol, *n*-hexane, chloroform, ethyl acetate and aqueous extracts respectively.

3.4.2. Hemolytic activity

Cytotoxic potential of *G. glabra* was investigated against human red cells through hemolytic assay. Ethyl acetate (19.46%) being the most active fraction in hemolysis. Antihemolytic potential of other fractions in ascending order was as: ethanol > methanol > aqueous > chloroform > *n*-butanol > *n*-hexane (Table 2). Hemolysis is mainly caused by oxidative stress that leads to erythrocytes lysis. *G. glabra* root extracts had up to 19.46% hemolytic ability. Current results are in agreement with the previous research that elucidated the 16% antihemolytic potential of *G. glabra* root extracts against human erythrocytes (Kim *et al.*, 2002). The cytoprotective efficacy of *G. glabra* root ingredients has future prospects in minimizing the hemolytic impediments.

3.5. Thrombolytic activity

Highest clot distortion activity was (35.4%) exhibited by methanol extract and rest of the fractions showed descending order as: chloroform < aqueous < *n*-butanol < ethyl acetate < ethanol < *n*-hexane (Table 2). Cardiovascular disorders are mostly caused by blood clot or thrombus formation at an alarming rate. Uncontrolled platelet accumulation can be life threatening. Anticoagulation potential of *G. glabra* root extracts was up to 35.5%. Literature survey indicated impressive thrombus inhibition (34.8%) by glycyrrhizin (Francischetti *et al.*, 1997). The present optimistic results reflect the usage of *G. glabra* root in form of herbal tincture as cardio protective and sport nutrition formulas.

3.6. Biofilm inhibition assay

For *P. multocida*, ethylacetate (72%) and for *S. aureus*, two fractions ethanol and *n*-butanol (51.57%) fractions showed optimal growth repression (Table 2). Descending trend of inhibition by other fractions against *P. multocida* was as: methanol > *n*-hexane > *n*-butanol > ethanol > chloroform > aqueous (Figure 2 a-b). Ascending order of *S. aureus* inhibition was as: ethyl acetate < aqueous < *n*-hexane < chloroform < methanol (Figure 2 c-d). Biofilm are microbial colonies adhering to a specific place that cause persistent chronic and tenacious

infections. Discovery of natural antimicrobial agents is needed to lessen their detrimental complexities. Optimum growth inhibition (72%, 51.57%) was depicted by *G. glabra* root extracts against *P. multocida* and *S. aureus*. Available data indicates no similar approach of evaluating anti-bacterial activity of *G. glabra* root extracts using *P. multocida* and *S. aureus* strains. *Current dynamic results* unveiled promising future of *G. glabra* root extracts to be used in toothpaste, cosmetics, chewing gum, beverages and in food items by improving the food safety assurance for consumers by killing infectious agents.

Table 2. Thrombolytic, hemolytic, anti-amnesic and anti-biofilm activities.

Fractions/ Extracts	% Inhibitions				
	Thrombolysis	Hemolysis	AChE	<i>S. aureus</i>	<i>P. multocida</i>
Methanol	35.4	3.8	5.2	50.12	57.05
Ethanol	4.1	3.4	5.5	51.57	47.68
<i>n</i> -butanol	7.9	8	5.3	51.57	49.05
<i>n</i> -hexane	3.2	10.6	5.49	27.19	54.84
Chloroform	12.9	7.6	5.46	45.50	45.57
Ethyl acetate	6.1	19.46	3.12	11.23	72
Aqueous	12.88	4.14	3.4	17.03	6.31
Control	50	82	59.5	82.2	83.79

Data is presented as percentage.

Positive control: citric acid (thrombolytic assay), Triton-X (hemolytic assay), physostigmine (AChE: acetyl cholinesterase inhibition), ampicillin (biofilm assay).

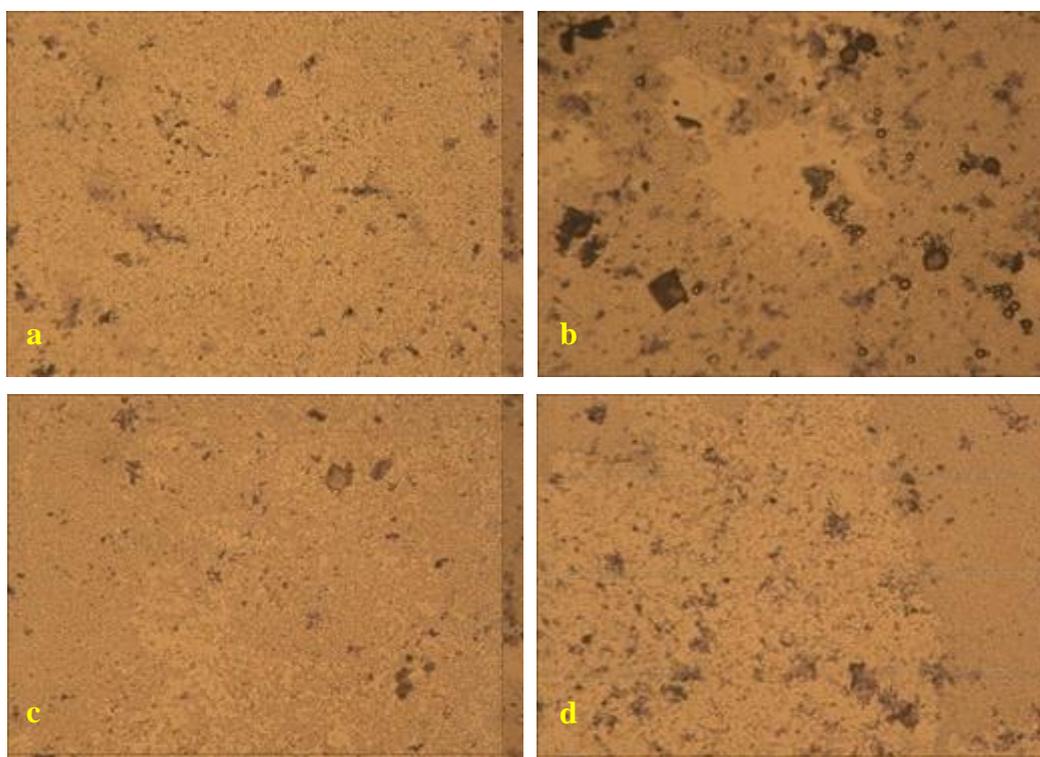


Figure 2. Antibiofilm assay-lowest and highest inhibition (a, b) *P. multocida* (c, d) *S. aureus*

4. Conclusions

On the basis of current study it is recommended that *G. glabra* root bioactive constituents are applicable to improve the food quality, safety, texture, taste, aroma and flavor. Since these *G. glabra* root extracts are well tolerated, relatively inexpensive and readily available, could provide complementary therapy in treatment of various diseases. However there is a need to further explore its bioactive constituent's to enhance human health status using animal trial.

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Conflict of interest

None to declare.

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