

Original Research

Received 09 March 2026

Revised 12 April 2026

Accepted 25 May 2026

Natural Resources for Human
Health 2026, Vol. 6 (15s): 1474–1495

KEYWORDS:

Glycyrrhiza glabra, phytochemicals,
antioxidant activity, HPTLC,
anticancer activity, MCF-7

“Phytochemical Characterization, Antioxidant Potential and In Vitro Anticancer Activity of Glycyrrhiza glabra L.”

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ABSTRACT: Cancer remains a leading global health challenge, necessitating the exploration of safer and more effective therapeutic agents. The present study evaluates the physicochemical properties, phytochemical composition, antioxidant potential, and in vitro anticancer activity of Glycyrrhiza glabra L. root extracts. Physicochemical analysis revealed total ash (7.92%), acid-insoluble ash (0.62%), water-soluble ash (1.11%), and moisture content (7.67%), indicating good quality and purity. Phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, terpenoids, and glycosides. Quantitative estimation showed high phenolic (46.15 mg/g) and flavonoid (39.81 mg/g) content. Antioxidant assays demonstrated significant activity, with aqueous extract exhibiting higher total antioxidant capacity (35.82 mM/g) and ferric reducing power compared to methanolic extract. Nitric oxide scavenging activity also increased with concentration. HPTLC fingerprinting confirmed the presence of multiple bioactive constituents. In vitro anticancer activity against MCF-7 breast cancer cell line using SRB assay indicated cytotoxic potential of the extract. The findings suggest that G. glabra is a promising source of natural antioxidants and anticancer agents, warranting further investigation.

INTRODUCTION

Cancer is a complex group of diseases characterized by uncontrolled cell proliferation, genetic mutations, and the ability to invade surrounding tissues and metastasize to distant organs. It remains one of the leading causes of mortality worldwide, with increasing incidence particularly among ageing populations. Despite advances in conventional therapies such as surgery, chemotherapy, and radiotherapy, treatment is often associated with adverse effects, drug resistance, and recurrence, highlighting the need for safer and more effective therapeutic strategies (Sakarkar & Deshmukh, 2011; Baskar et al., 2012).

Natural products, especially those derived from medicinal plants, have gained considerable attention as potential sources of anticancer agents. A significant proportion of modern drugs are plant-derived, including well-known compounds such as paclitaxel and vincristine. Medicinal plants are rich in phytochemicals such as alkaloids, flavonoids, phenolics, and terpenoids, which exhibit diverse biological activities including antioxidant, anti-inflammatory, and anticancer effects (Sofowora, 1993; Gilani & Rahman, 2005).

Glycyrrhiza glabra L. (licorice), a member of the Fabaceae family, is a widely used medicinal plant in traditional systems such as Unani medicine. Its roots and rhizomes are known for their anti-inflammatory, expectorant, antioxidant, and therapeutic properties, and are commonly used in the treatment of respiratory, gastrointestinal, and inflammatory disorders (Chevallier, 1996; Bruneton, 1995). The plant contains several bioactive constituents, including glycyrrhizin, flavonoids, and phenolic compounds, which contribute to its pharmacological potential.

Standardization of herbal drugs is essential to ensure their quality, safety, and efficacy. Physicochemical parameters such as ash values, moisture content, extractive values, and heavy metal analysis are commonly used for quality assessment, while advanced techniques like High-Performance Thin Layer Chromatography (HPTLC) enable fingerprint profiling of phytoconstituents. Additionally, evaluation of antioxidant activity is important, as oxidative stress plays a key role in the pathogenesis of cancer and other degenerative diseases (Mandal et al., 2009).

Therefore, the present study aims to investigate the physicochemical properties, phytochemical composition, antioxidant potential, and in vitro anticancer activity of *Glycyrrhiza glabra*, to explore its potential as a natural therapeutic agent.

MATERIAL AND METHODS

Collection of sample

The selected *Glycyrrhiza glabra* L. used in the present study were collected from the local market, Kalyan. It was further identified and authenticated from the Department of Botany, Agharkar Institute, Pune.

The dried *Glycyrrhiza glabra* L. roots was then powdered using mixer grinder and stored in air tight bottles and used for further quality control studies and other parameters were carried out.



Figure 1: *Glycyrrhiza glabra* L.

Quality Control Studies

The dried powder of *Glycyrrhiza glabra* L. was used to study physicochemical parameters such as total ash content, acid insoluble ash, water soluble ash, loss on drying, foreign organic matter, methanol and water soluble extractives and heavy metal content. The above parameters were studied as per standard method of API guidelines (API, 2006).

Ash values

Ash value is useful in determining authenticity and purity of drugs and also these values are important quantitative standards (Kokate et al., 2005).

For the estimation of total ash value, 2 g of dried plant powder was taken in a preweighed silica crucible and the crucible was incinerated in Muffle furnace by gradually increasing the temperature at 450°C till carbon free ash is obtained. Crucible was cooled, weighed and the procedure was repeated till it reached a constant value and the percentage of total ash was calculated.

For acid insoluble ash value, total ash obtained was boiled with 25 ml of 2N dilute hydrochloric acid for 5-10 minutes to remove acid insoluble materials and residue of acid insoluble ash. The insoluble matter was collected on an ash-less filter paper and washed with warm water, dried the filter paper, ignited in Muffle furnace. Crucible was cooled, weighed and the procedure was repeated till it reached a constant value and the percentage of acid insoluble ash was calculated.

For water soluble ash value, total ash obtained was boiled with 25 ml of distilled water for 5-10 minutes. The insoluble matter was collected on an ash-less filter paper, washed with warm water and ignited in Muffle furnace. The residue was weighed and then the percentage of acid insoluble ash was calculated with reference to the air dried drug.

Loss on drying

About 2 g of the powdered plant sample was weighed into a thin porcelain dish. Dried in the oven at 100°C. Cooled in a desiccators and weighed. The loss in weight was recorded as moisture (API, 2006).

Foreign organic matter

To identify the presence of unwanted matters in the powder, percentage of foreign matters is determined. 5 grams of dried plant powder is spread on a paper to form a thin layer. The sample was examined with unaided eye or using a magnifying lens (6x or 10x) and the foreign matter was separated and picked out. The separated matter was weighed and its percentage was determined (API, 2006).

Extractive values

Extractive values are useful for the evaluation of crude drug (Khandelwal, 2008). Methanol and water-soluble extractive values were determined to find out the amount of alcohol and water soluble components. For extractive value, 5 g of air-dried powdered sample was macerated with 100 ml of each solvent separately and kept on rotary shaker for 24 hrs. Using a whatmann No. 1 filter paper the extract was filtered and the 25 ml of the filtrate was evaporated to dryness and dried at 100°C to constant weight and percent w/w of extractive value was calculated.

Heavy metal determination

In the present work, arsenic, cadmium, lead and mercury were estimated from dried powder of *Glycyrrhiza glabra* L. by inductively coupled plasma atomic emission spectroscopy (ICP-AES) technique. The dried powder was digested in nitric acid and perchloric acid (4:1) and resultant residue were diluted in 2 % nitric acid and used for estimation.

Fluorescence study

The distinguishing characters of dried plant powder was identified by treating the powder with chemicals and finding characteristic fluorescence under visible and UV light. Small quantity of these peel powders were treated separately with different reagents and solvents like sodium hydroxide, ammonia, nitric acid, acetic acid, hydrochloric acid, iodine, ferric chloride, sulphuric acid, potassium dichromate, acetone, methanol, ethanol, chloroform, petroleum ether and n-butanol which were further observed under short wavelength at 254 nm, long UV wavelength at 366 nm and visible light and the changes in color of these powdered samples were noted down in order to study their fluorescence behavior (Kokoshi *et al.*, 1958).

Phytochemical analysis

To get an idea about the type of phytoconstituents present in the sample. Hence, the powdered samples of *Glycyrrhiza glabra* L. was subjected to qualitative and quantitative phytochemical analysis.

Qualitative phytochemical analysis

Preparation of extracts:

5 g of dried powder of *Glycyrrhiza glabra* L. was extracted in 50 ml of petroleum ether, chloroform, ethyl acetate, methanol, ethanol, butanol, acetone and distilled water. The content were filtered through Whatman filter paper No. 1 and filtrates were concentrated on water bath to half of the initial total volume. The above extracts were used to detect presence of various nutritional contents and phytoconstituents by following standard tests and the results were reported.

Qualitative phytochemical evaluation

Petroleum ether, chloroform, ethyl acetate, methanol, ethanol, butanol, acetone and distilled water extracts of dried powder of *Glycyrrhiza glabra* L. was tested for the presence or absence of various phytochemicals using standard methods described by Trease and Evans (1989) as well as Sofowora (1993).

Quantitative phytochemical estimation

The dried powder of *Glycyrrhiza glabra* L. quantitatively estimated for total carbohydrate content by anthrone method (Sadasivam and Manickam, 2008), reducing sugars by DNSA method (Sofowora, 1993), total amino acid by ninhydrin method (Harborne, 1998), total protein content by Lowry method (Lowry *et al.*, 1951), total phenolic content by Folin-ciocalteu method (Lobo *et al.*, 2010), total flavonoid content by aluminium chloride method (Hazra *et al.*, 2008) and total tannin content by ferric chloride reagent (Harborne, 1998).

Mineral determination

In the present work, sodium, calcium, copper, zinc, iron, potassium, manganese, magnesium, phosphorus and aluminium were estimated from dried powder of *Glycyrrhiza glabra* L. by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) technique. The dried powder was digested in nitric acid and perchloric acid (4:1) and resultant residue was diluted in 2% nitric acid and used for estimation.

HPTLC fingerprinting

Merck silica gel 60 F 254 TLC pre-coated plates were spotted with methanolic extract of *Glycyrrhiza glabra* L. using CAMAG Linomat 5 sample applicator. Ethyl acetate: Formic acid: Acetic acid: water (15:1:1:2) was used as the solvent system to resolve the phytochemical components. After derivatization with 10 % methanolic sulphuric acid reagent, the plates were scanned with CAMAG Scanner 5 equipped with winCATS Version 1.4.8 Planar Chromatography manager software. The study was carried out at Anchrom Enterprises, Mumbai.

Antioxidant analysis

Aqueous and methanolic extracts of dried powder of *Glycyrrhiza glabra* L. were evaluated for *in vitro* antioxidant activity. Antioxidant activity was carried out by Total antioxidant activity (Prieto *et al.*, 1999), Ferric reducing antioxidant power (Oyaizu, 1986) and Nitric oxide radical scavenging activity (Garraat, 1964). Total phenol content and total flavonoid content were estimated by using the methods of Kumar *et al.*, 2007 and Patel *et al.*, 2010 respectively.

In vitro anticancer activity

Experimental procedure for SRB assay

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 μ L at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO₂, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

Experimental drugs were solubilized in appropriate solvent at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100 μ g/ml, 200 μ g/ml, 400 μ g/ml and 800 μ g/ml with complete medium containing test article. Aliquots of 10 μ L of these different drug dilutions were added to the appropriate microtiter wells already containing 90 μ L of medium, resulting in the required final drug concentrations i.e. 10 μ g/ml, 20 μ g/ml, 40 μ g/ml, 80 μ g/ml.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 μ L of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 μ L) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength.

Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100.

Using the six absorbance measurements [time zero (T_z), control growth (C), and test growth in the presence of drug at the four concentration levels (T_i)], the percentage growth was calculated at each of the drug concentration levels.

Percentage growth inhibition was calculated as: $[T_i/C] \times 100 \%$

This study was carried out at ACTREC, Navi Mumbai.

RESULT AND DISCUSSION

Determination of Physico-Chemical Constants

The physicochemical constants viz., ash values, moisture content and extractive values helped in formulating pharmacopoeia standards to evaluate the peel powder sample.

Ash value

Ash value is a measure of the identity and purity of *Glycyrrhiza glabra* L. powder, since it is an indication of approximate measure of their chemical constituents. It is used to determine quality and purity of a crude drug and to establish the identity of it. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. These are present in definite amount in a particular crude drug. Hence, quantitative determination in terms of various ash values may help in their standardization.

Sometimes, inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects 'Total ash value'. Such variables are removed by treating with acid, as they are soluble in hydrochloric acid and acid insoluble ash is determined (Khandelwal, 2008).

Percentage of total ash content was found to be 7.92 ± 0.023 indicating presence of inorganic salts of carbonates, phosphates, silicates of sodium, potassium, calcium and magnesium. The percentage of acid insoluble ash content was found to be 0.62 ± 0.020 % indicating that very small amount of the inorganic component is insoluble in acid. Percentage of water soluble ash content was found to be 1.110 ± 0.015 % (Table 1 and Figure 2).

Loss on drying

The moisture content of the crude drug is responsible for its decomposition due to microbial attack or chemical changes since it provides suitable condition for the proliferation of microorganisms. This parameter was carried out to measure the amount of water and volatile compounds in a sample, when dried under specified conditions (API, 2006).

Loss on drying was found to be 7.67 ± 0.01 % in the powdered sample (Table 1 and Figure 2).

Table 1: Percentage of total ash content, acid insoluble ash content, water soluble ash content and Loss on drying in dried powdered sample

<i>Glycyrrhiza glabra</i> L. powder	Content in percentage
Total ash content	7.92 ± 0.023
Acid insoluble ash content	0.62 ± 0.020
Water soluble ash content	1.110 ± 0.015
Loss on drying	7.67 ± 0.01

Values are mean $\pm$ S.D of three readings

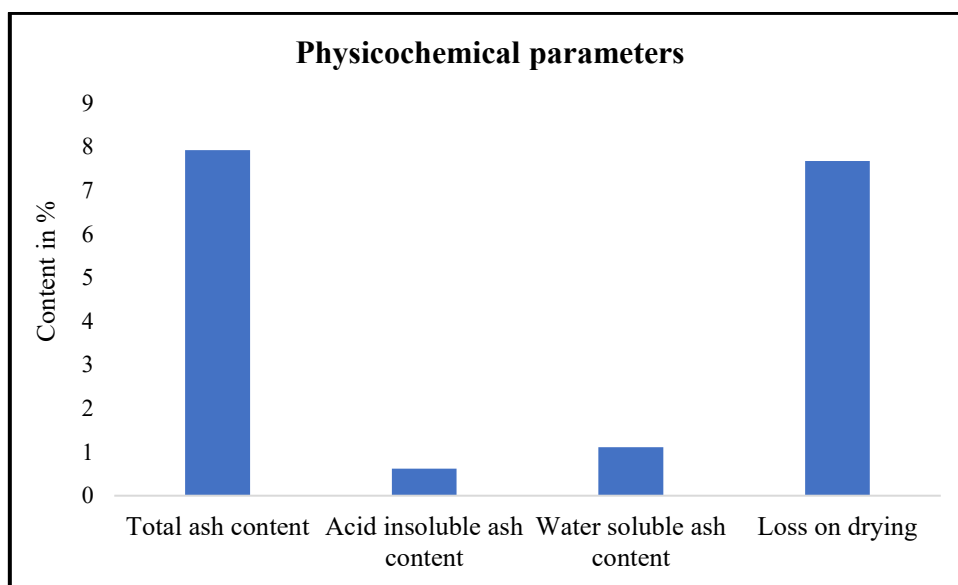


Figure 2: Percentage of total ash content, acid insoluble ash content, water soluble ash content and Loss on drying in dried powdered sample

Foreign organic content

The collected plant material was free from visible signs of contamination by molds or insects and other animal contaminants. It was without abnormal odour or any discolouration, etc. Thus, no foreign organic matter were found to be present.

Extractive value

The determination of extractable matter refers to the amount of constituents in plant materials which is extracted by specific solvents. It indicates the nature of constituents present in plant drug and also helps in detecting low grade exhausted plant materials (API, 2006).

In the present study, water and methanol soluble extractive values were determined in powder of *Glycyrrhiza glabra* L. Water soluble extractive value was found to be 25.89 ± 0.035 % and Methanol soluble extractive value was found to be 23.31 ± 0.040 % (Table 2 and Figure 3). The results showed high water soluble extractive value in comparison to the methanol soluble extractive value. It indicates the possibility of considerable amount of polar compounds and presence of large quantity of water soluble constituents such as sugar, glycosides, phenolics and tannins.

Table 2: Percentage of water soluble and methanol soluble extractive value in *Glycyrrhiza glabra* L. powder

<i>Glycyrrhiza glabra</i> L. powder	Content in percentage
Water soluble extractive value	25.89 ± 0.035
Methanol soluble extractive value	23.31 ± 0.040

Values are mean $\pm$ S.D of three readings

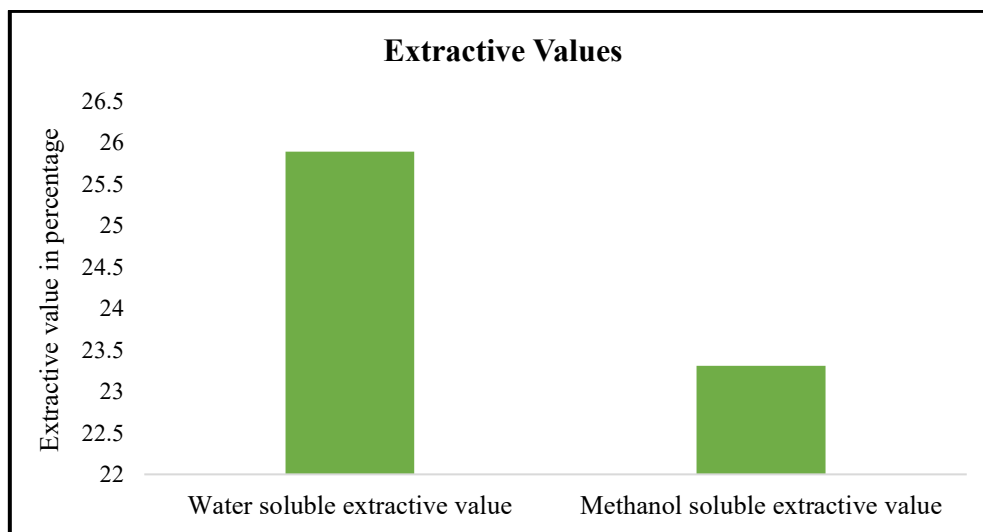


Figure 3: Water soluble and methanol soluble extractive value

Fluorescence analysis

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material. Some constituents show fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products, which do not visibly fluoresce in daylight. It is an important parameter of pharmacognostical evaluation. Fluorescence analysis helps in distinguishing the drug in powder form. Powder of *Glycyrrhiza glabra* L. was treated with chemicals under characteristic fluorescence, under visible light and UV light that can be useful in the detection of adulterants (Table 3).

Table 3: Fluorescence analysis of *Glycyrrhiza glabra* L. powder

Sr. No.	<i>Glycyrrhiza glabra</i> L. powder	Colour in Ordinary Light	Colour in UV Light with different wavelength	
			254 nm	366 nm
1.	Powder	Buff	Buff	Buff
2.	Powder + 1N HCl	Buff	Brown	Brown
3.	Powder + 1N NaOH	Yellow	Green	Green
4.	Powder + Conc. H ₂ SO ₄	Brown	Black	Black
5.	Powder + Conc. HNO ₃	Brown	Black	Black
6.	Powder + Iodine	Brown	Black	Black
7.	Powder + Ammonia	Yellow	Green	Green
8.	Powder + FeCl ₃	Yellowish Green	Black	Black
9.	Powder+ K ₂ Cr ₂ O ₇	Yellowish orange	Black	Black
10.	Powder + Water	Buff	Brown	Brown
11.	Powder + Acetone	Buff	Brown	Buff
12.	Powder + Acetic acid	No Change	Brown	Brown

13.	Powder + Methanol	No Change	Brown	Brown
14.	Powder + Ethanol	No Change	Brown	Brown
15.	Powder + Chloroform	Buff	Brown	Buff
16.	Powder+ Petroleum ether	No Change	Brown	Brown
17.	Powder + Butanol	No Change	Brown	Brown

Heavy Metal Content

Heavy metals are environmental pollutants and many of them are toxic even at very low concentrations. Pollution of the biosphere with toxic metals has accelerated dramatically since the beginning of the industrial revolution. The primary sources of this pollution are the burning of fossil fuels, the mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides and sewage (Memom *et al.*, 2001).

In many ways, living plants can be compared to solar driven pumps which can extract and concentrate several elements from their environment. From soil and water, all plants have the ability to accumulate heavy metals which are essential for their growth and development. These metals include magnesium, iron, manganese, zinc, copper, molybdenum and nickel. Certain plants also have the ability to accumulate heavy metals which have no known biological function. These include Cd, Cr, Pb, Co, Ag, Se and Hg. However, excessive accumulation of these heavy metals can be toxic to most plants and ultimately to animals and human beings. The ability to both tolerate elevated levels of heavy metals and accumulate them in very high concentrations has evolved both independently and together in a number of different plant species (Memon *et al.*, 2001).

In the present study, the heavy metal concentrations assessment was done for metals *viz.*, arsenic, cadmium, lead and mercury.

Arsenic

Toxic metal arsenic is not required by the body and cause serious effects upon exposure even at very low concentrations. No traces of Arsenic was found in *Glycyrrhiza glabra* L. (Table 4).

Cadmium

Cadmium was found to be absent in *Glycyrrhiza glabra* L. (Table 4)

Mercury

Mercury has a number of effects on humans, which includes disruption of the nervous system, damage to brain functions, DNA damage and chromosomal damage. It causes allergic reactions, resulting in skin rashes, tiredness and headaches. Mercury can cause sperm damage, birth defects and miscarriages. In *Glycyrrhiza glabra* L. powder no traces of Mercury was found (Table 4).

Lead

Lead is one of the most toxic metals. It is well known for its adverse effects on many parts of the body. Progressive exposure to lead results in a decrease in the performance of the nervous system and affects renal clearance. Lead was found to be absent in *Glycyrrhiza glabra* L. (Table 4).

Table 4: Heavy metal content in ppm in powder of *Glycyrrhiza glabra* L.

Powder	Heavy metal content in ppm			
	Lead	Arsenic	Cadmium	Mercury
	ND	ND	ND	ND
Permissible limits of heavy metal in ppm (API, 2006).	10	3	0.3	1

Key: ND- Not Detected

As per WHO guidelines quality of medicinal or herbal plant is mandatory before using for consumption (WHO, 2000). The above physico-chemical parameters will help in judging the purity and quality of the plant material. In the present work, data obtained from physicochemical parameters *viz.* ash value, extractive value, loss on drying and heavy metal content will be useful for maintaining the quality and can be considered as parameters to establish the authenticity of plant material.

Phytochemical Studies

A] Qualitative phytochemical evaluation

Aqueous, methanol, ethanol, petroleum ether, chloroform, ethyl acetate, butanol and acetone extracts of *Glycyrrhiza glabra* L. were studied for the presence of phytochemicals using standard qualitative test. Results showed the presence of alkaloids, phenols, flavonoids, terpenes, phytosterols, amino acids, proteins, carbohydrates, cardiac glycosides, steroids and tannins.

Carbohydrates, proteins, amino acids and reducing sugars were found to be present in aqueous extracts of *Glycyrrhiza glabra* L. Alkaloids and phenolics and tannins were found to be present in aqueous, methanolic, ethanolic extracts of *Glycyrrhiza glabra* L. Flavonoids were found to be present in aqueous, methanolic, ethanolic, petroleum and acetone extracts of *Glycyrrhiza glabra* L. Saponins were found to be present in aqueous extracts. Phlobatannins were absent in all the extracts of *Glycyrrhiza glabra* L. Anthraquinones were found to be present in methanolic, ethanolic and acetone extracts. Cardiac glycosides were found to be present in all the extracts of except in petroleum ether extract. Phytosterols were found to be present in acetone, ethyl acetate and chloroform extracts. Steroids and terpenoids were found to be present in all the extracts of except in petroleum ether extract.

B] Quantitative phytochemical estimation

Carbohydrate, protein, amino acid, reducing sugar, phenol, flavonoid and tannin were quantitatively estimated by using standard method from *Glycyrrhiza glabra* L.

Total carbohydrate content

Total carbohydrate content from *Glycyrrhiza glabra* L. was estimated by anthrone method. The linear equation for total carbohydrate content was found to be $y = 0.0045x$ with $R^2 = 0.9920$. The total carbohydrate content was $41.10 \pm 0.010\text{g}/100\text{g}$ (Table 5 and Figure 4).

Total reducing sugar content

Total reducing sugar content was estimated by DNSA method from the *Glycyrrhiza glabra* L. powder. The linear equation for total reducing sugar content was found to be $y = 0.4351x$ with $R^2 = 0.9912$. The total reducing sugar content was found to be $5.82 \pm 0.021\text{g}/100\text{g}$ (Table 5 and Figure 4).

Total amino acid content

Total amino acid content of *Glycyrrhiza glabra* L. was estimated by ninhydrin method. The linear equation for total amino acid content was found to be $y = 0.0056x$ with $R^2 = 0.9932$. The total amino acid content was found to be $1.70 \pm 0.012\text{g}/100\text{g}$ (Table 5 and and Figure 4).

Total protein content

Total protein content was estimated by Folin Lowry method from *Glycyrrhiza glabra* L. The linear equation for total protein content was found to be $y = 0.0016x$ with $R^2 = 0.9903$. The total protein content was found to be $13.061 \pm 0.001\text{g}/100\text{g}$ (Table 5 and Figure 4).

Total phenolic content

Total phenolic content was estimated by Folin- Ciocalteu method from *Glycyrrhiza glabra* L. The linear equation for total phenolic content was found to be $y = 21.516x$ with $R^2 = 0.9912$. The total phenol content was found to be $46.15 \pm 0.010\text{ mg}/\text{gm}$ (Table 6 and Figure 5).

Total flavonoid content

Total flavonoid content was estimated by ethanolic aluminium chloride method from *Glycyrrhiza glabra* L. The linear equation for total flavonoid content was found to be $y = 8.0190x$ with $R^2 = 0.9931$. The total flavonoid content was found to be 39.81 ± 0.011 mg/gm (Table 6 and Figure 5).

Total tannin content

Total tannin content in *Glycyrrhiza glabra* L. was estimated by $AlCl_3$ method. The linear equation for total tannin content was found to be $y = 8.0193x$ with $R^2 = 0.9928$. The total tannin content was found to be 15.12 ± 0.021 mg/gm (Table 6 and Figure 5).

Table 5: Total carbohydrate, Total reducing sugar, Total amino acid and Total protein content in *Glycyrrhiza glabra* L. powder

Sr. No.	Nutritional components	Content in g/100g
1.	Total carbohydrate content	41.10 ± 0.010
2.	Total reducing sugar content	5.82 ± 0.021
3.	Total amino acid content	1.70 ± 0.012
4.	Total protein content	13.061 ± 0.001

Values are mean of three readings

Figure 4: Total carbohydrate, Total reducing sugar, Total amino acid and Total protein content in *Glycyrrhiza glabra* L. powder

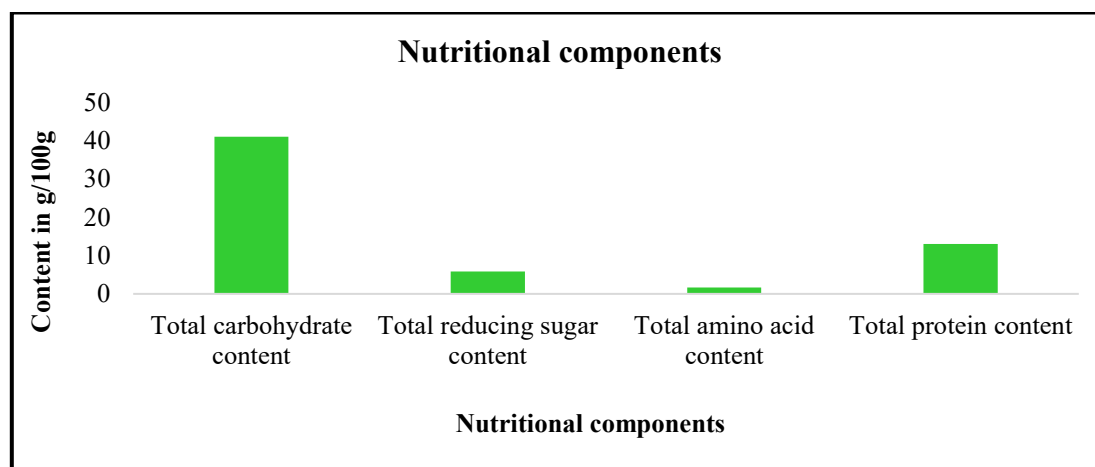


Table 6: Total phenolic, Total flavonoid and Total tannin content in *Glycyrrhiza glabra* L. powder

Sr. No.	phytoconstituents	Content in mg/gm
1.	Total phenolic content	46.15 ± 0.010
2.	Total flavonoid content	39.81 ± 0.011
3.	Total tannin content	15.12 ± 0.021

Values are mean of three readings

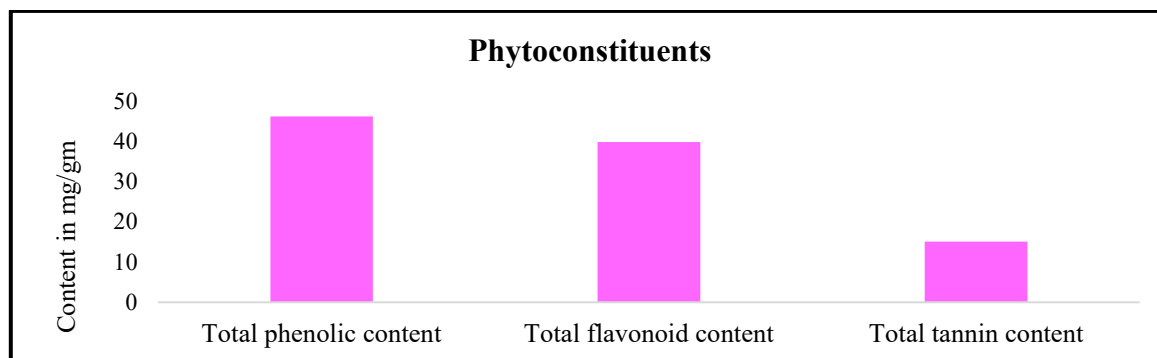


Figure 5: Total phenolic, Total flavonoid and Total tannin content in *Glycyrrhiza glabra* L. powder

Determination of mineral content

The content of different minerals were found out using ICP-AES. The results are given in table no 7 and Figure 6.

Table 7: Different mineral content in *Glycyrrhiza glabra* L. powder

Minerals	Content in ppm
Sodium	2940.67
Phosphorus	1109.9
Zinc	9.24
Copper	6.762
Magnesium	3283.74
Aluminium	733.25
Manganese	11.04
Potassium	7784.01
Calcium	11257.10
Iron	427.59
Chromium	ND

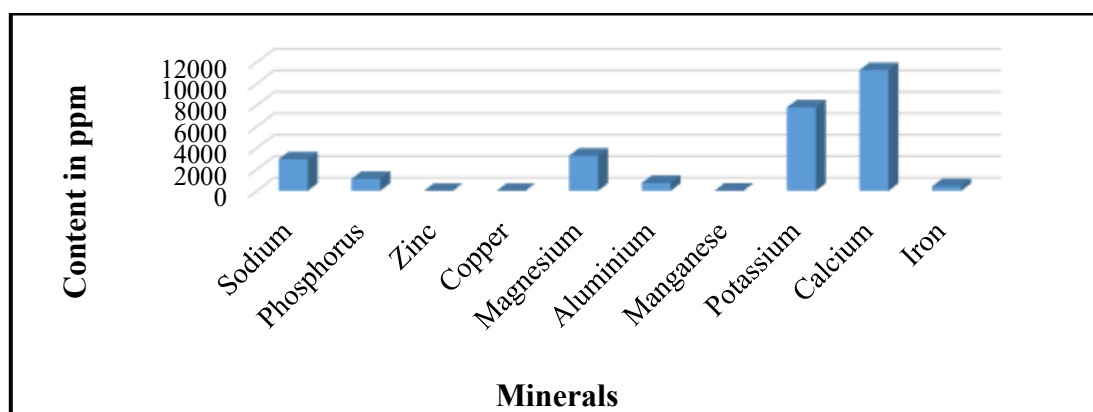


Figure 6: Different mineral content in *Glycyrrhiza glabra* L. powder

HPTLC analysis

In the present study, HPTLC analysis of methanolic extracts of *Glycyrrhiza glabra* L. was studied using Merck silica gel 60 F 254 TLC pre-coated plates as stationary plate.

Methanolic extract of *Glycyrrhiza glabra* L. showed 5 bands with R_f values 0.15, 0.23, 0.27, 0.84 and 0.97 at 254 nm (Table 14 and Plate 1). Methanolic extract of *Glycyrrhiza glabra* L. showed 7 bands with R_f values 0.17, 0.24, 0.28, 0.51, 0.54, 0.84 and 0.89 at 366 nm (Table 15 and Plate 2) and 7 bands with R_f values 0.17, 0.24, 0.28, 0.51, 0.54, 0.84 and 0.89 at white light after derivatization with 10% Methanolic sulfuric acid (Table 16 and Plate 3).

Table 8: Track Assignment of HPTLC analysis

Track Assignment					
Track	Vial ID	Description	Volume	Position	Type
1	SA2122096-01	Mulethi sample 500mg/10 ml Ethanol:water (7:3)	0.5 μ l	C1	Sample
2	SA2122096-01	Mulethi sample 500mg/10 ml Ethanol:water (7:3)	1.0 μ l	C1	Sample
3	SA2122096-01	Mulethi sample 500mg/10 ml Ethanol:water (7:3)	1.5 μ l	C1	Sample
4	SA2122096-01	Mulethi sample 500mg/10 ml Ethanol:water (7:3)	2.0 μ l	C1	Sample
5	SA2122096-01	Mulethi sample 500mg/10 ml Ethanol:water (7:3)	2.5 μ l	C1	Sample

Table 9: System Setup of HPTLC analysis

System setup:	
Software	Server LABSERVER, version 3.1.21109.3
ATS 4	S/N:250243 (NEW ATS4)
Chamber	N/A
Immersion	N/A
TLC Visualizer	S/N:150503 (VISUALIZER)

Table 10: Chromatography and plate layout of HPTLC analysis

Chromatography	
Plate layout:	
Stationary phase	Merck, TLC Al plates silica gel 60 F 254
Plate format	100 x 100 mm
Application type	User
Application	Position Y: 8.0 mm, length: 8.0 mm, width: 0.0 mm
Track	First position X: 15.0 mm, distance: 14.4 mm
Solvent front position	70 mm

Table 11: Application in HPTLC analysis

Application 1 - ATS 4 (S/N: 250243):	
Spray gas	Air
Sample solvent type	Water
Filling speed	8 µl/s
Predosage volume	200 nl
Retraction volume	200 nl
Dosage speed	50 nl/s
Filling quality	Standard
Rinsing cycles / vacuum	1 / 8 s
Filling cycles / vacuum	1 / 8 s
Rinsing solvent name	Methanol
Nozzle temperature	Unheated
Rack in use	Standard
Instrument diagnostics	Valid diagnostics
Step status	▲ Step was restored

Table 12: Development chamber for HPTLC analysis

Development 1 - Chamber:	
Tank	TTC 20x10
Mobile phase	Ethyl Acetate:Formic Acid:Acetic Acid:Water.15:1:1:2 (v/v/v/v).
Saturation time	20 min
Use saturation pad	true
Use smartALERT	false
Volume front through	5 ml
Volume rear through	5 ml
Drying time	5 min
Drying temperature	Room temperature

Table 13: Derivatization in HPTLC analysis

Derivatization 1 - Immersion:	
Reagent name	10% Methanolic sulfuric acid
Dipping speed	5
Dipping time	0 s
Reagent preparation	Place 180 ml of Methanol in a 200 ml glass bottle and cool it down in water-ice cubes- salt bath or in a freezer. To the ice-cold methanol add slowly and carefully 20 ml of concentrated sulphuric acid and mix well. Allow the mixture to cool to room temperature.
Heating	100 °C for 3 min, heated after

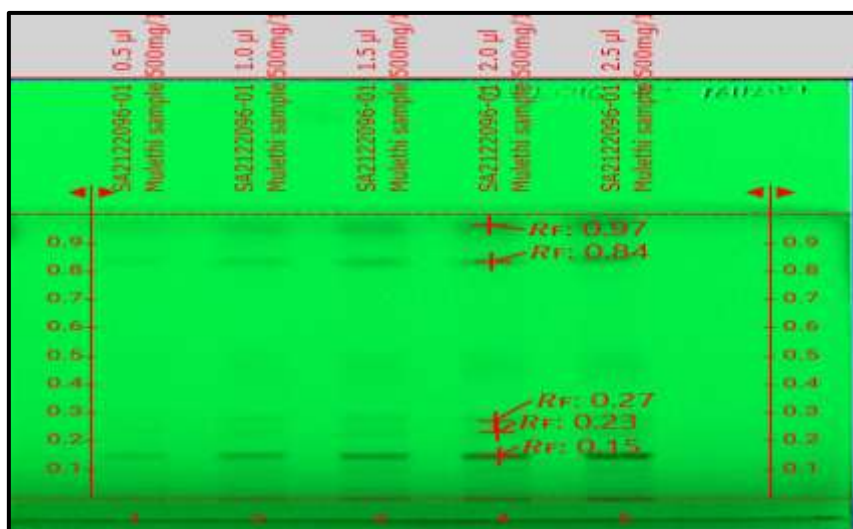


Plate 1: HPTLC fingerprinting of methanolic extract at 254 nm

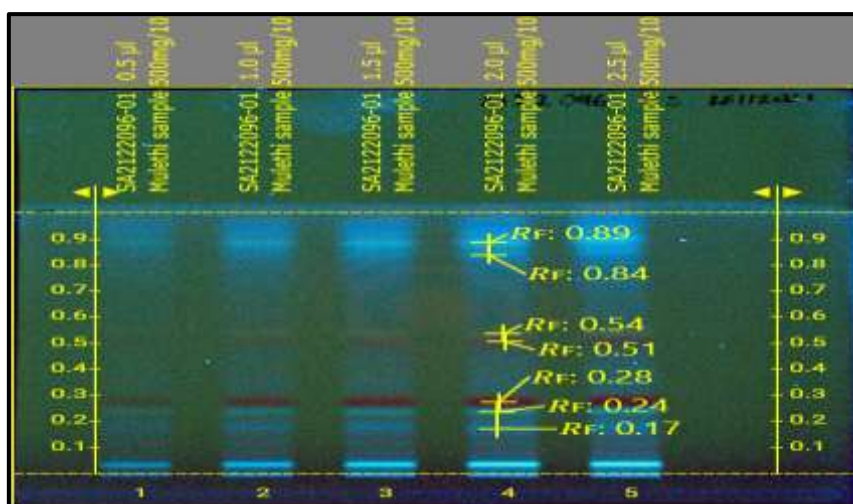


Plate 2: HPTLC fingerprinting of methanolic extract at 366 nm

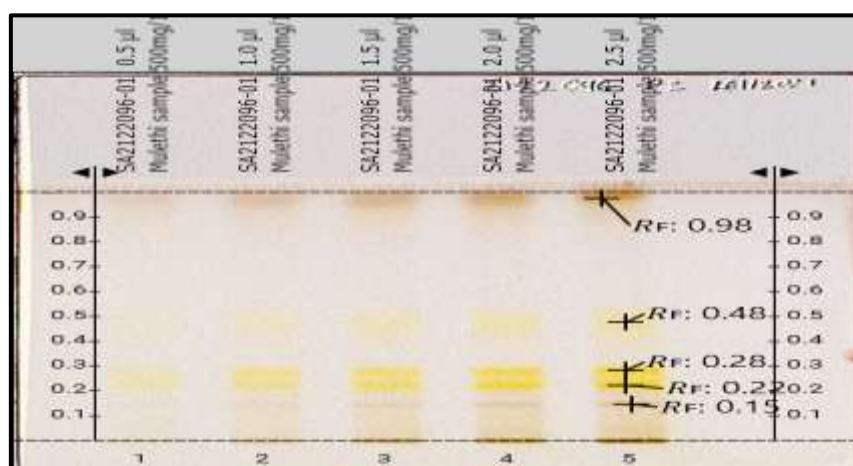


Plate 3: HPTLC fingerprinting of methanolic extract at 366 nm at white light after derivatization with 10% Methanolic sulfuric acid

Table 14: Image at R 254 -Respective track number, Rf values and intensity of prominent peaks

Sample name	Track No.	Rf	Intensity
<i>Glycyrrhiza glabra</i>	4	0.15	High
		0.23	Low
		0.27	Low
		0.84	Medium
		0.97	Medium

Table 15: Image at 366nm -Respective track number, Rf values, color and intensity of prominent peaks

Sample name	Track No.	Rf	Color	Intensity
<i>Glycyrrhiza glabra</i>	4	0.17	blue	Low
		0.24	Greenish blue	Medium
		0.28	Brick Red	High
		0.51	Brick Red	Low
		0.54	Pale yellow	Low
		0.84	Blue	Medium
		0.89	Aqua green	High

Table 16: Image at white light after derivatization with 10% Methanolic sulfuric acid -Respective track number, Rf values, color and intensity of prominent peaks

Sample name	Track No.	Rf	Color	Intensity
<i>Glycyrrhiza glabra</i>	4	0.17	blue	Low
		0.24	Greenish blue	Medium
		0.28	Brick Red	High
		0.51	Brick Red	Low
		0.54	Pale yellow	Low
		0.84	Blue	Medium
		0.89	Aqua green	High

RESULT AND DISCUSSION

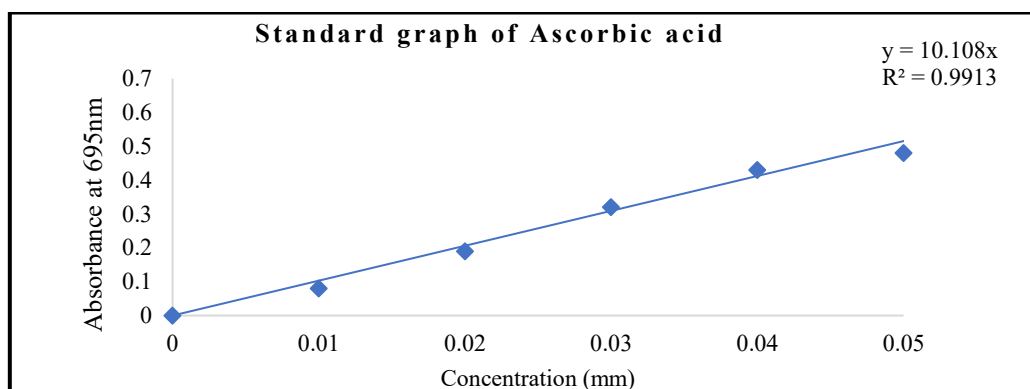
Total antioxidant activity

Total antioxidant activity is a quantitative assay, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The total antioxidant activity was evaluated by using phosphomolybdate reagent. This assay is based on the

reduction of Mo (VI) to Mo (V) in presence of antioxidant compounds and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH, which is measured at 695nm (Prieto, *et al.*, 1999).

Total antioxidant capacity of aqueous and methanolic extracts of *Glycyrrhiza glabra* L. was expressed as equivalent to ascorbic acid ($\mu\text{g/mL}$ of extract). The linear equation of ascorbic acid for total antioxidant activity was found to be $y=10.108x$ with $r^2=0.9913$ (Figure 7). The total antioxidant activity of *Glycyrrhiza glabra* L. was found to be 35.82 ± 0.23 and 29.41 ± 1.18 mM of ascorbic acid/ g dry weight of samples for aqueous and methanolic extracts (Table 17).

Figure 7: Standard calibration curve of Ascorbic acid for total antioxidant activity analysis



Values are mean $\pm$ S. D. of three determinants

Key: AE- Aqueous extract, ME- Methanolic extract

Table 17: Total antioxidant activity of aqueous and methanolic extracts of *Glycyrrhiza glabra* L.

Samples/Extracts	Total antioxidant activity (mM)
AE	35.82 ± 0.23
ME	29.41 ± 1.18

Ferric Reducing Antioxidant Power

The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom (Saha *et al.*, 2008). The presence of reductants (i.e. antioxidants) in plant extract causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue colour at 700 nm. Increase in absorbance at 700 nm reflects an increase in reductive ability. As the concentration of aqueous and methanolic extract of *Glycyrrhiza glabra* L. and standard (ascorbic acid) increases the ferric reducing antioxidant power increases. The reducing power was found to be higher in aqueous extracts of *Glycyrrhiza glabra* L. as compared to the methanolic extracts. (Table 18-19 and Figure 9).

Nitric oxide radical scavenging activity

Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signalling, and inhibition of platelet aggregation and regulation of cell mediated toxicity. Excess production of these radicals leads to production of peroxynitrite radicals by reacting with oxygen molecules. These peroxynitrite radicals lead to serious toxic reactions with biomolecules and cause renal injury. In the assay sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. As the concentration of *Glycyrrhiza glabra* L. and ascorbic acid increases the nitric oxide radical scavenging activity increases. The nitric oxide radical scavenging activity of aqueous extract of *Glycyrrhiza glabra* L. was found to be higher as compared to methanolic extracts (Table 20-21 and Figure 11).

Table 18: Ferric reducing antioxidant power (FRAP) of standard (Ascorbic acid)

Concentration (mg/ml)	Std. (Ascorbic acid)
0.01	0.14±0.011
0.02	0.34±0.01
0.03	0.52±0.01
0.04	0.66±0.008
0.05	0.79±0.01

Values are mean ± S. D. of three determinants

Figure 9: Ferric reducing antioxidant power (FRAP) of standard (Ascorbic acid)

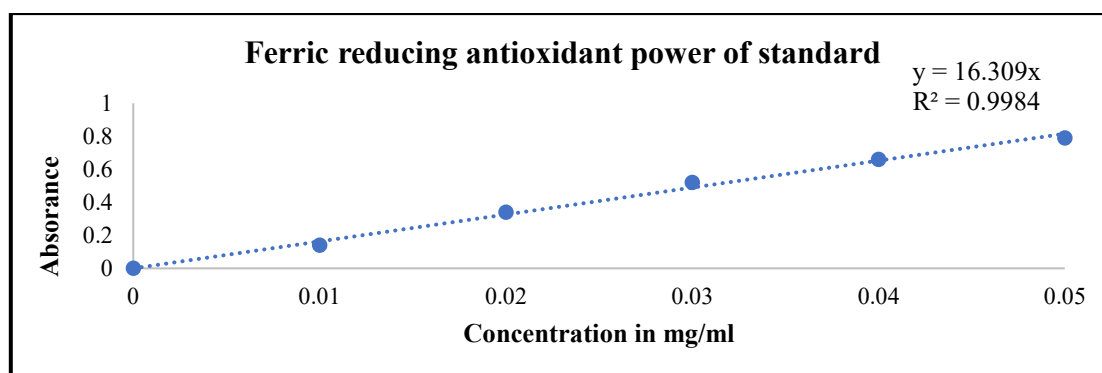


Table 19: Ferric reducing antioxidant power (FRAP) of *Glycyrrhiza glabra*

Concentration (mg/ml)	AE	ME
0.1	0.09 ± 0.007	0.07 ± 0.018
0.2	0.18 ± 0.013	0.15 ± 0.011
0.3	0.27 ± 0.018	0.21 ± 0.009
0.4	0.39 ± 0.010	0.32 ± 0.014
0.5	0.48 ± 0.017	0.41 ± 0.010

Values are mean ± S. D. of three determinants

Key: AE- Aqueous extract, ME- Methanolic extract

Table 20: Nitric oxide radical scavenging activity of standard (Ascorbic acid)

Concentration (mg/ml)	Std. (Ascorbic acid)
0.1	45.6±0.010
0.2	56.8±0.021
0.3	67.34±0.015
0.4	73.04±0.005
0.5	81.15±0.026

Values are mean ± S. D. of three determinants

Figure 11: Nitric oxide radical scavenging activity of standard (Ascorbic acid)

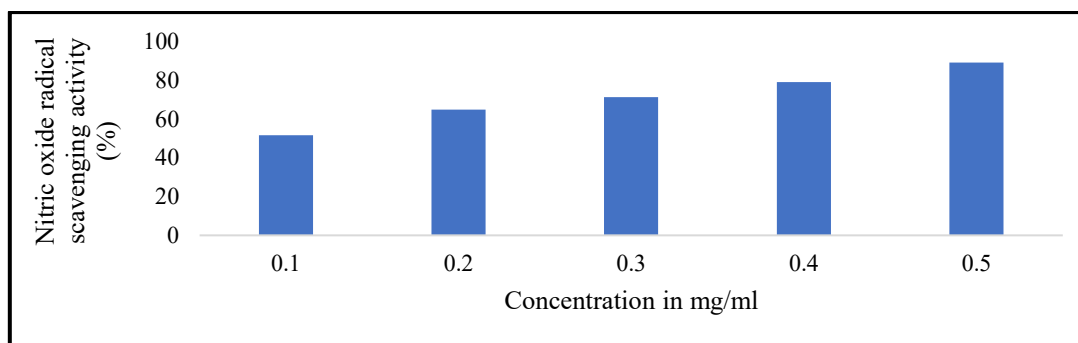


Table 21: Nitric oxide radical scavenging activity of aqueous and methanolic extracts of *Glycyrrhiza glabra* L.

Concentration mg/ml	AE	ME
0.2	14.11± 0.011	12.61± 0.010
0.4	21.18± 0.031	18.19± 0.014
0.6	32.26± 0.052	29.36± 0.021
0.8	47.31± 0.012	38.39± 0.033
1.0	51.63± 0.038	46.41± 0.007

Values are mean ± S. D. of three determinants

Key: AE- Aqueous extract, ME- Methanolic extract

Total phenol and flavonoid estimation

Phenolic and flavonoids comprise the largest group of plants secondary metabolite that have been reported to have multiple biological effects, including antioxidant property. The linear equation of Gallic acid for total phenol content was found to be $y=21.091x$ with $r^2=0.9913$. The total phenolic content of the aqueous and methanolic extract of *Glycyrrhiza glabra* L. was found to be 46.15 ± 0.010 and 42.20 ± 0.018 mg/g dry weight of samples respectively.

The linear equation of Quercetin for total flavonoid content was found to be $y=8.0190x$ with $r^2= 0.9981$. The total flavonoid content of the aqueous and methanolic extract of *Glycyrrhiza glabra* L. was found to be 39.81 ± 0.011 and 36.61 ± 0.009 mg/g dry weight of samples respectively (Table 22).

Table 22: Total phenolic content of aqueous and methanolic extracts of *Glycyrrhiza glabra* L.

Samples	Phenolic Content (mg/g)	
	AE	ME
Total phenolic content	46.15 ± 0.010	42.20 ± 0.018
Total flavonoid content	39.81 ± 0.011	36.61 ± 0.009

Values are mean $\pm$ S. D. of three determinants

Key: AE- Aqueous extract, ME- Methanolic extract

***In vitro* anticancer activity**

Anticancer activity of *Glycyrrhiza glabra* L. was evaluated with respect of standard drug, Adriamycin which was considered as a positive control in whole experiment. MCF-7 cell line was used to check *in vitro* anticancer activity. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. The result obtained from *in vitro* drug testing via SRB assay are showed in Table no 23 and Table no 24.

The results of microscopic examination of cytotoxic effects on selected cancer cell lines are shown in plate 4 to plate 8. This may be due to the presence of active compound in the plant powder. Further different concentrations of plant powder can be checked for anticancer activity.

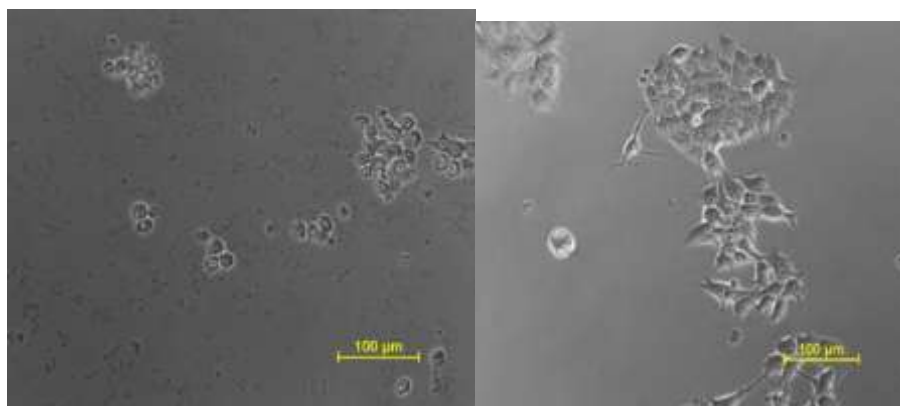


Plate 4: MCF-7 ADR Plate 5: MCF-7 Control

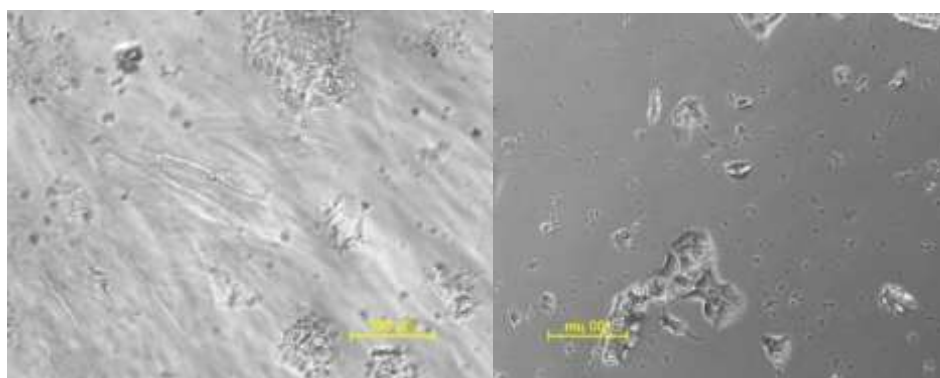


Plate 6: MCF-7 Glycyrrhiza glabra L. (Image 1) Plate 7: MCF-7 Glycyrrhiza glabra L. (Image 2)

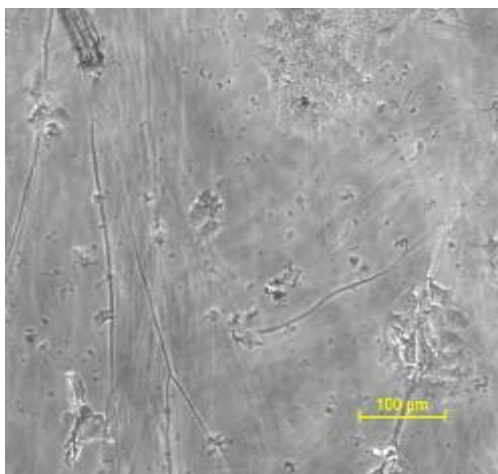


Plate 8: MCF-7 *Glycyrrhiza glabra* L. (Image 3)

CONCLUSION

Recently, antibiotics and most drugs on the market have shown unwanted symptoms and the emergence of resistant pathogenic microorganisms, toxic effects related to these drugs, and withdrawal issues restricting their use in many countries. Therefore, research on herbal plants has provided modern medicine with several useful chemical ingredients that have been used to manage various ailments. The increasing use of medicinal herbs among the general public has increased the need for scientific-based research to determine the mechanism of action of herbs. This is partly because these extracts are inexpensive and easily accessible. In ancient Ayurvedic System, more than 1250 preparations are described containing Yashtimadhu as one of its Constituents. In traditional Ayurvedic medicine, herbs were used as special foods, serving to eliminate the excesses as well as strengthen the deficiencies, restore and rejuvenate. The present study reveals that *Glycyrrhiza glabra* L. consists of different phytoconstituents like phenols, glycosides, saponins, flavonoids, steroids, tannins, alkaloids, and terpenes and is a good source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. The *in vitro* anticancer activity was found using SRB assay against MCF-7 cell line for breast cancer. Further different concentrations of plant powder can be checked for anticancer activity using different cell lines and may be a prominent candidature for the future anticancer drug.

Acknowledgement

The authors are grateful to the University of Mumbai for the grant provided, Department of Biotechnology, B. K. Birla College (Empowered Autonomous), Kalyan for providing the necessary facilities and support to carry out this research work. Department of Botany, Agharkar Research Institute, Pune, for the identification and Authentication of the plant material, Anchrom Enterprises, Mulund, for HPTLC analysis, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Kharghar, Navi Mumbai for the *in vitro* anticancer evaluation of *Glycyrrhiza glabra* L. The authors are thankful to all those who contributed directly or indirectly to the successful completion of this study.

REFERENCES

- [1] Ayurvedic Pharmacopoeia of India (2006). Appendices, department of Ayush Government of India, Ministry of Health And Family Welfare. 1(1).
- [2] Babior, B. M. (1978). Oxygen-dependent microbial killing by phagocytes. *The New England Journal of Medicine*. 298: 659-668.
- [3] Chen G, Zhu L, Liu Y, Zhou Q, Chen H, Yang. (2009), Isoliquiritigenin, a flavonoid from licorice, plays a dual role in regulating gastrointestinal motility *in vitro* and *in vivo*. *J. Phytother Res*. 23(4): pp 498-506.
- [4] Chin YW, Jung HA, Liu Y, Su BN, Castoro JA, Keller WJ, Pereira MA, Kinghorn AD. (2007), Anti-oxidant constituents of the roots and stolons of licorice (*Glycyrrhiza glabra*). *J. Agric. Food Chem*. 55(12): pp 4691-4697.
- [5] Cui YM, Ao MZ, Li W, Yu LJ. (2008), Effect of glabridin from *Glycyrrhiza glabra* on learning and memory in mice. *Planta Med*. 74(4): pp 377-380.

- [6] Fiore C, Eisenhut M, Krausse R, Ragazzi E, Pellati D, Armanini D, Bielenberg J. (2008), Antiviral effects of Glycyrrhiza species. *Phytother Res.* 22(2): pp 141-8.
- [7] Genovese T, Menegazzi M, Mazzon E, Crisafulli C, Di Paola R, Dal Bosco M, Zou Z, Suzuki H, Cuzzocrea S.(2009), Glycyrrhizin reduces secondary inflammatory process after spinal cord compression injury in mice. *Shock.* 31 (4): pp 367-375.
- [8] Garrat, D.C (1964). The quantitative analysis of Drugs, Japan: Chapman and Hall Ltd.
- [9] Harborne JB. (1998). Phytochemical method: A Guide to Modern technique of Plant Analysis. 3rd Edition, Chapman and Hall: New York.
- [10] Hazra B, Biswas S, Mandal N (2008). Antioxidant and free radical scavenging activity of *Spondias pinnata*. *BMC Complement. Altern. Med.* 8: 63.
- [11] Kamisoyama H, Honda K, Tominaga Y, Yokota S, Hasegawa S. (2008), Investigation of the anti-obesity action of licorice flavonoid oil in diet-induced obese rats. *Bioscience, Biotechnology, and Biochemistry*: 12 pp 3225-3231
- [12] Khandelwal K R (2008). Practical Pharmacognosy: Techniques and Experiments, 22nd ed India: Nirali Prakashan.
- [13] Kokashi CJ, Kokashi RJ, Sharma M. (1958). Fluorescence of powdered vegetable drugs in ultra-violet radiation. *J. Am. Pharm. Assoc.*; 47:715-717.
- [14] Kokate, C.K.; Purohit, A.P. and Gokhale, S.B. (2005). Pharmacognosy, 31st edition, Nirali Prakshan. 97-131.
- [15] Kumar, S.; Kumar, D.; Singh, N. and Vasisht, B.D. (2007). *In vitro* free radical scavenging and antioxidant activity of *Moinga oleifera* pods. *Journal of herbal medicine and toxicology*, 1: 17-22.
- [16] Kunle OF, Egharevba HO (2009). Preliminary studies on *Vernonia ambigua*: Phytochemistry and antimicrobial screening of the whole plant. *Ethnobotanical Leaflets*, 13: 1216-1221.
- [17] Lobo V., Patil A., Phatak A., Chandra N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews* 4(8). 118-126.
- [18] Lowry, O. H., and Bessey, O. A. (1951). *J. Biol. Chem.*, 163, 633.
- [19] Malekinejad H, Mirzakhani N, Razi M, Cheraghi H, Alizadeh A, Dardmeh F. (2011), Protective effects of melatonin and Glycyrrhiza glabra extract on ochratoxin A-induced damages on testes in mature rats. *Hum Exp Toxicol.*30 (2): pp 110-23.
- [20] Males Z, Medic-Saric M (2001). Optimization of TLC analysis of flavonoids and Phenolic acids of *Helleborus atrorubens* Waldst. et Kit. *J Pharm Biomed Anal* 24(3):353-9
- [21] Mandal, S.; Hazra, B.; Sarkar, R.; Biswas, S. and Mandal, N. (2009). *Hemidesmus indicus*, an age-old plant: study of its *in vitro* antioxidant and free radical scavenging potentials. *Pharmacology online.* 1: 604-614.
- [22] Memon, A.R.; Aktoprakligul; Zdemur, A. and Vertii. A. (2001). Heavy metal accumulation and detoxification mechanisms in plants. *Turk. J. Bot.* 25: 111-121.
- [23] Mendes-Silva W, Assafim M, Ruta B, Monteiro RQ, Guimarães JA, Zingali RB. (2003), Antithrombotic effect of Glycyrrhizin, a plant-derived thrombin inhibitor. *Thromb Res.* 112(1-2): pp 93-8.
- [24] Miller, D.M., and Aust, S.D. (1989). Studies of ascorbate-dependent, iron catalysed lipid peroxidation. *Arch. Biochem. Biophys.* 271, 113-119.
- [25] MG Rajesh, MS Latha, (2004), Protective activity of Glycyrrhiza glabra Linn. on carbon tetrachloride-induced peroxidative damage. *Indian Journal of Pharmacology.* 36(5): pp 284-287.
- [26] Minasian SM, Adamian TsI, Gevorkian ES. (2007), Changes of morphological indicators of blood from vibration and liquorice effect. *Russ Fiziol Zh Im I M Sechenova.* 93(9):1035-4
- [27] Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine. *Japanese journal of nutrition.* 44: 307-315.
- [28] Parle M, Dhingra D, Kulkarni SK. (2004), Memory-strengthening activity of *Glycyrrhiza glabra* in exteroceptive and interoceptive behavioral models.. *J Ethnopharmacol.* 91 (2-3): pp 361-5.
- [29] Patel, A. A.; Patel, A. and Patel, N.m. (2010). Estimation of flavonoid, polyphenolic and *in vitro* antioxidant capacity of leaves of *Tephrosia purpurca* L. (Leguminosae). *International Journal of Pharmaceutical Sciences and Research.* 1: 66-77.
- [30] P. Muralidharan, G. Balamurugan and Venu Babu (2009), Cerebroprotective effect of *Glycyrrhiza glabra* Linn. root extract on hypoxic rats. *Bangladesh J Pharmacol.* 4: pp 60-64.
- [31] Prieto P.; Pineda, M. and Aguilar, M.M. (1999). Spectrophotometric quantification of antioxidant capacity through the formation of phosphomolybdenum complex specific application to the determination of vitamin E. *Analytical Biochemistry.* 269: 337-341

- [32] Preethi R.M., Devanathan V.V., Loganathan M. (2010). Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. *Advances in Biological Research*, 4(2): 122-125.
- [33] Ram A, Mabalirajan U, Das M, Bhattacharya I, Dinda AK, Gangal SV, Ghosh B. (2006). Glycyrrhizin alleviates experimental allergic asthma in mice. *Int Immunopharmacol*. 6(9): pp 1468-77.
- [34] Roze L.V.; Chanda, A. and Linz, J.E. (2011). Compartmentalization and molecular traffic in secondary metabolism; a new understanding of established cellular processes. *Fungal genetics and Biology*. 48: 34-48.
- [35] Sadasivam, S. and Manickam, A. (2008). *Biochemical Methods*. 3rd Edn. New Age International Publisher, New Delhi.
- [36] Saha P., Mazumdar U. K., Haldar P. K., Sen S. K and Naskar S., (2008). Antihyperglycemic activity of *Lagenaria siceraria* aerial parts on streptozotocin induce diabetics in rats. *Original research article*, 40 (2): 49-50.
- [37] SB Kasture, SA Barhate, M Mohan. (2008), Glycyrrhiza glabra as an adjuvant in treatment of Parkinsonism and depression. *Planta Med*; 74 - PA310.
- [38] Shin YW, Bae EA, Lee B, Lee SH, Kim JA, Kim YS, Kim DH. (2007), In vitro and in vivo antiallergic effects of Glycyrrhiza glabra and its components. *Planta Med*. 73(3): pp 257-61.
- [39] Sofowara, A. (1993). A book entitled "Medicinal Plants and Traditional medicine in Africa". Published by Spectrum Books Ltd, Ibadan, Nigeria.
- [40] Song Z, Gong Y, Liu H, Ren Q, Sun X. (2011), Glycyrrhizin could reduce ocular hypertension induced by triamcinolone acetonide in rabbits. *Mol Vis*. 17: pp 2056-64.
- [41] Trease, G. E. and Evans, W. C. (1989). A book entitled "Pharmacognsy". 11th Edition. Published by Brailiar Tiridel Can. Macmillian Publishers.
- [42] Trivedi Hamaja S., Mohana Lakshmi, Jyothi M.Joy (2012). Pharmacognostic studies of the leaves of *Ficus nervosa* (Moraceae). *International Journal of Phytotherapy*, 2(1): 16-22.
- [43] WHO (2000). General guidelines for methodologies on research and evaluation of traditional medicine.
- [44] Zamansoltani F, Nassiri-Asl M, Sarookhani MR, Jahani-Hashemi H, Zangivand AA. (2009). Antiandrogenic activities of *Glycyrrhiza glabra* in male rats. *Int J Androl*; 32 (4): pp 417-22.