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COMPARATIVE PHYTOCHEMICAL & ANTIOXIDANT STUDY OF AQUEOUS EXTRACTS OF *GLYCYRRHIZA GLABRA* (MULETHI) & *PIPER LONGUM* (LONG PEPPER)

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ABSTRACT

Secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. The presence of these secondary metabolites in plants probably explains the various medicinal & antioxidant activities of these plants. It was a comparative study of phytochemical & antioxidant activity of aqueous extracts of *Glycyrrhiza glabra* (Mulethi) & *Piper longum* (Long pepper). The plant materials used were collected and grinded in mortar and pestle. Aqueous extracts was prepared by using sonicator and water bath then the extract of both the plants were investigated for the phytochemicals & *In-vitro* antioxidant activity by using different methods. Aqueous extract of *Glycyrrhiza glabra* have high of amount phytochemicals & high antioxidant activity than the aqueous extract of *Piper longum*.

Keywords: *Glycyrrhiza glabra*, *Piper longum*, Phytochemicals, Antioxidant activity.

INTRODUCTION

Secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. The most commonly encountered secondary metabolites of plants (phytochemicals) are saponin, tannins, flavanoides, alkaloids, terpenoid and glycosides. The presence of these Secondary metabolites in plants probably explains the various uses of plants for traditional medicine. Antioxidants help to prevent the free radical damage that is associated with cancer and heart disease. The potentially reactive derivatives of oxygen are known as reactive oxygen species (ROS) e.g. superoxide anions, hydrogen peroxide and hydroxyl, nitric oxide radicals, and play an important role in oxidative damage to various biomolecules including proteins, lipids and DNA, related to the pathogenesis of various important diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases and also

in the ageing process.¹⁻³ Licorice (*Glycyrrhiza glabra* L.) belongs to the Family Papilionaceae/Fabaceae. It is a traditional medicinal herb grows in the various parts of the world. Major active chemical constituent is Glycyrrhizin (Glycyrrhizic acid) responsible for its medical applications.¹⁰ It used in the treatment of psoriasis, to relieve 'Vata' and 'Kapha' inflammations, eye diseases, throat infections, peptic ulcers, arthritic conditions, and liver diseases in Indian Ayurveda system.¹¹ *Piper longum* belongs to the family Piperaceae. It is a common Indian dietary spice which has been shown to possess a wide range of therapeutic utilities in the traditional Indian medicines. It has been reported to possess immune-modulatory, antiasthmatic, hepatoprotective, hypocholesteremic and anti-inflammatory activities.² The present study was performed for phytochemical investigation of aqueous extracts of *Glycyrrhiza glabra* & *Piper longum* & *In-vitro* investigation of Antioxidant

activity of aqueous extracts of *Glycyrrhiza glabra* & *Piper longum* for various enzymatic and non-enzymatic antioxidants.

MATERIAL AND METHODS

Collection of Plant Material

The plant materials used were the roots of *Glycyrrhiza glabra* & dried fruits of *Piper longum* which were collected from local market of Paonta sahib and grinded in mortar and pestle.

Extraction of Plant Material

The plants (powdered form) taken for the study was stored under refrigerated condition till use. The samples were prepared by extraction of plant (powdered form) with distilled water by using sonicator and evaporating the aqueous mixtures on water bath and a crude extract was obtained from both plants.

Phytochemical Investigations of *Glycyrrhiza glabra* & *Piper longum*

Alkaloid

5g of each powdered sample extract was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 48 h. After filtration, the extracts were concentrated on a water bath to $\frac{1}{4}$ of the original volume. Concentrated ammonium hydroxide was added in drops to the extract until the precipitation was collected, washed with dilute ammonium hydroxide and then filtered. The residue obtained is the alkaloid, and was dried and weighed.⁴

Saponin

25g of each powdered sample were boiled in 25ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.⁴

Flavonoids

A portion of each powdered plant sample was separately heated with 10ml of ethyl acetate in a water bath for 3min. The mixtures were filtered and 4ml of each filtrate were shaken with 1ml of dilute ammonia solution. A yellow colour observation indicates the presence of flavonoids.⁴

Tannins

0.5g of each powdered sample were boiled in 20ml of water in a test tube and then filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colour.¹²

Glycoside

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycons) and a compound which is not a sugar. To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.⁸

Terpenoids

Four milligrams of each extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added solely and red violet color was observed for terpenoids.⁸

Reducing sugar

To 0.5 ml of each extract solution add 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Antioxidant Activity of *Glycyrrhiza glabra* & *Piper longum* Plant Extract

Assay of catalase activity

Catalase activity was assayed by the method of Sinha. The each enzyme extract (0.5 ml) was added to the reaction mixture containing 1ml of 0.01 M phosphate buffer (pH 7.0), 0.5 ml of 0.2 M H₂O₂, 0.4 ml H₂O and incubated for different time period. The reaction was terminated by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was

prepared by mixing 5% potassium dichromate with glacial acetic acid (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes and the absorbance was taken at 610 nm. Catalase activity was expressed in terms of moles of H₂O₂ consumed/min/mg protein.

Assay of peroxidase activity

The assay was carried out by the method of Addy and Goodman. The reaction mixture consisted of 3ml of buffered pyrogallol [0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0)] and 0.5 ml of 1% H₂O₂. To this added 0.1 ml enzyme extract and O.D. change was measured at 430 nm for every 30 seconds for 2 minutes. The peroxidase activity was calculated using an extinction coefficient of oxidized pyrogallol (4.5 liters/mol).

Assay of ascorbate oxidase activity

Assay of Ascorbate Oxidase activity was carried out according to the method of Vines and Oberbacher. The sample was homogenized [1:5 (w/v)] with phosphate buffer (0.1 M/ pH 6.5) and centrifuged at 3000 g for 15 min at 50°C. The supernatant obtained was used as enzyme source. To 3.0 ml of the substrate solution (8.8 mg ascorbic acid in 300 ml phosphate buffer, pH 5.6), 0.1 ml of the enzyme extract was added and the absorbance change at 265 nm was measured for every 30 seconds for a period of 5 minutes. One enzyme unit is equivalent to 0.01 O.D. changes per min.

Quantification of vitamins

The determination of ascorbic acid was carried out by the procedure given by Sadasivam and Manickam. The assay mixture for vitamin C consisted of 0.1 ml of brominated sample extract, 2.9 ml of distilled water, 1 ml of 2% DNPH reagent and 1-2 drops of thiourea. After incubation at 37°C for 3 h, the orange-red osazone crystals formed were dissolved by the addition of 7 ml of 80% sulphuric acid and absorbance was read at 540 nm after 30 minutes. Vitamin C concentration was expressed in terms of mg/g tissue.

RESULTS AND DISCUSSION

Phytochemical Screening of Plant Materials

Qualitative analysis carried out on each plant extract showed the presence of phytochemical constituents and the results are summarized in table1. The Phytochemical screening of aqueous extract of plant studied showed the presence of saponin, flavanoides, and steroids in aqueous extract of *Glycyrrhiza glabra* and alkaloids, reducing sugars, tannins, glycosides, and terpenoids were absent. Phytochemical test of aqueous extract of *Piper longum* showed the presence of alkaloids, tannins, and terpenoids where as reducing sugars, saponin, flavanoides, steroids and glycosides were absent.

Antioxidant Activity of Aqueous Extract of *Glycyrrhiza glabra* & *Piper longum*

The level of enzymatic and non-enzymatic antioxidants assessed in both plant extracts are collectively represented in table2& table3. The above results showed that the catalase activity of aqueous extract of *Glycyrrhiza glabra* (16.080 units/mg protein) was more than aqueous extract of *Piper longum* whose catalase activity was (14.05 units/mg proteins). Also the Peroxidase activity of aqueous extract of *Glycyrrhiza glabra* (10.22×10^3 units/mg protein) was more than aqueous extract of *Piper longum* (7.89×10^3 units/mg protein). Ascorbate Oxidase activity of *Glycyrrhiza glabra* (6.392 units/mg protein) was more than *Piper longum* (4.724 units/mg protein). Vitamin C content was high in aqueous extract of *Glycyrrhiza glabra* (1.762 mg/g) whereas in aqueous extract of *Piper longum* it was (0.163 mg/g).

DISCUSSION

Most of the people thought that spices, vegetables they used in their kitchen are only give them flavors to their food, but these also play a vital role in keeping us healthy and fit. These spices & vegetables contain various phytochemicals important to treat various diseases. The important new findings of this study are that we investigate

particular phytochemicals & antioxidants which are beneficial for the treatment of various diseases which are caused due to the oxidative stress like cancer and diabetes. Polyphenols such as tannins and flavanoides have been shown to have numerous health protective benefits, which include lowering of blood lipids.⁶ Thus these plants have been used to lower blood lipids content. The antioxidative properties of plants were related mostly to the presence of phenolic compounds, especially flavonoids.⁵ If we isolate these phytochemicals then it will be very helpful for the herbal drug industries to make new drugs and ultimately it helps the human beings.

CONCLUSION

The results from present study revealed that, the extract of both plants have significant amount of phytochemicals and antioxidant enzymes. The

wide use of these fruits in the Indian indigenous system of medicine as anti inflammatory and anti hepatotoxic may be in part due to their antioxidant potency. From the present study it has been concluded that aqueous extracts of *Glycyrrhiza glabra* & *Piper longum* are the good source of phytochemicals & anti-oxidants. But in *Glycyrrhiza glabra* both phytochemicals & anti-oxidant contents were high. So it is more beneficial for the treatment of various diseases which are caused due to the oxidative stress like cancer and diabetes.

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Table 1: Phytochemical constituents of *Glycyrrhiza glabra* & *Piper longum*

Phytochemical	<i>Glycyrrhiza glabra</i>	<i>Piper longum</i>
Alkaloids	–	+
Tannins	–	+
Reducing sugars	–	–
Terpenoids	–	+
Amino acids	+	+
Steroids	+	–
Glycosides	–	–
Flavanoides	+	–
Saponin	+	–

Table 2: Enzymatic antioxidant analysis of aqueous extract of *Glycyrrhiza glabra* & *Piper longum*

Sample	Catalase μ /mole of H ₂ O ₂ decomposed/min/g extract	Peroxidase IU/L	Ascorbate Oxidase μ mole/ml
Aqueous extract of <i>Glycyrrhiza glabra</i>	16.080	10.22×10^3	6.392
Aqueous extract of <i>Piper longum</i>	14.05	7.89×10^3	4.724
	1 unit = μ /moles of H ₂ O ₂ decomposed/ min/g extract	1 unit = μ moles pyrogallol oxidized/min	1 unit = 0.01 O.D change/min

Table 3: Non-enzymatic antioxidant analysis of aqueous extract of *Glycyrrhiza glabra* & *Piper longum*

Samples	Vitamin C (mg/g)
Aqueous extracts of <i>Glycyrrhiza glabra</i>	1.762
Aqueous extracts of <i>Piper longum</i>	0.163

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