Evaluation of antioxidant and phytochemical analysis of water extracted root powder of *Glycyrrhiza Glabra*

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Abstract

Glycyrrhiza glabra is a perineal herb containing chemical composition of glabridin and glycyrrhizic acid. Antioxidant substances cause to blocking of the oxidative stress of the human cells and phenolic and flavonoid also have been proved their effectiveness in chronic diseases. In this study, in vitro antioxidant activity, total phenolic and flavonoids content of hot and cold water extract of five different concentrations were determined by using spectrophotometric methods. Total Antioxidant activity of extracts was expressed with frap (ferric reducing antioxidant power) values of tannic acid equivalents/g dry weight. Antioxidant capacity of hot and cold water extract was 129.46±3.4 µmol/g and 87.95±4.1 µmol/g respectively. Assays of phenolic and flavonoid concentrations were expressed as tannic acid equivalents/g dry weight. Total phenolic content of hot extract was 51.46±8.05 mg /Tannic acid equivalent/g dry material; cold water extract was 37.68±0.50 mg Tannic acid equivalent/g dry material. Total flavonoid content in the hot water extract (144.02±3.52 mg Tannic acid equivalent/g dry material and cold water extract (109.26±1.82mg Tannic acid equivalent/g dry material). Hot water extracts of *Glycyrrhiza glabra* root powder showed the highest phenolic and flavonoid concentration and strong antioxidant activity.

Keywords: Antioxidant, flavonoid, *Glycyrrhiza glabra*, phenolic, Frap

Introduction

The study is invitro based on spectrophotometric method. Substances of free radicals are produced while normal cellular function. But excessive accumulation of the free radicals may leads to serious pathological conditions. Antioxidants are molecules which react safely with free radicals and neutralized them[1]. Protection of the free radical damage in the human cells can subside with the concentration of the phytochemicals in plants such as rich antioxidant, phenolic and flavonoids. Those chemical concentrations reduce the oxidative stress and prevent chronic diseases[2]. Synergistically effect of active flavonoids caused to therapeutic effect on metabolic disorders such as hyperlipidemia, diabetes and oxidative stress conditions [3]. Dietary intake of the rich phenolic contained food caused to the improving immune system. Hence phytochemicals having role of protective health from diseases [4].

Currently most researches helps to exploring natural antioxidant in food to utilized and remove the toxins in the body [5].

Glycyrrhiza glabra is a plant that belongs to the Leguminosae family. It is a hardy herb with height up to 2m and found in Sri Lanka under cultivation. The root and stem of the plant are used for medicinal purpose. The root of *G. glabra* has been extensively used in the treatment of many diseases in Siddha Medicine with valuable pharmacological actions. Rootsare used as a smooth muscle depressant, antimicrobial, antiviral, hypotensive, hepatoprotective, antiexudative, spasmolytic, antidiuretic, antiulcer, antimutagenic, antipyretics, antioxidant, anti-inflammatory, antinociceptive, expectorant as well as hypolipidemic and antiatherosclerotic (Sharma et al., 2001).

The roots are sweet and refrigerant which are used as an emetic in large doses, used for retention of urine as well as cough, bronchitis and hoarseness of voice. Externally it can use for the wounds and cuts to arrest the bleeding [6] . Root decoction is used for the falling of the hair and premature graying of the hair.

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Internal bleeding like epistaxis, hematemesis, rectal bleeding can be arrested by giving root powder mixed with ghee [7].

Organoleptic character of *Glycyrrhiza glabra* is *Suwai* (Taste) as *Inippu* (Sweet) ,*Thanmai* (*Veeriyam*) as *Seetham* (Cold) and *Pirivu* (*Vipakam*) is *Inippu* (Sweet) [8].

Objective of this study was to estimate the total antioxidant capacity, total phenolic and flavanoid content of root extract of *G. glabra* and to identify the difference in the effectiveness among the hot and cold water extract of *G. glabra*.

Methodology

Collection of the plant material and chemicals

The root of the *G. glabra* was collected from local market at Colombo and it was authenticated properly. The collected roots were washed thoroughly with distilled water and dried in sun shade. Finally, it was powdered and stored in dry cool air tight container.

Preparation of extract

Root powder was extracted with distilled water and prepared as hot and cold extracts. Cold water extract was prepared with 50 mg of root powder dissolved in 10 ml of distilled water (5mg/ml) and macerated well by using motor and pestle. Then it was centrifuged for 10 minutes in 10000 rpm. The supernatant was separated carefully and diluted to prepare concentration of 1 mg/ml, 2 mg/ml, 3mg/ml, 4mg/ml and 5 mg/ml.

Then hot water extract was prepared using undiluted extract by placing in a water bath at 1000 C for 5 minutes. Then it was allowed to cool to room temperature and centrifuged for 10 minutes at 10000 rpm. The supernatant was separated and the hot extract dilutions of 1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml and 5mg/ml were prepared. All prepared extracts were stored at room temperature.

Antioxidant property and phytochemical analysis

Total antioxidant capacity (Frap Assay)

Total antioxidant capacity was estimated spectrophotometrically according to the procedure described by Benzie and Strain (1996). Twenty microliters of extract (1g/L) was mixed with 1 ml mixed reagent and the absorbance at 593 nm was measured spectrophotometrically after incubating at room temperature for exactly 4 minutes, against a reagent blank. The absorbance of 1000 µM FeSO4 standard also was measured following the same procedure as for the samples. All the test and standard samples were done in duplicates and assay was carried out in replicates (3) separately for hot and cold extract. The ferric reducing antioxidant power was expressed in µmol/g dry weight.

Total phenolic content

The total phenolic content (TPC) in cold and hot water extracts were determined according to the method of Mc Donald et al., (2000) using the undiluted and diluted root extract (1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml, and 5mg/ml). Fifty microliter of each extract was mixed with 0.5ml of 10% Folin – Ciocalteu's reagent and 0.4ml sodium carbonate (7.5%). Then the tubes were vortexed and allowed to stand for 30 minutes at room temperature. Then the absorbance was measured at 765nm.Tannic acid solution (0.1g/l) was used as the standard. The TPC of extracts were expressed as mg tannic acid equivalent per gram of root powder on dry basis and all determinations were performed in duplicates and this assay were done three times for both cold and hot extracts of root powder.

Flavonoid assay

The total flavonoid content was determined by using a method described by Enujiugha, 2010[9]. Five concentrations of hot and cold extract were prepared to determine the flavonoid content of root of *G. glabra*. Briefly, 1.25ml distilled water and 75 µl 5% $NaNO_2$ were added to 250 µl of the extract. Then all the tubes were vortexed and allowed to stand at room temperature for 6 minutes. Then 150 µl 10% AlCl3 was added to the above mixture and allowed to stand at room temperature for 5 minutes. Finally, the absorbance was measured immediately after adding 0.5 ml of NaOH and 2.5 ml of Distilled water at 510 nm.

Tannic acid solution (1g/l) was used as the standard. The flavonoid content was expressed as mg tannic acid equivalent per gram of root powder on dry basis and all determinations were performed in duplicates and all the assays were in replicates of 3.

Statistical analysis

Results were presented as mean ±standard deviation. The significance effect within the groups was assessed by using independent sample t test using SPSS Software. $p< 0.05$ was considered as significant.

Results

Total antioxidant capacity

Antioxidant activity (µmol/L) increased with the plant extract concentration (Figure 1). Both hot and cold extracts had R2 > 0.99. Hence antioxidant activity was proportional to the extract concentration. Antioxidant activity of *G. glabra* in hot water extract was higher compared to cold water extract.

Independent samples t test indicates a significant ($p < 0.05$) difference in antioxidant capacity between the hot and cold water extracts (Table 2).

Total phenolic content

Total phenolic concentration increased with the plant extract concentration (Figure 2). Both hot and cold extract had R2 > 0.9. Hence phenolic concentration was proportional to the extract concentration. Total Phenolic concentration in hot water was higher than in cold water extracts.

Independent samples t test was performed to compare the mean phenolic content. Total phenolic content in hot water extract was higher than in cold water extracts and the difference was significant ($p < 0.05$) (Table 3).

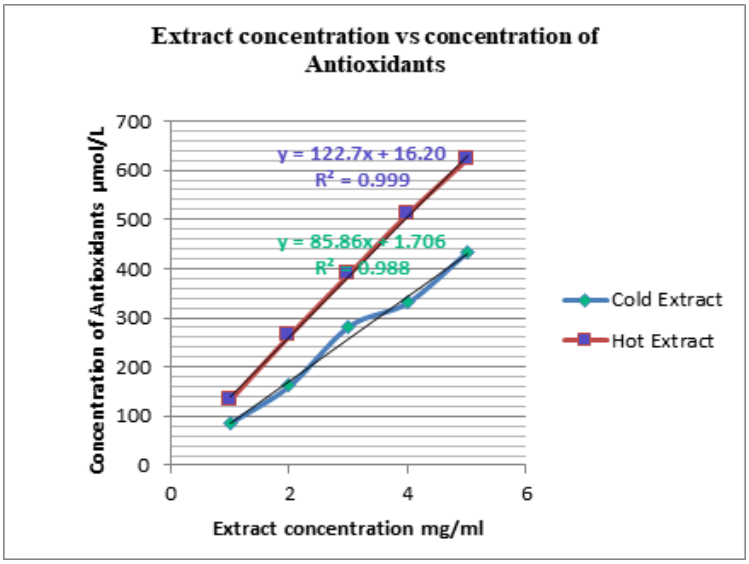

Figure 1: Total antioxidant capacity of hot and cold water extracts prepared with different amount of dry powder

Table 1: Independent samples T test comparison between the antioxidant capacity of hot and cold water extracts

Levene's Test for Equality of Variances		t test for Equality of means				
F	Sig	t	dt	Sig (2-Tailed)	Mean Difference	Std. Error Difference
.022	.887	-17.01	8	.000	-41.50	2.43
		-17.01	7.7	.000	-41.50	2.43

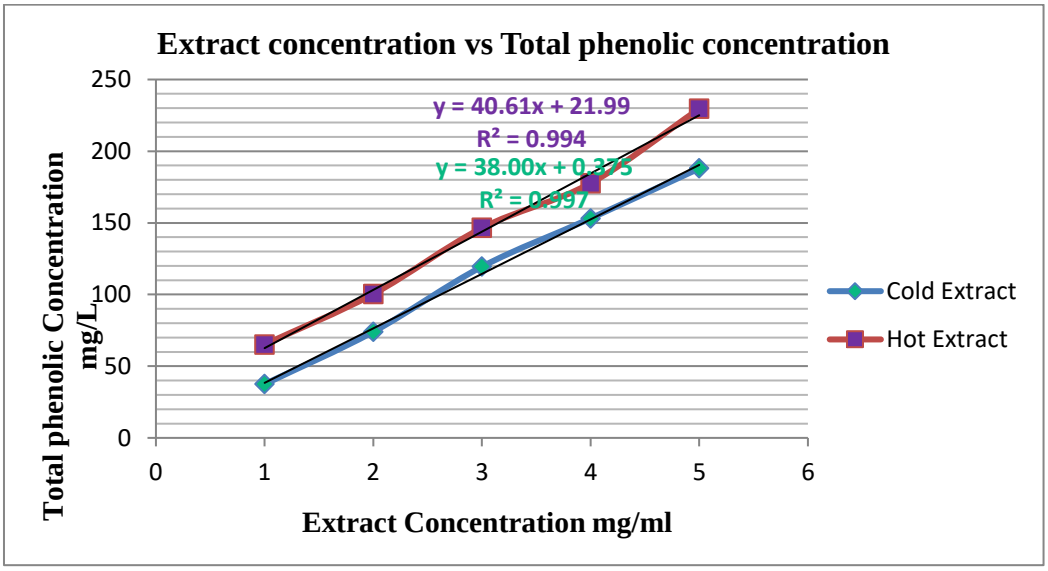

Figure 2: Total Phenolic concentration of hot and cold water extracts prepared with different amount of dry powder

Table 2: Independent samples T test comparison between the Total phenolic content in hot and cold extracts

Levene's Test for Equality of Variances		t test for Equality of means				
F	Sig	t	dt	Sig (2-Tailed)	Mean Difference	Std. Error Difference
4.71	.062	-3.81	8	.005	-13.78	3.61
		-3.81	4.03	.019	-13.78	3.61

Discussion

Phytochemical concentration has major property to protect against free radical damage in the cells. Most of the studies based on the phytochemicals have shown that plants have rich antioxidant capacity due to Vitamin A, C and E, phenolic compound such as flavonoids, Tannin and lignins. All of these compounds act as antioxidants and reduce the oxidative stress [2].

Flavonoids are one of the major micronutrients of the plants and active flavonoid component has synergistic effect on different biochemical pathways, which could contribute to the therapeutic effect on metabolic disorders such as hyperlipidemia, diabetes and oxidative stress conditions [3]. Plants containing flavonoids has biological activities due to their antioxidant properties and also modulation of lipid peroxidation. Other important chemical compounds of plant which contain phenolic, steroids and glycosides act individually or synergistically as antioxidants and those compounds may play major role as antihyperlipidemic substances [10].

Herbal medicines and medicinal plants are source of traditional medicines, folk medicines, food supplements and even modern medicines. In practice plant extractions are used as decoction, infusion and fluid extracts, tinctures or semisolid extract. Universal solvent of water is commonly used to extract plant products and water soluble phenols are important antioxidants [11].

The present study observed *G. glabra* is having antioxidant capacity, phenoilc and flavonoids in both hot water and the cold water extracts. Five different concentrations of hot and cold extract extracts (1mg/ml, 2mg/ml, 3mg/ml 4mg/ml and 5mg/ml) were used to analyze the ferric reduction antioxidant power, and evaluate the total phenolic and flavonoid

content of root of *G. glabra*. The phytochemicals concentration were in the order of 1mg/ml < 2mg/ml < 3mg/ml < 4mg/ml < 5mg/ml with hot water extract being higher than in cold water extract.

Recent studies have observed total phenolic content and antioxidant capacity are better extracted with hot water [12]. Flavonoids and phenolic substances protect atherosclerosis, hyperlipidemia, hyperglycaemia like metabolic disorders [13].

Conclusion

The results of the present study demonstrate that the root extract of *Glycyrrhiza glabra* has antioxidant capacity, with phenolic and flavonoid content higher in hot water extracts than cold water extracts.

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