Evaluation of antioxidant and urease inhibition activities of roots of *Glycyrrhiza glabra*

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Abstract: The object of this study is to determine the antioxidant activity of extracts from *Glycyrrhiza glabra* roots. The parent extract is methanolic extract while its sub fractions were prepared in ethyl acetate, chloroform, and n-butanol. The method based on scavenging activity and reduction capability of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH). Urease inhibition activities of these extracts were also evaluated. Chloroform fraction was the most effective antioxidant with 87.7% activity but the activity is less than the crude methanolic extract i.e. 90%. Chloroform fraction showed the same trend in reducing power as that in radical scavenging activity. However n- butanol extract was devoid of any activity when compared to standard BHA. Crude methanolic fraction and its sub-fractions were also screened for enzyme inhibition activities using jackbean urease as substrate. Significant anti urease activity i.e. 72 % was observed in the ethyl acetate fraction with respect to standard inhibitor thiourea.

Keywords: *Glycyrrhiza glabra,* 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH), radical scavenging, reduction capability, antioxidant, anti-urease.

INTRODUCTION

Glycyrrhiza glabra is a common herb of Mediterranean and Asian region. It is famous with other names as well like licorice and Sweet wood. The licorice belongs to pea family with subtropical habitat. Its oval leaflets, flat pods and purplish white flower clusters look very beautiful. It has extensive root system and its soft, fibrous main taproot with bright yellow interior is used for medicinal purpose (Olukoga & Donaldson, 1998). Traditionally it is used to treat liver diseases and is a major component of polyherbal formulations for the cure of hepatotoxicity (Rajesh *et al.,* 2000). The hypocholesterolemic and hypoglycemic activities of glycyrrhiza glabra root was reported in 1991 (Sitohy *et al.,* 1991). Isoflavone derivatives of glycyrrhiza glabra were reported to have protective role against oxidative stress (Haraguchi *et al.,* 2002).

Super oxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$), nitrogen oxide ($NO^{\cdot}$), peroxynitrite (ONO_2^-) and hypochlorous acid (HOCl) are well known reactive oxygen species (ROS) produced under oxidative stress that have been observed for their degenerative or pathologic role like aging (Bums *et al.,* 2001), cancer, coronary heart disease, Alzheimer's disease (Gay, 1990; Ames, 1983; Diaz *et al.,* 1997; Smith *et al.,* 1996), neurodegenerative disorders, atherosclerosis, cataracts and inflammation (Aruma, 1998). Against hazards of ROS, body has developed a defense mechanism based on antioxidants for cells and organ protection. The components of this defense system interact synergistically to neutralize ROS (Percival, 1998). Antioxidants both from exogenous and endogenous sources help maintain the oxido/redox balance (Mushtaq *et al.,* 2005). The efficiency of natural antioxidant system can be enhanced by intake of dietary antioxidant compounds (Halliwell, 1994; Terao *et al.,* 1994). Butylated hydroxytoulene (BHT) and butylated hydroxyanisole (BHA) are used in processed foods as synthetic antioxidants although some side effects are reported (Fukushima *et al.,* 1983; Yildirim *et al.,* 2001). Therefore this area desperately needs research for new effective antioxidants with minimal side effects.

The aim of present study is to evaluate roots of *Glycyrrhiza glabra* for their potential as antioxidant and urease inhibition activities. Various extracts of roots were used to determine its 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) radical scavenging and reduction capability. Additionally, urease inhibition of these root extracts was also evaluated.

MATERIALS AND METHODS

Plant Material & Preparation of Extracts:

The roots of *Glycyrrhiza glabra* was purchased from local market of Karachi and identified by the Chairman Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi. A sample was kept in the Herbarium (NF-035) of the department. The plant material was shade dried and 1 kg of it was chopped into small parts and then extracted at room temperature with 10 L methanol (three weeks × 3 times) and filtered. The filtrate was evaporated under reduced pressure at 45°C to

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yield 112.5 gm residue. For fractionation, a 625 mg of crude extract was dispersed in 500 ml of purified water and shaken with 500 ml of ethyl acetate, chloroform and butanol consecutively. All the fractions were evaporated under reduced pressure to obtain 190 mg ethyl acetate fraction, 165 mg chloroform fraction and 90 mg n-butanol fraction and used for enzyme inhibition and antioxidant activities.

Determination of DPPH radical scavenging activity

The free radical scavenging activity was measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH) (Cotelle *et al.*, 1996). DPPH solution (0.3mM) was prepared in ethanol. The activity was measured in different concentrations of each extract ranging 62.5 µg- 500 µg. 5 µl of different concentrations ranging (62.5 µg - 500 µg) of each extracted sample was mixed with 95 µl of DPPH solution in ethanol. The prepared dilutions were dispersed in 96 well plate and incubated at 37°C for 30 min. The absorbance was measured at 515 nm in microtitre plate reader (Spectramax Plus 384 Molecular Device, USA). The percent radical scavenging activity of root extracts was determined against methanol treated control.

$$\text{DPPH scavenging effect}\,(\%) = \frac{\text{Ac - As}}{\text{Ac}} \times 100$$

Where

Ac = Absorbance of Control (DMSO treated)

As = Absorbance of Sample

Determination of total reduction ability

Total reduction capability of different extracts of glycyrrhiza roots were estimated with some modifications in the reference method (Oyaizu, 1998). Phosphate buffer (250 µl, 0.2 M, pH 6.6) was mixed with 100 µl of each extract followed by the addition of 250 µL potassium ferricyanide [K_3 Fe-$(CN)_6$] (1%). The mixture was incubated at 50° C for 20 min. After incubation, 250µL Trichloro acetic acid (10 %) was added and the mixture was centrifuged for 10 minutes. Upper layer of solution (250 µl) was separated in another set of test tubes and mixed with equal volume of deionized water. 50 µL Ferric Chloride [$Fecl_3$] (0.1 %) was added to the aqueous solution and the absorbance was measured at 700 nm on spectrophotometer (Specord 200, Germany). Higher is the absorbance of reaction mixture stronger is the reducing power.

Evaluation of urease inhibition activity

Urease (Jack bean) solution (25 µl) was mixed with the 5 µl extracts (500 µg) and incubated at 30°C for 15 min. Aliquots were taken and immediately transferred to assay mixtures containing urea (100mM) in buffer (40 µl) and re-incubated for 30 min in 96 well plate. Urease activity was determined by indophenol method based on measuring of ammonia produced (Weatherburn, 1967). 50 µl each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µl of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to wells. Increase in absorbance was measured after 50 min at 630 nm on microtitre plate reader (Spectramax Plus 384 Molecular Device, USA). Final volume of reaction is 200 µl at pH 8.2 (0.01 M K_2HPO_4. $3H_2O$, 1mM EDTA and 0.01 M $LiCl_2$). All reactions were performed in triplicates. The standard used in this assay was Thiourea and percentage inhibitions were calculated by formula:

$$100 - \left(\frac{\text{OD test}}{\text{OD control}}\right) \times 100$$

RESULTS

1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) is a quick method used by most of the researchers to determine antioxidant activities. DPPH is a free radical that keeps its stability in aqueous or ethanolic solutions. It accepts an electron or hydrogen ion to become a stable diamagnetic molecule (Soares *et al.*, 1997). Therefore, DPPH is occasionally used as a substrate to determine antioxidant activity of compounds. In an experiment, change in absorbance of DPPH radicals was monitored that was indicated by discoloration from purple to yellow through free radical scavenging in various extracts of *Glycyrrhiza glabra*. Fig. 1 shows scavenging ability of various root extracts of *Glycyrrhiza glabra* compared with the standard BHA.

All fractions possess significant antioxidant activity except n-butanol fraction as shown in fig. 1. The highest activity of chloroform fraction was followed by ethyl acetate and n-butanol (87.7, 84.4 and 28.4%) respectively, although these values were lower than the antioxidant value (90.1 %) of crude extract.

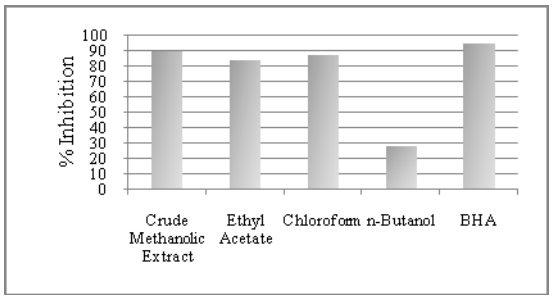

Fig. 1: Radical scavenging activity of BHA and various fractions of *Glycyrrhiza glabra.*

Fig. 2 shows comparison of radical scavenging effect of *Glycyrrhiza glabra* root extracts in chloroform, ethyl acetate, its crude methanolic extract and standard BHA at various concentrations (62.5, 125, 250 and 500 µg). The radical scavenging effect of chloroform fraction was 26, 66, 80, and 87% with respect to increasing concentrations showing that free radical scavenging activity increases in

dose dependant manner. The scavenging activity of ethyl acetate fraction on DPPH radical were observed as 77, 81, 86 and 84% at the concentration of 62.5, 125, 250 and 500 µg respectively. Interestingly crude methanolic extract of *Glycyrrhiza glabra* at the concentration of 62.5 µg, exhibited 91.3% scavenging activity as compared to standard (BHA) with 58.8% activity at the same concentration.

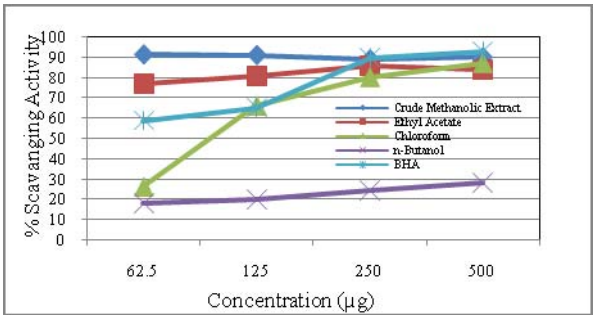

Fig. 2: Radical scavenging activity of BHA and various extracts of *Glycyrrhiza glabra* at various concentrations (62.5, 125, 250 and 500 µg).

In this study, the reductive ability of various extracts of *Glycyrrhiza glabra* and standard antioxidant (BHA) was measured by investigating the $Fe^{+3} \rightarrow Fe^{+2}$ transformation according to the method of Oyaizu and is shown in fig. 3. Again Crude methanolic extract shows the maximum reduction ability as compared to standard BHA, chloroform and ethyl acetate fractions. Reducing power of crude methanolic extract and its fractions followed the order: Crude Methanolic extract > BHA > Chloroform fraction > ethyl acetate fraction > n-butanol fraction. It has been reported that reducing power was associated with the antioxidant activity (Yen *et al.,* 1999).

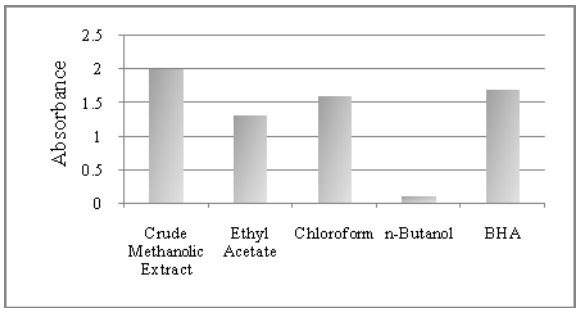

Fig. 3: Total reduction ability of various fractions of *Glycyrrhiza glabra* and BHA.

The Urease Inhibition activities of crude extract and its subsequent fractions of *Glycyrrhiza glabra* against jackbean Urease Enzyme are presented in fig. 4. The crude methanolic extract shows significant 64% inhibition against jackbean urease but the maximum urease inhibition activity was observed in ethyl acetate fraction that is 72 %. The inhibition of Urease activity evaluated for chloroform and n-butanol fraction were 42 % and 7.6 % respectively.

DISCUSSION

Recently urease inhibitor drugs are focused for their potent anti-ulcer role (Amtul *et al.,* 2002). Urease Activity has been shown as an important virulence determinant in the pathogenesis of many clinical conditions, detrimental for human and animal health as well as for agriculture (Mobley *et al.,* 1995; Calderon *et al.,* 2001). Therefore, strategies are being designed based on urease inhibition for the treatment of infection caused by urease producing bacteria. The crude methanolic extract and the fractions screened for urease inhibition activities can be considered as a source of natural urease inhibitors.

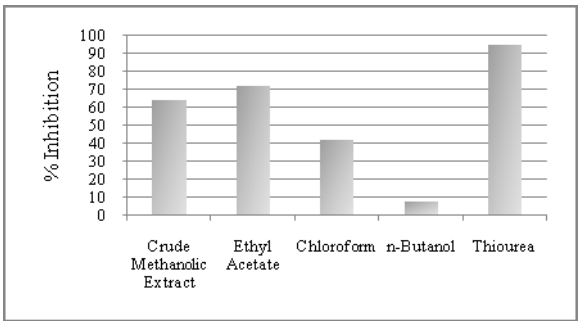

Fig. 4: Urease inhibition activity of crude extract of *Glycyrrhiza glabra* and its fractions.

The present study suggests that roots of *Glycyrrhiza glabra* is a potential source of antioxidants and urease inhibitors and the constituents may considered as a lead compound in the study of drug discovery and designing for ulcer treatment.

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