

Evaluation of the Antioxidant Capacity of Different Extracts of *Astragalus glaucacanthus*

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ABSTRACT

The issue of free radicals and their effects on biological systems is an important subject in Medicine. Antioxidants can protect against free radicals in biological systems. In recent years the use of synthetic antioxidants, like other chemical additives, due to potential toxicity and carcinogenicity is limited. Nowadays, most of studies in this field are use of new and safe antioxidants from plants, animals and microbes. The reserve supply of antioxidants to reduce oxidation stress effects is important. In this study, antioxidant activity of the *Astragalus glaucacanthus* extracts was determined by using free radical scavenging assay and regenerative properties of Fe³⁺ capacity and the synthetic antioxidant such as BHT and vitamin C were positive control. The methanol extract showed the highest antioxidant activity in all tests. The results showed significant antioxidant activity of *Astragalus glaucacanthus* with a rich source of antioxidant compounds.

KEYWORDS: Antioxidant, *Astragalus glaucacanthus*, DPPH, FRAP, BHT, Vitamin C

INTRODUCTION

The importance of plants in medicine remains even of greater relevance with the current global trends of shifting to obtain drugs from plant sources, as a result of which attention has been given to the medicinal value of herbal remedies for safety, efficacy, and economy [1,2]. The medicinal value of plants lies in some substances that produce a physiological action on the human body [3]. The plant is source of active compounds and plants act as antibacterial and antioxidants agents [4-5].

Astragalus glaucacanthus is from *Astragalus* genus and Fabaceae family [6]. The genus of *Astragalus* L. has 2000–3000 species in the world [7–8]. This genus is widely distributed throughout the temperate and arid regions of the world, and is 1500 species located in Asia, 500 species in North America 150 species in South America, and 120 species in Europe, but also on mountains in Africa [9, 10]. Species of *Astragalus* genus utilized as medicinal herbs against stomach ulcer, chronic bronchitis, cough, gynecological disorders, hypertension, diabetes and venomous bites of scorpion [11]. In this study, the inhibition of free radicals and ferric reducing ability in methanol and dichloromethane extracts of *Astragalus glaucacanthus* was investigated.

MATERIALS AND METHODS

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Chemicals and reagents

All chemicals and reagents were bought from Merck (Germany) compound.

-Plant material

The Plant material was collected in Jun 2014 from the North Khorasan Province Mountains of Iran.

- Preparation of plant extract

The aerial parts of the plants were dried under shade at room temperature and then cut into small pieces. About 160 g of sample was macerated in methanol and dichloromethane at room temperature for 48 h separately. Each solvent was allowed to remain in contact with plant material for 24 h, and replaced with fresh solvent four times. Removal of the solvents under vacuum at 40 °C gave the crude extracts.

- 1-2 Dipheny-picrylhydrazyl (DPPH) assay

The inhibitions of free radicals with methanol and dichloromethane extracts of *Astragalus glaucacanthus* were determined with DPPH method. This test depends on the reduction of purple DPPH to a yellow colored diphenyl picryl hydrazine. The maximum absorption of DPPH was measured at 517 nm. The different concentrations of each extracts were made in methanol (0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0.05 and 0.01) mg/mL and then 2.5 mL of samples was added to One mL of DPPH solution (0.3 mM). Methanol was blank. The mixtures react

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for 30 minutes at room temperature in darkness. The absorbance of samples was measured at 517 nm and Scavenging capacity of samples was prepared with the following equation:

$$\text{Scavenging capacity (\%)} = \frac{\text{absorbance of sample} - \text{absorbance of blank}}{\text{absorbance of blank}} \times 100$$

The tests were done in triplicate. The concentration of sample required to scavenge 50% of DPPH (IC₅₀) were determined by Biodatafit program [12].

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) of the extracts was measured by method of Xu. The FRAP reagent contained 2,4,6-tripyridyl-s-triazine (TPTZ) solution (10 mM) in 40 mM HCl, 20 mM FeCl₃·6H₂O and 0.3 M acetate buffer with pH 3.6. Three mL freshly prepared FRAP reagent mixed with 100 μl of each sample was incubated at 37 °C for 10 min in a water bath. After incubation, the absorbance was measured at 593 nm. Aqueous solutions of known Fe (II) concentration, in the range of 0-1 mM (FeSO₄·7H₂O), were used for calibration. FRAP values were expressed as mean ± standard error (SE) mmol Fe (II) per gram [13]. In this study, BHT and vitamin C considered as a positive control. The antioxidant power of extracts was weaker than vitamin C and BHT.

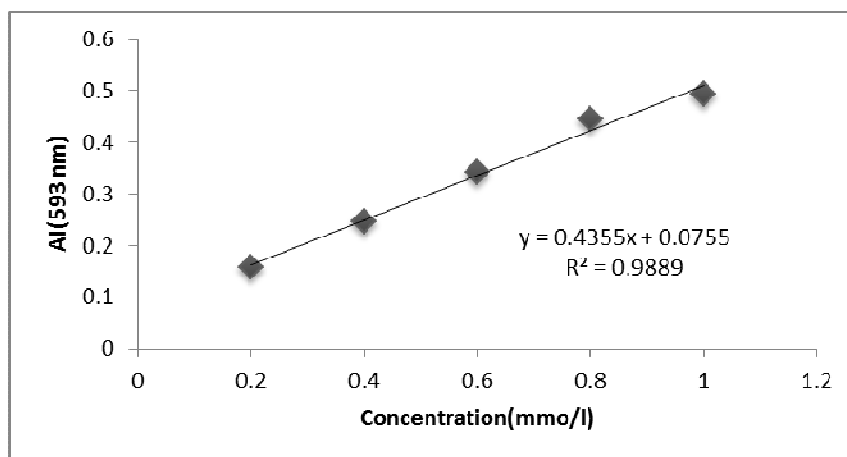


Fig. 1 Standard curve for Fe 2+ in FRAP assay

- Statistical analysis

All the assays were done triplicate. The results were expressed as means ± Standard deviation (SD). The statistical analyses were done with SPSS programmer.

RESULTS

As shown in figure 1, The methanol extract at all concentrations used, has the highest percentage of DPPH with amount of IC₅₀=0.196 and for dichloromethane extract was 0.536 μg/ml. Methanol and dichloromethane extracts of the leaves of *Astragalus glaucacanthus* were significantly less effect than Positive control such as BHT and VitC. In the investigation of Partasrasy [14] the amount of IC₅₀ for BHT was 0.004 mg/ml and in this study, the amount of IC₅₀for BHT was 0.003 mg/ml and there was not significantly different.

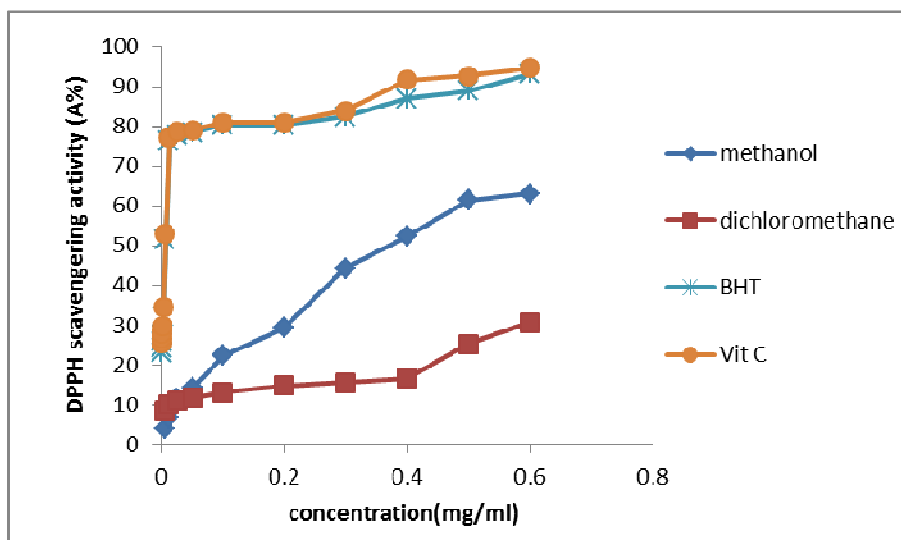


Fig. 2 Antioxidant activity for various extracts and positive control in DPPH method

Antioxidant activity of ferric ions was measured by reducing of ferric (II) to ferrous ions. As shown in Fig 3 In the result of FRAP assay, methanol extract at all concentrations with the highest amount of mmol Fe²⁺ per g extract (0.289) showed highest antioxidant activity in this method and dichloromethane extract was weaker than methanol extract with 0.177 mmol Fe²⁺ per g extract. Vitamin C and BHT was stronger than extracts. (0.554, 2.11 mmol Fe²⁺ per g sample).

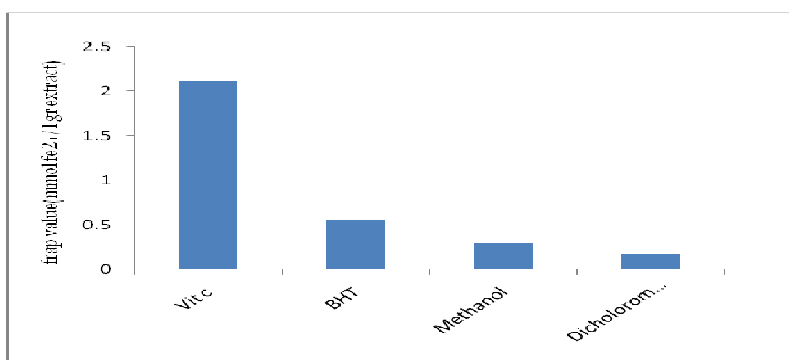


Fig 3: Comparison of antioxidant activity of methanol and dichloromethane extracts of *Astragalus glaucacanthus*, vitamin C, BHT in FRAP method

DISCUSSION

Many studies have used a variety of methods to determine the antioxidant capacity. Antioxidant activity affected to different conditions such as solubility into organic and aqueous phases, temperature, light intensity, and the oxidation conditions and the use of one method is not suitable for measuring antioxidant capacity [15].

In figure 1 and 2 there was significantly different in different concentration for evaluating of antioxidant activity and antioxidant activity was depending on concentration.

In other studies, IC50 for other species was 8.68 to 400.6 microgram per milliliter [16]. Concentrations and physicochemical properties of compounds were effective [17]. As a result, researchers have been observed difference between different extracts is depending on different polarity for solvents (as shown in Figure 1).

Extraction of antioxidant compounds from plants are depended on to the solubility of these compounds in various solvents .The polarity of solvents was key role in increasing he solubility of the compounds[18]. Researches indicate that high antioxidant activity of some extracts Such as methanol and ethanol extracts was due to high levels of phenolic compounds, because of a positive association between total phenols and antioxidant activity [19].

CONCLUSION

The results of this study indicate that the polarity of the solvents used to play a key role in increasing of antioxidant activity. In general, the amount of antioxidant for extracts can be concluded that the Methanol extract>the dichloromethane extract<BHT <Vit C. Further investigation of this plant is also used as for preservative and antioxidant compounds in industry, agriculture, food, Pharmaceutical and Cosmetics.

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