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Evaluation of linoleic acid oxidation in roots of *Astragalus glaucacanthus*

Somaye Langari[1], Esmaeil Ataye Salehi[2], Ameneh Mohammadi[3*]

[1]MSc of Department of Food Science and Technology, Quchan Branch, Islamic Azad University, Quchan, Iran
[2]Department of Food Science and Technology, Quchan Branch, Islamic Azad University, Quchan, Iran
[3]Natural Products and Medicinal Plants Research Center, North Khorsan University of Medical Sciences, Bojnurd, Iran.

Abstract : Astragalus genus is from Fabaceae family contains 3000 species in worldwide and it has hepatoprotective, immunestimulant, antiperspirant, tonic and diuretic properties and the dried roots of this genus have a long history of medicinal use in traditional Chinese medicine. Linoleic acid is the wild world polyunsaturated fatty acid and antioxidants are compounds that can inhibit lipid oxidation. The target of this study was evaluation of linoleic acid oxidation in roots of *Astragalus glaucacanthus* and also its phenolic content was evaluated. The total phenolic content in the methanol and dichloromethane extracts of *Astragalus glaucacanthus* was determined using Folin-Ciocalteu reagent and antioxidant activity of them was evaluated by betacaroten-Linoleic acid method. The results showed methanol extract had highest amount of phenolic content and at a concentration of 2 mg/ml; BHT, methanol and dichloromethane extracts of plant were shown to exhibit antioxidant activity levels of 87.8%, 16.2% and 3.0% respectively and there was a Significant differences ($P \leq 0.01$) in total phenolic content and antixodant activity between methanol and dichloromethane extracts. In conclusion we can conclude *Astragalus glaucacanthus* isn't a good antioxidant plant.
Keyword: *Astragalus glaucacanthus*, antioxidant, linoleic acid oxidation, phenolic content.

Introduction:

Astragalus genus is from Fabaceae family contains 3000 species in worldwide[1]. It was found in the central Asia, western North and South America[2]. *Astragalus* genus has hepatoprotective, immunestimulant, antiperspirant, tonic and diuretic properties and the dried roots of this genus have a long history of medicinal use in traditional Chinese medicine, for example they used as an immunomodulating agent in mixed herbal decoctions to treat common cold, diarrhea, fatigue and anorexia[3]. Active compounds in roots of Astragalus genus are polysaccharides, triterpene glycosides, flavanoids and isoflavanoids[4,5,6]. Inhibition of lipid oxidation in food industry is important[7]. Synthetic antioxidants such as butylated hydroxytoluene were added to food products but Natural antioxidants have recently increased because of negative effect of synthetic antioxidants[8].

Antioxidant activities of these compounds have been reported by many researchers and several antioxidants have already been developed[9].

Linoleic acid is the wild world polyunsaturated fatty acid and antioxidants are compounds that can inhibit lipid oxidation. One of the Iranian species of Astragalus genus is *Astragalus glaucacanthus* and the

target of this study was evaluation of antioxidant activities from roots of *Astragalus glaucacanthus* in a linoleic acid oxidation system and also its phenolic content was evaluated.

2. Materials and methods

2.1. Plant material

The Plant material was collected in Jun 2014 from the North Khorasan Province Mountains of Iran. The plant was identified from the Natural Products and Medical Sciences Research Center of Bojnurd University of Medical Sciences.

2.2. Preparation of plant extract

The dried plant (100 g) was macerated with dichloromethane (CH_2Cl_2) and methanol (MeOH) at room temperature for 24 °C. The extract was filtered and solvent was evaporated under vacuum at 40 °C to obtain 8.5 and 1.4 gram extract (yield 8.5 and 1.4%). The Extracts were stored at 4 °C until analysis.

2.3. Determination of total phenolic content

The total phenol content in the methanol and dichloromethane extracts of *Astragalus glaucacanthus* was determined using Folin-Ciocalteu reagent (10). In this method, 100 µL of extracts with 1000 mg/L concentrations were added to 100 µL Folin-Ciocalteau reagents (50%). After that, 2.8 ml H_2O and 2 mL of Sodium carbonate (Na_2CO_3) (2%) were added to tubes, and then the tubes were incubated for 60 min at room temperature. The absorbance was read at 720 nm. The analyses were performed in triplicates. The standard curve was prepared by gallic acid and total phenol contents were expressed as gallic acid equivalent (mg GA /g of dry extract)[10].

2.4. Determination of the antioxidant potential through the betacarotene/ linoleic acid system

For preparation of reagent, 20 µL of linoleic acid, 200 mg of tween 20, and 1 mg of Beta-carotene were added to 5 mL of chloroform and then were kept at 40 °C until all the chloroform had evaporated. After that, 50 mL of distilled water was added to the mixture and it was shaken. In each tube, 6 m L of the reagent was added to 50 µL of the extracts. 6 mL of the reagent with 50 µL of methanol was the control tube. After that the absorbance was read at 470 nm (Abs $_0$), and then tubes were placed in a water Bath at 50 °C for 2 h to catalyze the oxidation reaction and discoloring of beta-carotene. After 2 h, the absorbance of each tube was read again (Abs $_{120}$) (11). Antioxidant activity index (AAI) was carried out using Eq. (1):

$$AAI\% = 1 - \frac{(Abs0 - Abs\,120)sample}{(Abs0 - Abs\,120)control} \times 100 \qquad (1)$$

3. Statistical analysis

All data were reported as mean ± standard deviation of three replicates. The results were analyzed by the analysis of variance (ANOVA) and differences among means at 5% level ($P < 0.05$) were considered statistically significant. All statistical analyses were carried out using the software program STATISTICA 8 (StatSoft, Inc, USA).

4. Results and Discussion

The regression equation for determination of total phenolic contents was: Y=0.009X-0.007 (R^2=0.996). The total phenolic contents of methanol and dichloromethane extracts were shown in Table 1. In the present study, antioxidant potentials of extracts were determined by through the betacarotene/ linoleic acid system and butylated hydroxytoluene (BHT) was used as positive control (Table 1) and the variance analysis of results was shown in table 2. The results showed the methanol extract of plant had higher amount of total phenolic contents. Our result was similar to other literates and we can say the methanol solvent was better solvent than the others in extracting phenolic compounds[12]. In this study, antioxidant activity of methanol and dichloromethane extracts was evaluated with betacarotene/ linoleic acid method. Betacarotene/ linoleic acid test

is used to measure the ability of the antioxidant to inhibit the oxidative of lipids and fatty acids. The antioxidant compounds can inhibit the b-carotene-bleaching by neutralizing the linoleate-free radical[13]. This method is according to the loss of the yellow color of b-carotene due to its reaction with radicals produced by linoleic acid oxidation. The rate of b-carotene bleaching can be slowed down in the presence of antioxidants[14]. In this method, the absorbance of samples decreased rapidly without antioxidant whereas in the presence of an antioxidant, they retained their color and absorbance for a longer time. Therefore, the higher antioxidant activity of the phenolic compounds from plants suggests a biological agent in preventing the oxidative degradation of membrane lipids. In this study, the results were compared to BHT. Fig 1 shows the decrease in absorbance of b-carotene in the presence of plant extracts and BHT.

Table 1- Total phenolic contents and antioxidant activity of *Astragalus glaucacanthus* extracts

extract	Total phenolic (Gallic acid equivalents mg/g of dry extract)	AAI% (antioxidant activity index) in betacarotene/ linoleic acid system
Methanol	6.92±0.56	16.2%±1.32
Dichloromethane	5.02±0.13	3.0%±0.95
BHT		87.8%±0.24

Table 2. Variance analysis of phenol and antioxidant activity content of of *Astragalus glaucacanthus*.

Source of variations	Degrees of Freedom	Squares Average	F
Phenolic content of methanol extract	3	143.659**	0.001**
Phenolic content of dichloromethane extract	3	75.601**	0.00**
antioxidant activity index in betacarotene/ linoleic acid system (methanol extract)	3	78.320**	0.003**
antioxidant activity index in betacarotene/ linoleic acid system (dichloromethane extract)	3	27.00**	0.1**
antioxidant activity index in betacarotene/ linoleic acid system (BHT)	3	23126.520**	0.1**

** Significant differences (P≤0.01)

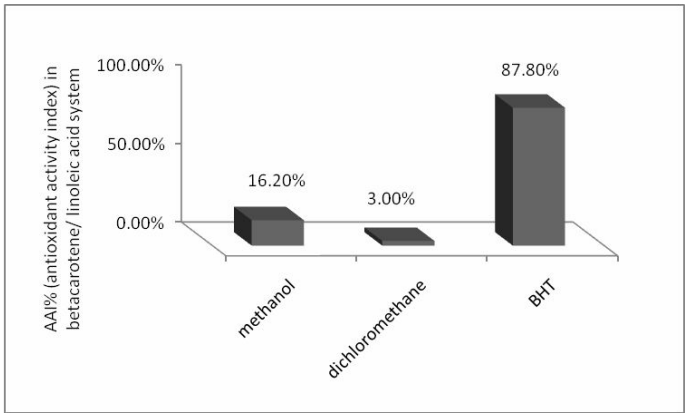

Fig 1. Inhibition of linoleic acid oxidation with extracts and BHT.

The results showed at a concentration of 2 mg/ml; BHT, methanol and dichloromethane extracts of plant were shown to exhibit antioxidant activity levels of 87.8%, 16.2% and 3.0% respectively. The variance analysis was shown in table 2 and the results showed, there was a Significant differences (P≤0.01) in total phenolic content and antioxidant activity between methanol and dichloromethane extracts of *Astragalus glaucacanthus*. In other research, the inhibition of free radicals and ferric reducing ability in methanol and dichloromethane extracts of *Astragalus glaucacanthus* was investigated and the results showed 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 dichloromethane extract < methanol extract < the < BHT <Vit C[15]. Our result also indicated that the methanol extract of *Astragalus glaucacanthus* had highest antioxidant activity in linoleicacid method. With comparison of our result with later study on this plant[15], we can conclude *Astragalus glaucacanthus* isn't a good antioxidant plant.

5. References

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