J. Agric. Food. Tech., 7(6)12-19, 2017

ISSN 2090 – 424X
Journal of Agriculture and Food Technology
www.textroad.com

Antioxidant Activity of Various Fractions Extracted from *Astragalus adscendens* Boiss & Haussk as Persian Manna

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ABSTRACT

Gavan-e-Gaz-angabin (*Astragalus adscendens* Boiss & Haussk) has high potential for producing of traditional medicine because this plant manufacture special manna called Gaz-angabin. The purpose of this research, the assessment of some biochemical factors of methanolic extract from different organs of Gavan-e-Gaz-angabin. The factors measured including antioxidant activity (DPPH radical scavenging as IC_{50}), polyphenol content (Folin-Ciocalteu reagent), flavones and flavonols, total flavonoid (Alumonium chloride method), total carbohydrate and total saponin on different parts (stem, leaves, flower, leaves-flower mix, root, manna) of this plant. The results indicated that, the maximum and minimum content of IC_{50} was observed in mix and stem samples respectively (7680.87 and 5475.75 µg/ml). While the highest and lowest proportions of total flavonoid were examined on flower and manna samples respectively (4.26 and 1.53 mg quercetin/g DW). On the other hand, the highest polyphenol content (13.73 mg Gallic acid/g DW) was obtained in flower sample whereas the lowest content (4.27 mg Gallic acid/g DW) relevant to stem tissue. Furthermore, the different organs of this plant, especially manna, has high level of carbohydrate. Likewise roots and stem have superlative value of saponin. Generally, the results revealed these biochemical factors were differing in various plant organs.
KEYWORDS: *Astragalus adscendens*, Biochemical factors, Persian Manna, Saponin.

1- INTRODUCTION

Astragalus L. is the extensive genus in the *Fabaceae* family and it's one of the widest genera in the plants, containing more than 2500 species. It is genus widespread all over the temperate area of the world (Massoumi 1995). Many species of *Astragalus* genus are used in traditional medicine for their hepatoprotective, antioxidative, immunostimulant virtues. It is also has antiviral and antibacterial activity (Luisa Pistelli *et al.* 2002). *Astragalus* palnts also inciting the body natural output of interferon (Lu and Fun 2011). Different class of chemical compounds have been explained in this genus such as polysaccharides, saponins and phenolics **(**Rios and Waterman 1997). Newly, plenty researchers have taken a great interest in *Astragalus* genus for their pharmacologically activity, phenolic concentrations, related total antioxidant potential and saponin content (Chen *et al.* 2011, Rios and Waterman 1997, Siahpoosh *et al.* 2010). *Astragalus* saponin I (*A. membranaceus*) used for several pharmacological targets, such as oxidative stress, prevention of diabetic nephropathy (DN) and etc.

Astragalus adscendens (Boiss. & Hausskn) a specialty plant in Iran, has been used in producing of special manna that called Gaz-angabin (Grami 1998). It is one of the oldest and most commonly used from different part of this plants in traditional Iranian medicine for example antispasmodic, antiheadache and their manna one of the resources of fructose (40%) that used for diabetics. It has been illustrated *Astragalus* polysaccharides are taking as a group of possible bioactive compound chip in to the useful effects of *Astragalus* (Niu *et al.* 2011). lately, polysaccharides derivated from different species have stimulated interest as provenance of new potential antioxidants, since published data showed that plant polysaccharides have powerful antioxidant operations (Zhong *et al.* 2010).

Different species of *Astragalus* have shown antioxidant activity, for example *A. membranaceus* root (AMT) or *A. polysaccharide* (APS) that used animal dietary (Zhong *et al.* 2012). Polysaccharides of AMT play important pharmacological roles in reducing oxidative stress (Ma *et al.* 1997). The most of extracts (aqueous, hydroalcoholic, Methanol and ethanol extracts) from medicinal plants are indicated excellent antioxidant potential to scavenge free radicals (Zhong *et al.* 2010). Although extensive research done by many researchers on *Astragalus* genus antioxidant effect such as *A. mongholicus* (Lu and Fun 2011, Rios and Waterman 1997, Sokmen 2009, Yu *et al.* 2005) but not any reported about this species. Analyses of Manna Gaz-angubin of this species that previously were measured are listed in table 1 (Samsam Shariat and Moatar 2003). The main goal of this study was to assessment the saponin and carbohydrate content, phenolic compounds, content of flavonoids and radical scavenging activity in methanolic extracts of different part of Gavan-e-Gaz-angubini for the first time from Iran.

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2- MATERIALS AND METHODS

2.1. Plant material and protocol

Different organs of Gavan-e-Gaz-angabini (*Astragalus adscendens*) were collected from a wild populations of Buin O Miandasht (Isfahan- Iran) region in the center of Iran (33° 08′ 58.89″ N and 50° 16′ 52.68″ E). Soil analyses of Gavan-e-Gaz-angubini habitat was reported in table 2. Different parts including root, stem, flower, leaves, manna and combination of leaves and flower (mixed sample) separately were dried on ambient room. Preparation of plant extracts for others parameters was done accordance with the method of Guo et al. (2008) and Krishnaiah et al. (2011) with minor revision. Plant material powdered (1 g) be weighted into a test tube, and then 5 ml solvent (70% hydroalcoholic) added. Afterwards, the solution was stirred alittle and the test tubes were put on shaker (200 rpm) for 24 h. Extracts were centrifuged for 15 min (6000 rpm), and supernatants were isolated and stored at 4 ˚C.

2.2. Antioxidant activity determination (DPPH radical scavenging)

Antioxidant potentioal was assessed by a spectrophotometric method procedure on the reduction of a methanol solution of DPPH using the method of Guo et al. (2008) and Krishnaiah et al. (2011) with some modifications. In the beginning, 100 µl of different extracts densitis were added to 5 ml of DPPH (0.004% methanol solution). The confection was shaken and were kept at ambient temperature for 40 min in the dark conditions. Then the absorbance of the samples and blank were read at 517 nm. The data were reformed for dilution and expressed in lM trolox per 100 g dry weight (dw). Deterrence of free radical, as percentage (I%) was appraised conforming to formula:

$I\% = (A_{blank} \_ A_{sample})/A_{blank} \times 100$

where A_{blank} was the absorbance of the control responce (without any extract), and A_{sample} is the absorption of treatments. Extract concentration obtaining 50% inhibition (IC_{50}) was camputed from the graph plotting inhibition percent versus extract density. Positive control treatment in this experiment was ascorbic acid and all experiments were carried out in triplicate.

2.3. Total phenolic compound Determination

Total phenolic contents of the extract were determined using the Folin–Ciocalteu agent conforming to Li et al. (2007) method with minor modifications and gallic acid used as standard. The extract solution (100µL) was admixed with 200µL of Folin–Ciocalteu defines (50%). The solution was permit to respond 3 min and then 1000µL aqueous solution of Na_2CO_3 (2%) was added immediately. After that, the mixtures was wiggled strongly and incubated for 45 min at ambient temperature, then was read at 760 nm. Total phenolic amounts were stated as mg gallic acid per g dry weight (mg GA/g DW).

2.4. Total flavonoid determination

Total flavonoid quantity were modulated matching to a colorimetric assessment with minor modification (Bouayed *et al.* 2009). Quercetine at different concentrations was prepared as standard. At the first, 300µL of $NaNO_2$ (5%) added to 1000µL different extract in a 10 ml test tube, and the mixtures were maintenance at room temperature for 6 min. In the following, 300µL of Al $(NO_3)_3$ (10%) was added to the mixture, and followed by adding 4 ml of NaOH (1 N) (methanol was used as solvent). Absorbance at 510 nm was deliberate after 15 min incubation. Total flavonoid quantity was calculated as mg quercetine /g dry weight (mg Q/g DW).

The flavone and flavenol content was used accordance to procedure explained by Popova et al. (2004). The results assessed as mg Quercetine/g dry weight.

2.5. Total saponin content measurement

Saponin quantity were measured by spume experiment. In this method 0.5 gr of different parts powder was added to 10 ml boiling water. After cooling shaker for ten second were done and then the height of spume was measured. The spume has a height of 0-10 cm and should be sustained for 10 minutes by adding HCL.

2.6. Total carbohydrates mensuration

Total carbohydrates quantity, were determined by Yemm and Willis (1954) manner. In this method 100 µl of each extract added to 3 ml anthron and then 10 min putted in benmari. After cooling the absorbance was measured at 625 nm. Results are presented as mg glucose/g dry weight.

2.7. Statistical analysis

Randomized complete design (RCD) was used as basic design with six treatments (different parts of plant organs) three replications. Statistical analysis was done by JMP 8.0 software package software and averages compared at 5% probability by Duncan multiple tests.

3. RESULTS AND DISCUSSION

3.1. Antioxidant activity

The antioxidant activity quantified by DPPH assay are presented in Fig. 1. The results demonstrated antioxidant activity significant differences ($p< 0.05$) between different extracts. Revealed that highest antioxidant activity (IC_{50}= 5475 µg/ml) and lowest antioxidant activity (IC_{50}=7680 µg/ml) were in mix sample and stem sample respectively . Antioxidant activity in some medicinal plants was between 1.93 to 730 µg/ml (Guo *et al.* 2008). Our results showed this plant has low antioxidant potential that may be due to lake solving of antioxidant compound on methanolic extract (Adiguzel *et al.* 2009). Furthermore, antioxidative activity of the different plants and tissue extract greatly pertain to experimental circumstance (Wong *et al.* 2006). Antioxidative valency affected by multiple factors, which cannot be thoroughly characterized with a single manner (Guo *et al.* 2008). Likewise, Sokmen et al. (2009) reported root ethanol extract of *A. macrocephalus* has high antioxidant capacity. Adıgüzel et al. (2009) reported methanol extract than hexan extract of different *Astragalus* species showed highest antioxidant activity. The use of various extracts from *Astragalus* showed ethyl acetate extracts has strong antioxidant activity (Lu and Fun 2011). Proficiency of the boiling water in antioxidant activity extracting from several Chinese plants was higher than methanol extracts (Wong *et al.* 2006). Briefly, the previous consequences represented that the *Astragalus* could be exploited as a rich origin of potentially natural antioxidants (Lu and Fun 2011).

3.2. Total phenolic compound

The total phenolic amount of six organs of *A. adscendens* were measured in this research. The quantity of total phenolic value differed significantly between various organs. The total phenolic values of different parts of gavan-e-gaz angabini, varied between 4.2 and 13.7 mg GA/g DW. The maximum concentration was measured in flower (13.7 mg GA/g DW) and the lowest content was in stem and mix samples (Fig. 2). The average phenolic compound of the tested organs treatments is 9.3 mg GA/g DW. Guo et al. (2008) reported average of total phenolic compound in different chines medicinal herbs were 90 mg/g DW. The extracts total phenolic content of *A. membranaceus* was reported 28.82 mg GA/g (Guo *et al.* 2008). Also total phenolic quantity from five Iranian medicinal plants was differed among 2.15 and 20.3 mg of GA/g DW (Bouayed *et al.* 2007). Comparison of 18 tonic Chinese medicinal herbs showing highest phenolic content was 443.79 in fruit of *Canarium album*. Phenolic compounds have different biologic actions including anti-inflammatory, anti-carcinogenic and cardiovascular protective results. These actions might be caused by their antioxidative acting (Li *et al.* 2007).

3.3. Total flavonoids

The concentrations of total flavonoids of five parts of this plant varied between 1.52 and 4.25 mg of Q/g DW. Leaves and flower containing the highest flavonoids concentration and stem, manna and mix having lowest content of flavonoids (Fig. 3). The flavone and flavonoid content showed in Fig. 4. The manna and flower of this plant have highest and lowest content of flavone and flavonoid respectively. The assembled flavonoids concentrations of some Iranian medicinal plants changing from 0.22 until 10.0 mg of CE/g DW (Bouayed *et al.* 2007).

3.5. Total saponin compounds

Saponin content of different organs of gavane- gaz angabin showing in Fig 5. Stem and roots of this plant and other plant in this genus have gum in centers of stem that called tragaganta. *Astragalus* saponin could use for cell development prevention in diverse cancer cell lines via adjustment of cell reproduction and apoptosis (Law *et al.* 2012). Same of the saponin compound show antifungal activity (Luisa Pistelli *et al.* 2002). The pharmacological activities of saponin-containing medicinal plants result in their marked therapeutic effects (Lovkova *et al.* 2001).

3.6. Determination of total carbohydrate

Results indicated the lowest (2824.37 mg/gr DW) and highest (102174.15 mg/gr DW) total carbohydrate with high difference were observed on flower and manna respectively (Fig. 6). Manna that produce by this plant (Gaz-angabin) is very sweetish because of its great fructose amounts, analogous to honey, with a 40% fructose and other polysaccharides sucrose, glucose, xylose, and mannose (Grami 1998). To some extent, Gaz-angabin importance could be due to its feasible performance as indigenous drug. Spectroscopic analysis (NMR) showed manna of this plant containing compounds α-D-Glucopyranose, β-D-Fructofuranose (Sucrose), β-D-Fructopyranose and oligosaccharides. Furthermore, Gaz-angubin viscosity has much higher than fructose causing presence of high molecular weight in this manna (Farahnaky *et al.* 2009).

Table 1: Analyses of Manna Gavan-e-Gaz-angubini (*Astragalus adscendens*) as Persian Manna (%)

Fructose	Polysaccharides	Sucrose	Ash	Humid	Insoluble in water	Mucilage	Tannins
41.2	31.6	2	2.26	15.2	4.72	3.02	0

Table 2. Soil Analyses of Gavan-e-Gaz-angubini (*Astragalus adscendens*) habitat

Depth (cm)	EC	pH	O.C	Total (N %)	P (p.p.m)	K (p.p.m)	Sand (%)	Silt (%)	Clay (%)	Texture
0-20	0.45	7.4	1.76	0.127	10.5	365	26.5	26	47.5	Clay
20-42	0.62	7.64	1.07	0.092	3	102	40.5	22	37.5	Clay-loam
42-100	0.36	7.87	0.72	0.062	3	50	60.5	20	19.5	Sandy-loam

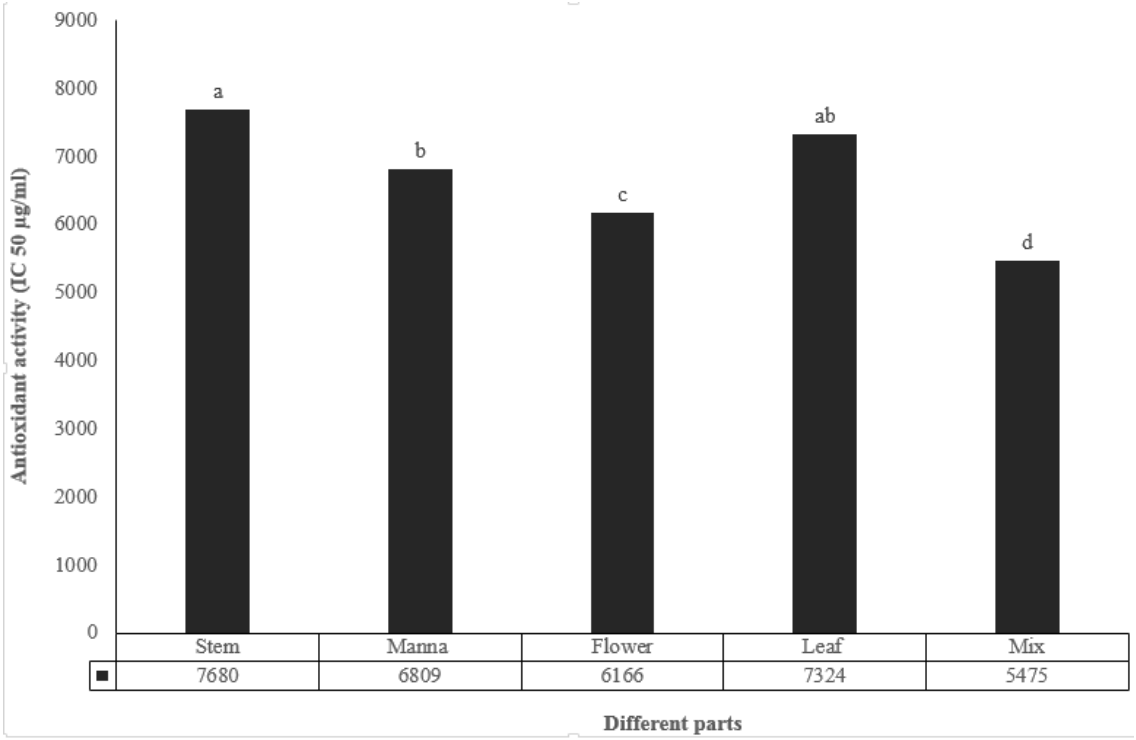

Fig 1. DPPH scavenging activities of different organs from *Astragalus adsendence*

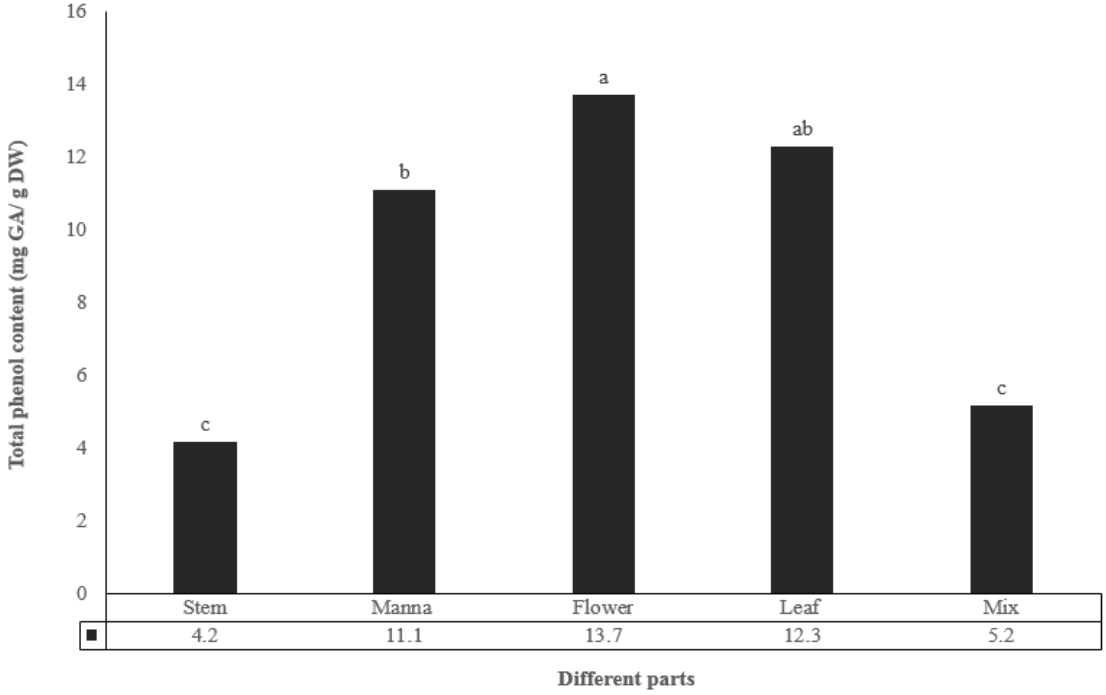

Fig 2. Total phenolic content of various organs from *Astragalus adsendence*

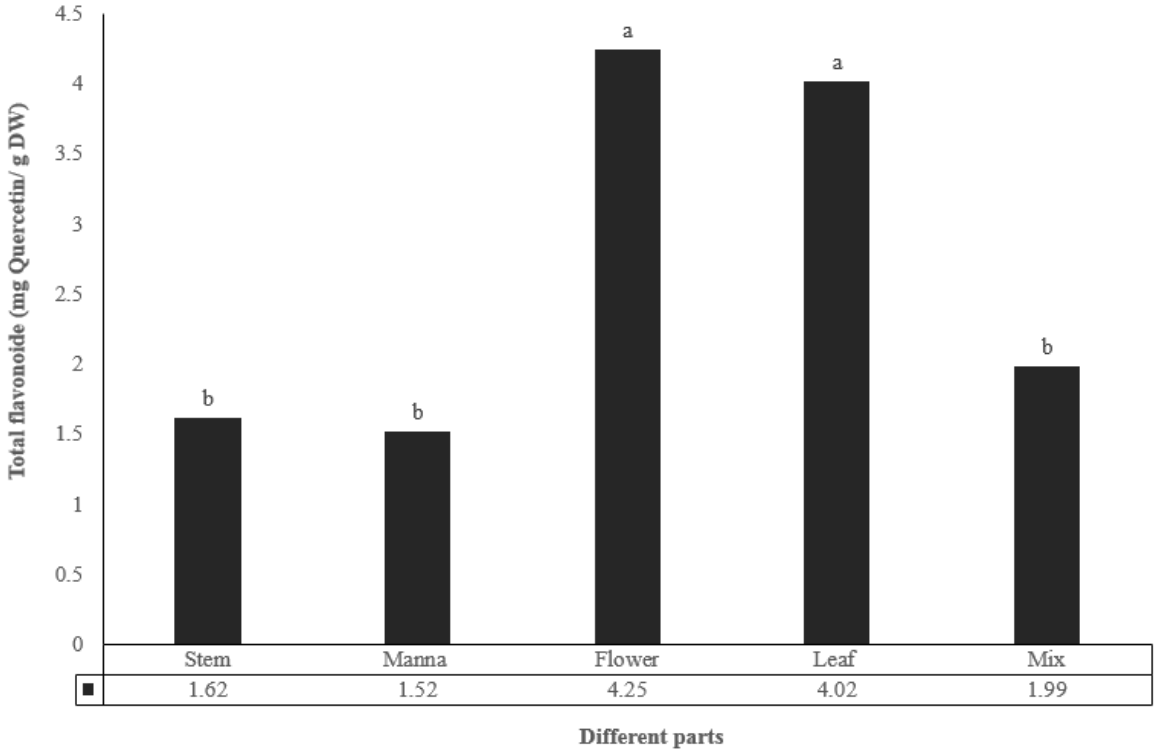

Fig 3. Total flavonoid contents of various organs from *Astragalus adsendence*

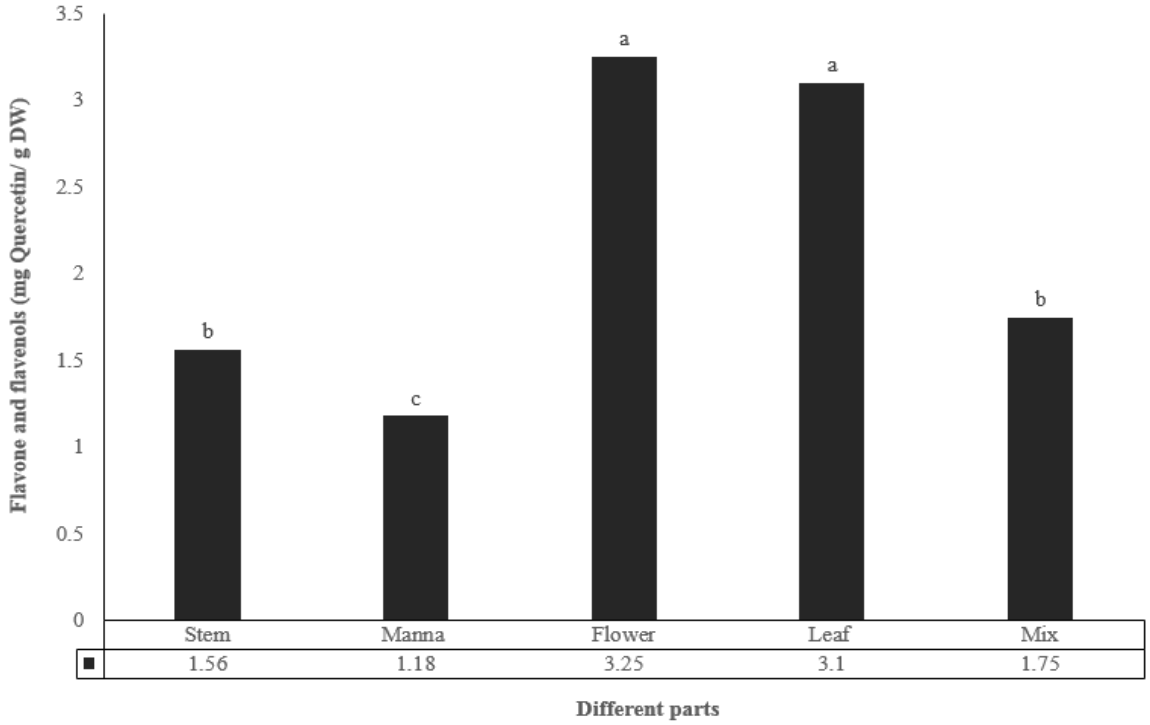

Figure 4. Flavone and flavenols contents of various organs from *Astragalus adsendence*

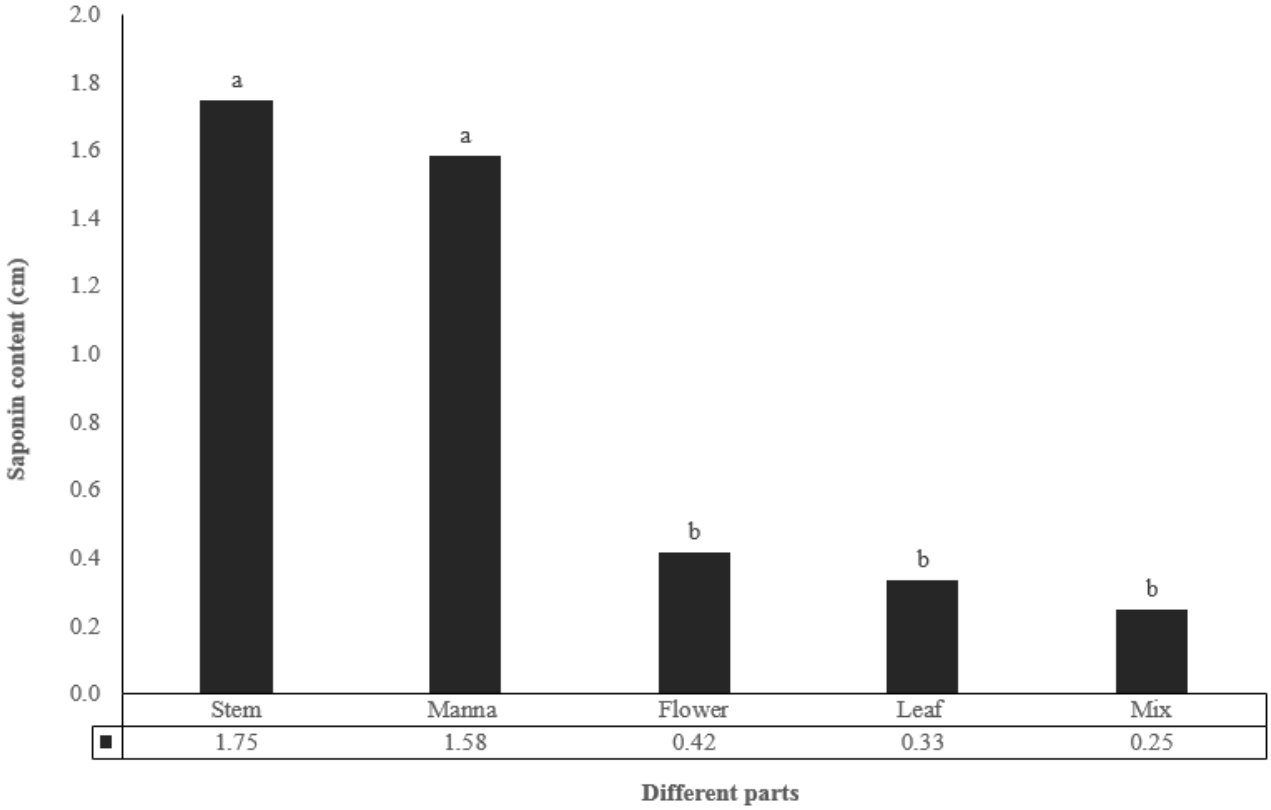

Fig 5. Saponin content of various organs from *Astragalus adsendence*

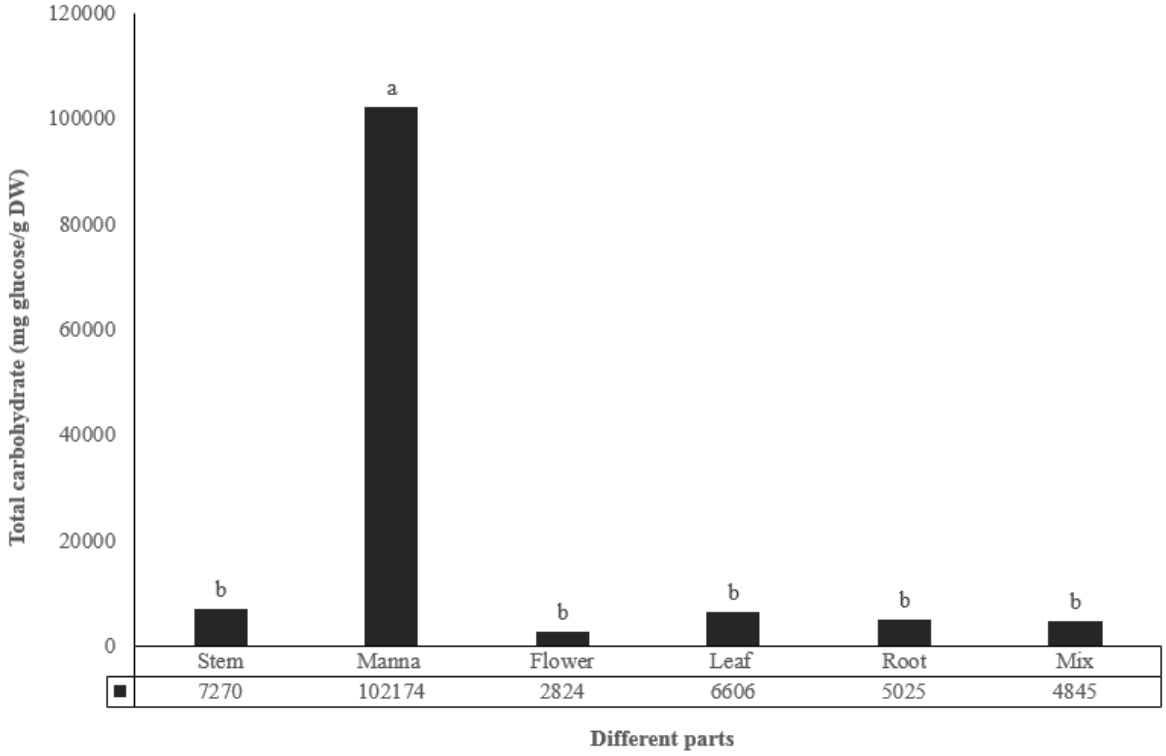

Fig 6. Total carbohydrate content of various organs from *Astragalus adsendence*

CONCLUSIONS

The present study, was investigated antioxidant potentially of the various organs from the *A. adsendence* (Gavan-e-gaz-angabin). Free radical scavenging activity in different organs was various. Comparison of phenolic compound and antioxidant activity of this species and other herbal plant showing that have high pharmaceutical potential. It is clear that content of this compound vary in different parts of same plant. Accordingly, the total phenolics, total flavonoids, flavone and flavenol showed the similar tendency in ranking: flower, leave, mix, manna and stem. The manna of this plant has a high carbohydrate content, it is suitable for food and pharmaceutical industries.
It is suggested the antioxidant activity by other methods such as ABTS also for as much as organs of this plant has very high carbohydrate and polyphenol content so other extract such as aqueous extract and polyphenol extract for evaluation of antioxidant capacity.

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