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CHEMICAL COMPOSITION OF *ASTRAGALUS*: CARBOHYDRATES AND MUCILAGE CONTENT

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Abstract

This study was conducted to determine the content of carbohydrates and mucilage in roots and leaflets of different *Astragalus* species for the first time from Iran. The content of reducing sugars, oligosaccharides and polysaccharides in roots of the species were determined spectrophotometrically. The content and sugar composition of the mucilages in leaflets was determined by gravimetry and gas-liquid chromatography, respectively. According to the results of this research, the content of sugars in roots and mucilage in leaflets depends on the factors such as the species and geographical location of the plants. Reducing sugars, oligosaccharides and polysaccharides content in roots of the species varied from 0.11-0.90, 2.21-7.27 and 1.3-7.33% dry weight, respectively. The mucilage content in leaflets of different species varied from 5.32 to19.24% dry weight.

Introduction

Astragalus L., (Fabaceae) is generally considered the largest genus of vascular plants with approximately 2500-3000 species (Podlech, 1986; Lock & Simpson, 1991). *Astragalus* is widely distributed in temperate regions of the Northern Hemisphere. The greatest number of species are found in the arid, continental regions of western North America (400 species) and central Asia (2000-2500 species). An additional 150 species are known from temperate South America and one species extends along the East African mountains to Transvaal, South Africa (Liston & Wheeler, 1994).

Mucilage, a class of polysaccharides, are high molecular weight biopolymers; commonly occurs in higher plants (Hadley, 1997). This class of natural products has received great attention of its importance in industry and medicine (Smith & Mntgomery, 1959: Kokate & Radwan, 1979). Polysaccharides, among other polymers, are frequently used in drug formulations, as binding agents, viscosity increasing agents, coating agents or as active ingredients (Vanlaeke, *et al*., 1989) and in food industries as suspending agent, thickener and stabilizer (Cottrell & Baird, 1980; Simpson & Conner-Ogrzaly, 1986; Franz, 1989). Mucilages have also some ecological functions. These compounds are of importance for the survival of the plant species under desert conditions. One of the best known functions is their greater water holding capacity (Ebrahimzadeh *et al*., 2000).

The interest in chemical constituents of various species of the genus *Astragalus* has been increasing during the last years. Many species of *Astragalus* have been investigated chemically e.g., for flavonoids, non-protein amino acid, saponins, alkaloids, nitro compounds, mucilage, sterols, proline content, phenolics etc., (Bisby *et al*., 1994; Ebrahimzadeh *et al*., 1999, 2000, 2001; Niknam & Ebrahimzadeh, 2002 a,b; Niknam *et al*., 2003).

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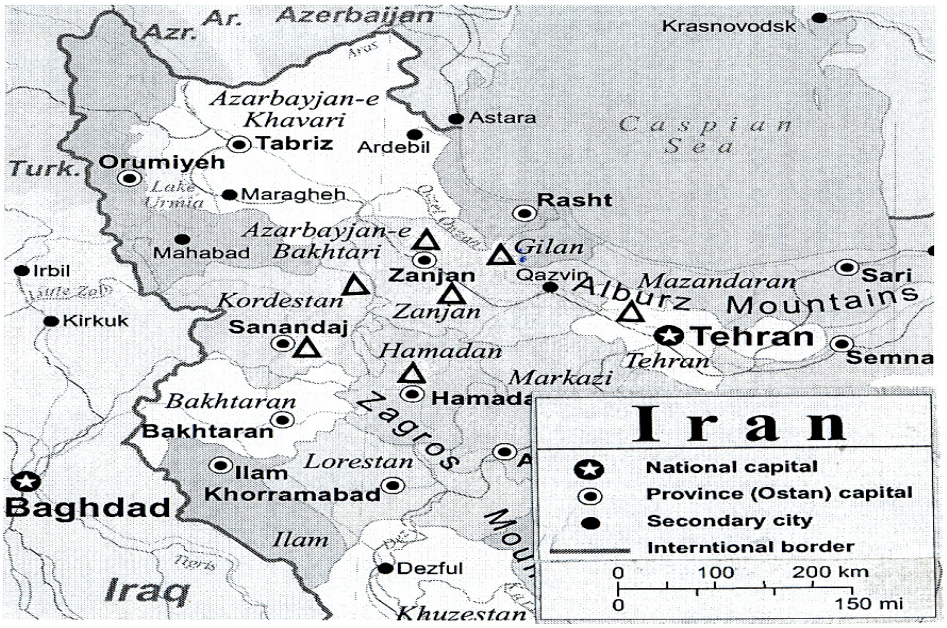

Fig. 1. Geographical map of the North west of Iran and the localities (Δ) from which the species were collected.

Many species of *Astragalus* are useful as forage plants, as ornamentals, to control erosion, restore overgrazed range, replace undesirable plants, provide palatable and nutritious forage for wildlife and domestic livestock (Williams, 1981), and provide useful sources for producing important drugs (Hirotani *et al*., 1994; Baratta & Ruberto, 1997).

As the *Astragalus* species are of greater economic significance, it is imperative that they should be extensively studied for biochemical characteristics. This study was therefore devoted to determine the carbohydrates content and the content and composition of mucilage of a number of the species of this genus from Iran for the first time.

Materials and Methods

Plant materials: Samples were collected from various regions of Iran and air dried in the shade. Geographical map of the region and the localities of the examined species are presented in Fig. 1. The specimens were identified by Dr. Ali Asghar Maassoumi of the Herbarium of Botanical Garden, Research Institute of Forests and Rangelands, Tehran, Iran and voucher specimens have been deposited in the Herbarium of the Botanical garden. The nomenclature of *Astragalus* species and section classification used herein are based on Maassoumi (1998). Roots and leaflets of the plant specimens were separated and then ground in a grinder.

Extraction: For determination of carbohydrates content, 0.5g of powder was extracted using 10 ml of ethanol-distilled water (8:2 V/V), by centrifuging twice and then collecting the supernatants. The residue from ethanol extraction was subsequently used for polysaccharide extraction by boiling water (Patumi *et al*., 1990).

Table 1. Carbohydrates content (%DW) in roots of *Astragalus* species from Iran.

Species	Distribution	Locality and date	R.S.*	O.S.*	P.S.*
A. ebenoides ssp. *ebenoides*	Iran: Endemic	Zanjan: 16.6.1997	0.28±0.00de**	5.41±0.07c	4.59±0.05d
A. submitis ssp. *submitis*	Iran: Endemic	Tehran: 28.5.1997	0.90±0.03a	2.84±0.08g	3.14±0.26hij
A. tortuosus DC.	Iran, Iraq, Turkey, Russia	Sanandaj: 18.6.1997	0.28±0.06de	3.89±0.03e	5.47±0.21c
A. caragana Fisher and C.A. Meyer	Iran, Iraq, Turkey, Russia	Tehran: 28.5.1997	0.90±0.02a	6.05±0.13b	3.72±0.17fg
A. parvistipulus Rech. f.	Iran, Afghanistan, Pakistan	Zanjan: 15.6.1997	0.23±0.02ef	3.57±0.06e	4.12±0.91def
A. aegobromus Boiss. and Hohen.	Iran, Iraq, Turkey, Russia	Tehran: 28.5.1997	0.25±0.03def	2.21±0.50i	3.10±0.06hij
A. basilicus Podlech and Maassoumi	Iran: Endemic	Loshan: 28.5.1997	0.40±0.03c	2.56±0.07ghi	1.30±0.01l
A. alyssoides Lam.	Iran, Iraq, Russia	Zanjan, Bijar Road: 16.6.1997	0.28±0.00de	2.59±0.32ghi	2.86±0.20ij
A. alyssoides Lam.	Iran, Iraq, Turkey, Russia	Zanjan, Soltaniieh: 15.6.1997	0.16±0.02gh	3.10±0.10f	2.70±0.01j
A. glumaceus Boiss.	Iran: Endemic	Zanjan: 16.6.1997	0.13±0.02h	2.68±0.00gh	4.10±0.15def
A. paralurges Bunge	Iran: Endemic	Zanjan: 15.6.1997	0.17±0.01gh	2.33±0.11hi	4.30±0.17de
A. monspessulanus ssp. m*onspessulanus*	Iran, Turkey, Russia	Sanandaj: 17.6.1997	0.11±0.03h	2.59±0.07ghi	5.67±0.14c
A. monspessulanus ssp. m*onspessulanus*	Iran, Turkey, Russia	Zanjan-Geidar:15.6.1997	0.13 ±0.00h	2.50±0.01ghi	3.30±0.11ghi
A. monspessulanus ssp. *monspessulanus*	Iran, Turkey, Russia	Zanjan-Bijar Road: 16.6.1997	0.54±0.06b	2.30±0.08hi	3.83±0.07efg
A. eugenii Grossh.	Iran, Russia	Sanandaj: 16.6.1997	0.19±0.03fg	6.13±0.67b	5.93±0.19c
A. molis Bunge	Iran, Iraq, Russia	Zanjan: 16.6.1997	0.24±0.00ef	3.76±0.07e	3.79±0.50efg
A. iranicus Bunge	Iran, Iraq, Russia	Hamadan: 16.6.1997	0.27±0.01de	4.88±0.03d	4.15±0.13ef
A. iranicus Bunge	Iran, Iraq, Turkey, Russia, India, Libya	Zanjan: 15.6.1997	0.16±0.00gh	3.55±0.16e	7.33±0.49a
A. angustifolius ssp. *angustifolius*	Iran, Turkey, Libya	Sanandaj: 16.6.1997	0.33±0.02d	5.83±0.08c	3.51±0.16gh
A. strictipes Bornm.	Iran: Endemic	Tehran: 28.5.1997	0.40±0.06c	2.33±0.04ghi	1.90±0.08k
A. siliquosus ssp. *siliquosus*	Iran, Iraq, Turkey, Russia	Sanandaj: 17.6.1997	0.59±0.05b	7.27±0.31a	2.00±0.10k

*R.S: Reducing sugars; O.S.: Oligosaccharides; P.S.: Polysaccharides

**The values are the mean of three determinations ± SD. Means in columns followed by different letters are significantly different at $P \leq 0.05$.

Leaflets of the specimens were collected and after washing in water, treated with boiling 96% ethanol for 5 min., and dried in an oven at 60°C for 48h. Dried leaflets were powdered and passed through a sieve with 0.25mm pore size. Two grams of powdered material was mixed with 200 ml of distilled water acidified with HCl (pH 3.5) and mucilage was extracted in water bath (90 - 95 °C) for 12h with frequent stirring. The solution was then filtered with muslin in a buchner funnel. The extract was concentrated to about 50 ml in vacuum rotary evaporator then centrifuged (3700g, 15 min). The solution was kept over night at 4°C. The precipitated mucilage was separated by filtering through Whatman No. 541 filter paper, dried to constant weight at 40°C for 48h and determined gravimetrically (Karawya *et al*., 1980).

Hydrolysis of mucilage:

The crude mucilage collected from filter paper was hydrolysed by 1N H_2SO_4 and purified according to Karawya *et al*., (1980). The hydrolysis solution was neutralized in each case with $BaCO_3$ according to Moyna & Difabio (1978). The hydrolysate was evaporated under reduced pressure and low temperature. The monosaccharides formed by hydrolysis were analysed quantitatively and qualitatively by Gas - Liquid Chromatography (GLC) of their trimethylsilyl (TMS) ether derivatives.

Derivatization and GLC: The TMS ether derivatives of authentic monosaccharides (Sigma) and monosaccharides formed from mucilages were prepared as described by Sweeley *et al*., (1963). Monosaccharide identity of different mucilages was determined by comparison with the retention times of the authentic monosaccharides. The ralative compositions of the individual monosaccharides in the mucilage of each species were calculated from the ralative peak areas of the GLC chromatograms. A Shimadzu GC-16 A, equipped with a flame ionization detector and 1.6m x 3.2 mm i. d. glass column packed with SE - 30 5% was used. Nitrogen was used as carrier gas at a flow rate of 50 ml min^{-1}. Flow rates of hydrogen and air were 55 and 400 ml min^{-1}, respectively. The temperatures of the injector and detector block were both 280°C. The analysis was performed using temperature programming from100°C to 260°C at a heating rate of 4°C min^{-1}

Quantitative determination of carbohydrates: Shimadzu UV-Visible recording spectrophotometer (UV-160) with 10 mm-matched quartz cells was used for absorbance measurement. Total carbohydrate content of polysaccharides and alcohol soluble extracts was estimated by the method of Dubois *et al*., (1959).

Satistical analysis: The data determined in triplicate were analysed by analysis of variance (ANOVA) using MSTAT-C (Version 1.42). The significance of differences was determined according to DMRT. *P* values ≤ 0.05 were considered to be significant.

Results and Discussion

The content of reducing sugars, oligosaccharides and polysaccharides in roots of 17 species of *Astragalus* collected from different parts of Iran is given in Table 1. Reducing sugars, oligosaccharides and polysaccharides content in roots of the species varied from 0.11-0.90, 2.21-7.27 and 1.3-7.33% dry weight, respectively.

In addition to the carbohydrate content variation among the species, the significant variation of the carbohydrate content in a species between sites was also observed. Analysis of variance (ANOVA) showed significant differences among the localities (Table 1). Variations in the content of plant metabolites appears be the result of many

Table 2. Mucilage content in leaflets of some *Astragalus* species from Iran.

Species	Distribution	Locality and date of collection	Mucilage content (%DW)
A. submitis ssp. *submitis*	Iran: Endemic	Tehran: 28.5.1997	11.69±0.26d*
A. aegobromus Boiss. and Hohen.	Iran, Iraq, Turkey, Russia	Tehran: 28.5.1997	14.17±0.31c
A. semilunatus Podlech	Iran, Russia	Sanandaj: 19.6.1997	5.32±0.38h
A. nervistipulus Boiss.	Iran: Endemic	Sanandaj: 19.6.1997	11.79±0.20d
A. pauxillis Maassoumi and Ghahremani	Iran: Endemic	Zanjan: 17.6.1997	7.58±0.33g
A. sciureus Boiss. and Hohen.	Iran: Endemic	Tehran: 28.5.1997	10.67±0.29*e
A. sciureus Boiss. and Hohen.	Iran: Endemic	Tehran: 28.5.1997	9.93±0.20*f
A. monspessulanus ssp. *monspessulanus*	Iran, Turkey, Russia	Sanandaj: 19.6.1997	18.51±0.30b
A. iranicus Bunge	Iran, Iraq, Russia	Zanjan: 17.6.1997	19.24±0.55a
A. glochideus Boiss.	Iran, Russia	Loshan: 28.5.1997	11.69±0.29d

*The values are the mean of three determinations ± SD. Means in columns followed by different letters are significantly different at $P \leq 0.05$.

** These two samples were collected at the same day from different localities in Zanjan.

Table 3. Monosaccharide composition (%GLC) of the mucilages obatined from leaflets of *Astragalus* species[*].

Species	Glu	Fru	Gal	Ara	Xyl	Fuc	Rha	GluA	GalA
A. submitis ssp. *submitis*	34	5.3	13.2	13	11	5.3	7.6	10.6	-
A. aegobromus Boiss. and Hohen.	19.3	-	11.2	10	9.1	4.1	11	22.5	12.7
A. semilunatus Podlech	3	22	13.7	7	-	-	15.4	7.1	5.1
A. pauxillis Maassoumi and Ghahremani	27.3	5.1	17	21.1	5.7	5.1	7.1	11.6	-
A. sciureus Boiss. and Hohen.	24.1	6.5	13.8	16.5	8.8	5.7	10	10.4	4.3
A. monspessulanus ssp.	15.8	-	35.1	17.2	9.8	3.6	10.2	7.2	-
A. monspessulanus iranicus Bunge	20.6	2.5	11.4	12.1	11.6	6	8.7	15.4	11.5
A. glochideus Boiss.	34	-	13.2	13	11	5.3	7.6	10.5	-

[*]Monosaccharide composition of mucilage obtained from *A.nervistipulus* Boiss. was not presented because of sample loss.

factors. There may be a genetic component to such a variation (Bowers & Stamp, 1992), but the genotype can be modified by a variety of biotic and abiotic features. For example, seasonal changes in biochemical characteristics are caused by shifting patterns of resource allocation that reflect different physiological demands associated with growth, defense and reproduction. At the same time, a diversity of environmental stresses contributes to spatial variation within and among populations (Waterman & Mole, 1994). Clearly, in order to determine factors contributing to the variability of carbohydrates content of a species between localities, manipulative field or greenhouse experiments are necessary.

Mucilage content and monosacchride composition of the mucilages obtained from 9 species of *Astragalus* are presented in Table 2 and 3, respectively. The mucilage content in leaflets of different species varied from 5.32 to19.24% of dry weight. Relative monosaccharide composition of the mucilages varied depending on the species. The mucilages of the examined species composed of 7-9 different sugar residues. The main sugars varied according to the species investigated. In 5 species, glucose was the major sugar and in other 3 species fructose, galactose or glucoronic acid were the major ones. In 7 out of 8 examined species, glucose percentage was high (15.8- 34%). Only, in *A. semilunatus* glucose was detected in trace (3%). Fucose was detected in low concentrations in mucilage of 7 out of 8 species. Glucoronic acid was determined in mucilage of all the examined species and galactoronic acid was detected in mucilage of 4 species.

The results of this study along with the results of other investigations (Ebrahimzadeh *et al*., 1999; Ebrahimzadeh *et al*., 2000; Niknam & Ebrahimzadeh, 2002a,b; Niknam *et al*., 2003) can be used for selection of the desirable species for medicinal and forage purpose to restore overgrazed range, control erosion and replace undesirable plants.

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