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MUCILAGE CONTENT AND ITS SUGAR COMPOSITION IN *ASTRAGALUS* SPECIES FROM IRAN

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Abstract

Mucilage content and its sugar composition in leaflets of 15 species of *Astragalus* from Iran were examined. The mucilage content of different species varied from 3.44 to 23.56% dry weight. Mucilage content in some of the species of this genus is comparable with that of the richest sources of mucilage. The mucilages obtained from different species, all showed the presence of glucose, galactose, arabinose, xylose, fucose and rhamnose but in different amounts. In mucilages of 12 species glucose was the major monosaccharide and in 3 species galactose was the main constituent. The uronic acids were detected in 12 of the 15 species.

Introduction

Mucilages, a class of polysaccharides, are high molecular weight (200000 and more) biopolymers which commonly occur in higher plants (Hadley, 1997) and this class of natural products has received much attention since it is of great importance in industry and medicine (Smith & Montgomery, 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. In *Carrichtera annua* and *Anastatica hierochuntica* from the Negeve desert highlands of Israel the main effect of mucilage in winter is in water retention and soil particle contact while in summer day may allow damage repair and priming for the germination process (Gutterman & Shem-Tov, 1997). These compounds are of importance for the survival of the plant species under desert conditions (Gutterman & Shew-Tov, 1996). One of the best known function is their water holding capacity. In certain plants such as *Tsuga canadensis* water evaporation is retarded by a layer of mucilage on the cell walls (Levitt, 1980). *Mesembryanthemum chilense* (Aizoaceae) is a succulent plant which grows in sandy areas. *Notocactus ottensis* (Cactaceae) grows well on barren rock. These species occupy different and very specialized ecological niches. All have in common the need of systems capable of quick absorption of running rain water (or in *Tillandsia aeranthos*, of external humidity), and supplementary systems to slow the loss of this absorbed water. The water - soluble polysaccharides are part of this system.

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Table 1. Taxonomic and locality data and mucilage content of *Astragalus* species from Iran.

Section	Species	Locality and date of collection	Distribution	Mucilage* content (% DW)
Ammodendron Bunge	*squarrosus*	Kashan: 1997	Iran, Russia, and Libya	17.43
Ankylotus Bunge	*commixtus* Bunge	Zanjan: 19.6.1997	Iran, Afghanistan Turkey, Russia and Pakistan	15.55
Anthylloidei DC.	*tortuosus* DC.	Sanandaj:20.6.1997	Iran, Iraq, Turkey and Russia	5.37
Astragalus	*caragana* Fisher and C.A. Meyer	Zanjan: 28.5.1997	Iran, Turkey and Russia	8.18
Caprini DC.	*basilicus* Podl. and Maass.	Loshan: 28.5.1997	Iran: Endemic	13.16
Caraganella Bunge	*parvistipulus* Rech.f.	Zanjan: 18.6.1997	Iran, Afghanistan and Pakistan	14.09
Hololeuce Bunge	*alyssoides* Lam.	Zanjan: 19.6.1997	Iran, Iraq and	3.44
Hymenostegis Bunge	*chrysostachys* Boiss.	Sanandaj:20.6.1997	Iran, Iraq, Turkey and Russia	7.68
Hymenostegis Bunge	*glummaceous* Boiss.	Zanjan: 19.6.1997	Iran: Endemic	13.44
Malacothrix Bunge	*mollis* Bieb.	Zanjan: 19.6.1997	Iran, Iraq, Turkey, Russia, India and Libya	11.99
Malacothrix Bunge	*eugenii* Grossh	Sanandaj:20.6.1997	Iran and Russia	23.56
Melanocercis Bunge	*angustifolius* ssp. *angustifolius* Lam.	Sanandaj:20.6.1997	Iran, Turkey and Libya	19.18
Onobrychoidei DC.	*strictipes* Bornm	Taleghan:28.5.1997	Iran: Endemic	9.06
Ornithopodium Bunge	*schistosus* Boiss. and Hohen.	Zanjan: 28.5.1997	Iran: Endemic	15.62
Theiochrus Bunge	*siliquosus* ssp. *siliquosus*	Taleghan:28.5.1997	Iran, Iraq, Turkey and Russia	13.31

*The values represented in this Table are means of three determinations.

Mucilaginous polysaccharides represent the chemical counterpart of the water retention system. The synthesis of monosaccharides would represent an increment in the osmotic pressure that eventully would disrupt the development of the normal cell. By polymerization this can be avoided. On the other hand, the polymer has to be easily degraded if it is to be useful as a water reservoir for periods of stress. The acidic polysaccharides fill this need by having easily degraded branches united to a more stable backbone that can survive till the next growth period (Moyna & Tubio, 1977).

Contact reduction is a mechanism by which plant roots are able to mobilize manganese from insoluble oxides in the rhizosphere. Contact between the root surface and soil is established and develops when the mucilages produced by root cells form gel upon their interaction with soil (Uren, 1993). Mucilages may be important factors in the tolerance of certain plant species to toxicity of aluminium (Rengel, 1990; Archambault *et al.*, 1996) and copper (Brown *et al.*, 1988). A new allelopathic substance has also been isolated from mucilage of germinated Cress (*Lepidium sativum* L.) seeds (Masegawa *et al.*, 1992; Kosemura *et al.*, 1993).

Composition of mucilage has been proposed as a rough guide to grouping species within the family (Moyna & Difabio, 1978), Chemotaxonomic aspects of the chemistry of Acacia gum exudate has also been studied (Anderson & Dea, 1969).

Terminology in the field of plant polysaccharides such as gums and mucilages which was vague though colourful is currently yielding to more precise designations. A gum has the subjective attribute of being gummy, a mucilage of being mucilaginous. The terms gum and mucilage have often been used interchangeably, although the pharmacist generally consider a mucilage as being a solution of a gum (Franz, 1989).

Mucilages have complex structures and their precise structure is unknown in most of the plants. A detailed examination of mucilage from Canola (*Brassica campestris* L.) showed that it was composed of 8.2% moisture, 18.9% protein, 31.5% carbohydrate and 29.5% ash (Sharafabadi, 1987). Analysis of yellow mustard (*Sinapis alba* L.) crude mucilage revealed carbohydrate as the major component (80-90%) with ash (1.7 - 15%) and protein (2-2.4%) as minor constituents (Cui *et al.*, 1993).

Since mucilages find wide application and have ecological importance, it was considered worthwhile to investigate plant species as new sources for their mucilage content and for the composition of constituent monosaccharides in the mucilage. The mucilage content and its sugar composition in 15 different species of *Astragalus* from Iran is given in this report which has not been described before.

Materials and Methods

Plant material: Samples were collected from different regions of Iran and air dried in the shade. The specimens were identified by one of us (A.A. Maassoumi) and voucher specimens were deposited in the Herbarium of the Botanical Garden Tahran. Taxonomic and locality data of the materials used for extraction of mucilage are listed in Table 1. The nomencalture of *Astragalus* species and section classification used herein is based on Maassoumi (1998).

Table 2. Monosaccharide composition of mucilages obtained from leaflets of *Astragalus* species.

	Glu	Fru	Gal	Ara	Xyl	Man	Fuc	Rha	GluA	GalA
A. squarrosus	37.5	-	28.5	9.9	9.1	-	3.0	12.0	-	-
A. commixtus Bunge	21.4	-	40.7	14.2	10.3	0.9	2.5	9.9	-	-
A. tortuosus DC.	34.6	-	15.9	9.6	6.0	4.0	2.3	4.9	13.2	9.4
A. caragana Fisher and C.A. Meyer	3.6	4.0	40.3	16.9	7.4	-	5.7	7.9	10.3	4.0
A. basilicus Podl. and Maass.	5.5	-	31.8	11.3	7.9	-	3.1	7.0	20.7	12.5
A. parvistipulus Rech.f.	27.9	4.1	11.0	9.7	5.1	4.6	2.1	3.4	8.5	4.7
A. alyssoides Lam.	36.5	4.8	12.8	17.3	8.5	-	5.8	8.8	5.3	-
A. chrysostachys Boiss.	33.6	-	11.3	15.0	9.6	7.7	4.5	7.8	8.4	2.2
A. glummaceous Boiss.	32.6	2.6	11.8	12.3	7.0	-	3.7	4.7	16.4	8.9
A. mollis Bieb.	33.5	2.8	16.7	13.7	8.4	1.9	3.7	6.5	14.8	-
A. eugenii Grossh	60.5	6.2	18.4	6.3	-	-	5.1	3.5	-	-
A. angustifolius ssp. *angustifolius* Lam.	38.5	-	12.7	10.1	12.2	-	3.2	17.1	6.1	-
A. strictipes Bornm	31.6	5.2	13.6	13.1	10.7	-	4.7	8.7	4.2	1.5
A. schistosus Boiss. and Hohen.	27.4	5.1	11.7	11.1	8.6	-	5.1	12.9	13.7	4.4
A. siliquosus ssp. *siliquosus*	27.7	4.1	12.2	10.0	7.3	-	4.1	6.9	19.8	9.9

Sample preparation and extraction: 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 0.25 mm sieve. 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 passing 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.

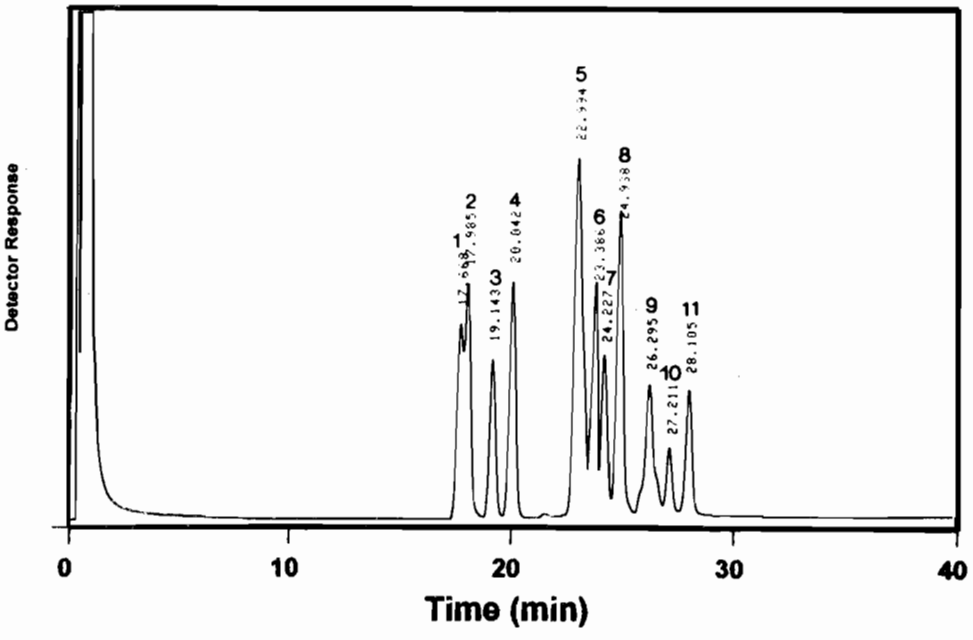

Fig. 1. GLC analysis of authentic monosaccharides as their TMS ether derivatives performed on a 5% SE-30 glass column.

For conditions see Materials and Methods. Peak identities are: 1 = L(+) Arabinose; 2 = L(+)Rhamnose; 3 = L(-) Fucose; 4 = D(+) Xylose; 5 = D(+) Mannose; 6 = D(-) Fructose; 7-D(+) Galactose; 8 = D(-) Glucose; 9 = D-Galacturonic acid; 10 = L(-) Glucose; 11 = D-Glucuronic acid.

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 relative peak areas of the GLC chromatograms. A Shimadzu GC-16 A, equipped with a flame ionization detector and 1.6 m 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 remperatures of the injector and detector block were both 280°C. The analysis was performed using temperature programming from 100°C to 260°C at a heating rate of 4°C min^{-1}

Results and Discussion

Mucilage content of leaflets of 15 species of Astragalus collected from different regions of Iran (Table 1) showed that content of mucilage varied from 3.44 to 23.56% dry weight where *A. alyssoides* Lam., had the lowest and *A. eugenii Grossh* highest

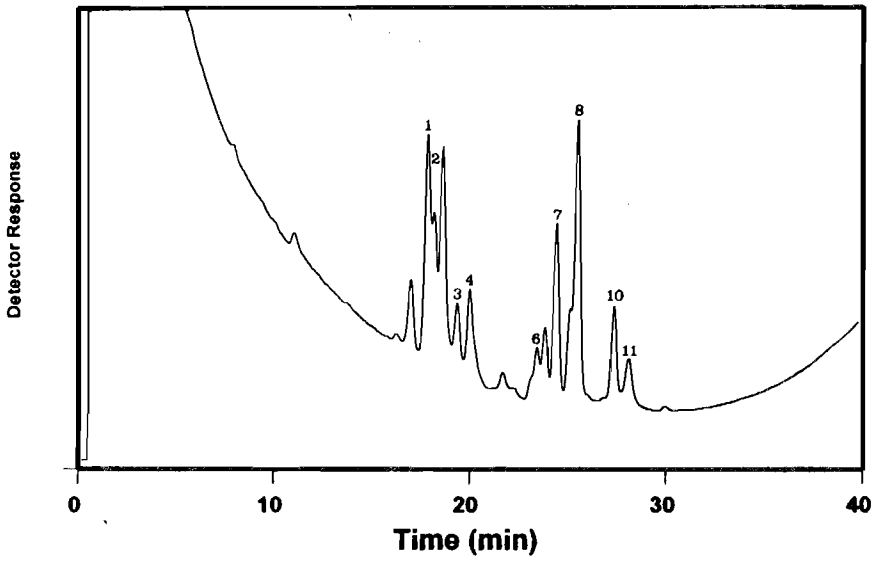

Fig. 2. GLC chromatogram of the monosaccharide constituents of the mucilage obtained from the leaflets of *A. alyssoides* Lam./Peak identified.

mucilage content. The content of mucilage of some of the species is comparable with that of *Plantago ovata* Forsk seeds (Movafeghi, 1993). This species of Plantago, also called isabghul in Hindi, is one of the most important sources of mucilage (Sharma & Koul, 1986).

The standard monosaccharides were separately derivatized to the TMS ethers and chromatographed. GLC chromatogram of the mixture of eleven monosaccharides, showing their retention times and the order of their elution is given in Fig. 1. The hydrolysis products from the original mucilages as TMS ether derivatives were analysed by GLC. The TMS ether peaks for various sugars were identified by comparison with authentic sugars. Monosaccharide composition of the mucilages obtained from different species of *Astragalus* are presented in Table 2. Two representative GLC chromatograms of the monosaccharide constituents of the mucilage obtained from *A. alyssoides* Lam., and *A. siliquosus* sp. *siliquosus* are given in Figs. 2 and 3, respectively, Relative monosaccharide compositon of mucilages of different species varied depending on the species. The mucilages of the examined species composed of 6-10 different sugar residues. *A. parvistipulus* Rech. f. was the only species in which all of the tested monosaccharides were detected in its mucilage with 6 of them viz., fructose, xylose, mannose, fucose, rhamnose and galacturonic acid in traces. The main sugars varied according to the species investigated (Table 2). Quantitative analysis showed differences among mucilages from various species of *Astragalus*. In 12 of 15 species, glucose was the major monosaccharide, followed by other monosaccharides depending on the case. In mucilages of *A. commixtus* Bunge, *A. caragana* Fisher & Meyer and *A. basilicus* Podl. & Maass, galactose was the major constituent, where mucilage of *A.*

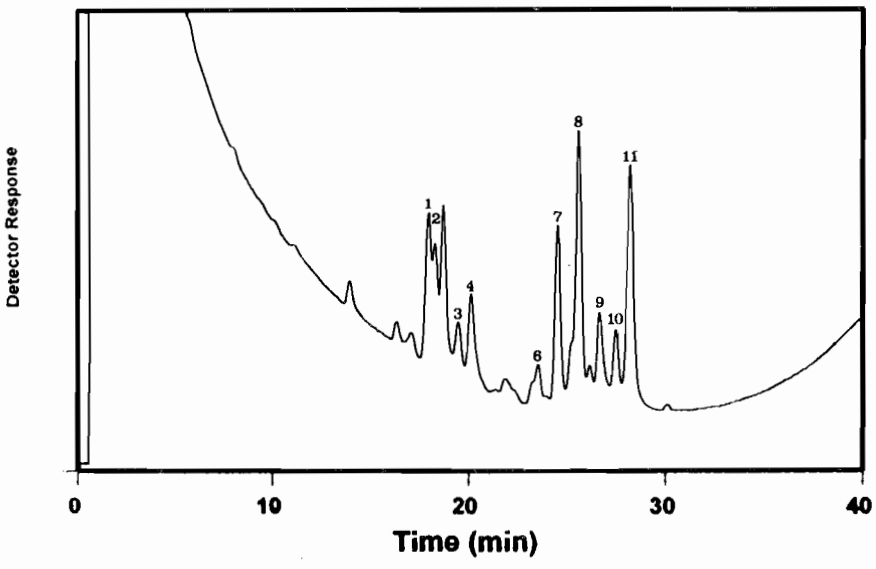

Fig. 3. GLC chromatogram of the monosaccharide constituents of the mucilage obtained from the leaflets of *A. siliquosus* ssp. siliquosus. Peak identified.

commixtus Bunge was abundant in glucose (21.4%) and those of *A. caragana* Fisher & Meyer and *A. basilicus* Podl. & Maass were poor in glucose. Fructose was determined in mucilages of 9 species only in low concentrations (2.8 - 6.2%). Low concentration of mannose was detected only in mucilages of 4 species. Thus fructose and mannose are not common constituents in mucilages of *Astragalus*. Fucose in low concentration (2.1-5.8%) is common in mucilages of all the examined species. In *A. squarrosus, A. commixtus* Bunge and *A. eugenii* Grossh, mucilages were neutral and did not contain uronic acids. In rest of the examined species uronic acids were detected. In 9 species, both glucuronic acid and galacturonic acid were determined and in 3 species only glucuronic acid was detected. Mucilages of *A. chrysostachys* Boiss., and *A. glumaceous* Boiss., both from section *Hymenostegis* Bunge were acidic derivatives. Mucilage of *A. mollis* Bib., from section *Malacothrix* Bunge, was acidic and contained glucuronic acid (14.8%), but that of *A. eugenii* Grossh was neutral. It has been suggested that acidic mucilage acts as a counterion in ion storage and secretion by glands of the halophyte *Ceratostiya plumbaginoides* (Borchert, 1989).

Comparing the gelling properties of the mucilages obtained from *Astragalus* species, the exceptional position of *A. siliquosus* sp. *siliquosus*, could be demonstrated. Mucilage extract of this species is solidified after cooling when its volume is reduced to about 30-40 ml. No other mucilage extracts showed this property. This mucilage contained both glucuronic acid (19.8%) and galacturonic acid (9.9%) and after glucose (27.7%), glucuronic acid was the major monosaccharide followed by galactose, arabinose, galacturonic acid, xylose, rhamnose and fructose. The gelling power and viscosity of pectin solutions has been attributed to the number of galacturonic acid units in the molecule (Tyler *et al.*, 1988).

There does not appear to be any previous report regarding the presence and composition of mucilage in these and many other species of *Astragalus*. Immune-stimulating polysaccharides have been determined in herbal drugs including *A. gummifer* and *A. mongholicus* (Franz, 1989). Induction of hairy-root from *A. gummifer* and *A. monogholicus* and mucilage production by the hairy-root from *A. gummifer* and *A. monogholicus* and mucilage production by the hairy-root cultures has been reported (Isa, 1991; Ionkova *et al.*, 1993). Other reports are on the therapeutic properties of polysaccharides derived from *Astragalus* (Mao *et al.*, 1988; McLaughlin. 1981).

The gum and mucilage have often been used interchangeably (Franz, 1989). Several species of *Astragalus* are the sources of gum. Gum tragacanth is an exudate from *A. gummifer*, or other Asiatic species of *Astragalus* (Tyler, 1988; Anderson & Grant, 1989), found in the dry and mountainous regions of Iran, Syria and Turkey. For the collection of gum, the plants are incised and the gum which exudes after drying collected. Iran produces the highest quality gum on commercial scale. Gum tragacanth consists of a complex mixture of acidic polysaccharices containing galacturonic acid, galactose, fucose, xylose and arabinose (Cottrell & Baird, 1980).

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