

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 to 19.24% 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 & 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. 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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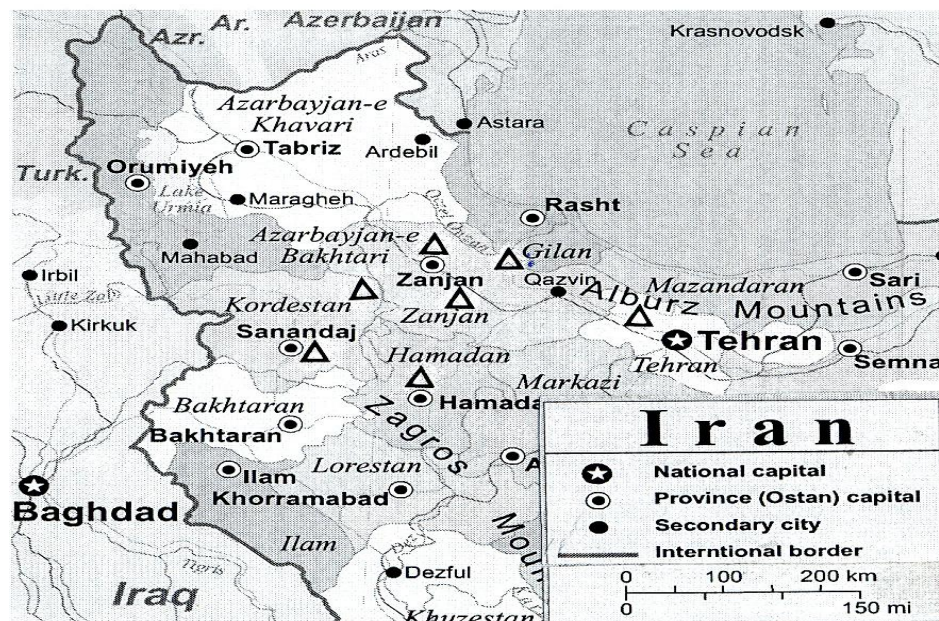


Fig. 1. Geographical map of the North west of Iran and the localities (Δ) from which the species were collected.

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).

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 H₂SO₄ and purified according to Karawya *et al.*, (1980). The hydrolysis solution was neutralized in each case with BaCO₃ 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 relative 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 1.6m 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 from 100°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).

Statistical 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 to be the result of many

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 to 19.24% 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 glucuronic 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. Glucuronic acid was determined in mucilage of all the examined species and galacturonic 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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