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PHENOLICS CONTENT IN *ASTRAGALUS* SPECIES

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Abstract

The content of total phenolics in roots, leaflets and seeds of a number of *Astragalus* species from Iran was examined. Phenolics content of the aqueous and methanolic extracts of the species were determined spectrophotometrically. Phenolics content depend on factors such as the species, geographical location of the plants and the organs. Moreover, the content of phenolics depends on the extraction solvents. Phenolics content in roots and leaflets of the species using aqueous extracts varied from 0.13-0.80 and 0.63-5.16% dry weight, respectively while using methanolic extracts ranged from 0.25-0.95 and 0.52-3.75% dry weight, respectively. Generally, the content of phenolics in leaflets was higher than that of the roots and seeds. In this paper the content of the phenolics in various species of *Astragalus* is reported for the first time.

Introduction

Phenolic compounds (phenolics) are widely distributed plant constituents.They have traditionally been believed to play an important role in plant-herbivore interactions (Feeny, 1976; Swain, 1979). Indeed, phenolics can play a role in virtually any interaction a plant can have with its environment, biotic or abiotic. In terms of the biotic environment, these interactions may be within the autothrophic level (allelopathy), or with consumers of either living or dead plant material. With regard to the consumers of living plants, both pathogens and herbivores can be involved. At the ecosystem level, phenolics can mediate interactions that directly or indirectly link autothrophs to each other, to saprophytes, to pathogens, or to herbivores and their predators (Waterman & Mole, 1994).

Plant phenolics have been implicated in food selection by many species of animals as diverse as elephants (Jachmann, 1989) and ants (Seaman, 1984). According to the results of food selection studies, most herbivores avoid consuming levels of phenolics that are in excess of their normal diet; this is particularly true where tannins are involved (Mole & Waterman, 1987; Cork & Foley, 1991). Some phenolics exert subtle and non-lethal influence on herbivores. For instance, the isoflavonoid genistein present in clover, acts as oestrogen analogues and reduce the fertility of sheep grazed on pasture containing this species (Harborne, 1988).

One of the classic plant-animal interactions where there is a well established role for phenolics is that of plant pollination. Here, flavonoid-based pigments, together with carotenoids (some of which can also be phenolic), play a privotal role in the determination of flower colour and thus pollinator preferences. The flavonoids important in flower colour determination are both those that absorb in the wavelength range visible to human observer and those which absorb in the ultraviolet (UV) range visible to many insects such as bees. In the first of these groups we are mostly concerned with the anthocyanidins (Waterman & Mole, 1994). Tannins, a group of phenolics, are important economically as agents for the tanning of leathers and for certain medicinal purposes. Medicinal values of phenolics are well known for a long time (Farnsworth, 1966).

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Astragalus L. (Fabaceae) is generally considered the largest genus of vascular plants with an estimated 2500-3000 species (Podlech, 1986; Lock & Simpson, 1991). *Astragalus* is widely distributed in temperate regions of the Northern Hemisphere. The greatest numbers 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).

Species of *Astragalus* from Europe, Asia and North Africa are among the herbs which fill every conceivable ecological niche from warm and dry deserts to timberline in their native habitat. Many species of *Astragalus* are useful as forage plants, to control erosion, as ornamentals (Williams, 1981) or as medicinal plants (Hirotani *et al.*, 1994; Baratta & Ruberto, 1997). Native and introduced forage plants are used to restore overgrazd range, control erosion, replace undesirable plants, provide palatable and nutritious forage for wildlife and domestic livestock, and provide useful sources for producing important drugs.

The interest in chemical constituents of various species of the genus *Astragalus* has been increasing during the recent years. Many species of *Astragalus* have been investigated chemically for flavonoids, non-protein amino acid, saponins, alkaloids, nitro compounds, mucilages, sterols etc., (Bisby *et al.*, 1994; Ebrahimzadeh *et al.*, 1999; Ebrahimzadeh *et al.*, 2000; Ebrahimzadeh *et al.*, 2001).

The present report describes the total phenolics content of a number of the species of the genus from Iran.

Materials and Methods

Plant materials: Samples were collected from various regions of Iran and air dried in the shade. The specimens were determined by Dr. Ali Asghar Maassoumi from Herbarium of Botanical Garden, Research Institutes of Forests and Rangelands, Tehran, Iran and voucher specimens deposited at the Herbarium of the Botanical garden. The nomenclature of *Astragalus* species and section classification used herein is based on Maassoumi (1998). Roots and leaflets of the plant specimens were separated and then ground in a grinder. Mature seeds of five species of *Astragalus* received from collections were ground to a fine powder in a small mortar and pestle.

Extraction: Two methods were used for the extraction of phenolics from 0.5 g ground samples. In the first method phenolics were extracted with boiling water (distilled H_2O) for 30 min., (Ranganna, 1986). In the second method phenolics were extracted with MeOH-H_2O (80:20) at 70°C water bath for 3h (modified from Conde *et al.*, 1995). The suspensions of water extraction were filtered and the aqueous solutions were used for quantitative determination. The suspensions of methanolic extraction were filtered, the MeOH was removed by vacuum distillation and then the aqueous solutions were used for quantitative determination.

Quantitative determination: Shimadzu UV-Visible recording spectrophotometer (UV-160) with 10 mm-matched quartz cells was used for absorbance measurement.

Total phenolics content were determined by the Folin-Denis method (Folin & Denis, 1912; Waterman & Mole, 1994). In this procedure appropriate volumes of aqueous

solutions were diluted to final volume of 17 ml by distilled H_2O then 1 ml of Folin reagent and 2 ml of saturated solution of sodium carbonate were added. After 30 min., the absorbance was measured at 760 nm. Aqueous solutions of tannic acid (0-6.25 µg/ml) were used as standards for plotting working curve (Ranganna, 1986).

Results and Discussion

The content of total phenolics in roots of 20 species and in leaflets of 21 species of *Astragalus* using two commonly used solvents for extraction of phenolics i.e., water and methanol, varies according to the solvent used for extraction (Table 1). This is similar to the reports of Waterman & Mole (1994). The phenolics content in roots using methanolic extracts was higher than that of the aqueous extracts that of the phenolics content in roots of *A. glumaceus* Boiss., (Table 1). However, the highest and lowest amount of phenolics were observed in roots of *A. eugeni* Grossh., (from section *Malacothrix*) and *A. semilunatus* Podlech (from section *Caprini*), respectively. The phenolics content in roots of *Astragalus* species using aqueous and methanolic extracts, varied from 0.13 to 0.80 and 0.25 to 0.95% dry weight, respectively. In the case of *A. sciureus* Boiss. & Hohen. (collected from Tehran) and *A.iranicus* Bunge (collected from Zanjan) only the phenolics content using aqueous extract was determined. In some other cases the phenolics content in roots was not determined for lack of the root samples.

In contrast to the results of phenolics determination in roots, the phenolics content of leaflets using aqueous extracts was higher than that of the methanolic extracts. The phenolics content in leaflets of *Astragalus* species using aqueous and methanolic extracts varied from 0.63 to 5.16 and 0.52 to 3.75% dry weight, respectively. Thus, the phenolics content of the *Astragalus* species in leaflets was much higher than that of roots. The only exception was *A. eugenii* Grossh., (from section *Malacothrix*), in which the phenolics content in roots was higher than that of the leaflets. This species had the highest amount of the phenolics in its roots (0.80 to 0.95% dry weight using aqueous and methanolic extracts, respectively).

Higher amount of phenolics in leaflets in comparison to that of roots, may be attributed to the presence or absence of light that affects the phenolics content of the organs. There is a well established positive relationships between the intensity of solar radiation and the quantity of phenolics produced by plants. Generally there is a rise in total phenolics in plants grown in sunny situations relative to shady ones, but it can be seen at the intra-individual level by comparing plant parts exposed to different amounts of light (Mole *et al.*, 1988). An adaptive interpretation of this response in terms of plant physiological needs is that the phenolics are produced as a way of reducing the photodestruction of exposed tissues. This is seen as being particularly likely where UV light is being absorbed (Del Moral, 1972).

The content of phenolics in seeds of five *Astragalus* species using methanolic extracts varied from 0.24 to 0.66% dry weight (Table 2) which was lower than those of both roots and leaflets (Table 1).

To date no data are available on the comparative study of the total phenolics in *Astragalus* species. Aynechi *et al.*, (1981), Sabahi *et al.*, (1985) and Fazly Bazzaz *et al.*, (1997) reported on the survey of Iranian plants including a number of *Astragalus* species for the presence of saponins, alkaloids, flavonoids and tannins with visual colourimetric techniques.

Table 1. Phenolics content (%DW) in roots and leaflets of *Astragalus* species from Iran using two solvents (Water and 80% methanol). The values shown in bold represent the lowest and highest content of the phenolics using both the extraction solvents. The values are the mean of three determinations.

Section	Species	Distribution	Locality and date of collection	Content in root		Content in leaflet	
				H_2O	MeOH	H_2O	MeOH
Ammodendron	*sqarrosus*	Iran, Russia, Libya	Kashan: *1995*	-	-	*1.53*	-
Anthylloidei	*ebenoides* ssp. *ebenoides*	Iran: Endemic	Zanjan: 16.6.1997	0.27	0.30	-	-
Anthylloidei	*submitis* ssp. *submitis*	Iran: Endemic	Tehran: 28.5.1997	0.40	0.43	1.48	1.27
Anthylloidei	*tortuosus* DC.	Iran: Iraq, Turkey, Russian	Sanandaj: 18.6.1997	0.36	0.55	1.02	0.82
Astragalus	*caragana* Fisher and C.A. Meyer	Iran, Iraq, Turkey, Russia	Tehran: 28.5.1997	0.34	0.44	1.44	1.115
Caraganella	*parvistipulus* Rech.f.	Iran, Afghanistan, Pakistan	Zanjan: 15.6.1997	-	-	0.97	0.88
Caprini	*aegobromus* Boiss. and Hohen.	Iran, Iraq, Turkey, Russia	Tehran: 28.5.1997	0.23	0.32	1.98	1.52
Caprini	*basilicus* Podlech and Maassoumi	Iran: Endemic	Loshan: 28.5.1997	0.28	039	1.67	1.44
Caprini	*semilunatus* Podlech	Iran, Russia	Zanjan: 15.6.1997	0.13	0.25	1.37	1.22
Hololeuce	*alyssoides* Lam.	Iran, Iraq, Russia	Zanjan: Bijar Road: 16.6.1997	0.32	0.39	0.67	0.60
Hololeuce	*alyssoides* Lam.	Iran, Iraq, Russia	Zanjan, Soltaniiejh: 15.6.1997	-	-	1.12	0.98
Hymenostegis	*chrysostachys* Boiss.	Iran, Iraq, Turkey, Russia	Sanandaj: 18.6.1997	0.22	0.35	-	-
Hymenostegis	*glumaceus* Boiss.	Iran: Endemic	Zanjan: 6.6.1997	0.39	0.36	5.16	3.75
Hymenostegis	*pauxillis* Massoumi and Ghahremani	Iran: Endemic	Zanjan: 15.6.1997	-	-	1.80	1.48
Hymenostegis	*paralurges* Bunge	Iran: Endemic	Zanjan: 15.6.1997	0.28	0.36	-	-
Hymenostegis	*sciureus* Boiss. Hohen.	Iran: Endemic	Tehran: 28.5.1997	0.26	-	0.79	0.75
Hymenostegis	*sciureus* Boiss. Hohen.	Iran: Endemic	Loshan: 28.5.1997	-	-	1.39	1.33
Incani	*monspessulanus* spp. *monspessulanus*	Iran, Turkey, Russia	Sanandaj: 17.6.1997	0.24	0.26	2.59	-
Malacothrix	*eugenii* Grossh.	Iran, Russia	Sanandaj: 16.6.1997	0.80	0.95	0.63	0.52
Malacothrix	*iranicus* Bunge	Iran, Iraq, Russia	Sanandaj: 16.6.1997	0.33	0.42	-	-
Malacothrix	*iranicus* Bunge	Iran, Iraq, Russia	Hamadan: 16.6.1997	0.71	-	1.83	-
Malacothrix	*mollis* Bunge	Iran, Iraq, Turkey, Russia, Indian, Libya	Zanjam: 15.6.1997	0.32	0.42	1.33	0.83
Melanocercis	*angustifolius* spp. *angustifolius*	Iran, Turkey, Libya	Sanandaj: 16.6.1997	-	-	1.89	1.51
Onobrychoidei	*strictipes* Burnm.	Iran: Endemic	Tehran: 28.5.1997	0.30	0.44	1.39	1.03
Ornithopodium	*glochideus* Boiss.	Iran, Russia	Loshan: 28.5.1997	0.39	0.48	1.52	1.27
Ornithopodium	*schistosus* Boiss. and Hohen.	Iran: Endemic	Tehran: 28.5.1997	0.78	0.91	1.42	-
Theiochrus	*siliquosus* spp. *siliquosus*	Iran, Iraq, Turkey, Russia	Tehran: 28.5.1997	0.37	0.45	-	1.77
Theiochrus	*siliquosus* spp. *siliquosus*	Iran, Iraq, Turkey, Russia	Sanandaj: 17.6.1997	-	-	1.98	1.65

Table 2. Phenolics content (%DW) in seeds of *Astragalus* species using 80% methanol as the extraction solvent. The values are mean of three determinations.

Section	Species	Distribution	Locality and date of collection	Phenolics content
Adiaspastus	*aureus* Willd	Iran, Turkey, Russia	E. Azarbayejan: 1996	0.66
Ammodendron	*squarrosus*	Iran, Russia, Libya	Kashan: 1997	0.57
Hymenostegis	*chrysostachys* Boiss.	Iran, Iraq, Turkey, Russia	Hamadan: 1997	0.48
Hymenostegis	*lagopoides* Lam.	Iran, Iraq, Turkey, Russia	W. Azarbayajan, 1997	0.65
Rhacophorus	*strictifolius* Boiss.	Iran, Iraq, Turkey, Russia	Zanjan: 1997	0.24

Regarding the solvent selection for extraction of phenolics, if a plant is being extracted prior to total phenolics analysis then clearly one wants to use a solvent in which every phenolics in the plants will dissolve, this is perhaps too much to expect, but some solvents can approach this ideal. Some phenolics like those present in creosote bush resin are insoluble in water but soluble in non-polar solvents. Others, such as some phenolic glycosides are most soluble in water. It is generally accepted that in a survey of a large number of different species it is not possible to optimize for each of them so an arbitary selection may have to be made (Waterman & Mole, 1994). Thus we used two most commonly used solvents for the extractions.

In this research, in addition to the phenolics content variation among the species and the organs, the significant variation of the content of phenolics in a specific organ of a species between sites has been observed. The phenolics content of *A. alyssoides* Lam., and *A. iranicus* Bunge was significantly influenced by the locality (Table 1; the statistical data have not been presented). Variation in the content of plant secondary metabolites is the results of many factors. There may be a genetic component to such a variations (Bowers & Stamp, 1992), but the genotype can be modified by a variety of biotic and abiotic features. For example, seasonal changes in biochemistry 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 contribute to spatial variation within and among populations (Waterman & Mole, 1994; Dudt & Shure, 1994). In order to determine factors contributing to the variability of phenolics concentration of a species between locality, a field or greenhouse experiments are necessary.

The results of the present study alongwith the results of other investigations (Ebrahimzadeh *et al.*, 1999; Ebrahimzadeh *et al.*, 2000) can be used for the selection of desirable species as forage, to restore overgrazed range, control erosion, replace undesirable plants. Moreover, this study illustrates that the content of phenolics, an important group of secondary metabolites, varies among the species, the plant parts and geographical location of the species.

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