Research Article

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Quantitative and qualitative study of phenolics in leaves of Astragalus species growing in various regions of Iran.

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Highlights

- Astragalus glumaceus, A. caraganae, and A. siliqusus species have the content of the most phenolic compounds among species collected from various regions of Iran.
- Gas-liquid chromatography analysis has shown that syringic acid, coumaric acid, caffeic acid, and dihydroxy benzoic acid are the predominant phenolic compounds in all the Astragalus species collected from different regions of Iran.

ABSTRACT

Astragalus L. is the largest genus in the Fabaceae family and Iran is considered as one of the most important centers of the genus. In this study, phenolic contents were investigated in the leaves of ten Astragalus species, using spectrophotometric and GLC methods. The study showed that the highest level of phenolics and flavonols were detected in A. glumaceus and A. aegobromus species, respectively. Further, the greatest content of phenolic acids and flavonoids were observed in A. caraganae. Besides, A. siliqusus had the highest level of o-diphenols. Gas-liquid chromatography (GLC) analysis of phenolic compounds showed that syringic acid, p-coumaric acid, caffeic acid, and dihydroxy benzoic acid were predominate phenolic acids in these species. The present study revealed that Astragalus species used in this experiment are a good source of phenolic compounds and antioxidants, and therefore, can be introduced as potent natural sources for medicinal and industrial purposes.

Keywords : Astragalus species, phenolic compounds, Gas-liquid chromatography analysis

INTRODUCTION

The family Leguminosae (Fabaceae) contains 40 tribes subdividing into approximately 750 genera and 18000 species (Benchadi et al. 2013). Astragalus L. is the largest genus in this family and one of the largest genera of vascular plants on Earth, comprising 2000-3000 species and with more than 250 taxonomic sections in the world (Pistelli, 2002). Astragalus plants are annual or perennial stemmed herbs or small shrubs (up to 150-200 cm), growing from underground roots. Various parts of Astragalus species are also used in traditional medicine in Bulgaria, Russia, and other European and Asiatic countries. The investigations showed that several species of Astragalus L. have attracted significant attention due to diuresis, anhidrotic, and tonic effects (Shang et al., 2018). The genus Astragalus contained a wide range of bioactive secondary metabolites such as saponins, flavonoids, polysaccharides, nitro-compounds, indolizidine alkaloids, and seleniferous derivatives. Phenolics compounds have received much attention because of their antioxidant behavior and advantageous effect on degenerative diseases, such as cardiovascular, cancer, and etc. (Liu, 2003, Ahmadi et al., 2019 and 2022). Moreover, these compounds are used

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in industrial applications, for example, as natural colorants and preservatives for foods and in the production of paints and cosmetics. According to Harborne et al. (1980), phenolic compounds are grouped into the following categories: 1. phenols, phenolic acids, phenylacetic acids; 2. cinnamic acids, coumarins, isocoumarins and chromones; 3. lignans; 4. ten group of flavonoids ; 5. lignins; 6. tannins; 7. benzophenones, xanthones, and stilbenes; 8. quinones; 9. betacyanins. Most phenolic compounds are found in nature in a conjugated form, mainly with a sugar molecule (Carrasco-Pancorbo et al., 2005).

The aim of this work was to quantify the phenolic compounds of various Astragalus species belonging to different sections in Iran. Based on our knowledge, there is still limited research on Astragalus, and to date, there is no report on phenolic compounds in leaves of these species from Iran.

MATERIALS AND METHODS

Chemicals

Colorimetric and GLC standards were purchased from Sigma Chemical Company. Sodium carbonate, sodium nitrite, sodium molibdate, sodium acetate, aluminum chloride, potassium acetate were obtained locally. Methanol, acetonitrile were purchased from Merck Chemical Company.

Plant materials

The leaves of ten Astragalus species were collected from various regions of Iran. The names of these species are mentioned on the herbarium sheets, and the nomenclature and section classification used herein is based on Maassoumi (1998). Samples were dried at room temperature (20-25 °C) and then were analyzed for the content of phenolic compounds.

Phenolic compound extraction

For extraction of flavonols and phenolic acids, 0.1 g of dry powdered matter with 80% methanol was boiled three times (6 h each time) under reflux. But for phenolics, flavonoids and condensed tannins, 0.1 g dry matters were mixed with 5 ml of 80% methanol at room temperature for 48 h. Also for o-diphenols, 0.1 g dry powder matter was mixed with 10 ml of 50% methanol at room temperature for 48 h. Then, all extracts were filtered through a filter paper (Wathman, No. 4) and evaporated in vacuum in order to dryness. The extract was filtered and stored at 4 °C. A UV–Vis spectrophotometer (UV-160, Shimadzu, and Tokyo, Japan) was used for determination of phenolic compounds.

Total phenolic content

The total phenolic content was determined using the method given by Ranganna (1986) involving Folin–Denis reagent and tannic acid as standard. Plant extract (1 ml) was mixed with 1 ml of Folin–Denis reagent, 1 ml of 21% (w/v) sodium carbonate and 2 ml distilled water. Total phenolics were determined after 30 min of incubation at room temperature. Absorbance was measured at 760 nm with a spectrophotometer. The amount of total phenolics was expressed as percentage dry weight (DW).

Total o-diphenol content

Total o-diphenol contents were measured using the method described by Carrasco-Pancorbo et al. (2005). 0.5 ml of a 5% solution of sodium molybdatedihydrate in ethanol/water (50:50 v/v) was added to 0.2 ml of leaves methanol/water (50:50 v/v) extract. Then the mixture was vortexed, vigorously. The absorption was measured at 370 nm after 15 min. The amount of total o-diphenols is expressed as percentage DW. Gallic acid was used as standard.

Total flavonoid content

Aluminum chloride colorimetric method was used for flavonoids determination (Chang et al. 2002). Methanol extracts (0.5 ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water and kept at room temperature for 30 min.The absorbance of the reaction mixture was measured at 415 nm. Total flavonoids were expressed in term of equivalent amount of quercetin.

Total flavonols contents

Total flavonol content was estimated using the method described by Akkol et al. (2008) with some modification. In brief, 0.5 ml of ethanolic extract were mixed with 2 ml of 2% aluminum chloride and 6 ml of 5% sodium acetate and afterward, was left at room temperature for 150 min. The absorbance of the reaction mixture was read at 440 nm. The amount of total flavonols is expressed as percentage DW. The calibration curve range was 0.015-0.5 mg ml-1 rutin.

Total phenolic acids contents

Total phenolic acid was measured using the Arnow reagent [sodium molybdate 10% (w/v) and sodium nitrite 10% (w/v)] following the method of Matkowski et al. (2008) with some modification. Methanol extracts (1 ml) was mixed with 1 ml of 0.1 M HCl, 1 ml of Arnow reagent, 1 ml 1 M NaOH, and the volume was made up to 10.0 ml with H2O. The absorbance was read immediately at 490 nm. The results were expressed as caffeic acid equivalent.

Total condensed tannins contents

Total condensed tannins were determined using the modified vanillin assay described by Sun et al. (1998). 50 µl of properly diluted sample was added to 3 ml of reagent A (1% (w/v)

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Vanillin in methanol) and 2.5 ml of reagent B (9N H2SO4 in methanol) and then an absorbance reading in 500 nm after 15 min at 35 °C. The total condensed tannins were expressed as percentage DW.

Derivatization and Gas-liquid chromatography

The trimethysilyl (TMS) ether derivatives of authentic phenolics and total phenolics extracts were prepared as described by Saitta et al. (2002). Ten mg of each authentic phenolic compound was dissolved by 1 ml of anhydrous Pryidin, 0.2 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane. A Shimadzu GC-16A equipped with a flame ionization detector and 1.6 m × 3.2 mm i.d. glass column packed with 5% SE-30 was used. Operation temperatures were 100 °C for the oven and 270 °C and 280 °C for the injector and detector, respectively. Flow rates of hydrogen and air were 55 and 400 ml min-1, respectively. The peaks were identified by comparison of the retention times with authentic standards. 1 µl of extracts were used for injection.

Statistical analysis

All experiments were carried out in three replicates and presented as mean ± standard error of the mean using SPSS version 21.0. The data were statistically analyzed by one-way ANOVA and Duncan test. The level of statistical significance was set at $p \leq 0.05$.

RESULT

The content of phenolic compounds (total phenolics, total o-diphenols, total flavonoids, total flavonols, total phenolic acids, and total tannins) in the leaves of Astragalus species is presented in Table 1. As shown in Table 1, the amount of total phenoics and o-diphenols in leaves ranged from 0.48 to 1.22% DW and 0.01 to 0.90% DW, respectively. The highest level of phenolics and o-diphenols was found in A. glumaceus and A. siliqusus, respectively. While the lowest amount of phenolics and o-diphenols was detected in A.sciureus and A. paralurges, respectively. There were significant differences in the amount of phenolics and o-diphenols compound of this species.

Table 1 : Total phenolics, total o-diphenols, total flavonoids, total flavonols, total phenolic acids, and total tannins in leaves of ten Astragalus species. Means ± SE of three replicates. Different letters indicate significant differences ($P < 0.05$).

Species	Total Phenolics (%DW)	Total o- di Phenolics (%DW)	Total Flavonoids (%DW)	Total Flavonols (%DW)	Total Phenolic acids (%DW)	Total Tannins (%DW)
A. glochideus	0.74±0.01c	0.41±0.01cd	0.47±0.03c	0.14±0.01h	1.98±0.01ab	0.05±0.02b
A. siliqusus	0.59±0.01f	0.90±0.02a	0.73±0.04b	1.26±0.03d	1.32±0.16cde	0.04±0.02b
A. strictipes	0.71±0.02d	0.40±0.05cd	0.55±0.03c	1.49±0.05c	1.75±0.08abc	0.08±0.01ab
A. caraganae	0.65±0.01e	0.63±0.02bc	0.88±0.03a	1.95±0.04b	2.07±0.03a	0.06±0.01ab
A. aegobromus	0.88±0.01b	0.76±0.03ab	0.16±0.02de	2.20±0.02a	1.15±0.13de	0.05±0.01ab
A. schistosus	0.73±0.01c	0.40±0.01cd	0.57±0.03c	1.16±0.01e	1.54±0.03bcd	0.09±0.01a
A. submitis	0.65±0.01e	0.23±0.03de	0.27±0.01d	0.97±0.02f	0.97±0.06e	0.05±0.01ab
A. sciureus	0.48±0.01h	0.07±0.01e	0.03±0.02e	0.91±0.04f	1.29±0.03cde	0.05±0.01ab
A. glumaceus	1.22±0.02a	0.50±0.01c	0.52±0.03c	0.91±0.04f	1.17±0.02de	0.08±0.01ab
A. paralurges	0.52±0.01g	0.01±0.01e	0.04±0.01e	0.48±0.04g	0.96±0.12e	0.07±0.01ab

Total flavonoid can be determined in the sample extracts by reaction with potassium acetate, followed by the development of coloured flavonoid-aluminum complex formation using aluminum chloride. Among ten species tested in this study, it was determined that flavonoid contents were between 0.03 - 0.88% DW and the flavonol amount ranged between 0.14-2.2% DW. The greatest amount of flavonoids and flavonols were detected in A. caraganae and A. aegobromous, respectively.

The results of the Gas-liquid chromatography (GLC) analysis on extract from leaves of the ten Astragalus species were shown in Table 2. In all cases, there were numerous peaks that were not identified because of lack of suitable standards. The

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investigation revealed that all tested Astragalus species have a wide variety of phenolic acids and flavonoids profiles in their leaves. Identification of phenolic acids and flavonoids by standards revealed that all of studied Astragalus species contain syringic acid. p-comaric acid, caffeic acid, and dihydroxy benzoic acid were observed in most of the studied species. Cinnamic acid, gallic acid, and flavone were seen in few species. Furthermore, all species studied, except three species (A. strictipes, A. aegobromus, and A. sciureus) have coumaric acid. A. glumaceus, A. caraganae, and A. siliqusus have the highest number of total phenolic acid and flavonoid compounds. A. aegobromus and A. sciureus have the lowest number of phenolic acids compounds in their leaves. Caffeic acid found in five species and naringenin, vannilic acid, and frulic acid were found just in some of Astragalus species (A. glumaceus and A. strictipes).

Table 2 : Phenolics in leaves of Astragalus species. Compounds are expressed as both percentage of total compounds (GLC %) and mg. 100-1 gr DW.

Phenolic compounds		Dihydroxy benzoic acid	Syringic acid	Cinnamic acid	Coumaric acid	Caffeic acid	Gallic acid	Vannilic acid	Ferulic acid	Flavon	Naringenin
A. glochideus	(%GlC)	ND	77.88	ND	13.08	ND	ND	ND	ND	ND	ND
	Mg/100 g DW	ND	101.49	ND	9.52	ND	ND	ND	ND	ND	ND
A. siliqusus	(%GlC)	0.469	43.19	ND	4.31	0.579	0.646	ND	ND	0.403	ND
	Mg/100 g DW	0.64	109.24	ND	6.09	0.87	0.72	ND	ND	6	ND
A. strictipes	(%GlC)	1.86	64.04	ND	ND	0.573	ND	ND	5.89	ND	ND
	Mg/100 g DW	3.66	234.07	ND	ND	1.48	ND	ND	16.29	ND	ND
A. caraganae	(%GlC)	0.329	80.364	0.359	1.365	0.272	ND	ND	ND	ND	ND
	Mg/100 g DW	0.66	297.79	0.36	2.82	0.6	ND	ND	ND	ND	ND
A. aegobromous	(%GlC)	ND	83.91	ND	ND	ND	ND	ND	ND	ND	ND
	Mg/100 g DW	ND	207.06	ND	ND	ND	ND	ND	ND	ND	ND
A. schistosus	(%GlC)	ND	77.44	ND	7.05	ND	ND	ND	ND	ND	ND
	Mg/100 g DW	ND	76.73	ND	4.06	ND	ND	ND	ND	ND	ND
A. submitis	(%GlC)	ND	77.62	ND	2.13	ND	1.01	ND	ND	ND	ND
	Mg/100 g DW	ND	209.69	ND	3.22	ND	1.16	ND	ND	ND	ND
A. sciureus	(%GlC)	ND	58.48	ND	ND	ND	ND	ND	ND	ND	ND
	Mg/100 g DW	ND	68.76	ND	ND	ND	ND	ND	ND	ND	ND
A. glumaceus	(%GlC)	7.26	34.06	0.29	4.14	1.07	ND	1.66	ND	0.23	0.77
	Mg/100 g DW	39.62	345.13	0.81	23.44	6.48	ND	11.73	ND	13.92	56.24
A. paralurges	(%GlC)	ND	80.22	ND	0.97	0.65	ND	ND	ND	ND	ND
	Mg/100 g DW	ND	178.14	ND	1.2	0.86	ND	ND	ND	ND	ND

DISCUSSION

Folin-Denis reagent was used to determine total phenolics in the leaf extract of Astragalus. This reagent oxidizes phenolates, resulting in the production of complex molybdenum-tungsten blue (Naczk and Shahidi, 2004). Some previous investigations

revealed that total phenolic content during the growth cycles of plants is significantly influenced by many intrinsic factors such as plant species (genetic), parts of the plants, and extrinsic factors such as environmental conditions (e.g. light, temperature, irrigation, soil, exposure to diseases and pests, and nutrients), harvest season, cultural practices, handling and storage, and drying methods (Alirezalu et al., 2018 Tavassoli and Djomeh, 2011). Regarding the above-mentioned factors in the current study, we observed that phenolics content varies in different species of Astragalus collected from different regions of Iran. Variation in total phenolics content has been previously reported in roots and leaflets of 21 species of Astragalus using two solvents (Niknam and Ebrahimzadeh, 2002). Further, some studies suggest that the flavonoids content depends on plant varieties and environmental factors (García-Mateos et al., 2013).

Variation in the phenolic acid content would be expected to be dependent on the nutritional status of the plant and environmental conditions (Ndhlala et al., 2007). There are many reports of biological and pharmaceutical properties of polyphenols such as phenolic acids including antioxidant, free radical-scavenging abilities, anti-cancer, anti-bacterial, antiviral and anti-inflammatory (Báidez et al., 2007). Antioxidant metabolites such as phenolic compounds and flavonoid play an important role in reducing the negative effects of ROS.

In summary, our investigation showed that the most total phenolics were observed in A. glumaceus, A. caraganae, and A. siliqusus species. Thus, these species can be promising candidates for further phytochemical and chromatographic studies to isolate and identify the compounds related to antioxidant activity, and therefore, can be used for medicinal and industrial purposes.

Authors' contribution : Dr. Mahboobe Ghanbarzadeh performed the experiments, analyzed data, and wrote the manuscript. Dr. Vahid Niknam has designed and supervised the entire work. Dr. Hasan Ebrahimzadeh, Dr. Faezeh Fazeli, Dr. Fatemeh Shaki, Dr. Tayebeh Ahmadi and Sahar Hassannejad helped with the experiments. All authors have read and approved the final manuscript.

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Conflict of Interest : The authors declare that they have no conflict of interest.

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