

## Phytochemical profile, antioxidant properties and protein contents of *Astragalus tenuifoliosus* seeds

Maher Mahmoudi<sup>1,2\*</sup>, Fayçal Boughalleb<sup>2</sup>, Mahmoud Mabrouk<sup>3</sup>, Raoudha Abdellaoui<sup>2</sup>

<sup>1</sup>Laboratory of Rangeland Ecosystems and Valorization of Spontaneous Plants and Associated Microorganisms (LR16IRA03), Arid Regions Institute, University of Gabes, Medenine, Tunisia.

<sup>2</sup>Laboratory of Plant, Soil and Environment Interactions (LR21ES01), Faculty of Sciences of Tunis, University of Tunis El-Manar, Tunis, Tunisia.

<sup>3</sup>Platform Advances Analysis, Institute of Arid Regions, University of Gabes, Medenine, Tunisia.

\*Corresponding author: Maher Mahmoudi, Postal address : Institut des Régions Arides, Route de Djorf km 22.5, Médenine 4119, Tunisie. e-mail: [mahmoudi.maher@fst.utm.tn](mailto:mahmoudi.maher@fst.utm.tn); Phone number (+216 97 77 44 39).

Received August 14<sup>th</sup>, 2022; Accepted December 14<sup>th</sup>, 2022.

DOI: <http://dx.doi.org/10.29356/jmcs.v67i1.1854>

**Abstract.** Fabaceae seeds are reported to be used for varying medicinal and pharmaceutical purposes. However, knowledge of the nutritive value of *Astragalus tenuifoliosus* seeds is largely based on very limited data and remains unexplored, we report here the protein content, phenolics as well as the antioxidant potential of plant seeds to give adequate information on its suitability as a new source of natural bioactive compounds. The protein content was determined using the Kjeldahl and Bradford assays. The phytochemical contents were evaluated, and the extracts were further subjected to high-performance liquid chromatography-electrospray ionization – mass spectrometry (HPLC-ESI-MS) analysis. The antioxidant potential was evaluated using the total antioxidant capacity and the free DPPH radical scavenging activity. The results obtained from the protein analysis showed that the total content was 59.43 % of the dry matter basis. The globulins constituted the dominant fraction and followed by albumins, glutelins, and prolamins. The phytochemical investigation showed considerable amounts of polyphenol, flavonoid and condensed tannin amounts. The LC-ESI/MS analysis revealed the presence of 18 phenolics including 8 phenolic acids and 10 flavonoids mostly predominated by quinic acid (255.4  $\mu\text{g g}^{-1}$  DW), *p*-coumaric acid (65.39  $\mu\text{g g}^{-1}$  DW), quercetin (97.21  $\mu\text{g g}^{-1}$  DW), and cirsiolol (29  $\mu\text{g g}^{-1}$  DW). The seeds possessed strong antioxidant potential evidenced by their DPPH radical scavenging activities and total antioxidant capacity. The obtained findings contribute to the limited bibliographic information concerning *A. tenuifoliosus* seeds and represent a starting point to evaluate its potential as a valuable source of proteins, natural antioxidants, and safe bioactive compounds.

**Keywords:** *Astragalus tenuifoliosus* seeds; proteins; globulins; quinic acid; LC-ESI/MS.

**Resumen.** Las semillas de las Fabaceae se utilizan para diversos fines medicinales y farmacéuticos. Sin embargo, el conocimiento del valor nutritivo de las semillas de *Astragalus tenuifoliosus* se basa en gran medida en datos muy limitados y sigue sin explorarse. Aquí se reporta el contenido de proteínas, fenoles y el potencial antioxidante de las semillas de plantas para brindar información adecuada sobre su idoneidad como nueva fuente de compuestos bioactivos naturales. El contenido de proteína se determinó utilizando los ensayos de Kjeldahl y Bradford. Se evaluaron los contenidos fitoquímicos y los extractos se sometieron a análisis de cromatografía líquida de alto rendimiento, ionización por electropulverización y espectrometría de masas (HPLC-ESI-MS). El potencial antioxidante se evaluó utilizando la capacidad antioxidante total y la actividad de captación de radicales libres DPPH. Los resultados obtenidos del análisis de proteína mostraron que el contenido total fue

de 59.43% con base en el peso seco. Las globulinas constituyeron la fracción dominante, seguidas por las albúminas, las glutelinas y las prolaminas. La investigación fitoquímica mostró cantidades considerables de polifenoles, flavonoides y taninos condensados. El análisis mediante LC-ESI/MS reveló la presencia de 18 fenoles, incluidos 7 ácidos fenólicos y 11 flavonoides, predominando en su mayoría el ácido quínico (255.4  $\mu\text{g g}^{-1}$  DW), el ácido p-cumárico (65.39  $\mu\text{g g}^{-1}$  DW), la quercetina (97.21  $\mu\text{g g}^{-1}$  DW), y el cirsiol (29  $\mu\text{g g}^{-1}$  DW). Las semillas poseen un fuerte potencial antioxidante, evidenciado por sus actividades de eliminación de radicales DPPH y su capacidad antioxidante total. Los hallazgos obtenidos contribuyen a la limitada información bibliográfica sobre las semillas de *A. tenuifoliosus* y representan un punto de partida para evaluar su potencial como fuente valiosa de proteínas, antioxidantes naturales y compuestos bioactivos seguros.

**Palabras clave:** *Astragalo tenuifoliosus*; ácido quínico; globulinas; proteínas; LC-ESI/MS; semillas.

## Introduction

*Astragalus*, of the Fabaceae family, is the largest genus of vascular plants including about 2438 species, which are classified into 10 subgenera and 130 sections according to Maassoumi [1], and 2900 species and 136 sections according to Podlech and Zarre [2]. It is widely distributed in arid and temperate regions of the Northern Hemisphere and South America principally in Europe, Asia, and North America [3]. Within this genus, several species are recognized for potential antiperspirant, diuresis, detoxification, and tonic effects [4,5]. Fabaceae plants are cultivated throughout the world and consumed in various dishes, and they have been frequently used in foods, pharmaceutical industries, medicines, cosmetics, tea flavoring agents, coffee substitutes, and sources of natural gums [6].

Their seeds are recognized as nutritious food and are a rich source of protein, fat, carbohydrates, vitamins, and microelements [7]. Also, they are a source of several bioactive compounds with antioxidant properties, including phenolic acids and flavonoid compounds [8-10]. *Astragalus tenuifoliosus* Desf. also called *Astragalus algerianus* E. Sheld. is socioeconomic valuable species distributed in the Mediterranean/Sahara regional transition zone; grassland [11], especially in Algeria, Morocco, Tunisia, and Spain [12]. The plant contributes to soil fertilization and stabilization by enhancing nitrogen fixation [13].

The vegetable proteins of several legume crops are widely commercialized and were used for many industrial purposes including gelation and dough visco-elastic properties [14]. However, proteins are becoming more limited and expensive. The search for new sources has become an important research trend in recent decades [9-15] and they have been discussed as important compounds in the Fabaceae seeds [9-16]. The importance of polyphenolic compounds has largely increased due to their higher nutritive values and their beneficial health effects. Several classes of phenolics such as phenolic acids, flavonoid compounds, and their derivatives are the focal chemical compounds occurring widely in foods, plants, and formulated herbal products [17]. The antioxidant capacity of polyphenols and their derivatives play a crucial role in preventing several diseases related to reactive oxygen species (ROS) effects such as cellular aging, cancer, mutagenesis, rheumatoid arthritis, coronary heart, Alzheimer's, and Parkinson's diseases [18]. There is a great deal of evidence indicating that a high intake of food rich in dietary antioxidants is significantly correlated with a low risk of several diseases related to ROS effects such as cancer and heart disease [19]. Indeed, the functional food industry includes the incorporation of antioxidants to prevent the oxidation process and also to protect the body against several ROS-related diseases [20]. The unpleasant odor of food has been attributed to the oxidation of unsaturated fatty acids while the antioxidants retard and even reduce the development of the oxidation process. The antioxidative properties of natural sources are due to the active phytochemical compounds normally present in plants. Besides, the growing interest in the investigation of safe and natural antioxidants in food processing that substitute synthetic ones has fostered research on seeds and the screening of raw materials for identifying new antioxidants [21].

In continuation of our studies on the *Astragalus* species, herein we wish to report the antioxidant potential as well as the protein content of *A. tenuifoliosus*. The aim of the present study was thus to determine the crude and storage protein amounts, to evaluate the phytochemical contents as well as the antioxidant properties of the seeds of this plant.

## Experimental

### Material and methods

#### Collection and preparation of plant sample

Seeds of *Astragalus tenuifoliosus* were collected in July 2015 at the Dhaher Douz region (South of Tunisia; coordinates: 33°14'21.60"N, 9°19'53.86"E, at 112 m a.s.l.). The plant was authenticated and identified by Dr. Raoudha Abdellaoui (Laboratory of rangeland ecosystems and valorization of spontaneous plants and associated microorganisms, Arid Regions Institute, Medenine, Tunisia). The plant material was washed, dried, and reduced to a fine powder.

#### Chemical reagents

The standard solutions were of high-performance liquid chromatography (HPLC) grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA) and Loba Chimie Ltd (Mumbai, India).

#### Total protein contents (Kjeldahl method)

Crude protein contents were determined using the Kjeldahl method [22]. Briefly, a sample of 100 mg of defatted seeds was digested with 6 mL of concentrated sulfuric acid for 4 hours at 400 °C in the presence of potassium sulfate as a catalyst until the digest solution clears. After that, a volume of 20 mL of 40 % sodium hydroxide solution was added to the digested solution and the mixture was heated until all ammonia was distilled. The NH<sub>3</sub> was trapped in a flask containing a standard boric acid solution and drops of methyl red and the distillate was titrated against standard 0.1 N HCl. The conversion factor used to convert the Kjeldahl nitrogen value (N) to protein amount was 6.25 [23] and the protein content was calculated as follows: % protein = % N × 6.25. The crude protein content was expressed as mg g<sup>-1</sup> DW.

#### Antioxidants

##### Preparation of methanolic extracts

The extraction of phenolic compounds occurred in hydromethanolic solution in a flask protected from light at 40 °C for 24 h. The mixture was centrifuged at 4500 revolutions per minute (rpm) for 15 min and filtered using a syringe filter (0.2 µm hydrophilic polytetrafluoroethylene membrane). The dried extract was stored at -20 °C until use [9].

##### Total phenolic content

Total phenolic content estimated according to the Folin-Ciocalteu method [24]. A volume of 125 µL of seed extracts was added to 125 µL of Folin-Ciocalteu reagent and followed by the addition of 1250 µL of Na<sub>2</sub>CO<sub>3</sub> (7 %) and the mixture was made up to 3 mL with distilled water. The tubes were incubated in the dark for 90 min and the absorbance was measured at 760 nm. A gallic acid standard curve was used to express the phenol contents in mg of gallic acid equivalents (mg GAE g<sup>-1</sup>DW).

##### Total flavonoid contents

The flavonoid content of *Astragalus* seed extracts was determined according to the aluminium chloride method [24-25]. For this assay, 160 µL of samples were added to 75 µL of NaNO<sub>2</sub> (7%) followed by the addition of 150 µL of freshly prepared AlCl<sub>3</sub> (10 %) and 500 µL of NaOH (1 M). The final volume was made up to 3 mL with distilled water and the absorbance was measured at 510 nm. A quercetin standard curve was used to express the flavonoid contents in mg of quercetin equivalents (mg QE g<sup>-1</sup> DW).

##### Condensed tannin content

The tannin content was estimated using the methodology described by Broadhurst and Jones [26]. A volume of 25 µL of samples was added to 1.5 mL of vanillin solution 4 % followed by the addition of 750 µL of concentrated sulfuric acid. The tubes were incubated in the dark at room temperature for 15 min and the absorbance was measured at 500 nm. A catechin standard curve was used to express the tannin contents in mg of equivalents catechin (mg CE g<sup>-1</sup> DW).

### High-performance liquid chromatography-electrospray ionization – mass spectrometry (HPLC-ESI-MS)

HPLC-MS analyses were conducted on seed extracts of *A. tenuifoliosus* using a Shimadzu UFLC XR system (Kyoto, Japan), equipped with an electrospray ionization source (ESI), two LC-20ADXR pumps, a SIL-20AXR autosampler, an SCL-10A system controller, a CTO-20 AC column oven, and a DGU-20AS degasser. The phenolic compounds were separated on Bio Wide Pore C18 column (150 mm×3 mm, 3 μm) using two mobile phases A and B that consisting of 0.1 aqueous formic acid v/v (phase A) and 0.1 % methanolic formic acid v/v (phase B). Following the linear gradient elution: 0–14 min, from 10 % to 20 % B; 14–27 min, from 20 % to 55 % B; 27–37 min, from 55 % to 100 % B; 37–45 min, 100 % B; and 45–50 min 10 % B. The MS was operated in negative ion mode with a scanning range from *m/z* 35 to *m/z* 500 and following the condition: capillary voltage of -3.5 v, a nebulizing gas flow of 1.5 L/min, a dry gas flow rate of 15 L/min, a DL (dissolving line) temperature of 280°C, a block source temperature of 400°C, and a voltage detector of 1.35 V. Data acquisition and processing were performed using Shimadzu LabSolutions software ver.5.42. (Shimadzu, Kyoto, Japan). Quantification and identification of individual compounds were performed by comparing their retention time and mass spectra with those of reference standards and results were expressed as μg per gram of dry weight of the seed powder (μg g<sup>-1</sup> DW) [9,15].

### Total antioxidant capacity (TAC)

The phosphomolybdenum assay as described by Prieto *et al.* [27] was applied to evaluate the total antioxidant capacity. Briefly, a volume of 200 μL of each extract was mixed with 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). After incubation at 95°C for 90 min, the absorbance was measured at 700 nm and the TAC was expressed as mg equivalents of gallic acid per gram of dry weight (mg GAE g<sup>-1</sup> DW).

### DPPH radical scavenging activity

The free radical DPPH scavenging activity of *Astragalus* seeds extracts was evaluated according to Sánchez-Moreno *et al.* [28]. In brief, 50 μL of sample extract at various concentrations was added to 1.95 mL of DPPH methanolic solution (0.025 g L<sup>-1</sup>). After that, the solution was well mixed using a vortex mixer and allowed to stand in the dark at room temperature for 30 min. Simultaneously, a negative control was prepared by adding 50 μL of methanol to 1.95 mL of DPPH solution. The DPPH radical inhibition was determined at 515 nm. The percentage of the ability to scavenge the DPPH radical was calculated as follows: [(Abs control-Abs sample)/Abs control] × 100. Where Abs control refers to the control absorbance and Abs sample refers to the sample absorbance.

## Results and disussion

### Total protein content (Kjeldahl method)

The results for crude protein content, determined by the Kjeldahl method, clearly indicated that *A. tenuifoliosus* seeds are very rich in protein and the content was 59.4% on a dry weight basis (Table 1) which was consistent with our previous study that reported high crude protein amounts in the seeds of *A. gombiformis* (52.21 %), *A. caprinus* (54.35 %), and *A. Armatus* (54.74 %) [9].

**Table 1.** Total and storage protein class amounts in *A. tenuifoliosus* seeds.

		Contents	
		mg g <sup>-1</sup>	%
Soluble Protein fraction	Albumins	98.15±3.4	23.52
	Globulins	221.25±17.18	53.03
	Prolamins	28.22±4.11	6.76
	Glutelins	69.53±2.46	16.66
	Total	417.15±28.75	
Protein content (Kjeldahl)		59.4±2.39	

Moreover, El Naga and Abou Rizk [29] investigated the protein contents in Egyptian *Astragalus* species and they were in the range of 30.7—44.8 % for *A. macrocarpus* and *A. caprinus* respectively. Moreover, Smith Jr *et al.* [30] studied the protein contents in *A. cicer* (39.9 %) and *A. jalcati* (41.0 %). Indeed, the obtained amount was similar to that of *A. panduratus* (52.5 %) reported by Van Etten *et al.* [31]. Proteins have been discussed as important compounds in the Fabaceae seeds. However, these results differ from the studies of Bakoğlu *et al.* [32], who reported lesser amount for the most common Fabaceae such as *Vicia ervilia* (20.09 %), *Trifolium aureum* (28.6 %), *Lotus corniculatus* (21.2 %), *Trifolium pretense* (25.4 %), and *Onobrychis fallax* (26.1 %). In addition, this content is substantially higher than that reported for cereal seeds such as maize, triticale, and wheat with values ranging from 8.4 to 14.8 % [33].

### Soluble protein fractions

The examination of the obtained data showed that *A. tenuifoliosus* seeds are rich in proteins (Table 1). Globulins are the major proteins; they form 53.03 % of total protein (221.25 mg g<sup>-1</sup>), followed by albumin and glutelins, which form 23.52 % and 8.55 % respectively (98.15 and 69.53 mg g<sup>-1</sup>). The prolamin fraction presented only 6.76 % (28.22 mg g<sup>-1</sup>). This profile is consistent with those of *A. armatus*, *A. caprinus*, and *A. gombiformis* [9]. However, *Calicotome villosa* and *Lotus creticus* seeds, Leguminosae species, were rich in globulins and albumin [9-34]. This result has further strengthened our hypothesis that *A. tenuifoliosus* could be a natural source of proteins. Due to their functional properties, the vegetable proteins of several legume crops are widely commercialized for many industrial purposes [14]. The albumin and globulin proteins are considered biologically more active proteins and are involved in the physical prosperities of several industrial products such as emulsification and foaming performance [35].

### Total polyphenol, flavonoid, and condensed tannin contents

The total polyphenol content in the seeds of *A. tenuifoliosus* was performed according to the Folin-Ciocalteu method and the results were summarized in Table 2. The TPC was found to be 6.5 mg GAE g<sup>-1</sup> DW. The identified contents are within the range of other Tunisian *Astragalus* species including *A. caprinus* (3.4 mg GAE g<sup>-1</sup> DW), *A. armatus*, and *A. gombiformis* (6.5 mg GAE g<sup>-1</sup> DW). Moreover, the quantification revealed that the methanol extracts of seeds are rich in flavonoids and the content was 2.67 mg QE g<sup>-1</sup> DW. While the TFC contents fluctuated between 1.24 and 5.15 mg QE g<sup>-1</sup> DW for seeds of *A. gombiformis* and *A. armatus*, respectively. The condensed tannin content was found to be 15.05 mg CE g<sup>-1</sup> DW, within the range of that reported for *A. caprinus* (12.07 mg CE g<sup>-1</sup> DW) and *A. armatus* (23.21 mg CE g<sup>-1</sup> DW). The available information on the phenolic contents of the studied *Astragalus* seeds is very sparse. Niknam and Ebrahimzadeh [36] assessed the total polyphenol contents in several *Astragalus* species that ranged from 0.24 to 0.66 % DW. The obtained amounts are substantially higher than those reported for other legumes, such as soybeans (0.9 mg GAE g<sup>-1</sup> DW) [37]. The detected phenolic contents in the seeds of *Astragalus* are interesting; this gives their important nutritional and therapeutic value.

### Antioxidant activity

The free radical DPPH scavenging activity and total antioxidant capacity methods were selected to evaluate the *in vitro* antioxidant potential of *A. tenuifoliosus* seeds based on their popularity in antioxidant assays (Table 2). The DPPH value was found to be 13.1 % while the TAC value was 7.19 mg GAE g<sup>-1</sup> DW. The DPPH radical scavenging activities were in the range of 6.23—23.77 % while the TACs ranged from 3.88 to 6.44 for *A. caprinus* and *A. gombiformis*. Our finding is well supported by a previous report investigating the antioxidant activity of some *Astragalus* species [38]. Also, a previous study reported high antioxidant potential for *A. armatus* explaining their high antibacterial effect [39]. Moreover, the data obtained from the current study were in agreement with previous work that reported high antioxidant activity of the leaf extracts of *A. gombiformis* [40].

**Table 2.** Total polyphenol, flavonoid, condensed tannin contents; DPPH scavenging activities; and total antioxidant capacity of *A. tenuifoliosus* seeds.

	Total polyphenol content (mg GAE g <sup>-1</sup> DW)	Total flavonoid content (mg QE g <sup>-1</sup> DW)	Condensed tannin content (mg CE g <sup>-1</sup> DW)	DPPH assay (%)	Total antioxidant capacity (mg GAE g <sup>-1</sup> DW)
Contents	6.5 ± 0.02	2.67 ± 0.81	15.05 ± 1.78	13.1 ± 2.53	7.19 ± 0.58

Data expressed as means ± standard deviation. mg GAE g<sup>-1</sup> DW: mg gallic acid equivalents/g dry weight, mg QE g<sup>-1</sup> DW: mg quercetin equivalents/g dry weight, mg CE g<sup>-1</sup> DW: mg catechin equivalents/g dry weight, DPPH:  $\alpha, \alpha'$ -diphenyl- $\beta$ -picrylhydrazyl.

### HPLC-ESI-MS analysis

The *Astragalus* plant extracts possessed high biological activities, including antioxidant antiviral, antibacterial, antitumor, anti-inflammatory, hepatoprotective, immunomodulatory, and hepatoprotective effects [41] all of which are mainly well correlated with the phytochemical and bioactive properties of the plant. The seeds of *A. tenuifoliosus* were found to contain considerable flavonoid and phenolic acid levels.

Eighteen bioactive molecules were identified and quantified including 8 phenolic acids and 10 flavonoids (Table 3). Total phenolic contents were found to be 471.45  $\mu\text{g g}^{-1}$  DW. While the total phenolic acids were 338.47  $\mu\text{g g}^{-1}$  DW and the flavonoids were 132.98  $\mu\text{g g}^{-1}$  DW. A higher quinic acid content was obtained in seed extract (255.4±5.19  $\mu\text{g g}^{-1}$  DW) followed by *p*-coumaric acid (65.39  $\mu\text{g g}^{-1}$  DW), trans-ferulic acid (7.78  $\mu\text{g g}^{-1}$  DW), syringic acid (3.25  $\mu\text{g g}^{-1}$  DW), gallic acid (2.94  $\mu\text{g g}^{-1}$  DW), protocatechuic acid (1.87  $\mu\text{g g}^{-1}$  DW), 3,4-di-*O*-caffeoylquinic acid (1.03  $\mu\text{g g}^{-1}$  DW), and caffeic acid (0.82  $\mu\text{g g}^{-1}$  DW). Concerning the flavonoid compounds, quercetin and cirsiol were identified as the major components (79.21 and 29  $\mu\text{g g}^{-1}$  DW, respectively). However, catechin (+), quercetin-3-*O*-galactoside, rutin, quercetin-3-*O*-rhamnoside, naringenin, and apigenin were characterized at low amounts (< 1  $\mu\text{g g}^{-1}$  DW). No data were found in the literature on the identification of phenolic compounds in *A. tenuifoliosus*; however, we identified in our previous work phenolic acids and flavonoids in the seed extracts of *A. gombiformis*, *A. caprinus*, and *A. armatus* [9]. Moreover, the obtained data from the current study were in agreement with previous work that investigated the phenolics of three Tunisian *Astragalus* species in which quinic acid was the main phenolic acid for the three species and *A. armatus* possessed the highest amount of *p*-coumaric acid while *A. caprinus* and *A. armatus* showed important levels of cirsiol. While caffeic acid, syringic acid, 3,4-di-*O*-caffeoylquinic acids, and acacetin were found to be identified only in *A. armatus* at low amounts. Indeed, quercetin-3-*O*-galactoside, quercetin-3-*O*-rhamnoside, quercetin, kaempferol, naringenin, and apigenin were identified in the extracts at a low level. Compared to other legumes, quinic acid, quercetin-3-*O*-galactoside, and luteolin were identified as the greatest compounds in the extracts of *Calicotome villosa* seeds [34]. Several investigations have shown that quinic acid has biological properties, including anti-inflammatory activity, hepatoprotective, and antioxidant [42] and it is also required for several drugs [43].

**Table 3.** Content of phenolic acids and flavonoids compounds in *A. tenuifoliosus* seeds detected by LC/ESI-MS.

No	Compounds	Linear range (mg L <sup>-1</sup> )	Rt (min)	MW	Ionization forms	M-H] <sup>-</sup> and [2M-H] <sup>-</sup> m/z	Contents ( $\mu\text{g g}^{-1}$ DW)
1	Quinic acid	0.01 - 2	2.273	191	[M-H] <sup>-</sup>	191	255.4±5.19
2	Gallic acid	0.005 - 5	4.029	169	[M-H] <sup>-</sup>	169	2.94±0.01
3	Protocatechuic acid	0.005 - 5	7.011	153	[M-H] <sup>-</sup>	153	1.87±0.3
4	Catechin (+)	0.01 - 5	11.041	289	[M-H] <sup>-</sup>	163	0.41±0.01
5	Caffeic acid	0.01 - 5	14.696	182	[M-H] <sup>-</sup>	179	0.82±0
6	Syringic acid	0.01 - 2	16.361	198	[M-H] <sup>-</sup>	197	3.25±0.23
7	<i>p</i> -coumaric acid	0.05 - 7.5	21.11	164	[M-H] <sup>-</sup>	163	65.39±1.01
8	Trans ferulic acid	0.01 - 10	23.356	194	[M-H] <sup>-</sup>	193	7.78±0.08
9	Quercetin-3- <i>O</i> -galactoside	0.05 - 5	24.336	463	[M-H] <sup>-</sup> , [2 M-H] <sup>-</sup>	464, 927	0.9±0.03

10	Rutin	0.01 - 5	24.334	609	[M-H] <sup>-</sup> , [2 M-H] <sup>-</sup>	609, 1219	0.83±0.01
11	3,4-di-O-caffeyoquinic acid	0.05–5.0	25.312	515	[M-H] <sup>-</sup> , [2 M-H] <sup>-</sup>	609, 1219	1.03±0.06
12	Quercetin-3-O-rhamonoside	0.01 - 2	26.988	447	[M-H] <sup>-</sup> , [2 M-H] <sup>-</sup>	448, 863	0.93±0.03
13	Quercetin	0.05 - 2	32.243	301	[M-H] <sup>-</sup> , [2 M-H] <sup>-</sup>	301, 603	97.21±0.45
14	Naringenin	0.01 - 10	34.085	271	[M-H] <sup>-</sup>	579	0.61±0.01
15	Apigenin	0.05–1.0	34.922	269	[M-H] <sup>-</sup>	270	0.15±0
16	Luteolin	0.05 - 2	35.287	285	[M-H] <sup>-</sup> , [2 M-H] <sup>-</sup>	286, 571	2.05±0.11
17	Cirsiliol	0.01 - 2	35.826	329	[M-H] <sup>-</sup>	329	29±0.47
18	Cirsilineol	0.01 - 10	38.901	343	[M-H] <sup>-</sup>	343	1.22±0.04
<b>Total phenolic acids</b>							<b>338.47</b>
<b>Total flavonoids</b>							<b>132.98</b>
<b>Total phenolics</b>							<b>471.45</b>

Data expressed as means ± standard deviation. *Rt*: retention times, MW: molecular weight.

## Conclusion

To our knowledge, this is the first study reporting the proteins and antioxidants from *A. tenuifoliosus* seeds. From the research that had been undertaken, it is possible to conclude that *A. tenuifoliosus* seeds were a potential and natural source of protein and bioactive compounds. Storage proteins showed that globulins constituted the major fraction followed by albumin and glutelins however the prolamin fraction presented at low amounts. *A. tenuifoliosus* seeds were found to have significant antioxidant activities, high flavonoids, and phytochemical content thus 8 phenolic acids and 10 flavonoids were characterized. Quinic acid, *p*-coumaric acid, trans-ferulic acid, quercetin and cirsiliol were identified as major phenolics. The free availability, as well as the easy accessibility of the present species in the rural areas and the arid regions, side by side with the obtained data, led us to conclude that *Astragalus* seeds are promising for use as a new source of bioactive compounds and natural antioxidants. Our results are encouraging and should be validated by further analysis including the quantitative determination of the amino acids by HPLC complying with FAO recommendations.

## Acknowledgements

We are grateful to the technical staff of the Arid Regions Institute, Medenine (IRA), for their help with the conduct of these experiments.

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