

## Phenolic, Flavonoid Contents and Antioxidant Activity of Methanolic and Aqueous Extracts of Different Parts of *Astragalus fasciculifolius* and Evaluation Antibacterial Activity of Methanolic Gum Extract

Najmeh Khademi pour<sup>1</sup>, Anoushe Sharifan<sup>1\*</sup>, Hosein Bakhoda<sup>2</sup>

1- Department of Food Science and Technology, Science and Research Branch, Islamic Azad University, Tehran, Iran

\*Corresponding author (a\_sharifan@srbiau.ac.ir)

2- Department of Mechanization, Science and Research Branch, Islamic Azad University, Tehran, Iran

### Abstract

*Astragalus fasciculifolius* belongs to the genus *Astragalus* and the legume family. The distribution of this plant is in Southwest Asia. So far, no detailed studies have been conducted on this plant. This study tried to extract different parts of *Astragalus fasciculifolius* (gum, aerial parts, and roots) using two solvents of water and methanol. The extracts' content of total phenolic and flavonoid compounds and antioxidant activity (DPPH, ABST, CUPRAC, PMB, and FRAP) were evaluated, and the correlation between total phenolic compounds and antioxidant activity or Pearson test was investigated. The results showed that the methanolic gum extract had the highest antioxidant activity as well as the highest content of total phenol ( $22.30 \pm 1.30$  mg GAEs/g extract) and total flavonoids ( $11.30 \pm 0.87$  mg Routine (RUE)/g), which was significantly different from the other parts extracts ( $P \leq 0.05$ ), and the correlation between total phenolic compounds and antioxidant activity was also significant ( $P \leq 0.05$ ). According to the results, it was found that methanolic gum extract has antimicrobial activity and the MIC and MBC of *Clostridium perfringens* were lower than *Pseudomonas aeruginosa*. Based on the findings of this study, *Astragalus fasciculifolius* gum has the potential to be used in food, pharmaceutical, and health industries.

Received: 2021.12.19

Revised: 2022.03.23

Accepted: 2022.04.10

Online publishing: 2022.04.11

### Keywords

Antimicrobial activity

Antioxidant activity

*Astragalus fasciculifolius*



© 2022, Research Institute of Food Science and Technology. All rights reserved.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution 4.0 International (CC-BY 4.0). To view a copy of this license, visit (<https://creativecommons.org/licenses/by/4.0/>).

### Introduction

Since ancient times, medicinal plants have been applied to prevent and treat some diseases, and all or part of these plants have been used for medicinal purposes; today, medicinal plants play an essential role in modern treatment, and the tendency to use herbal medicines is increasing worldwide (Vasfilova & Vorob'eva, 2020).

The World Health Organization (WHO) has stated that more than 80% of the world's population uses medicinal plants or their derivatives (WHO, 2019), and more than 50% of new drugs are based on medicinal plants or their active compounds. In addition, medicinal plants can be used as antimicrobial and antioxidant compounds in the food,

pharmaceutical, and health industries (Teng & Shen, 2015). In recent decades, efforts have accelerated to find antioxidant compounds of plant origin in order to replace existing synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Leng *et al.*, 2018). Phytochemical compounds found in plants include flavonoids and other phenolic compounds. (Jaganath & Crozier, 2010). The relationship between phenolic compounds in plants and their bioactivity potentials has been proven (Nguyen Viet *et al.*, 2021). Phenolic compounds in plants can give hydrogen to free radicals in chemical reactions and prevent oxidation progression (Zhong & Shahidi, 2015), and they also show their effectiveness by activating antioxidant enzymes and chelating metals (Kamath *et al.*, 2015).

*Astragalus* is the most prominent genus of flowering plants belonging to the Fabaceae family. It has about 3,000 species found mainly in central and southwestern Asia (Jaradat *et al.*, 2017). *A. Fasciculifolius* also belongs to the genus *Astragalus*, which grows wild and has demonstrated high ecological values in soil conservation, animal nutrition, carbon sequencing potential, and medicinal properties (Shahid & Rao, 2015).

The pharmacological properties of *Astragalus fasciculifolius* have been numerous and include anti-cancer (Huang *et al.*, 2012), anti-inflammatory (Lu *et al.*, 2013), antiviral, and antibacterial (Huang *et al.*, 2008). More than 140 chemical compounds have been identified in the *Astragalus* genus, such as cyclovartan triterpene glycosides, flavonoids, and various polysaccharides (Li *et al.*, 2014). The main constituents of glycosides are saponin, phenolic compounds, and polysaccharides (Pistelli *et al.*, 2003).

Many studies are underway on the antioxidant effects of bioactive compounds of known and unknown plants worldwide (Sarikurkcu & Zengin, 2020; Zhang *et al.*, 2020), and some research is focused on *Astragalus*. Phytochemical and biological

properties of 4 species of *Astragalus* evaluated by Jaradat *et al.* (2017). Arumugam *et al.* (2019) examined phenolic profile, antioxidant activity, and enzymatic inhibition of methanolic extracts of different parts of *Astragalus ponticus* Pall. Sarikurkcu *et al.* (2020) investigated the phytochemical, antioxidant, inhibitory activities of tyrosinase and  $\alpha$ -amylase of ethanolic extracts from three different species of *Astragalus* (*A. gymmolobus* Fisch., *A. leporinus*, and *A. Onobrychis*).

Since no comprehensive research on phenolic profile, antioxidant, and antimicrobial of *Astragalus fasciculifolius* has been done so far. In this study, it was aimed to investigate the phenol and flavonoid, phenolic profile, antioxidant and antimicrobial activities of methanol (MeOH), and aqueous extracts obtained from three different parts (Aerial parts, root, and gum) of *Astragalus fasciculifolius*.

## Materials and methods

### Plant collection

*Astragalus fasciculifolius* was collected from the cultivation pastures of this plant in the north of Hormozgan-Iran (25° 24' 28°.53"N 52° 44' 59.14"E) and a Botanist approved the genus and species of this plant of Hormozgan University. Plants were harvested on 18 July 2020, and the root, aerial parts, and gum of the plant were separated; after that, the *Astragalus fasciculifolius* were cleaned and dried in the shade at room temperature and proper airflow for 72 h and were grounded into an adequate powder particles size using an industrial mill. The plants were stored in the refrigerator at 4 to 6 °C.

### Preparation and extraction of *Astragalus fasciculifolius* extract

For aqueous sample extraction, 5 g of each sample (aerial parts, roots, and gum) was mixed with 200 mL of distilled water, heated to 40 °C, and stirred simultaneously for 20 min. The above solution was filtered with a Whatman filter paper (Number 1),

and the filtered extract was kept at 2 to 6 °C.

For methanolic extract, the extraction was carried out with the soxhlet extraction method; for this, 20 g of the dried powder of sample (root, aerial parts, and gum) was packed into a thimble and then extracted using 250 mL of methanol (Loba Chemie, India) as a polar protic solvent. The extraction was kept going until decolorized the siphon solvent; it took 8 h in this research, and the operation was performed at 60 °C. Then, the obtained extract was warmed in a water bath at 30 to 40 °C, and then, the residual solvent was evaporated under vacuum. The resulting extract was stored at 2 to 6 °C (Jaradat *et al.*, 2017).

#### **Determination of total phenol content (mg Gallic acid/g)**

The spectrophotometric method calculated total phenol compounds in the extract samples (Robbins, 2003). An aqueous solution of methanolic extracts (each plant part separately) extracted as 1 mg/mL was prepared. 0.5 mL of the extract was mixed with 2.5 mL of 10% aqueous soluble folic acid and 2.5 mL of 7.5% aqueous NaHCO<sub>3</sub> solution. The above mixture was kept for 45 min at a constant temperature of 45 °C. A spectrophotometer read the absorbance of each sample at a wavelength of 765 nm. Gallic acid was the standard sample, and the absorption curve was drawn. Based on the adsorption read at the gallic acid concentration, total phenol compounds were expressed as mg of GAE/g of extract (Jaradat *et al.*, 2017).

#### **Determination of total flavonoids content (mg Routine/g)**

Routine standard solution (100 mg) was used and dissolved in 10 mL of distilled, and then its volume was increased to 100 mL. The solution was used to draw a standard curve. 0.5 mL of the extract was mixed with 3 mL of methanol, 0.2 mL of Aluminum chloride 10% (Sigma Aldrich, Germany), 0.2 mL of 1 M potassium acetate (Sigma Aldrich, Germany), and 5 mL of distilled water. Samples were kept

at room temperature for 30 min. The uptake of each sample was measured at 415 nm. Based on the Routine (MP-Biomedical, USA) uptake curve's calibration, the amounts of flavonoids in mg RUE/g extract were reported (Singh *et al.*, 2015).

#### **Antioxidant activity**

##### **Evaluation of antioxidant activity by free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH)**

The method presented by Jaradat *et al.* (2017) was used to evaluate the antioxidant activity. For this purpose, the concentration of 1 mg/mL of ethanol was prepared from the extract and the standard solution of Trolox. Working solutions were prepared at concentrations of 1, 2, 3, 7, 10, 20, 30, 40, 50, 80 and 100. The solutions were mixed with methanol and DPPH reagents in equal proportions. The prepared solutions were placed at room temperature for 30 min in a dark cabinet, and finally, antioxidant activity was read using a spectrophotometer at 517 nm wavelength and expressed as the following formula.

$$\text{DPPH inhibition activity} = \frac{A-B}{A} \times 100 \quad (1)$$

A and B are the optical density of the samples and blank (Jaradat *et al.*, 2017).

##### **Evaluation of antioxidant activity by IC<sub>50</sub> inhibition (%)**

The antioxidant half-maximal inhibitory concentration (IC<sub>50</sub>) of aqueous and organic *Astragalus fasciculifolius* extracts were assessed according to Jaradat *et al.* (2017). The antioxidant activity was presented as the percentage of inhibition.

##### **Evaluation of antioxidant activity by ferric ion reduction capacity**

For this, the Benzie & Strain (1996) method was used. 1 mL of the FRAP reagent was added to 0.002 mL of extract (1 mg/mL) and placed at 37 °C for 5 min. The adsorption was read at 593 nm. The calibration curve was constructed using

FeSO<sub>4</sub>. 7H<sub>2</sub>O (Sigma-Aldrich, Germany) concentrations (0.1, 0.4, 0.8, 1, 1.2 and 1.4 mm).

#### **Evaluation of antioxidant activity by phosphomolybdenum test**

The method expressed by Prieto *et al.* (1999) was used, and for this purpose, 0.2 mL of the extract was mixed with 2 mL PMB reagent and held at 95 °C for 90 min, and then antioxidant activity was read at 695 nm wavelength and reported as Trolox equivalent.

#### **Evaluation of the radical inhibitory activity of 2,2-azino-bis(3-ethylbenzothiazyl-6-sulfonic acid) cations**

For producing radical 2,2-azino-bis(3-ethylbenzothiazyl-6-sulfonic acid) (ABTS), a solution reaction of 7 mm ABTS with 2.45 mm potassium sulfate was used. The resulting solution was placed in darkness and at room temperature for 12-16 min. Before starting the assay, the ABTS solution was diluted with methanol, and then the sample solution (1 mL) was added to the ABTS solution (2 mL), and the vertex style was uniform. Sample adsorption was read at 734 nm after incubation for 30 min at room temperature. Cationic radical inhibition activity of ABTS was expressed as the equivalent of Trolox (Zengin *et al.*, 2015).

#### **Evaluation of antioxidant activity by cupric ion reducing activity (CUPRAC)**

0.5 mL of the extract (1 mg/mL methanol solvent) was added to the reaction mixture containing copper chloride, neocapron, and ammonium acetate buffer. After 30 min of storage at room temperature, the adsorption of samples was read at 450 nm wavelength. CUPRAC was expressed as the equivalent of Trolox (Apak *et al.*, 2006).

#### **Determination of the lowest minimum inhibitory concentration (MIC) and the minimum bactericide concentration (MBC)**

The dilution method in the tube was applied to calculate the MIC. A set of 12 test tubes was used to determine the MIC

for Anzarut extracts. 9 tubes for testing different dilutions of each extract and 1 tube as a negative control (containing diluted extract plus Mueller Hinton broth culture medium) and 1 tube as a positive control (containing microbial suspension plus Hinton Broth culture medium) and 1 tube containing methanol, microbial suspension, and Mueller Hinton broth culture medium was also used to ensure the growth of bacteria in the methanol-containing medium used for extraction. Add 100 µL of the prepared bacterial suspension to each dilution of the extract (8, 16, 32, 64, 128, 256, 512 and 1024 mg/mL), and tubes containing 2 µL of culture medium, dilution of the extract, and bacterial suspension store at 37 °C for 24 h. After 24 h, the tubes were examined for turbidity due to the growth of inoculated bacteria. Samples were taken from all tubes in which no bacterial growth was observed and cultured by spread method to determine the MBC of the extracts. For this purpose, 100 µL of the tubes that showed no bacterial growth, the tube containing the lowest concentration of the extract, and the lack of bacterial growth will be observed in the relevant plate, is considered the MBC of that extract and the first tube, Where turbidity was not observed and completely transparent, it will be considered MIC (Hemeg *et al.*, 2020).

#### **Statistical analysis**

Comparison of mean of effective compounds (total flavonoids and total phenols contents) and antioxidant activity of different parts (roots, leaves, and gums) extracts of *Astragalus fasciculifolius* were carried out in triplicate for each sample, and the data were analyzed by SPSS software (version 26) and using one way ANOVA test. The results were presented as mean±SD. The Pearson test was performed by the correlation between total phenol compounds and antioxidant activities, and the results were reported with errors of 5 and 1%.

## Results and discussion

### Bioactive compounds

This study tried to examine the antioxidant activities of the methanol and water extracts of different parts of *Astragalus fasciculifolius*. As well known, it is necessary to find a relation between the antioxidant potential and bioactive compounds of the samples to find an assumption about the bioactive material responsible for the antioxidant activity. Furthermore, total phenolic and flavonoids contents of the *Astragalus fasciculifolius* extracts were clarified.

Due to differences in species of one genus, differences in the harvest period, plant maturity, different parts, and heterogeneity of test conditions, the amount of calculated phenolic compounds may be different; this result can be seen in the studies of other researchers (Shahrani *et al.*, 2021).

Considering the importance of solvent type in the extraction process, the findings showed that solvent type had a significant effect on the content of active compounds.

The phenolics and flavonoid content were as mg gallic acid (mg GAEs/g extract) and rutin (mg RUE.s/g extract), respectively. Table (1) and Fig. (1) show the extract's bioactive compounds (phenolics and flavonoids contents). The methanolic gum extract of *Astragalus fasciculifolius* (MGE) had the maximum

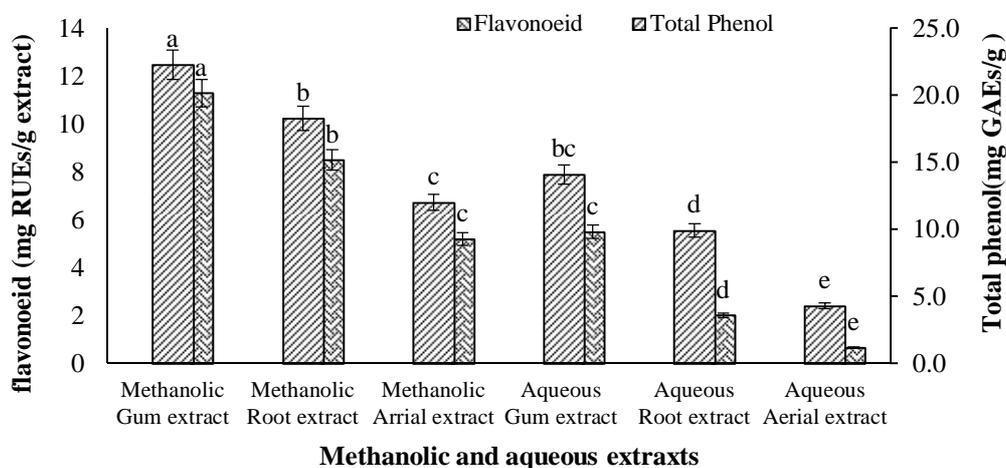
total phenol content (22.30 mg GAEs/g extract). Also, MGE extract had the most flavonoids content (11.3 mg REs/ g). Furthermore, among the different parts of *Astragalus fasciculifolius* research, it was found that the poorest phenolics and flavonoid content belong to aerial parts extracted by water (ARE) (4.27 mg GAEs/g extract and 0.65 mg RUEs/g extract).

Moreover, as Table (1) shows, the methanolic extract has more phenolics and flavonoids than aqueous extracts.

According to our findings, it could be seen that flavonoids are a majority part of the total phenolic content in extracts. Some studies have reported total phenolics in other *Astragalus* species (Sarikurkcu & Zengin, 2020; Zhang *et al.*, 2020).

According to Fig. (1), it is clear that the most phenolic and flavonoids content had correlated to the extracted from the gum, root, and aerial parts of the *Astragalus fasciculifolius*, respectively, and methanol extracts had more phenolic and flavonoid contents than water extracts.

Shahrani *et al.* (2021) examined the *Astragalus fasciculifolius* gum extract and stated that the content of phenolic compounds was 36.35 mg (GAL acids/g) which is nearly close to the amount of total phenol reported in the current study.



**Fig. 1.** Phenolic and flavonoid compounds in methanolic and aqueous different parts of *Astragalus fasciculifolius*

**Table 1.** The phenolic, flavonoid content, and antioxidant activity of the aqueous and methanolic extracts of three parts of *Astragalus fasciculifolius*

Extract solution	Part	Phenol (mg gallic acid/g)	Phelavonooid (mg Rutine/g)	DPPH (%)	IC <sub>50</sub>	FRP	ABST (Trolox equivalent)	PMB (Trolox equivalent)	CUPRIC (Trolox equivalent)
Methanol	gum	22.3±1.30 <sup>a</sup>	11.30±0.78 <sup>a</sup>	42.32±1.80 <sup>a</sup>	5.84±0.91 <sup>d</sup>	28.004±0.76 <sup>a</sup>	6.52±0.02 <sup>a</sup>	7.55±0.21 <sup>a</sup>	4.07±0.50 <sup>a</sup>
	root	18.3±0.80 <sup>b</sup>	8.50±0.40 <sup>b</sup>	36.23±1.90 <sup>a</sup>	5.94±0.14 <sup>d</sup>	18.02±1.24 <sup>b</sup>	4.61±0.48 <sup>b</sup>	5.81±0.11 <sup>b</sup>	3.12±0.25 <sup>b</sup>
	aerial	15.4±1.70 <sup>c</sup>	5.21±0.12 <sup>c</sup>	20.21±1.24 <sup>ab</sup>	8.92±0.23 <sup>d</sup>	15.37±0.35 <sup>bc</sup>	2.76±0.35 <sup>d</sup>	2.91±0.63 <sup>c</sup>	2.76±0.11 <sup>c</sup>
water	gum	12.59±0.44 <sup>c</sup>	2.26±0.09 <sup>d</sup>	21.68±0.29 <sup>ab</sup>	5.51±0.60 <sup>b</sup>	18.60±0.154 <sup>b</sup>	3.52±0.20 <sup>c</sup>	3.86±0.23 <sup>c</sup>	2.80±0.21 <sup>c</sup>
	root	9.91±0.28 <sup>d</sup>	2.04±0.05 <sup>d</sup>	17.85±1.73 <sup>ab</sup>	7.51±0.61 <sup>c</sup>	13.36±1.42 <sup>c</sup>	2.20±0.21 <sup>e</sup>	3.00±0.52 <sup>d</sup>	2.03±0.21 <sup>d</sup>
	aerial	4.27±0.59 <sup>e</sup>	0.65±0.01 <sup>e</sup>	12.01±1.22 <sup>b</sup>	10.13±1.21 <sup>a</sup>	7.37±0.35 <sup>d</sup>	1.80±0.30 <sup>e</sup>	2.76±0.21 <sup>e</sup>	1.43±0.44 <sup>e</sup>

\* The results are the average of three repetitions±standard deviation

\* Different English lowercase letters in each column indicate a significant difference ( $P \leq 0.05$ ).

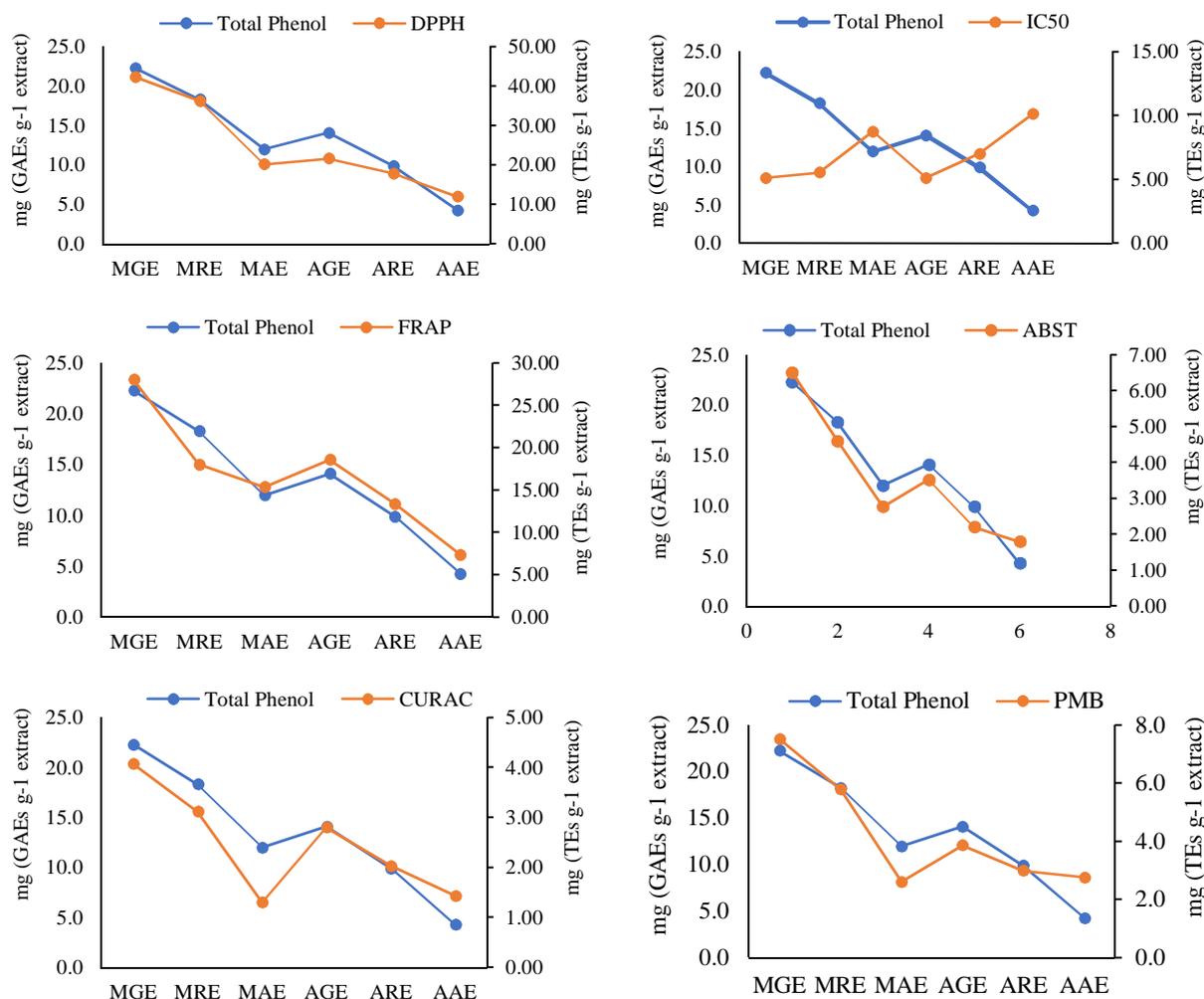
### Antioxidant activity

According to Table (1), where phenolic and flavonoid compounds of extracts are presented, it is clear that methanolic gum extract is more prosperous than other parts of both flavonoids and phenolics. Furthermore, Table (1) also showed that the most antioxidant activity had belonged to methanolic gum extract. The amount of DPPH, for example, methanolic gum extract, was 42.32±1.80 (mg TE/g extract), and this amount for methanolic extracts of roots and aerial was 36.23±1.90 and 20.21±1.24 (mg TE/g extract), respectively. The aqueous aerial parts extract had the lowest amount of DPPH activity. The sample of methanolic gum extract also had the lowest IC<sub>50</sub>, and the highest IC<sub>50</sub> was related to the aqueous extract sample of aerial parts.

The sample extracts' reducing powers were examined with CUPRAC and FRAP tests (Table 1). According to the results, it was shown that the methanolic gum extract had the highest antioxidant activity in those tests (4.07±0.50 and 28.04±0.76 mg TE/g extract) respectively. It has been detected that the reduction power of *Astragalus fasciculifolius* extracts was found to correlate with its bioactive composition. It was seen that the maximum antioxidant activity in the phosphomolybdenum test belongs to methanolic gum extract with 7.50±0.20 mg TE/g extract. The findings resulted in the Duncan test stated that the difference between the antioxidant activities (CUPRAC, FRAP, and Phosphomolybdenum) of methanolic gum extract with other samples were significantly different, and the lowest antioxidant activity in the expressed tests was related to the aqueous extract sample of plant parts of *Astragalus fasciculifolius*. The ABST finding has shown that methanolic gum extract has had the highest activity in the ABST test and that sample has had statistical differences with other samples. Moreover, methanolic root extract and water gum extract (4.60±0.40 and 43.52±0.20 mg TE/g extract). The correlations between all tests and total phenol values were examined (Table 2).

**Table 2.** Correlations among phenolic compounds and assays

	DPPH	IC <sub>50</sub>	FRPA	ABST	PMB	CUPRAC
Phenol	0.831**	-0.676**	0.856**	0.960**	0.959**	0.870**
DPPH		-0.547*	0.693**	-0.692**	0.679**	0.642**
IC <sub>50</sub>			-0.763**	-0.698**	-0.697**	-0.873**
FRP				0.918**	0.837**	0.841**
ABST					0.962**	0.899**
PMB						0.940**

\* Significant at  $P < 0.05$ .\*\* Significant at  $P < 0.01$ .**Fig. 2.** Correlation between antioxidant capacity and the total phenol of methanolic and aqueous different parts of *Astragalus fasciculifolius*. Methanolic Gum Extract (MGE), Methanolic Root Extract (MRE), Methanolic Aerial Extract (MAE), Aqueous Gum Extract (AGE), Aqueous Root Extract (ARE), Aqueous Aerial Extract (AAE).

The correlation between total phenol and DPPH activity was 0.81. For other tests, ABST was 0.960, and according to Table (2), the correlation between total phenol and IC<sub>50</sub> was reported as -0.676, which showed a reverse relation together. The correlation indexes between the extracts' total phenol compounds and the

activity in the CUPRAC and FRAP tests reported as 0.870 and 0.856, respectively (Table 2). Our findings agree with the results of Lizcano *et al.* (2010). Wang *et al.* (2010), Locatelli *et al.* (2011), Zhang *et al.* (2015), and Sarikurkcu & Zengin (2020) reported a significant correlation between total phenol compounds and

antioxidant activities. According to our searches so far, there is no literature for the composition and antioxidant activity of *Astragalus fasciculifolius*, so these findings have high value for other research.

Plant sources' phenolic composition has high antioxidant attributes cause of their ability to scavenge free radicals, and active oxygen mediates. Flavonoids, considered a secondary metabolic class of natural compounds known as vitamin P, account for 60% of total phenols in plants. Humans usually eat them and exhibit mind anti-inflammatory, anti-allergic, and anti-cancer activities (Jaradat *et al.*, 2017).

In similar research, Jaradat *et al.* (2017) reported that 4 *Astragalus* species had an antioxidant activity of aqueous, methanol, acetone, and dichloromethane extracts. Moreover, the aqueous, methanol, acetone, and DCM of *A. boeticus* had the highest antioxidant activity. Furthermore, the result showed that methanol polar protic organic was the best organic solvent as our findings. Also, Sarikurcu *et al.* (2020) examined phytochemical analysis and biological activity of *Astragalus gymnolobus*, *A. leporinus var hirsutus*, and *A. onobrychis*, they expressed that *A. gymnolobus* and *A. onobrychis* are richer than *A. leporinus var. hirsutus* in flavonoids and phenolics content. Arumugam *et al.* (2019) worked on phenolic profile, antioxidant and enzyme inhibitory potential of methanolic extract from different parts of *Astragalus ponticus* Pall showed the total antioxidant activity

was recorded in the leaf extract followed by root and stem.

#### Methanolic gum extract's antimicrobial activity

The antimicrobial effect of methanolic and aqueous extracts of different parts of *Astragalus fasciculifolius* screened on *Pseudomonas aeruginosa* and *Clostridium perfringens*. Table (3) indicated that the methanolic extracts of different parts of *Astragalus fasciculifolius* showed bioactivity inhibitory against the growth of microbial pathogens presented in this study. The methanolic gum extract exhibited higher bioactivity (MIC 64 and MBC 128 mg/mL) against *Pseudomonas aeruginosa* growth than *Clostridium perfringens* (MIC 128 and 256 mg/mL). Methanolic root and aerial extracts showed MIC and MBC of *Pseudomonas aeruginosa* 128 and 256 mg/mL, respectively. MIC and MBC were examined for aqueous gum extract 256 and 512 mg/mL against *Pseudomonas aeruginosa* and could not have bactericidal and bacteriostatic activity on *Clostridium perfringens*. All methanolic extracts of different parts of *Astragalus fasciculifolius* exhibited antimicrobial activity in an increasing order against the growth of *Pseudomonas aeruginosa* and *Clostridium perfringens*. According to bioactive compounds (total phenol and flavonoid), extracts containing high total phenol had the most antimicrobial effect, and different cell membrane structures were affected by bioactive compounds in plants (Gyawali & Ibrahim, 2014; Ramli *et al.*, 2017).

**Table 3.** Antimicrobial activity of methanolic and aqueous extracts of different parts of *Astragalus fasciculifolius*

Type of bacteria	MIC (mg/mL)			MBC (mg/mL)			
	Gum	Root	Aerial	Gum	Root	Aerial	
Methanolic	<i>Pseudomonas aeruginosa</i>	64	128	128	128	256	256
	<i>Clostridium perfringens</i>	128	256	256	256	512	512
Aqueous	<i>Pseudomonas aeruginosa</i>	256	nd	nd	512	nd	nd
	<i>Clostridium perfringens</i>	nd	nd	nd	nd	nd	nd

Some researchers have indicated that plants' phytochemicals have antimicrobial activities (Abreu *et al.*, 2012). The species of the genus *Astragalus* have been used in traditional medicine globally (Jaradat *et al.*, 2017). Around 804 species (65%) of this *Astragalus* genus has been indicted in Iran; these plants have many bioactive compounds like tannins and flavonoids responsible for antioxidant and antimicrobial activities (Nosrati *et al.*, 2019). Jaradat *et al.* (2017) reported that some aqueous extracts of *Astragalus* showed the highest antibacterial activity, while the methanolic extract showed the highest antifungal and antioxidant activities. Phytochemical analysis cleared several secondary metabolites, such as alkaloids, polyphenols, flavonoids, anthraquinones, coumarins, saponins, tannins triterpenes, and steroids. Several molecules are effective on pathogenic microorganisms (Ibrahim *et al.*, 2019). Being such metabolites in some plant extracts can present a primary explanation for their antimicrobial potential. Other studies on antimicrobial agents derived from natural plant extracts (Kang & Song, 2021; Son *et al.*, 2017; Woo *et al.*, 2020) have shown that the primary mode of action by which phenolic compounds in plant extracts exert their antimicrobial activity is to damage the cell membrane, consequently increasing membrane permeability (Gyawali & Ibrahim, 2014; Ramli *et al.*, 2017).

### Conclusions

Our research studied the total phenol, flavonoid contents, and antioxidants activities of different parts of *Astragalus fasciculifolius*. Since these species are

endemic to the southwest of Asia, the informs resulting for *Astragalus fasciculifolius* are considered to be much crucial to future research. The result showed that the extracts by methanol solvent had more phenolic compounds than water solvent. Furthermore, the gum extract had the highest phenol and flavonoid, and methanolic gum extract also had the most potent antioxidant activity among samples. According to our findings, methanolic gum extract has shown the most amounts in DPPH, ABST, CUPRAC, FRAP, and phosphomolybdenum, and the lowest IC<sub>50</sub> was related to gum methanolic extract. The correlation between phenol contains and all methods of analyzing antioxidant activity was significant. According to the current study's findings, it was concluded that the methanolic gum extract of *Astragalus fasciculifolius* could be used in the food, cosmetic, and medical industries.

### Author contributions

**Najmeh Khademi pour:** Data collection, Data analysis, Writing the draft of the manuscript, Data analysis and interpretation, Presenting the research idea and study design, Revising and editing the manuscript; **Anousheh Sharifan:** Data analysis and interpretation, Presenting the research idea and study design, Supervising the study, Approval of the final version; **Hossein Bakhoda:** Data analysis, Data analysis and interpretation, Presenting the research idea and study design.

### Conflict of interest

There is no conflict of interest based on the writers.

### References

- Abreu, A. C., McBain, A. J., & Simões, M. (2012). Plants as sources of new antimicrobials and resistance-modifying agents. *Nat Prod Rep*, 29(9), 1007-1021. <https://doi.org/10.1039/c2np20035j>
- Apak, R., Güçlü, K., Özyürek, M., Esin Karademir, S., & Erçağ, E. (2006). The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *International journal of food sciences and nutrition*, 57(5-6), 292-304. <https://doi.org/10.1080/09637480600798132>

- Arumugam, R., Kirkan, B., & Sarikurku, C. (2019). Phenolic profile, antioxidant and enzyme inhibitory potential of methanolic extracts from different parts of *Astragalus ponticus* Pall. *South African Journal of Botany*, 120, 268-273. <https://doi.org/10.1016/j.sajb.2018.07.002>
- Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, 239(1), 70-76. <https://doi.org/10.1006/abio.1996.0292>
- Gyawali, R., & Ibrahim, S. A. (2014). Natural products as antimicrobial agents. *Food control*, 46, 412-429. <https://doi.org/10.1016/j.foodcont.2014.05.047>
- Hemeg, H. A., Moussa, I. M., Ibrahim, S., Dawoud, T. M., Alhaji, J. H., Mubarak, A. S., . . . Marouf, S. A. (2020). Antimicrobial effect of different herbal plant extracts against different microbial population. *Saudi Journal of Biological Sciences*, 27(12), 3221-3227. <https://doi.org/10.1016/j.sjbs.2020.08.015>
- Huang, C., Xu, D., Xia, Q., Wang, P., Rong, C., & Su, Y. (2012). Reversal of P-glycoprotein-mediated multidrug resistance of human hepatic cancer cells by Astragaloside II. *Journal of Pharmacy and Pharmacology*, 64(12), 1741-1750. <https://doi.org/10.1111/j.2042-7158.2012.01549.x>
- Huang, X., Wang, D., Hu, Y., Lu, Y., Guo, Z., Kong, X., & Sun, J. (2008). Effect of sulfated astragalus polysaccharide on cellular infectivity of infectious bursal disease virus. *International Journal of Biological Macromolecules*, 42(2), 166-171. <https://doi.org/10.1016/j.ijbiomac.2007.10.019>
- Ibrahim, W. A., Marouf, S. A., Erfan, A. M., Nasef, S. A., & El Jakee, J. K. (2019). The occurrence of disinfectant and antibiotic-resistant genes in *Escherichia coli* isolated from chickens in Egypt. *Veterinary world*, 12(1), 141. <https://doi.org/10.14202/vetworld.2019.141-145>
- Jaganath, I. B., & Crozier, A. (2010). Dietary flavonoids and phenolic compounds. *Plant phenolics and human health: biochemistry, nutrition, and pharmacology*, 1, 1-50. <https://doi.org/10.1002/9780470531792.ch1>
- Jaradat, N., Adwan, L., K'aibni, S., Zaid, A. N., Shtaya, M. J., Shraim, N., & Assali, M. (2017). Variability of chemical compositions and antimicrobial and antioxidant activities of *Ruta chalepensis* leaf essential oils from three Palestinian regions. *BioMed research international*, 2017. <https://doi.org/10.1155/2017/2672689>
- Kamath, S. D., Arunkumar, D., Avinash, N. G., & Samshuddin, S. (2015). Determination of total phenolic content and total antioxidant activity in locally consumed food stuffs in Moodbidri, Karnataka, India. *Advances in Applied Science Research*, 6(6), 99-102.
- Kang, J.-H., & Song, K. B. (2021). Antimicrobial activity of honeybush (*Cyclopia intermedia*) ethanol extract against foodborne pathogens and its application in washing fresh-cut Swiss chard. *Food control*, 121, 107674. <https://doi.org/10.1016/j.foodcont.2020.107674>
- Leng, B., Yuan, F., Dong, X., Wang, J., & Wang, B. (2018). Distribution pattern and salt excretion rate of salt glands in two recretohalophyte species of *Limonium* (Plumbaginaceae). *South African Journal of Botany*, 115, 74-80. <https://doi.org/10.1016/j.sajb.2018.01.002>
- Li, X., Qu, L., Dong, Y., Han, L., Liu, E., Fang, S., . . . Wang, T. (2014). A review of recent research progress on the astragalus genus. *Molecules*, 19(11), 18850-18880. <https://doi.org/10.3390/molecules191118850>
- Lizcano, L. J., Bakkali, F., Ruiz-Larrea, M. B., & Ruiz-Sanz, J. I. (2010). Antioxidant activity and polyphenol content of aqueous extracts from Colombian Amazonian plants with medicinal use. *Food Chemistry*, 119(4), 1566-1570. <https://doi.org/10.1016/j.foodchem.2009.09.043>
- Locatelli, M., Epifano, F., Genovese, S., Carlucci, G., Končić, M. Z., Kosalec, I., & Kremer, D. (2011). Anthraquinone profile, antioxidant and antimicrobial properties of bark extracts of *Rhamnus catharticus* and *R. orbiculatus*. *Natural product communications*, 6(9), 1275-1280.
- Lu, J., Chen, X., Zhang, Y., Xu, J., Zhang, L., Li, Z., . . . He, X. (2013). Astragalus polysaccharide induces anti-inflammatory effects dependent on AMPK activity in palmitate-treated RAW264. 7 cells. *International Journal of Molecular Medicine*, 31(6), 1463-1470. <https://doi.org/10.3892/ijmm.2013.1335>
- Nguyen Viet, D., Le Ba, V., Nguyen Duy, T., Pham Thi, V. A., Tran Thi, H., Le Canh, V. C., . . . Tuan Anh, H. L. (2021). Bioactive compounds from the aerial parts of *Hypericum sampsonii*. *Natural Product Research*, 35(4), 646-648. <https://doi.org/10.1080/14786419.2019.1586690>
- Nosrati, F., Fakheri, B., Solouki, M., Mahdi Nezhad, N., & Valizadeh, M. (2019). Analysis of some phytochemical characteristics of *Astragalus fasciculifolius* Boiss. in natural habitats of South Sistan and Baluchistan Province, Iran. *Iranian Journal of Medicinal and Aromatic Plants Research*, 35(1), 68-79. <https://doi.org/10.22092/IJMAPR.2019.121991.2327> (in Persian)

- Pistelli, L., Giachi, I., Lepori, E., & Bertoli, A. (2003). Further saponins and flavonoids from *Astragalus verrucosus* Moris. *Pharmaceutical biology*, 41(8), 568-572. <https://doi.org/10.1080/13880200390501370>
- Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*, 269(2), 337-341. <https://doi.org/10.1006/abio.1999.4019>
- Ramli, S., Radu, S., Shaari, K., & Rukayadi, Y. (2017). Antibacterial activity of ethanolic extract of *Syzygium polyanthum* L.(Salam) leaves against foodborne pathogens and application as food sanitizer. *BioMed research international*, 2017. <https://doi.org/10.1155/2017/9024246>
- Robbins, R. J. (2003). Phenolic acids in foods: an overview of analytical methodology. *Journal of agricultural and food chemistry*, 51(10), 2866-2887. <https://doi.org/10.1021/jf026182t>
- Sarikurkcü, C., Sahinler, S. S., & Tepe, B. (2020). *Astragalus gymmolobus*, *A. leporinus* var. *hirsutus*, and *A. onobrychis*: phytochemical analysis and biological activity. *Industrial crops and products*, 150, 112366. <https://doi.org/10.1016/j.indcrop.2020.112366>
- Sarikurkcü, C., & Zengin, G. (2020). Polyphenol profile and biological activity comparisons of different parts of *Astragalus macrocephalus* subsp. *finitimus* from Turkey. *Biology*, 9(8), 231. <https://doi.org/10.3390/biology9080231>
- Shahid, M., & Rao, N. (2015). New records for the two Fabaceae species from the United Arab Emirates. *J. New Biological Reports*, 4(3), 207-210.
- Shahrani, M., Asgharzadeh, N., Toriki, A., Asgharian, S., & Lorigooini, Z. (2021). *Astragalus fasciculifolius* manna; antinociceptive, anti-inflammatory and antioxidant properties in mice. *Immunopathologia Persa*, 7(1), e02. <https://doi.org/10.34172/ipp.2021.02>
- Singh, R., Sharma, S., & Sharma, V. (2015). Comparative and quantitative analysis of antioxidant and scavenging potential of *Indigofera tinctoria* Linn. extracts. *Journal of integrative medicine*, 13(4), 269-278. [https://doi.org/10.1016/S2095-4964\(15\)60183-2](https://doi.org/10.1016/S2095-4964(15)60183-2)
- Son, H.-J., Kang, J.-H., & Song, K. B. (2017). Antimicrobial activity of safflower seed meal extract and its application as an antimicrobial agent for the inactivation of *Listeria monocytogenes* inoculated on fresh lettuce. *LWT-Food Science and Technology*, 85, 52-57. <https://doi.org/10.1016/j.lwt.2017.06.063>
- Teng, Z., & Shen, Y. (2015). Research progress of genetic engineering on medicinal plants. *Zhongguo Zhong yao za zhi= Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica*, 40(4), 594-601.
- Vasfilova, E., & Vorob'eva, T. y. (2020). Little-known medicinal plants with a widespectrum of pharmacological action under the conditions of introduction in the Middle Urals. *BIO Web of Conferences*,
- Wang, J., Zhang, Q., Zhang, Z., Song, H., & Li, P. (2010). Potential antioxidant and anticoagulant capacity of low molecular weight fucoidan fractions extracted from *Laminaria japonica*. *International Journal of Biological Macromolecules*, 46(1), 6-12. <https://doi.org/10.1016/j.ijbiomac.2009.10.015>
- WHO. (2019). *WHO global report on traditional and complementary medicine 2019*. World Health Organization.
- Woo, H. J., Kang, J. H., Lee, C. H., & Song, K. B. (2020). Application of *Cudrania tricuspidata* leaf extract as a washing agent to inactivate *Listeria monocytogenes* on fresh-cut romaine lettuce and kale. *International Journal of Food Science & Technology*, 55(1), 276-282. <https://doi.org/10.1111/ijfs.14305>
- Zengin, G., Uysal, S., Ceylan, R., & Aktumsek, A. (2015). Phenolic constituent, antioxidative and tyrosinase inhibitory activity of *Ornithogalum narbonense* L. from Turkey: A phytochemical study. *Industrial crops and products*, 70, 1-6. <https://doi.org/10.1016/j.indcrop.2015.03.012>
- Zhang, H., Shao, Y., Bao, J., & Beta, T. (2015). Phenolic compounds and antioxidant properties of breeding lines between the white and black rice. *Food Chemistry*, 172, 630-639. <https://doi.org/10.1016/j.foodchem.2014.09.118>
- Zhang, S., Hou, J., Yuan, Q., Xin, P., Cheng, H., Gu, Z., & Wu, J. (2020). Arginine derivatives assist dopamine-hyaluronic acid hybrid hydrogels to have enhanced antioxidant activity for wound healing. *Chemical Engineering Journal*, 392, 123775. <https://doi.org/10.1016/j.cej.2019.123775>
- Zhong, Y., & Shahidi, F. (2015). 12 - Methods for the assessment of antioxidant activity in foods | This chapter is reproduced to a large extent from an article in press by the authors in the *Journal of Functional Foods*. In F. Shahidi (Ed.), *Handbook of Antioxidants for Food Preservation* (pp. 287-333). Woodhead Publishing. <https://doi.org/10.1016/B978-1-78242-089-7.00012-9>

## محتوای فنولی، فلاونوئیدی و فعالیت آنتی‌اکسیدانی عصاره‌های متانولی و آبی بخش‌های مختلف گیاه انزروت و ارزیابی فعالیت ضدباکتریایی عصاره صمغ متانولی

نجمه خادمی پور<sup>1</sup>، انوشه شریفان<sup>1\*</sup>، حسین باخدا<sup>2</sup>

1- گروه علوم و صنایع غذایی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران  
\* نویسنده مسئول (a\_sharifan@srbiau.ac.ir)

2- گروه مکانیزاسیون کشاورزی، واحد علوم و تحقیقات، دانشگاه آزاد اسلامی، تهران، ایران

### چکیده

گیاه انزروت از تیره *آستراگالوس* و خانواده حبوبات است. پراکندگی این گیاه در جنوب غربی آسیا می‌باشد و تاکنون مطالعه‌های منسجمی روی خواص آنتی‌اکسیدانی و ضد میکروبی این گیاه انجام نشده است. در این تحقیق سعی شده است که عصاره قسمت‌های مختلف گیاه انزروت (صمغ، اندام هوایی و ریشه) با استفاده از دو حلال آب و متانول استخراج شود و محتوای ترکیبات فنولی و فلاونوئیدی کل و فعالیت آنتی‌اکسیدانی (1 و 1-دی فنیل-2-پیکریل هیدرازیل (DPPH)، 2 و 2-آزینو-بیس (3-اتیل بنزوتیازولین-6-سولفونیک اسید) (ABST)، فعالیت کاهنده یون مس (CUPRAC)، فسفومولیدنم (PMB) و ظرفیت کاهش یون آهن (FRAP)) عصاره‌ها مورد بررسی قرار گیرد، ارتباط بین ترکیبات فنولی کل و فعالیت آنتی‌اکسیدانی نیز با آزمون پیرسون بررسی شد. نتایج نشان داد که عصاره‌های استخراج شده با حلال متانول دارای ترکیبات زیست‌فعال بالاتر و فعالیت آنتی‌اکسیدانی قوی‌تری هستند. عصاره صمغ استخراج شده با حلال متانول، بیشترین فعالیت آنتی‌اکسیدانی و نیز بیشترین محتوای فنول کل ( $22/30 \pm 1/30$  میلی‌گرم گالیک اسید بر گرم عصاره) و فلاونوئید کل ( $11/30 \pm 0/87$  میلی‌گرم رول بر گرم عصاره) داشت که در مقایسه با عصاره سایر قسمت‌ها تفاوت معنی‌داری داشت ( $P \leq 0/05$ )، نتایج نشان داد که همبستگی بین ترکیبات فنولی کل و فعالیت آنتی‌اکسیدانی نیز معنی‌دار بود ( $P \leq 0/05$ ). با توجه به نتایج، مشخص شد که عصاره صمغ انزروت دارای فعالیت ضد میکروبی می‌باشد و میزان غلظت بازدارندگی و حداقل غلظت کشندگی باکتری کستریدیوم پرفرنژنس نسبت به باکتری *سودوموناس اثرورژینوزا* کمتر بود. براساس یافته‌های این مطالعه، مشخص شد که صمغ انزروت قابلیت استفاده در صنایع غذایی، دارویی و بهداشتی را دارد.

**واژه‌های کلیدی:** انزروت، فعالیت آنتی‌اکسیدانی، فعالیت ضد میکروبی