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VARIATION ON BIOLOGICAL ACTIVITY AND PHYTOCHEMICAL CHARACTERISTICS OF GUM TRAGACANTH EXUDATE FROM *Astragalus gossypinus* AND *A. parrowianus*

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Abstract. Antibacterial and antioxidant activities, total phenolic content, and protein content of gum tragacanth harvested from different populations of *Astragalus gossypinus* and *Astaragalus parrowianus* (Fabaceae) from three provinces in central to southwestern Iran were investigated. Total phenolic amount of aqueous solution of gum tragacanth was determined using Folin-Ciocalteu method. Antioxidant activity of the aqueous solution of gum tragacanth was evaluated by measuring DPPH. The antibacterial activity of the aqueous solution of gum tragacanth against four bacteria was determined by serial dilution assay. Results indicated that there were significant differences in amount of total phenol, antibacterial and antioxidant activities among different populations of two species. The highest amounts of total phenol of gum tragacanth were obtained from the Shahrekord and the Khomeyn populations for *A. parrowianus* (237 and 235 mg GAE·g^{-1} gum tragacanth, respectively). The highest protein content was obtained from the populations of *A. parrowianus* and the lowest amount of protein content was obtained from the Shahrekord population of *A. gossypinus*. The highest antioxidant activity was obtained the Shahrekord population for both species (IC_{50} = 0.345, and 0.419 mg·ml^{-1}), and the Khomeyn population for *A. gossypinus* (IC_{50} = 0.511 mg·ml^{-1}). The aqueous solutions of the gum tragacanth studied indicated moderate-to-good inhibitory activities (MICs = 0.125 to 0.250 mg·ml^{-1}) against four bacteria, especially against *Listeria monocytogenes*. In conclusion, gum tragacanth from some populations of *A. gossypinus* and *A. parrowianus* could be an important dietary source of protein and total phenolic compound with antioxidant capacity and antibacterial activity.

Key words*:* Iranian gum tragacanth, antioxidant activity, antibacterial activity, total phenolic, protein

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INTRODUCTION

Astragalus L., as the largest genus in Angiosperms with about 2500 to 3000 species in the world, belongs to the tribe Astragaleae of Papilionoideae in the family Fabaceae [Ranjbar and Karamian 2002a, Ranjbar and Karamian 2003a]. This genus occurs primarily in cold to warm arid and semiarid mountainous regions of the Northern Hemisphere and South America [Chaudhary et al. 2008]. The *Astragalus* genus is the most species-rich and diverse genus in Central Asia and Southwestern Asia [Ranjbar and Karamian 2002b, Ranjbar and Karamian 2003b]. The genus is most diverse in the Irano-Turkish region of Southwestern Asia (1000–1500 spp.), the Sino-Himalayan Plateau of south central Asia (ca. 550 spp.) and the Great Basin and Colorado Plateau of Western North America (ca. 450 spp.). In addition, the center of origin and diversity of *Astragalus* is Eurasia, specially the drier mountainous parts of Southwestern and South-Central Asia and the Himalaya [Maassoumi 1998, Lock and Schrire 2005, Wojciechowski 2005]. The *Astragalus* genus, with the common Persian name of 'Gavan', consists of about 800 species of herbaceous annual and perennials, and shrubs in Iran [Mozaffarian 2008]. This genus consists of eight subgenera and about 85 sections which grow wild in many regions of Iran. Some species of *Astragalus* have botanical, industrial, food, and pharmaceutical interest due to its characteristic scent [Ghasemi Pirbalouti 2009]. In addition, some species of the *Astragalus* genus have a major role on soil conservation and provide forage for livestock grazing in summer during dry season in semiarid lands in Iran [Ghasemi Pirbalouti 2010].

Gum tragacanth, as an important source in food, pharmaceutical, and chemical industries, obtains from the stems and branches of Asiatic species of *Astragalus* [Weiping 2000, Azarikia and Abbasi 2010] such as *Astragalus gossypinus* and *Astaragalus parrowianus* that both species wild grow in the alpine of Southwestern Iran. Gum tragacanth has been known and used for thousands of years. Gum tragacanth is a branched, heterogeneous, and anionic carbohydrate with high molecular weight [Weiping 2000] that consists of two major fractions: water-insoluble component (bassorin) and water-soluble component (tragacanthin) [Mohammadifar et al. 2006, Balaghi et al. 2011]. Different species of *Astragalus* have various ratios of the two fractions, different chemical compositions and also varying physicochemical properties [Balaghi et al. 2010, Balaghi et al. 2011]. Gum tragacanth is widely used in the food industry as a stabilizer, emulsifier and thickener in food industry, pharmaceutics and cosmetics [Weiping 2000, Abbasi and Rahimi 2006, Weiping and Branwell 2000]. It has been reported that it inhibits the growth of cancer cells and it could regulate blood sugar in diabetic patients.

The characteristics of medicinal plants are known to be affected by genetic, environmental factors, and their interaction effects [Ghasemi Pirbalouti et al. 2013a, Ghasemi Pirbalouti et al. 2014]. Bioclimatic preferences along with geographic distances play a major role in ecotype differentiation [Ghasemi Pirbalouti et al. 2013b] that affect plant constituency. In addition, identification of variation in phytochemical properties and biological activity in different ecotypes and the effect of environmental factors on quality and quantity of gum tragacanth are important. Yet, to our knowledge, no documents are available dealing with the variation of antibacterial and antioxidant activities, total phenolic content and protein content of gum tragacanth harvested from wild populations of *Astragalus gossypinus* and *Astaragalus parrowianus* due to the growth environment. The main objective of this study was to evaluate content of phenolic com-

pounds, antioxidants and antibacterial activities of the gum tragacanth harvested from *A. gossypinus* and *A. parrowianus* and to evaluate them as potential sources of natural antioxidants and antimicrobial.

MATERIALS AND METHODS

Chemicals and reagents. Gallic acid, nutrient broth, saturated Na_2CO_3, and ethanol used in this study were purchased from Merck Co. (Darmstadt, Germany). The Folin-Ciocalteu reagent, the 1,1-diphenyl-2-picryl-hydrazil (DPPH), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Co. (Steineheim, Germany).

Plants material and origin. Samples of two species of Iranian gum tragacanth exuded by *Astragalus parrowianus*, and *A. gossypinus*, collected from wild populations of the plants (at least 7 years old) growing in alpine regions of southwestern Iran were used in this study. The plants were tapped with a knife by making careful oblique incision in the taproot and the bark of the branches (fig. 1). After two days, the gum readily exuded from these cuts in the form of 'ribbons' that became brittle on drying [Balaghi et al. 2011]. In total, three replicate samples of 20 plants were gathered from three natural habitats in three provinces between June and July 2012 (tab. 1). Plant identities were confirmed by Dr. H. Shirmardi (Research Center for Agricultural & Natural Resources, Shahrekord, Iran). The raw gum was grounded using a Moulinex food processor (Moulinex International, Spain) and sieved. Powdered gum with mesh size between 300 and 500 μm was used in this study. The crude gum tragacanth powder (1 g) was dissolved in 1 L deionized water on a magnetic stirrer with hot plate at 50°C during 30 min.

Table 1. Geographical and climate of natural habitats of *Astragalus gossypinus* and *A. parrowianus*

Regions	Province	Altitude (m)	Latitude	Longitude	P* (mm)	T (°C)	pH	E.C. ($dS \cdot m^{-1}$)	O.C (%)	Sand (%)	Silt (%)	Clay (%)
Khomeyn	Markazi	1810	33–35°	50–51°	240	16	7.64	0.18	0.49	28	29	43
Khonsar	Isfahan	2300	33–34°	50–51°	303	14	7.60	0.54	0.42	30	22	48
Shahre-kord	Chaharmahal va Bakhtiari	2070	31–32°	49–51°	318	12	7.73	0.49	0.45	32	44	24

* – P – annual precipitation (mm), T – average temperature (°C), E.C. – electrical conductivity ($dS \cdot m^{-1}$), O.C. – organic carbon (%). Meteorological information was obtained from weather stations located within the study area and the surrounding zone; each value in the mean of 10 to 15 year data. Soil characteristics are based on average of samples taken from three farms in each region

The chosen collection regions were in different geographic areas and included areas in which differences in physical characteristics of the plant accessions were observed [Mozaffarian 2013]. Each sample was labeled and the location was recorded using a global positioning system (GPS, Vista Garmin) receiver. The physical and chemical characteristics of the soil, including pH, electrical conductivity (EC), organic carbon (%OC), and texture, at the sample collection sites were determined (tab. 1) along with climatic conditions as recorded by the nearest meteorology station.

Fig. 1. The plants tapped with a knife by making careful oblique incision

Determination of total phenolic compounds. The total amount of phenolic compounds in each aqueous solution of gum tragacanth was determined using the Folin–Ciocalteu method following procedure of Singleton and Rossi [1965] with some modifications. Briefly, A 0.5 ml of the sample was mixed with 2.5 ml of Folin- Ciocalteu's phenol reagent for 5 min at 37°C, 2 ml of saturated Na_2CO_3 (7.5%) was added, and the mixture was brought to 10 ml with the addition of deionized, distilled water. The mixture was maintained at room temperature in the dark for 120 min and then the absorbance was measured at 765 nm against a reagent blank using a Perkin-Elmer Lambda UV/Vis spectrophotometer. Gallic acid was used as the reference standard and the total phenolic content was expressed as mg of gallic acid equivalents per gram of gum tragacanth on dry basis (mg GAE·g^{-1} gum).

Protein content. Nitrogen content of the gums was determined using the standard methods of AOAC [2006]. The protein content was calculated from percentage nitrogen by means of the factor (N × 6.25) established recently [Debon and Tester 2001].

Antibacterial test. Antibacterial activity of the aqueous solution of gum tragacanth was tested using clinical isolates of four bacterial strains, the Gram-positive bacteria (*Bacillus cereus* and *Listeria monocytogenes*) and the Gram-negative bacteria (*Pseudomonas aeruginosa* and *Salmonella typhimurium*). The bacteria, originally obtained from chicken meat samples, were provided by the Food Microbiology Laboratory, Veterinary Medicine Faculty, (I.A.U.) Iran and had been positively identified using PCR-RFLP along with conventional morphological and biochemical tests. The population of each bacterial strain was increased by culturing in an overnight Nutrient broth (NB) at 37°C. To quantify the antibacterial activity of the aqueous solution of gum tragacanth, bacteria populations were prepared for testing by adjusting each population to 1.0 McFarland standards (1.0×10^7 CFU·mL^{-1}), using a spectrophotometer (Eppendorf, AG, Germany). Minimum inhibitory concentrations (MIC) were determined using the broth-serial dilution method following standardized methods [CLSI 2012]. Subsequent test concentrations were made in a series of two-fold dilutions to develop concentration levels of 16 to 500 µg·mL^{-1} in sterile, 10 mL test tubes containing NB. The minimum bactericidal concentration (MBC) of each sample was determined according to the MIC values by transferring 5 µL from MIC tubes to agar plates and incubating at 37°C for 24 h. All experimental tests were replicated three different times.

Antioxidant test. The DPPH radical scavenging activity of the aqueous solution of gum tragacanth was determined using the method proposed by Hung et al. [2005]. The aqueous solution of gum tragacanth (100 µL) at concentrations of 8, 16, 32, 62.5, 125, 250, and 500 µg·mL^{-1} were mixed with 3.9 mL an equal volume of 0.2 mM ethanol solution of DPPH. The disappearance of DPPH was followed spectrophotometrically at 515 nm beginning immediately after mixing and incubation for 30 min at room temperature. The absorbance of the DPPH radical without antioxidant against a control measured daily. Control contained methanol instead of the antioxidant solution while blanks contained methanol instead of DPPH solution. The amount of the sample necessary to decrease the absorbance of DPPH by 50% (IC_{50}) was calculated graphically. The percentage inhibition was calculated according to equation 1:

$$\% \text{ inhibition} = \frac{AC_0 - AA_t}{AC_0} \times 100,$$

where AC_0 is the absorbance of the control at t = 0 min and AA_t is the absorbance of the antioxidant at t = 30 min. All measurements were replicated three times.

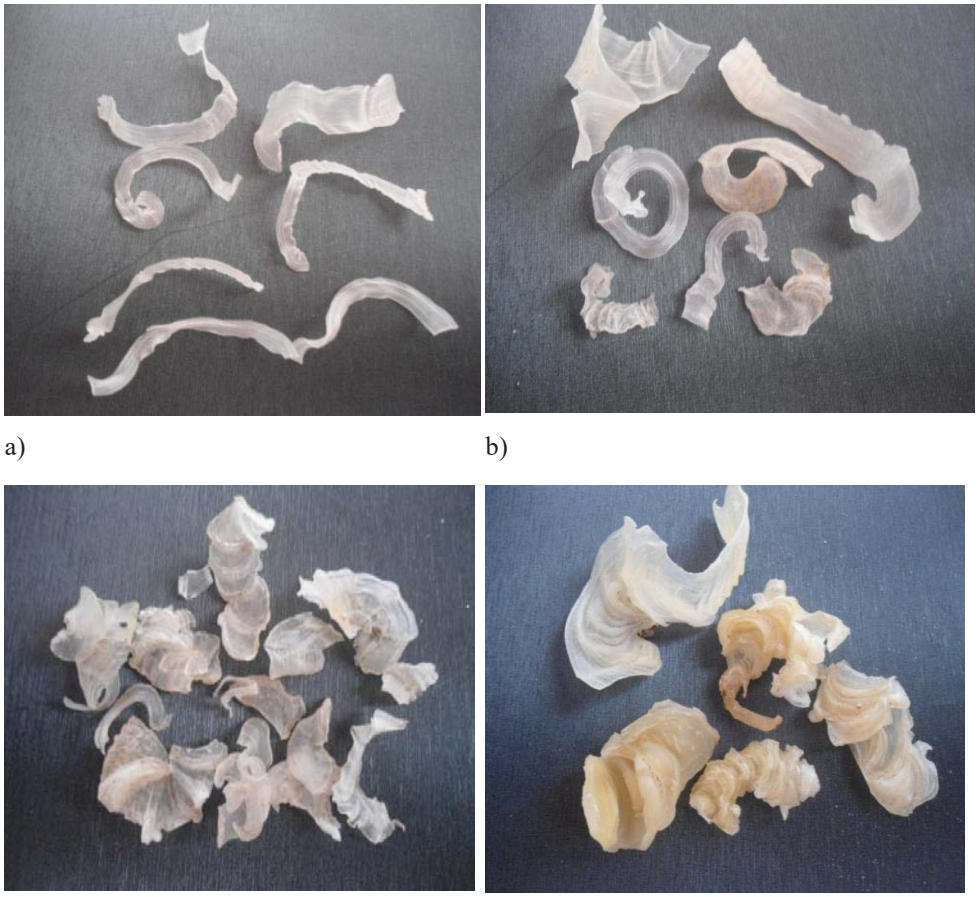
Statistical analysis. Data were analyzed as a one-way analysis of variance with three replications using the SPSS 19.0 (SPSS Inc., Chicago) statistical software. Means of total phenolic, and protein contents, the antioxidant activity (IC_{50}) were compared using Duncan's multiple range test at $p \leq 0.05$ level.

RESULTS AND DISCUSSION

Physical properties. According to a method [Asadian and Barati 2006], the samples of gum tragacanth were ranked into four types:

1. The samples of straight and slender (ribbon) gum tragacanth (> 3 cm length) harvested from the Khonsar population of *A. gossypinus* (fig. 2a);

2. The samples of twisted gum tragacanth (1–3 cm length) harvested from the Khomeyn population of *A. gossypinus* (fig. 2b);



a) b)

c) d)

Fig. 2. Ranking of appearance properties for Iranian gum tragacanth exuded by *Astragalus parrowianus*, and A. *gossypinus*; a) straight and slender (ribbon) gum tragacanth (> 3 cm length) from the Khonsar population of *A. gossypinus*; b) twisted gum tragacanth (1–3 cm length) from the Khomeyn population of *A. gossypinus*; c) flake gum tragacanth (1 < cm length) from the Shahrekord population of *A. gossypinus*, d) yellow and round gum tragacanth from different populations of *A. parrowianus*

3. The samples of flake gum tragacanth (1 < cm length) harvested from the Shahrekord population of *A. gossypinus* (fig. 2c);

4. The samples of yellow and round gum tragacanth harvested from different populations of *A. parrowianus* (fig. 2d).

Chemical properties. Total phenolic content. Phenolic compounds are an integral part of the human diet and could be helpful against cancers, arteriosclerosis, ischemia, and inflammatory disease, which are caused by exposure to oxidative stress [Caillet et al. 2006]. A significant difference ($p \leq 0.01$) for total phenolic content was measured among the aqueous solutions of gum tragacanth (tab. 2). The maximum total phenolic content was obtained from the Shahrekord and the Khomeyn populations for *A. parrowianus* (237 and 235 mg GAE·g^{-1} gum tragacanth, respectively), and the lowest amount of total phenolic was achieved from the Khonsar population of *A. gossypinus* with 102 mg GAE·g^{-1} gum tragacanth (tab. 2). In total, the total phenolic content in the aqueous solution of gum tragacanth harvested from *A. parrowianus* (mean = 219.3 mg GAE·g^{-1} gum tragacanth) was higher than the aqueous solution of gum tragacanth harvested from *A. gossypinus* (mean = 136.8 mg GAE·g^{-1} gum tragacanth). The differences in the total phenolic content among the samples could be attributed to the geographic origin of the plant and genetic diversity in two species.

Table 2. Antioxidant activity, protein and total phenolic contents of the aqueous solutions of gum tragacanth from the various populations of *A. gossypinus* and *A. parrowianus*

Species	Population	Protein content (%)	Total phenolic (mg GAE·g^{-1} extract)	IC_{50} (mg·g^{-1})
A. gossypinus	Khonsar	0.066 b	101.78 ±11.41 c	0.75 ±0.13 ab
A. parrowianus		2.76 a	185.37 ±10.77 ab	1.03 ±0.31 b
A. gossypinus	Khomeyn	0.067 b	235.15 ±14..29 a	0.69 ±0.17 ab
A. parrowianus		2.91 a	159.89 ±12.32 abc	0.51 ±0.09 a
A. gossypinus	Shahrekord	0.063 b	148.83 ±9.29 bc	0.41 ±0.06 a
A. parrowianus		2.84 a	237.36 ±20.11 a	0.34 ±0.07 a
BHT †	–	–	–	0.29 ±0.05 a
ANOVA	–	$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.05$

† – butylated hydroxytoluene as a chemical antioxidant (positive control). Values in column having similar letter are not statistically different at $p \leq 0.05$

Protein content. A significant difference ($p \leq 0.01$) for protein content was measured among the aqueous solutions of gum tragacanth (tab. 2). The highest protein content was obtained from the populations for *A. parrowianus* (2.76–2.91%), and the lowest amount of protein content was obtained from the Shahrekord population of *A. gossypinus* with 0.063% (tab. 2). Similarly, Balaghi et al. [2010] reported that gum tragacanth from *A. parrowianus* (3.05 g·100 g^{-1}) had the highest level of protein and while the protein content in gum tragacanth from *A. gossypinus* was lower than other *Astragalus* species. The protein content of *A. gossypinus* was the lowest content that is similar to previously reported results for protein content of some other hydrocolloids like agar, carrageenan, and gum Karaya [Mohammadifar et al. 2006, Balaghi et al. 2010].

Biological activity. DPPH radical scavenging activity. The potential antioxidant activity of the aqueous solutions of gum tragacanth was determined by the scavenging activity of the stable free radical DPPH. This is a quick, reliable and reproducible method to assess the *in vitro* antioxidant activity of pure compounds as well as plant extracts [Mosquera et al. 2007]. The effect of antioxidants on DPPH is based on their ability to donate a hydrogen atom to DPPH, thus converting the radical into a stable molecule [Diouf et al. 2009]. The lower IC_{50} value indicates a stronger ability of the extract to act as a DPPH scavenger while the higher IC_{50} value indicates a lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction. In our study, the antioxidant activity of the aqueous solutions of gum tragacanth from the various populations of *A. gossypinus* and *A. parrowianus* was expressed as IC_{50} with values from 0.34 to 1.02 mg·ml^{-1} that indicating the aqueous solutions of gum tragacanth act as moderate to good DPPH scavenger (tab. 2). Significant differences ($p < 0.05$) in IC_{50} values were found for the populations of both species. A comparison of all the aqueous solutions in the DPPH assay indicated that the aqueous solution of gum tragacanth from the Shahrekord population for *A. parrowianus* with the highest total phenolic content was the most effective free radical scavenging agents (tab. 2). The total phenolic in this gum tragacanth provided substantial antioxidant activity.

Table 3. Antibacterial activity (MICs and MBCs) of the aqueous solutions of gum tragacanth from the various populations of *A. gossypinus* and *A. parrowianus* and two chemical antibiotics against four bacteria

Pathogens	*A. gossypinus*		*A. parrowianus*		*A. gossypinus*		*A. parrowianus*		*A. gossypinus*		*A. parrowianus*		†Fl	Am
	population													
	Khonsar						Khomeyn				Shahrekord			
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MIC
B. cereus	500	>500	500	>500	500	>500	500	>500	500	>500	500	>500	62	32
L. monocytogenes	250	500	125	250	125	250	125	250	500	>500	500	>500	62	62
P. aeruginosa	500	>500	500	>500	500	>500	500	>500	500	>500	500	>500	125	125
S. typhimurium	500	>500	500	>500	250	500	250	500	500	>500	500	>500	125	125

†Fl – flumequine, Am – ampicillin; µg·ml^{-1}

Antibacterial activity. The aqueous solutions of gum tragacanth from the various populations of *A. gossypinus* and *A. parrowianus* demonstrated relatively inhibitory activities against the pathogenic bacteria tested, the MICs and MBCs of the tested samples are presented in Table 3. Results indicated that the different bacteria species demonstrated different levels of sensitivity to the aqueous solutions of gum tragacanth. The MICs of the aqueous solutions of gum tragacanth were within concentration ranges from 0.125 to 0.500 mg·ml^{-1}, and the respective MBCs were from 0.25 to > 0.50 mg·ml^{-1}. Generally, the aqueous solutions of gum tragacanth indicated moderate to good inhibitory activities against four bacteria. The highest antibacterial activity was obtained from the aqueous solution of gum tragacanth from the Khomeyn

populations for *A. parrowianus* against four bacteria, especially *L. monocytogenes*. Probably, in the present study the phenolic compounds are responsible of the antibacterial activity of the aqueous solution of gum tragacanth. The aqueous solution of gum tragacanth from the Khomeyn population for *A. parrowianus* with the highest total phenolic content (235 mg GAE·g^{-1} gum tragacanth) had the highest antibacterial activity. The mechanisms by which plant extracts and secondary metabolites can inhibit microorganisms vary. Phenolic compounds can act at two different levels: the cell membrane and cell wall of the microorganisms [Taguri et al. 2006]. They can interact with the membrane proteins of bacteria by means of hydrogen bonding through their hydroxyl groups which can result in changes in membrane permeability and cause cell destruction. Phenolic compounds can also penetrate into bacterial cells and coagulate cell content [Tian et al. 2009].

CONCLUSIONS

The present study is apparently the first report of quantitative total phenol profile, antioxidant and antibacterial activities of the aqueous solution of gum tragacanth from various populations of *A. gossypinus* and *A. parrowianus*. The gum tragacanth exuded from both species is ordinarily used for food industry, pharmaceutics, and cosmetics purposes and also as health foods. The results of the current study demonstrated that the aqueous solution of gum tragacanth harvested from *A. parrowianus*, with the maximum total phenolic content had the highest antioxidant and antibacterial activities. Phenolic compounds present in the gum tragacanth exuded from the plant are responsible for its effective free radical scavenging, antioxidant and antibacterial activities. The gum tragacanth of *Astragalus*, especially harvested from *A. parrowianus*, is effective for inhibition of microbial pathogens and so could be used as a natural antibacterial agent. In final, the use of gum tragacanth of *A. gossypinus* and *A. parrowianus* in foods (especially, dairy foods), cosmetics and drugs, requires the identification of the bioactive compounds to perform further studies on their mechanism of action.

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ZRÓŻNICOWANIE AKTYWNOŚCI BIOLOGICZNEJ I CECH FITOCHEMICZNYCH WYDZIELINY GUMY TRAGAKANTOWEJ *Z Astragalus gossypinus* I *A. parrowianus*

Streszczenie. Badano działanie antybakteryjne i antyoksydacyjne, całkowitą zawartość fenoli i zawartość gumy trragakantowej w różnych populacjach *Astragalus gossypinus* i *Astaragalus parrowianus* (Fabaceae) z trzech prowincji w środkowym i południowo-zachodnim Iranie. Określono całkowitą zawartość fenoli wodnego roztworu gumy tragakantowej przy użyciu metody Folin-Ciocalteu. Antyoksydacyjne działanie wodnego roztworu gumy tragakantowej oceniono, mierząc DPPH. Antybakteryjne działanie wodnego roztworu gumy tragakantowej w odniesieniu do czterech bakterii ustalono za pomocą testu seryjnych rozcieńczeń. Na podstawie wyników badań stwierdzono, że istnieją istotne różnice w całkowitej ilości związków fenolowych oraz działaniu antybakteryjnym i antyoksydacyjnym w różnych populacjach obydwu gatunków. Największą całkowitą zawartość związków fenolowych gumy tragakantowej otrzymano z populacji Shahrekord i Khomeyn dla *A. parrowianus* (odpowiednio, 237 i 235 mg GAE·g^{-1} gumy tragakantowej). Największą zawartość białka otrzymano z populacji Shahrekord dla *A. gossypinus*. Największą aktywność antyoksydacyjną uzyskano dla populacji Shahrekord dla obydwu gatunków (IC_{50} = 0,345 i 0,419 mg·ml^{-1}) oraz Khomeyn dla *A. gossypinus* (IC_{50} = 0,511 mg·ml^{-1}). Wodny roztwór gumy tragakantowej wskazywał umiarkowane do dobre-

go działanie inhibicyjne (MICs = 0,125 do 0,250 mg·ml^{-1}) względem czterech bakterii, zwłaszcza *Listeria monocytogenes*. Podsumowując, guma tragakantowa z niektórych populacji *A. gossypinus* i *A. parrowianus* może być ważnym żywieniowym źródłem białka i związków fenolowych o zdolnościach antyoksydacyjnych i antybakteryjnych.

Słowa kluczowe: irańska guma tragakantowa, działanie antyoksydacyjne, działanie antybakteryjne, całkowita zawartość fenoli, białko

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