Bulgarian Chemical Communications, Volume 53, Issue 3 (pp. 307 - 312) 2021 *DOI: 10.34049/bcc.53.3.5351*

Phytochemical composition and antioxidant activity of Algerian *Astragalus gombo* stems

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Received: November 19, 2020, Revised: February 18, 2021

Astragalus gombo obtained from Ouargla, Algeria, is first studied in this research for antioxidant activity. Our aim was to evaluate total polyphenol, flavonoid, tannin contents and antioxidant capacity of *Astragalus gombo* stems. Total polyphenol, flavonoid and tannin contents of *Astragalus gombo* stems varied from 4.174 ± 0.034 mg GAE/g DW to 0.213 ± 0.001 mg GAE/g DW, 0.706 ± 0.002 and 0.011 ± 0.000 mg QE/g DW and 0.098 ± 0.005 and 0.0084 ± 0.0001 mg CE/g DW, respectively. The antioxidant activity was measured by two methods: inhibition of DPPH$^{\bullet}$ and total antioxidant capacity by phosphomolybdate assay (PM). The results showed that the extracts display an excellent antioxidant capacity. An excellent relationship between antioxidant activity and total polyphenol content manifested that the phenolic compounds were the most important antioxidant components in the extracts.

Keywords: *Astragalus gombo*; Antioxidant; DPPH$^{\bullet}$; Total polyphenol content

INTRODUCTION

The medicinal use of plants is very old, the researchers demonstrate that remedial use of medicinal plants is known since 4000 - 5000 B.C. and Chinese first employed the natural herbal composition as treatment. In India, sources of use of plants as medicine can be found in Rig-Veda, which is said to be written between 1600-3500 B.C. [1]. The expression of medicinal plants comprises a different kind of plants and several of these plants possess medicinal activities [2]. These medicinal plants are rich of ingredients, which can help in developing drugs.

The genus *Astragalus* is the biggest in the Fabaceae family, with more than 2500-3000 species [3]. The species are spread in Central Asia, South and North America and North and South Africa. One of these species, *Astragalus gombo* bunge, is present in Septentrional Algerian Sahara. It is a perennial plant, common and endemic, frequently used as animal fodder [4]. Astragalus roots are used in traditional medicine as an anti-tonic. It has also been used to treat diabetes, leukemia and treating female irregular menstruation and amenorrhea [5]. Some astragalus species like *Astragalus mongholicus* and *A. membranaceus* are used in traditional Chinese medicine due to their anti-cancer properties [5, 6].

Free radicals are atoms or molecules with an unpaired electron and are important intermediates in natural processes, involving regulating biological functions such as vasodilation, and neurotransmission. However, an excessive amount causes many diseases like cancer [7].

Free radicals are not stable and react rapidly with other compounds, aiming to take the necessary electron to achieve stability. The human body possesses a complex system of enzymatic and non-enzymatic antioxidant defenses which neutralize the deleterious consequences of free radicals [8]. The term antioxidant designates any substance which has a low concentration compared to oxidizable substrates, inhibits or delays the oxidation process [7]. The antioxidants play an important role in the protection from free radicals by donation of hydrogen or electron, which quench the free radicals [9].

The interaction of flavonoids with many radicals has been used in various studies to evaluate the essentials of antioxidant capacity [10].

The aim of this work is to measure the total content of phenolic, flavonoid and tannin compounds of stems *Astragalus gombo* bunge and study the correlation between them and the antioxidant capacity.

MATERIAL AND METHODS

Chemicals and reagents

Folin-Ciocalteu reagent, ascorbic acid, gallic acid, catechin, vanillin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sulfuric acid, butylated hydroxytoluene (BHT) were purchased from Sigma Aldrich; ammonium molybdate, sodium carbonate,

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hydrochloric acid, sodium dihydrogen phosphate, were provided by Biochem Chempharma. All other chemicals and solvents used in this research were of analytical grade.

Instrumentation

The proposed work was carried out on a UV/Vis spectrophotometer SPECTROSCAN 80 DV. All weighing was done on an electronic analytic balance. Plant extracts preparation was carried out by using a rotary evaporator ISOLAB GmbH for evaporating the solvent.

Plant collection

Plant stems were collected from the region of Oued N'ssa [32°23'N 5°21'E] (Ouargla, Algeria, arid climate). The collection was in the mornings in the beginning of March 2018. Professor Abdelmadjid Chahma from the University of Ouargla, Algeria, identified the specimen. Stems were cut into small parts and stored in paper containers at 25 °C in darkness until required for use.

Preparation of the extracts

Stems of *Astragalus gombo* were macerated at room temperature with petroleum ether for 24 h, and then the plant was air-dried until all the solvent was evaporated. After that the stems were macerated again with MeOH-H_2O (80:20, v/v) for 24 h three times. After filtration, the filtrate was evaporated until dryness, subsequently the dried filtrate was dissolved with H_2O and successively extracted using different organic solvents dichloromethane, ethyl acetate and butanol. These extracts, as well as the rest water fraction were concentrated under reduced pressure and then redissolved with minimum of methanol or water and kept at 4°C.

Phytochemical screening

The crude extract of stems was used for carrying out the following phytochemical tests using standard procedures to identify the various constituents described in the literature [11-13].

Determination of total phenolic content (TPC)

The total phenolic content in all stems extracts of *Astragalus gombo* was determined spectrophotometrically according to the Folin-Ciocalteu method [14]. Briefly, 0.1 ml of the extract sample was mixed with 0.5 ml of a 10 % Folin-Ciocalteu reagent (diluted in distilled water 1/10). After 5 min, 2.0 ml of 20 % Na_2CO_3 were added, and the mixture was incubated for 30 min at 25°C in the dark. Absorbance at 760 nm was measured. The TPC was determined using linear regression equation obtained from the calibration curve of gallic acid. The content of total phenolic compounds was determined as mean±SD (n=3) and expressed as mg gallic acid equivalent GAE/g of dry weight (DW) of plant.

Determination of total flavonoid content (TFC)

The content of total flavonoids was measured using the aluminum chloride colorimetric assay [15]. Briefly, 0.5 ml of the extract was added to 0.5 ml of 2 % $AlCl_3$ ethanol solution. After 30 min incubation at 25°C, the absorbance at 430 nm was measured. The content of total flavonoids was estimated as mean ± SD (n = 3) and expressed as mg quercetin equivalent QE/g of dry weight (DW).

Determination of total tannin content (TTC)

The total tannin content of the *Astragalus gombo* stems was determined by using the vanillin-acidified method [16]. 3 ml of a freshly prepared solution of vanillin (4% w/v vanillin solution in ethanol) and 1.5 ml of concentrated HCl were added to 0.4 ml of extract sample. After 15 min of incubation, the absorbance at 500 nm was measured. The content of total tannin was estimated as mean ± SD (n = 3) and expressed as mg catechin equivalent CE/g dry weight (DW).

Antioxidant activity

Evaluation of total antioxidant capacity by phosphomolybdate assay (PM). Phospho-molybdate assay was used to estimate the antioxidant activity of the samples [17]. The different extracts and fractions of *Astragalus gombo* were used. Various concentrations of extracts (0.1 ml) were mixed with 1 ml of reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulfuric acid). The mixture was kept at 95 ºC for 90 min. The absorbance was read at 695 nm against blank after cooling the tubes at 25°C, using ascorbic acid as a positive control, and the results were expressed in mM as the ascorbic acid equivalent antioxidant capacity AEAC. The following equation was employed to determine the values of AEAC:

$$AEAC = \frac{k_{extract}}{k_{ascorbic\ acid}}$$

where, $k_{extract}$: slope of the curve *absorbance = f(1/dilution number)*, $k_{ascorbic\ acid}$: slope of the curve *absorbance = f(concentration of ascorbic acid).*

DPPH radical scavenging activity. The power to scavenge the free radical DPPH• was estimated as the decrease in absorbance at 517 nm according to

[10]. Sample solution (1.5 ml) at various concentrations was added to methanolic DPPH (1.5 ml of a 0.25 mM solution) . The mixture was incubated for 30 min in dark, and then the absorbance was measured at 517 nm against a control. The antioxidant capacity of the extract was expressed as an IC_{50} value defined as the concentration (mg/ml) of the extract that inhibited $DPPH^{\bullet}$ radicals by 50%. The following equation was employed to determine the % DPPH radical scavenging activity:

$$\% \text{ DPPH radical scavenging} = \frac{(control\ absorbance - extract\ absobance)}{(control\ absorbance)}\ x100$$

RESULTS AND DISCUSSION

The recovery percentage of extractable compounds of stems ranged from 4.8852 to 0.1387%. The extraction yields of dichloromethane and of ethyl acetate were low, however, the yield of butanol extraction was significant. The results revealed that the highest yield extracts were obtained by the crude (methanol) (4.8852%) and butanol (1.2947%). This can be related to the richness of the plant in polar compounds. The variation in the yields of extracts could be attributed to the difference in solvent polarities used, which also plays a key role in increasing the solubility of phytochemical compounds.

The curative properties of *Astragalus gombo* are probably due to the existence of various secondary metabolites like polyphenols, flavonoids, coumarins, saponins, quinones, tannins, etc. The phytochemical screening of the extracts from the stems of *Astragalus gombo* is illustrated in Table 1.

The results of phytochemical tests detected the presence of flavonoids, tannins, protein, glycosides, terpenoids, coumarins, saponins and quinones. The existence of these compounds has been proved and six new cycloartane-type triterpene glycosides have been isolated in *Astragalus gombo* [18].

Table 1. Phytochemical screening of extracts from stems of *Astragalus gombo*

Phytochemical components	Stems
Flavonoids	++
Steroids	-
Tannins	+
Saponins	+++
Terpenoids	+
Alkaloids	-
Glycosides	++
Cardenolides	-
Protein	+
Quinones	+
Polyphenols	+++
Coumarins	+++

+ = Present, - = Absent

Another study [19] revealed the presence of protein in the aerial parts of *Astragalus gombo* from a semi-aride region in Algeria. On the other hand, Kim *et al.* [20] isolated protein of *Astragalus membranaceus* which acts as an allergen.

The richness of the *Astragalus* species in polysaccharides and saponins explains the use of this plant for their hepatoprotective, immune-stimulant, antioxidative, and antiviral properties [21].

In addition, this plant contains coumarins which are used as anticoagulants [22], antioxidants, antimicrobial (antiviral, antifungal, and anti-parasitic), anticancer, antidiabetic, analgesic, anti-neurodegenerative, and anti-inflammatory agents [23].

Preliminary tests have shown the existence of phenolic compounds. These compounds have a wide range of biological activities [24]. Therefore, our choice focused on this family of compounds that were obtained by the methods of extraction of polyphenols. The results are shown in Table 2.

The TPC of the various stems extracts is expressed in terms of GAE and is presented in Table 2. The TPC were calculated using the following linear regression equation derived from the standard curve of gallic acid: $y = 3.326x$, $R^2 = 0.992$, where y is absorbance and x is the concentration of gallic acid in g/L. Total phenolics ranged from 4.174 ± 0.034 mg GAE/g DW to 0.213 ± 0.001 mg GAE/g DW (Table 2). The highest content was found in the aqueous phase of stems; the lowest content was registered in the ethyl acetate phase of stems.

Ethyl acetate was applied to extract medium polar flavonoids and glycosides while butanol and water were utilized for extracting polar compounds like phenolic acids, aglycones. The TFC of the various crude extracts are expressed in terms of QE and are presented in Table 2. The TFC were calculated using the following linear regression equation obtained from the standard plot of quercetin: $y = 36.37x$, $R^2 = 0.999$, where y is absorbance and x is the concentration of quercetin in g/L.

Table 2. Yields of the extracts, total phenolic, flavonoid, tannin and antioxidant activity of various extracts of *Astragalus gombo*

Extracts	Yield %	TPC mg GAE/g DW	TFC mg QE/g DW	TTC mg CE/g DW	IC_{50} mg/ml	AEAC mM
Crude	4.8852	3.104±0.011	0.548±0.001	0.029±0.001	1.586±0.011	271.5±0.3
Dichloromethane	0.1387	0.254±0.001	0.011±0.000	0.008±0.000	0.073±0.005	099.1±1.8
Ethyl acetate	0.1508	0.213±0.001	0.518±0.002	0.008±0.000	0.300±0.026	099.2±1.1
Butanol	1.2947	1.209±0.008	0.706±0.002	0.039±0.003	0.616±0.021	194.7±22
Aqueous	ND	4.174±0.034	0.593±0.002	0.098±0.005	0.890±0.080	229.1±1.4
BHT	/	/	/	/	0.004±0.000	004.9±1.5
Vitamin C	/	/	/	/	0.009±0.000	/

ND, not determined

TFC of the crude extract and the fractions of *Astragalus gombo*, expressed as quercetin equivalent per gram dry weight mg QE/g DW, was between 0.706± 0.002 and 0.011 ± 0.000 mg QE/g DW. The highest content was found in butanol stems extracts; the lowest content was registered in dichloromethane stems extracts. Total tannin equivalents of the various extracts are expressed in terms of catechin equivalent and are presented in Table 2. The TTC were calculated using the following linear regression equation obtained from the standard plot of catechin: $y = 4.378x$, $R^2 = 0.997$, where y is absorbance and x is the concentration of catechin in mg/mL. TTC in the fractions and the crude extract of *Astragalus gombo*, varied between 0.098 ± 0.005 and 0.0084±0.0001 mg CE/g DW. The highest content was found in aqueous stems fractions, while the lowest was found in dichloromethane stems fractions.

The role of an antioxidant is to scavenge free radicals. One mechanism out of which this is done includes donating hydrogen to a free radical and hence its reduction to an unreactive species. Addition of hydrogen removes the odd electron feature, which is responsible for radical reactivity. The antiradical activity of ten crude extracts of *Astragalus gombo* leaves and stems was investigated by radical scavenging methods such as DPPH$^{\bullet}$.

The scavenging effect of different concentrations of *Astragalus gombo* leaves and stems extracts on the DPPH$^{\bullet}$ free radical was compared with that of a standard antioxidant, (ascorbic acid and BHT). The results, expressed as IC_{50} (mg/ml), are shown in Table 2.

The method of DPPH$^{\bullet}$ is based on the reduction of the stable radical DPPH$^{\bullet}$ with a violet color to DPPH-H with a yellow color. The disappearance of the violet color can be monitored spectrophotometrically at 517 nm. The values of IC_{50} varied between 1.586±0.011 and 0.073±0.005 mg/ml (Table 2). The best activity was found in the dichloromethane stems fraction with an IC_{50} value of 0.073±0.005 mg/ml followed by ethyl acetate with an IC_{50} value of 0.3±0.026 mg/ml. BHT and ascorbic acid showed a good antiradical activity, better than all extracts.

The total antioxidant activity of the different extracts of *Astragalus gombo* was measured by the phosphomolybdenum method, which is based on the reduction of Mo (VI) to Mo (V).

The formation of green phosphate/Mo (V) compounds was measured at 695 nm. Total antioxidant activity of all extracts varied between 271.5 ± 0.3 and 99.1 ± 1.8 mM. Crude fraction had a strong antioxidant activity with a value of 271.5 ± 0.3 mM followed by aqueous stems extract with a value of 229.1 ± 1.4 mM. The lowest antioxidant activity was recorded in the dichloromethane fraction with a value of 99.1 ± 1.8 mM.

Recently interest in food phenolics has highly increased, for their antioxidant capacity and their possible beneficial effects in human health, such as in the treatment and prevention of cancer, cardiovascular disease, and other pathologies [25]. Phenolic compounds undergo a complex redox reaction with the phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu reagent [26]. Under alkaline conditions, phenolic compounds reduce the Folin reagent to form a blue color; however, the assay is *not specific for phenolic compounds*. Tryptophan, ascorbic acid, thiols, redox-active metal ions, and nucleotide bases all reduce the Folin reagent and increase the final values [27].

Flavonoids, as a class of polyphenolics, are one of the most diverse, and most extensively present in plants. Numerous studies have established positive effects of flavonoids as potent antioxidants. These compounds exert a wide range of anticancer effects [28].

We have observed that phenolic and flavonoid contents are higher in the polar fractions (aqueous

and butanol fractions), which may indicate that these polyphenol compounds are more hydroxylated and/or glycosydated. The content of phenolic or flavonoid compounds in the fractions was affected by their solubility in the solvent used for extraction. Polar fractions had more polyphenols than non-polar fractions. Other studies showed that the content of phenolics in leaflets in different *Astragalus* species was higher than that in roots and seeds [29].

Comparing our results with previous studies specific for *Astragalus gombiformis* we noted that the values of IC_{50} obtained by Teyeb *et al*. [30] are higher. By way of example, for the aerial parts they give 0.473 mg/ml; and for the roots - 0.626 mg/ml. We recall that the extraction system used in this study is methanol.

Other studies on the *Astragalus chrysostachys* Boiss. roots show that the inhibition of DPPH$^{\bullet}$ is better than our results - in ethyl acetate phase 0.0146 mg/ml and in butanol phase - 0.0517 mg/ml [31].

Antioxidant capacity assays may be in general classified as single electron transfer (SET) and hydrogen atom transfer (HAT) based assays. SET assays measure the capacity of an antioxidant in the reduction of an oxidant. Phosphomolybdenum assay method is based on the redox antioxidant reaction.

Relationships among antioxidant capacity estimates and TPC, TFC, TTC

In the current research, the extracts of stems give an excellent correlation between TTC and TPC with a correlation coefficient R^2 = 0.85 while the extracts give a medium correlation between TPC and TFC with R^2 = 0.46. Moreover, the extracts of stems give a medium correlation between TFC and TTC with R^2 = 0.48. The TTC and TFC give a medium correlation with the antioxidant activity (especially with IC_{50}). On the contrary, a good correlation is shown between TPC and the antioxidant activity especially with IC_{50} (R^2 = 0.78).

Zhang *et al*. [32] estimated the antioxidant activity of a number of extracts from *Astragalus complanatus* obtained under different extraction conditions. They found that the antioxidant capacity, measured by DPPH$^{\bullet}$ test, well correlates to the TPC of the corresponding extracts. The high correlation coefficient (0.95) indicates that the total phenolics in the extracts were the major free radical scavenging compounds.

In addition, a good correlation is shown of TPC, TFC and TTC with the reduction capacity of Mo (VI) to Mo (V). Furthermore, according to some studies, the antioxidant activity depends on the structural conformation of the phenolic compounds. The latter is generally influenced by the phenolic compounds in the samples and by the different mechanisms involved in the radical–antioxidant reactions. These compounds may have a wide set of chemical structures that could react with radicals by hydrogen donation and/or electron transfer. There is an excellent correlation between the two methods of antioxidant activity determination (PM and DPPH$^{\bullet}$) (R^2 = 0.94).

CONCLUSION

In the present study, application of different solvents to extract antioxidant compounds from *Astragalus gombo* stems was investigated. Our results suggest that *Astragalus gombo* bunge could be a potential source of compounds with strong antioxidant potential. In addition, a high content of phenolics and tannin in this plant was determined.

This study indicated that the aqueous fraction has higher phenolic and tannic contents than other fractions. In addition, it exhibited strong antioxidant capacities in PM assay. All extracts exhibited strong reduction of Mo(VI) to Mo(V) comparable to the commercial BHT antioxidant. That is why the *Astragalus gombo* bunge is a suitable plant for treatment of many diseases.

Acknowledgements: *The authors gratefully acknowledge the support provided by Mr Mohamed Hadjadj, Director of VPRS Laboratory, University of Ouargla, Algeria.*

Conflict of interest: *All authors declare no conflict of interest.*

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