Physicochemical Characteristics, Anti-Lipase and Antioxidant Activities of Polysaccharide Extracted from *Astragalus spinosis* Grown in the Northern Region of Saudi Arabia

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Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Northern border region of Saudi Arabia is one of the richest areas of traditional plants. This study was designed with an interest to work on the polysaccharide extracted from *Astragalus spinosis* leaves (PLAS) collected from rafha province.

Methods: Polysaccharide was isolated by hot water extraction, followed by ethanol precipitation. The isolated crude polysaccharide contains 62.43% ±2.09% carbohydrate and 0.29 ± 0.07% protein. The physicochemical characteristics, such as chemical composition, humidity, foaming capacity, solubility as well as water and oil holding capacity were evaluated. The structural feature of polysaccharide was studied through Fourier transform infrared (FT-IR) analysis and scanning electron microscopy.

Results: Polysaccharide extracted from *Astragalus spinosis* leaves showed good inhibitory lipase and antioxidant activities. It was observed that the total antioxidant capacity, the 1,1-diphenyl-2-

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1. INTRODUCTION

Saudi Arabia has a hot desert climate in most parts of the country [1]. Different plants grow abundantly in various parts of Saudi Arabia and are used as medicinal plants. Astragalus spinosis is an example of this category of plants. Astragalus is a large genus of about 3,000 species of herbs and small shrubs, belonging to the legume family Fabaceae and the subfamily Faboideae. It is the largest genus of plants concerning the described species. Al-Snafi 2018, described in his review the traditional uses of two different species of Astragalus which are Astragalus hamosus and Astragalus tribuloides [2]. They were used in treating irritation of the mucous membranes, nervous affections, and catarrh. Astragalus tonifies the lungs and was used in cases of frequent colds and shortness of breath.

Biopolymers such as polysaccharide isolated from natural products have shown many biological properties as compared with synthetic drugs [3]. Previous studies have demonstrated that polysaccharide, one of the major active molecules from the roots, seeds or leaves of plants possess a wide range of pharmacologic functions including antitumor, immunoregulatory, antioxidant, hypoglycemic and cardioprotective activities [4]. The biological activities of polysaccharide vary with the location of cultivation, the part of the plant used for the extraction of polysaccharide, the season of the plant collected, and the plant extraction method.

Hajji M et al 2019, discovered a novel polysaccharide from Periploca laevigata root barks that demonstrated appreciable in vitro antioxidant potential and high antibacterial activity against several Gram (+) and Gram (-) strain [5]. A novel cold-water-soluble polysaccharide was extracted from Astragalus membranaceus by Liu AJ et al 2018 [6]. This novel polysaccharide has an underlying application as natural antitumor agents. Other polysaccharides extracted and purified from Astragalus cicer L have important antioxidant activities. These antioxidant properties were evaluated based on the determination of scavenging activities against DPPH and ABTS free radicals, as well as ferric reducing power [4].

However, to date, no earlier studies have been conducted in Saudi Arabia to explore the biological activities of polysaccharide extracted from Astragalus spinosis. To our knowledge, there are no reports available on the physicochemical constituents and antioxidant activities elucidation of polysaccharide extracted from Astragalus spinosis. Hence, the present study aimed to extract, structurally and biologically characterize of polysaccharide from Astragalus spinosis. The results could be used for further investigation of the structure-activity relationship and development of applications of Astragalus spinosis polysaccharide.

2. MATERIALS AND METHODS

2.1 Materials

Astragalus spinosis leaves were collected from Rafha Province desert in Northern Border region of Saudi Arabia kingdom.

2.2 Preparation of Astragalus spinosis Polysaccharide

Preparation of Astragalus spinosis leaves polysaccharide. The crude polysaccharide was isolated by hot water extraction, followed by ethanol precipitation, as described in a previous study [7].

2.3 Determination of Physicochemical Properties of Astragalus spinosis Polysaccharide

2.3.1 Solubility

The solubility of PLAS in water was evaluated. It was measured at 100°C according to the method as described by [8].
2.3.2 Carbohydrate content

The total sugar content of the samples was determined by the phenol-sulfuric acid method using glucose as a standard [9].

2.3.3 Protein content

Bradford method was used to determine the content of protein using BSA (bovine serum albumin) as the standard [10].

2.3.4 Quantitative determination of sulfate groups

Sulfate groups concentration were determined by BaSO₄ turbidity method [11], and formula for calculating degree of substitution is:

\[ DS = 1.62 \times S\% - 1.02 \times S\% \]

Where % was the percentage of sulfate groups in the sample.

2.3.5 Moisture and total ash content

The PLAS water content was performed in an oven at 105 °C. Approximately 150 mg of the sample were dried, until constant weight was reached (sample dry weight) [12].

The moisture content of PLAS was determined by measuring the mass loss of the sample in triplicate and expressed as follows:

\[ MC(\%) = \left( \frac{W₀ - W₁}{W₀} \right) \times 100 \]

Where \( W₀ \) and \( W₁ \) are respective masses (g) of the sample before and after drying.

The total ash content of PLAS was evaluated using 150 mg of dried powder of the leaves, followed by incinerating at a temperature not more than 450°C until it became completely white. The crucible was cooled and weighed. This process was continued until constant weight was obtained. The total ash content was calculated in triplicate as mg/g of the dried material and the amount was expressed in a percentage.

2.3.6 Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC)

The PLAS samples (10 mg) were mixed in 10 mL of distilled water or 5 mL of corn oil to measure the WHC and OHC, respectively. The mixture was stirred and incubated at room temperature for 1 h and then centrifuged at 5000×g for 15 min. The ratio between the weight of the tube content after draining and the weight of the polysaccharide samples was determined. Finally, the capacity (%) was reported as grams of water or oil bound per gram of the polysaccharide on a dry basis [13].

2.3.7 Foaming capacity and stability

Foam capacity (FC) and foam stability (FS) of PLAS were determined according to the method described by Bayar N et al., 2017 [14]. Briefly, 10 mL of polysaccharide solution at a concentration of 0.5% (w/v) was homogenized in a centrifuge tube for 10 min using vortex at room temperature. FC and FS of PLAS were expressed and calculated as follows:

\[ FC(\%) = \left( \frac{VT - V₀}{V₀} \right) \times 100 \]  
\[ FS(\%) = \left( \frac{VT - V₀}{V₀} \right) \times 100 \]

Where \( VT \) is the total volume after whipping; \( V₀ \) is the volume before whipping and \( V_t \) is the total volume after the solution is left at room temperature for 30 min.

2.3.8 Emulsifying properties

The emulsifying properties of PLAS were assayed according to the previously method reported by Han PP et al., 2014 [15]. Briefly, olive and corn oil (3 mL) were added to 3 mL of PLAS solution in a test tube and stirred in a vortex for 5 min. All emulsions were prepared and left at room temperature for 1, 24 and 168 h to determine E₁, E₂₄ and E₁₆₈, respectively. After 1 hour, the emulsion index (E₁) was determined as given below depending on the assay: \( E₁ = \frac{hₑ}{hₜ} \times 100 \), where \( hₑ (mm) \) is the height of the emulsion layer and \( hₜ \) is the overall height of the mixture.

All samples were stored at room temperature. All tests were performed in triplicate. The same assay was repeated after 24 h and 168 h.

2.3.9 Light Microscopy of Emulsions

A light microscope (Olympus CX40, Olympus, Tokyo, Japan) was used to examine and photograph the emulsion after 1, 24 and 168 h storage at 25 °C through a ×40 objective lens.

2.3.10 Surface tension measurements

The surface tension of the water-soluble polysaccharide was measured at different concentrations (1.0, 2.0, 3.0, 4.0, 5.0 mg/ml),
with a surface tensiometer (Krusch GmbH, Hamburg Germany), at room temperature of 25 ± 2 °C. Measurements were repeated three times for every sample [16].

2.3.11 Fourier transform infrared (FT-IR) spectrophotometer analysis

The infrared spectroscopy of PLAS was tested using Perkin-Elmer FT-IR instrument. It was used to determine the different sulfate, carboxyl and hydroxyl groups present in the samples [17]. One part of the extract was mixed with ninety nine parts of a dried potassium bromide (KBr) separately and then compressed to prepare a salt disc. The absorbance of PLAS was read between 650 to 4150 cm⁻¹.

2.3.12 Analysis of scanning electron microscopy (SEM)

The polysaccharide was coated with a thin layer of gold using a sputter coater, and then determined by JSM-6480A scanning electron microscope.

2.4 Determination of Bioactive Activities of Astragalus spinosis Polysaccharide

2.4.1 Anti-lipase activity of PLAS

The inhibitory capacities of the polysaccharide on pancreatic lipase were evaluated [18]. Briefly, various concentrations of the polysaccharide solution (0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0 mg/L, 1.0 mL), soybean oil as a substrate (1.0 mL), 5 mL phosphate buffer solution (0.1 mol/L, pH 7.2) and 1 mL of lipase solution (0.71 mg/mL, pig pancreatic Type II) were combined and incubated at 37°C for 1h. The mixture was transferred to a boiling water bath for 5 min and then centrifuged (4800 g, 10 min) (L600, Cence Test Instrument Co., Ltd., Hunan, China). The amount of free fatty acid in the reaction mixture was measured by titration with 0.05 mol/L NaOH and phenolphthalein as an indicator. The activity of pancreatic lipase was determined by measuring the production rate of free fatty acids. All analyses were carried out in triplicate.

2.4.2 Determination of antioxidant activities of Astragalus spinosis polysaccharide

2.4.2.1 Total antioxidant capacity (TAC)

To determine the total antioxidant capacity of PLAS at different concentrations, 0.3 mL of the sample was added with 3.0 mL reagent solution and mixed (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample was incubated at 95°C in the water bath for 90 min. Finally, the antioxidant capacity of the PLAS sample was measured at 695 nm [19].

2.4.2.2 DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of PLAS was determined using a previously assay with slight modifications [20]. DPPH solution (2.0 mL, 0.1 mM in ethanol) was mixed with a PLAS solution (2.0 mL) at various concentrations. After mixing rapidly, the mixture was incubated at room temperature for 30 min in the dark, and the absorbance was measured at 517 nm. The Distilled water was used as the blank control, and BHA was used as the positive control. The DPPH radical scavenging activity (RSA) was calculated according to the following equation:

\[
\text{DPPH RSA} \, \% = \left[1 - \frac{(A_2 - A_1)}{A_0}\right] \times 100\%
\]

\(A_2\) : the absorbance of a mixture of the sample and the DPPH solution
\(A_1\) : the absorbance of the sample without the DPPH solution
\(A_0\) : the absorbance of the DPPH solution without the sample

2.4.2.3 ABTS⁺ radical-scavenging activity

The 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid radical cation (ABTS⁺) was produced by reacting ABTS⁺ (7 mmol/L) with 140 mmol/L potassium persulfate solution (890 μL) and the mixture was incubated in the dark at room temperature for 12–16 h. Radical scavenging activity of the tested samples was measured by mixing 0.1 mL of the different concentrations of crude polysaccharides with 1.9 mL of diluted ABTS⁺ solution, and the absorbance was measured after 6 min [21]. The scavenging activity was calculated using the following Equation (1):

\[
\text{Scavenging rate} \, \% = \left[1 - \frac{(A_2 - A_1)}{A_0}\right] \times 100\%
\]

Where \(A_0\) is the absorbance of the control, \(A_1\) is the absorbance of the samples addition and \(A_2\) is the blank absorbance without ABTS⁺ solution.

2.5 Statistical Analysis

Statistical analyses will be performed with SPSS ver. 17.0, professional edition using ANOVA analysis at a p level=0.05.
3. RESULTS AND DISCUSSION

3.1 Chemical Compositions of PLAS

The chemical compositions of PLAS were evaluated (Table 1). The extraction yields of PLAS were determined as 70.16 ± 2.09% which were higher than that of the previous study [22]. High carbohydrate, low protein and sulfate contents were observed in PLAS (62.43 ± 1.2%, 0.29 ± 0.07% and 0.68 ± 0.15% respectively). In addition, moisture and ash contents were determined (2.45 ± 0.86% and 16.73 ± 0.81% respectively). The results suggest that the method used to extract PLAS had a significant effect on the content of carbohydrate.

The solubility of PLAS was 62.49 ± 1.13%. PLAS have a strong affinity to water molecules. This is due to the presence of multi-OH groups. However, this also leads to a strong interaction among polysaccharide molecules via hydrogen bonding [23].

3.2 Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC)

Water holding capacity is the total amount of water retained by dry polysaccharide. As shown in Table 1, PLAS have a high WHC (28.25± 1.05%). This result was significantly higher than the results obtained for the seagrass Cymodocea nodosa (10.73 g/g) [24] and for the Nostoc flagelliforme (27.82 g/g) [13]. Water uptake of polysaccharide may encourage microbial growth resulting in fecal bulking and output [25,26].

Oil holding capacity OHC is the amount of oil retained by the polysaccharide. According to the result mentioned in Table 1, PLAS samples showed a high OHC (48.35± 0.25%). It was higher than the OHC of polysaccharide extracted from red algae Gigartina pistillata (1.32 g/g) [27] and Ulva prolifera (15.09 g/g) [28]. The capacity of polysaccharide to directly bind fat may contribute to its overall hypolipidemic activity [13]. These results demonstrate that PLAS might be a suitable for use in the food and pharmaceutical industries.

3.3 Foaming Capacity and Stability of PLAS

The foaming capacity and stability of PLAS were evaluated using different concentrations (0.5, 1.0 and 2.0%). At 0.5%, PLAS showed the lowest foaming capacity and stability with the value of 50% and 75% respectively. These results were higher than those obtained with the pectin extracted from Opuntia ficus indica cladodes (FC 30% and FS 25%) at the same concentration [29]. The effects of the increasing PLAS concentration from 0.5% to 2.0% on the foam capacity and stability were observed in Fig 1. PLAS showed a very high foam capacity and stability at 2.0% which reached 80% and 90% respectively. This was confirmed by Ropers MH et al., 2008 in their previous study [30]. They concluded that the improvement of the foaming properties of pectin by the formation of large aggregates was probably not the only parameter affecting foam properties. However, it was related also to the concentration in pectin leading to the best foaming properties.

Table 1. The physicochemical characteristics of the investigated PLAS

| Polysaccharide yield (%) | 70.16 ± 2.09 |
|--------------------------|---------------|
| Total carbohydrate content (%) | 62.43 ± 1.2 |
| Protein content (%) | 0.29 ± 0.07 |
| Sulfate content (%) | 0.68 ± 0.15 |
| Moisture (%) | 2.45 ± 0.86 |
| Ash (%) | 16.73 ± 0.81 |
| Solubility (%) | 48.35 ± 0.25 |
| Water-holding capacity (WHC) (%) | 28.25 ± 1.05 |
| Oil-holding Capacity (OHC) (%) | |

Values are mean ± standard deviation (n=3)

3.3.1 Emulsifying activity of PLAS with corn and olive oils

Emulsion is considered an excellent system for delivering nutraceuticals. The emulsions of PLAS were prepared using corn and olive oils and compared. The emulsification indexes were determined after 1h, 24h and 168 h. As shown in Fig. 2a, the high emulsification indexes, indicate the effect of PLAS on the emulsifying activity. The emulsification indexes of PLAS used with corn oil was 88% after 24 h when it reached 68% with olive oil. PLAS formed stable emulsions with each oil as they did not break within 168 h. It was observed that the emulsions prepared with corn oil exhibited the higher emulsification indexes. This was confirmed by the micrographs for each emulsion as in Fig. 2b. The smaller, distributed droplets were observed in the emulsions of PLAS prepared with corn oil, which had higher emulsification indexes, whereas the larger droplets were found with olive oil.
Fig. 1. Foaming capacity (FC) and stability (FS) of PLAS

Fig. 2.a. Emulsification activity prepared with olive and corn oils. All emulsions were prepared and left at room temperature for 1, 24 and 168 h to determined E1, E24 and E168, respectively

Fig. 2.b. Photomicrographs of emulsions prepared with olive and corn oils. All emulsions were prepared and left at room temperature for 1, 24 and 168 h to determined E1, E24 and E168, respectively
The high emulsifying activity of polysaccharide could be explained by the protein content linked to their structure [31]. This was confirmed also by Shen, S-G et al., 2019 in their previous study [13]. Proteins mainly stabilized emulsion droplets with electrostatic repulsive forces [32]. The emulsifying ability of PLAS to stabilize emulsions between water and hydrophobic compounds might promote its applications in numerous industrial areas [33].

3.3.2 Scanning electron microscopy (SEM) micrograph of PLAS

The microstructure and surface topography of PLAS were evaluated using scanning electron microscopy (SEM) micrograph method. SEM can provide an idea about the three dimensions and reality of polysaccharide. As observed in Fig. 3, PLAS were flaky with a smooth and transparent surface. The size of the fragmented segments was different. This finding was similar to the microstructure of the polysaccharides extracted from Novel Sorghum bicolor (L.) seed [34].

3.3.3 Surface tension measurements of PLAS

The properties of surface active polysaccharide is their capacity to lower the interfacial tension of their solvents. The surface tension of PLAS was studied as a function of the concentration (1.0, 2.0 and 3.0 mg/mL). It was decreased with the increase of PLAS concentrations. It was 60 mN/m at 1.0 mg/mL to be 20 mN/m at 5.0 mg/mL (Fig. 4). A similar effect was observed for the sucrose and dextran solutions in the previous study [35]. This may be explained by the presence of hydroxyethyl (hydrophobic part) and hydroxyl groups or carboxyl (hydrophilic part) in the structure of polysaccharide which can be adsorbed and oriented at liquid–liquid interfaces to reduce efficiently the interfacial tension and to promote the formation of a nanoparticle dispersion system.

Fig. 3. Scanning electron microscopy (SEM) micrograph of water-soluble polysaccharide PLAS at different magnifications: 50 µm magnification (a), 100 µm magnification (b)

Fig. 4. Surface tension of water-soluble polysaccharide PLAS as a function of concentration
3.3.4 Ultraviolet and Fourier transform infrared (FT-IR) spectrophotometer analysis of PLAS

The ultraviolet–visible spectrum of PLAS was observed in Fig. 5a. The absorption at 260 and 280 nm were very low, indicated that they contained few proteins and nucleic acid impurities. This was consistent with results obtained by Bradfords method.

The FT-IR spectrum of PLAS was analysed in the range of 650-4150 cm⁻¹. PLAS showed a typical absorption peaks (Fig. 5b). The strong peak at approximately 1000 cm⁻¹ was related to the presence of C–O–C and C–O–H stretching vibration resulted from pyranose ring [35-36]. The second strong peak at around 3350 cm⁻¹ was originated from O–H stretching. A weak band at 2900 cm⁻¹ was attributed to the stretching vibrations of C–H. The band at approximately 1600 cm⁻¹ determine the carboxylate ion (–COO–) of uronic acid [37]. According to the previous study [38], peaks at 823 and 918 suggested the presence of α-glycoside and β-glycoside.

3.4 Inhibitory Effects on Lipase

Hyperlipidemia is considered as an excess of the pancreatic lipase in the blood. High levels of lipase may indicate a problem related to the pancreas. Thus, inhibitory of lipase activity may reduce the risk of pancreatitis. In this study, PLAS exhibited a high lipase inhibitory activity (Fig. 6). The inhibitory activities increased at low polysaccharide concentrations (1.5 mg/mL-2.0 mg/mL). The lipase activity was totally inhibited at 2.5 mg and 3.0 mg/mL. These results are similar to those found by [39].
Fig. 6. Anti-lipase activity of water-soluble polysaccharide PLAS as a function of concentration

3.5 Antioxidant Activities of PLAS

3.5.1 Determination of Total Antioxidant Capacity (TAC)

The antioxidant activity of polysaccharide from plant extracts have received an extensive study and attention. Total antioxidant capacity (TAC) of PLAS at various concentrations of samples was used to determine this biological activity. As shown in Fig. 7a, TAC increased with increasing concentrations of samples. It was around 20 µmol/mL at 0.25 mg/mL and becomes 97 µmol/mL at 2.0 mg/mL. These results indicated that the high antioxidant capacity of PLAS is closely related to the concentration of polysaccharide.

3.5.2 DPPH radical scavenging activity

Oxygen-derived free radicals are harmful to human health [40]. Free radicals are highly reactive molecules with unpaired electrons. DPPH radical scavenging activity of PLAS was evaluated to confirm this antioxidant ability of polysaccharide extracted from *Astragalus spinosis*. This activity was determined using different concentrations of samples (Fig. 7b). Both PLAS and BHA (positive control) showed scavenging abilities for DPPH radicals over the concentrations of 0.2–2.0 mg/mL. The ability of PLAS and BHA to scavenge DPPH increase proportionally to the concentrations of samples. PLAS showed a weak activity at low concentration (0.2 mg/mL) which was 16.87% concentrations relative to positive control (81.17%). However, PLAS have comparable activity as that of positive control at high concentration; it was 73% and 93% at 2.0 mg/mL respectively.

The scavenging activity of this polysaccharide was lower than BHA at low concentrations. It may be explained that PLAS was more complex than BHA with presence of impurities in crude extract that interfere with the reaction. These results agreed with those observed by Zhu, Y et al regarding three purified polysaccharide isolated from Chinese Huaishan-yams [41].

Compared to the scavenging rate of polysaccharide extracted from *Cucurbita moschata* seeds [42], which showed 45% scavenging rate at 1.0 mg/mL, PLAS exhibited 70 % at the same concentration. This suggests that PLAS had strong antioxidant activity.

3.5.3 ABTS radical scavenging activity

Polysaccharide is one of the bioactive components in natural products. One of the biological activities of PLAS is ABTS radical scavenging activity. As shown in Fig. 7c, PLAS exhibited a high ABTS scavenging rate. This activity was proportional to the concentration of polysaccharide. As it was observed with DPPH radical scavenging activity, PLAS showed a weak ABTS radical scavenging activity at low concentration relative to the positive control. The activity increased at high concentration and becomes comparable to the positive control. At 2.0 mg/mL the ability of PLAS to scavenge ABTS was 93% when it was 98% for α tocopherol. Chen, L et al., 2018 isolated a sulfated polysaccharide from *Ascophyllum nodosum* with 40 % scavenging activity at 2.0 mg/mL which is lower than scavenging ability of PLAS [43].
Fig. 7.a. Total antioxidant capacity (TAC) of PLAS

Fig. 7.b. DPHH radical scavenging activity

Fig. 7.c. ABTS radical scavenging activity of PLAS
As we can see, the antioxidant activities of PLAS were high at 2.0 mg/mL. The antioxidant abilities of polysaccharide were not influenced by a single factor, but rather a combination of several factors including the MW and chemical composition as well as the method of extraction [44,45].

4. CONCLUSION

In conclusion, the functional properties of polysaccharide extracted from Astragalus spinosis leaves were investigated. These include physicochemical, emulsifying, foaming, water and oil-holding capacity as well as surface tension properties. The microstructure of PLAS were evaluated using scanning electron microscopy. The FT-IR spectrum of PLAS and inhibitory lipase activity were analysed. The results showed that PLAS was a candidate for use in the nutraceuticals. PLAS exhibited excellent antioxidant activities in the scavenging ABTS and DPPH radicals in vitro as well as total antioxidant capacity. As a result, PLAS seems to be a good source of the natural antioxidant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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