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Antidiabetic potential of mucilage fraction extracted from *Astragalus gyzensis* seeds

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ABSTRACT: The objective of the current work is to extract a new mucilage fraction from *Astragalus gyzensis* Bunge. seeds, which are collected from the El-Oued province (septentrional Algerian Sahara) and evaluated for their antidiabetic potential. The mucilage fraction is obtained using hot water extraction followed by alcoholic precipitation of polysaccharides by cold ethanol (96%). The primary investigation was performed by describing the main structural features of the extract through colorimetric assays, Fourier-transform infrared spectroscopy and thin-layer chromatography analysis using two systems. Biological activity was also monitored by antidiabetic activity by testing the inhibition of α-amylase and α-glucosidase enzymes in vitro. The extraction yield was 20.69%. The chemical composition mainly consisted of 78.60±0.29% carbohydrates, among them 63.92±0.67% neutral sugar, 15.78±0.76% uronic acid, 8.08±0.04% proteins and 2.57±0.05% phenolic compounds. The results obtained by thin-layer chromatography analysis showed the dominance of mannose and galactose. Fourier-transform infrared spectrum showed characteristic bands expected galactomannans. The investigations highlighted the antihyperglycemic effect in a dose-dependent manner by the inhibition of the α-amylase enzyme (IC50=0.8±0.005 mg/mL). These factors make it suitable for the industrial application of dietary supplement fiber made for diabetic individuals.

Keywords: *Astragalus gyzensis* Bunge; Mucilage; Antidiabetic; Galactomannans; Dietary supplement fiber.

1. INTRODUCTION

Ethnopharmacology serves as one of the best sources to find a plant molecule which can be used as a template or a lead compound for creating a drug. The traditional medical system continues to play a key role in health care [1]. Floral species earned a prominent place in pharmaceutics because of their therapeutic efficacy and the availability of their potentially active natural chemical constituents [2]. Polysaccharides isolated from plant sources have attracted a great deal of attention in the biomedical area, specifically for their broad spectrum of therapeutic properties and relatively low toxicity. Dietary fibers play a vital role in the prevention of various diseases [3]. Most plants from arid regions belong to the Fabaceae family, which is one of the main botanical families [4]. It is also a commonly used plant family in traditional African medicine [5]. Astragalus is likely the largest and most abundant genus of vascular plants on Earth, comprising nearly 2500–3000 annual and perennial species distributed in all continents, mainly around the Northern Hemisphere, Western North America, South America, Central Asia and tropical East Africa. However, it is not found in Australia. There are numerous species of Astragalus growing in North Africa and the Mediterranean, with 15 species found in the Sahara of Algeria [6]. The plants have been extensively analyzed for three main groups of biologically active compounds: polysaccharides, flavonoids and saponins [7]. *Astragalus gyzensis* Bunge. is traditionally used in North African medicine to treat snakebites. In Algeria, *A. gyzensis* is quite common in the deserts and is locally known as «Dlilia» [8].

Reactive oxygen species (ROS) are highly reactive molecules derived from the metabolism of oxygen. They are often byproducts of biological reactions. *In vivo*, some ROS play positive roles in cell physiology, but they may also damage cell membranes and DNA, inducing oxidation that causes membrane lipid peroxidation and decreased membrane fluidity [9]. On the other hand, many diseases, such as cardiovascular diseases and diabetes, are associated with oxidative stress [10]. Type 2 diabetes is a serious metabolic disorder characterized by defects in the control of blood glucose [11]. Like other nutrients, carbohydrates are mostly digested in the small intestine. However, it is the salivary amylase in the mouth that begins 5% of the initial breakdown of carbohydrates. The glucosidase enzymes (maltase, lactase and sucrase) secreted by intestinal mucosa complete the breakdown of oligosaccharides into monosaccharide units, which are then absorbed by the body and transported to the liver through the portal vein. The body uses these monosaccharides as a direct source of energy [12]. Therapeutic treatments include commercial insulin and oral hypoglycemic medication that help control a patient's glycaemia. Both physical activity and proper diet are key factors in managing diabetes. Dietary fibers, especially soluble fibers, stand out as an important part of a healthy diet directed at treating diabetes due to their ability to increase peripheral insulin sensitivity and reduce blood lipid levels [13]. Reduced postprandial hyperglycemia is one treatment method for early-stage diabetes. This is accomplished by suppressing the carbohydrate-hydrolyzing enzymes, α-glucosidase and α-amylase in the digestive system, to prevent glucose absorption. As a result, inhibitors of these enzymes slow the absorption of glucose, dampening the postprandial plasma glucose spike [14]. The hypoglycemic and antidiabetic effects of several plants used as traditional antidiabetic remedies have been proven, and the mechanisms of hypoglycemic activity in these plants has been studied effectively [15]. Moreover, increasing evidence has confirmed that polysaccharides with different structures from various plants possess different inhibitory activities of α-amylase and α-glucosidase [16]. Antidiabetic drugs combat diabetes by reducing hyperglycemia and its complications through different modes of action, one being improved peripheral utilization of glucose and another that increases antioxidant activity which scavenges free radicals formed due to hyperglycemia [17].

To our knowledge, the chemical composition and biological benefits of the water-soluble polysaccharides extracted from *Astragalus gyzensis* Bunge. seeds (PSAG) have not been reported. This work was done to evaluate if the water-soluble polysaccharides could resist the carbohydrate hydrolyzing enzymes and reduce hyperglycemia. The goals of our work were the isolation and partial characterization of the mucilage fraction using colorimetric assays, UV-vis Scanning, Fourier Transform Infrared spectroscopy (FT-IR) and Thin Layer Chromatography analysis (TLC), and the *in vitro* evaluation of the biological activities by antioxidant and antihyperglycemic activities, which were tested against α-amylase and α-glucosidase enzymes.

2. MATERIALS AND METHODS

2.1. Raw material and chemicals

The pods of *A. gyzensis* were collected in March and April 2019 at their full maturity from Hassi Khalifa in the El-Oued province (septentrional Algerian Sahara). The identification of the plant specimen was confirmed by Dr. Slimani Noureddine (Faculty of Biology, University Echahid Hamma Lakhdar, El-Oued, Algeria). The pods were dried in the shade away from sunlight and moisture for three weeks. The seeds were then manually isolated from the dry pods and stored in kraft paper bags at room temperature. Standard monosaccharides (arabinose, rhamnose, galactose, glucose, mannose, glucuronic acid and galacturonic acid), metahydroxydiphenyl, trifluoroacetic acid (TFA), α-amylase, α-glucosidase, acarbose, *p*-nitrophenyl α-D-glucopyranoside (*p*-NPG), 2-chloro-p-nitrophenylα-D-maltotrioside (CNPG3), and 2,2'-diphenyl-1-picrylhydrazyle (DPPH) were purchased from Sigma-Aldrich in Germany. All other chemicals used were of an analytical grade.

2.2. Polysaccharides extraction procedure

The polysaccharides extraction procedure followed the method of Addoun et al. [18] with a slight modification. *A. gyzensis* seeds were ground to a fine powder using an agate mortar and pestle. Ten grams of unground seeds were extracted by hot maceration using distilled water (20% w/v) at 70°C for two hours with moderate stirring (450 rpm). The highly viscous dispersion was put through a fine filter to remove residual debris. The same process was repeated three times before it was centrifugated at 4000 rpm for 15 minutes. The macerate was concentrated (1/3V) by a rotary evaporator at 65°C. The polysaccharides were precipitated by adding three volumes of cold ethanol (96%) and refrigerating for 24 hours in a 4°C environment. The pellet was recovered after centrifugation (4000 g, 4°C, 15 minutes), then washed with acetone multiple times. Finally, the polysaccharides fractions obtained (PSAG) were dried at 50°C for 48 hours, then crushed into a fine powder (<3 mm) by a mechanical blender.

Figure 1. *Astragalus gyzensis* Bunge. (a) *Astragalus gyzensis* Bunge. plant (b) *Astragalus gyzensis* Bunge. seeds (April 2019).

2.3. Chemical Determination

2.3.1. Determination of total sugars content

The total sugar content was evaluated by phenol-sulfuric acid method using Glc as the standard [19] with a small modification. Two hundred microliters of each dilution of PSAG was mixed with 200 µL of 5% aqueous solution of phenol in a test tube. Subsequently, 1 mL of concentrated sulfuric acid was rapidly added to the mixture. After allowing the test tubes to stand for ten minutes, they were vortexed for 30 seconds then placed for 30 minutes in a water bath at 90°C for color development. Lastly, light absorption was used at 490 nm.

2.3.2. Determination of neutral sugars content

The neutral sugar levels were measured by 1,3-dihydroxybenzen method using Glc as the standard [20] with a minor alteration. Two hundred microliters of each dilution of PSAG was mixed with 200 µL of 0.6% aqueous solution of resorcinol in a test tube. One milliliter of concentrated sulfuric acid was added rapidly to the mixture. The test tubes were left to rest for ten minutes, then vortexed for 30 seconds and placed in a water bath for 30 minutes at 80°C for color development. Then, light absorption was used at 450 nm.

2.3.3. Determination of the uronic acids content

The uronic acid content was quantified by m-hydroxydiphenyl assay using Gal. A as the standard [21] with a slight modification. Two hundred microliters of each dilution of PSAG was mixed with 1.2 mL of a 0,12M solution of tetraborate of sodium in a test tube. After ten minutes, the test tubes were vortexed for 30 seconds and placed in a water bath for five minutes at 100°C. Next, the test tubes were removed from the water bath and 20 µL of 0,15% solution of m-hydroxydiphenyl was added, Then, light absorption was used at 520 nm.

2.3.4. Determination of the proteins content

Protein content was estimated by Bradford assay using bovine serum albumin as reference [22] with a minor change. First, 500 µL of each dilution of PSAG is mixed with 500 µL of Bradford reagent. Then, they are vortexed for 30 seconds and placed in a dark, in room temperature environment for 30 minutes. Then, light absorption was used at 595nm.

2.3.5. Determination of the phenol content

The phenolic content was evaluated with the Folin-ciocalteu reagent using gallic acid as the standard [23] with a slight alteration. One milliliter of each dilution of PSAG was mixed with 0.5 mL of Folin-ciocalteu reagent (diluted ten times with distilled water), then the mixture was left in the dark for five minutes. Next, 2 mL of sodium carbonate (7.5%) was added to the tube, stirred, and stored in the dark at laboratory temperature for 30 minutes. The final absorbance of the solution obtained was read at λ=765 nm.

All test reference solutions were prepared in an identical manner, except that the volume of the extract was replaced with distilled water. The results were calculated according to the standard curve and expressed in triplicates (means ±SD, n=3).

2.4. Spectroscopic analyses

Ultraviolet-visible spectra of 2.5 mg/mL of PSAG solution (dissolved in distilled water) was scanned with a UV-visible (Shimadzu-1800) scanner using a wavelength of 200–900 nm at 25°C. Fourier transform infrared spectra of PSAG powder (2 mg) was measured according to the spectrum of FT-IR (Nicolet iS5,

Thermo Fisher Scientific) in the spectral range 400–4000 cm^{-1}. The spectrum performed a smoothing and a correction of the baseline using Origin Pro8 software.

2.5. Monosaccharide composition by Thin Layer Chromatography (TLC)

PSAG monosaccharides content was determined through its hydrolysis by TFA 2M at 100°C for four hours [24] followed by thin layer chromatography (TLC) analysis. Standard solutions and hydrolyzed polysaccharides were applied in a Silica gel 60 F254 chromatoplate. After elution with System 1; ethyl acetate, pyridine, water, n-butanol, acetic acid in the proportions 5/4/4/10/2 [25]. System 2; Chloroform, n-butanol, methanol, acetic acid, water in the proportions 4.5/12.5/5/1.5/1.5 [26]. The plates were dried at 100°C for two minutes and detected with nigrum. The R_f values for the separated spots were calculated and compared with R_f values of the pure standards.

2.6. Antihyperglycemic activity

The antihyperglycemic activity of PSAG was investigated by evaluating the inhibition of both α-amylase and α-glucosidase activities.

2.6.1. Inhibition of α-amylase activity

The inhibition of α-amylase activity was estimated using the methods of Kumar et al. [27] and Kajaria et al. [28] with a slight modification. One hundred eighty microliters of each dilution from 0.1–5 mg/mL of PSAG was added to dry test tubes, with acarbose as the positive control and PBS as the negative control. Then, 90 µL of α-amylase solution (5 IU/L) was added to each tube. The reaction mixtures were pre-incubated for 15 minutes at 37°C. Next, 500 µL of the substrate CNPG3 solution (0.5 mg/mL) was added using gentle stirring, followed by incubation for ten minutes at 37°C. The absorbances were measured at λ=405 nm using a (Shimadzu-1800 spectrophotometer).

2.6.2. Inhibition of α-glucosidase activity

The inhibition of α-glucosidase activity was estimated using the methods of Bisht et al. [29] and Qian et al. [30] with a slight difference. First, 500 µL of α-glucosidase solution (2IU/L) was introduced to dry test tubes with 100 µL of each dilution from 0.1–5 mg/mL of PSAG, using acarbose as the positive control and PBS as the negative control. The mixture was pre-incubated for 15 minutes at 37°C. Then, 100 µL of the substrate *p*-NPG solution (4 mM) was added. The tubes were shaken and incubated for 20 minutes at 37°C. One milliliter of Na_2CO_3 (0.2 M) was added to stop the reaction and the absorbances were measured at λ=405 nm using a (Shimadzu-1800 spectrophotometer). The results of the inhibition of both α-amylase and α-glucosidase activities was expressed using the equation of Telagari et al. [31]:

$$\text{Inhibition (\%)} = ((A_{control}-A_{sample})/A_{control})\times 100$$

All the experiments were done in triplicate.

2.7. Antioxidant activity by DPPH radical scavenging assay

The antioxidant activity of PSAG and ascorbic acid were evaluated by using the DPPH procedure described by Delattre et al. [32]. One milliliter of each dilution from 0.1–5 mg/mL of PSAG or ascorbic acid was added into 1 mL of a DPPH solution at 0.1 mM in ethanol. The solution was aggressively stirred then incubated for 30 minutes at room temperature (25°C) in obscurity. The absorbance was measured at 517 nm using a (Shimadzu-1800 spectrophotometer). The DPPH inhibition (%) was calculated using the equation below:

Inhibition (%) = (1 - ($A_{sample}/A_{control}$)) × 100

where A_{sample} and $A_{control}$ are the absorbances of the sample and ultra-pure water, respectively.

2.8. Statistical analysis

The data were analyzed via Origin Pro8 software and Microsoft Excel 2007.

3. RESULTS AND DISCUSSION

3.1. Extraction yield of PSAG

Our method was quite effective in the extraction of polysaccharides. The mucilage fraction isolated from *Astragalus gyzensis* Bunge. seeds were amorphous white powders. The extraction yield was 20.69% w/w. Regarding the extractions procedure, this number of polysaccharides is a higher yield compared to the seeds of other plants in the Fabaceae family, such as fenugreek (10%) [33], *Alhagi maurorum* Medik. (12.58%) [34], and *Caesalpinia ferrea* Mart. seeds (9%) [35]. However, it is lower than the yield from *Senna tora* seeds (35%) [36]. Seed gums are mostly obtained from leguminous plants, the endosperms of the seeds mainly responsible for water solubility [37]. Moreover, this value was higher than amounts of polysaccharides found in other seeds species of the Astragalus genus such as *A. lehmannianus* (4,8%), *A. sericeocanus* (3,6%), *A. danicus* (3,4%), *A. cicer* (5,9%), *A. alpinus* (0,6%) and *A. tibetanus* [38, 24, 39, 40, 41]. Some seeds species collected in the same Saharan zone reported higher values than these, such as *A. armatus* (4.21%) [42] and *A. gombo* (6.8%) [43].

3.2. Chemical composition of PSAG

The mucilage fraction is mainly composed of (78.6%) total sugar. This high sugar content confirmed the efficiency of the extraction process [44], among them (63.92%) neutral sugar and (15.78%) uronic acid. Neutral sugar contents are consistent with the biochemical composition of other polysaccharides extracted from *Astragalus* sp. [24, 38, 39, 40-42]. While the uronic acid content was higher than the values described for other polysaccharides seeds of *A. armatus* and *A. Gombo* [42, 43], these changes in biochemical compositions could be attributed to differences in species and the harvest area. Both PSAG extracts contained traces of proteins (8.08%) and polyphenols compounds (2.57%). The elimination of proteins might be difficult, especially when conjugate proteins are present [44]. The use of protein separation techniques can lead to the degradation of polysaccharides and cause them to lose their native structure, because the glycoprotein protein is linked covalently to the polysaccharides moiety [45].

Table 1. Chemical composition of polysaccharides from *Astragalus gyzensis* Bunge. Seeds.

Total sugar (% w/w)	Neutral sugar (% w/w)	Uronic acid (% w/w)	Proteins (% w/w)	Phenolic compounds (% w/w)
78.60 ± 0.29	63.92 ± 0.67	15.78 ± 0.76	8.08± 0.04	2.57±0.05

3.3. Spectroscopic analyses

3.3.1. UV-visible Spectrum Scanning

The absorption spectra (UV-visible) of the PSAG extract are presented in figure 2. As illustrated, PSAG reaches UV absorption peak at 210 nm, which corresponds with the absorption of the polysaccharides and a larger absorption peak between 250–300 nm, indicated by the low amount of proteins and/or nucleic acids impurities in the extract, which is consistent with previous results [46, 47]. There was no absorption at 620 nm, suggesting the pigment was completely removed as the result described by Tang et al. [44].

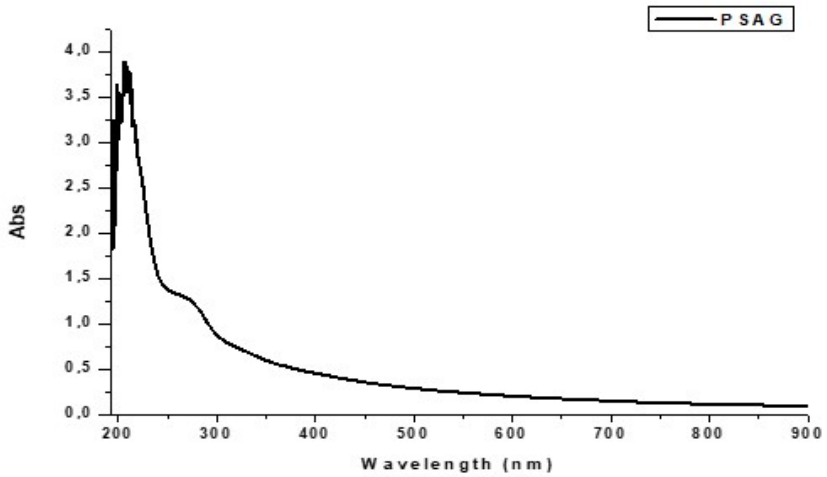

Figure 2. Uv-vis absorption spectra of PSAG (polysaccharides extracted from *Astragalus gyzensis* Bunge. seeds).

3.3.2. FT-IR analysis

FT-IR analysis is a significant tool for obtaining first-hand knowledge and preliminary identification of biopolymers from diversified sources. Galactomannan as a biopolymer has been characterized using FT-IR previously in literature [33]. Figure 3 represents FT-IR spectra of PSAG in the frequency range between 400 cm^{-1}–4000 cm^{-1}.

The IR spectra of polysaccharides showed the peaks at 814 cm^{-1} and 871 cm^{-1} are related to the presence of anomeric configurations (α and β conformers) and glycosidic linkages attributed to α-D-galactopyranose and β units -D-mannopyranose units, respectively [35]. The absorption peaked in 1029.99 cm^{-1}, and in 1149.57 cm^{-1} showed that the constituent sugar cycles in PSAG belong to the pyranose cycle, which are the absorption peaks generated by the vibration of the COC ether bond [48]. The broad band between 1198 cm^{-1} and 983 cm^{-1} results from the stretching vibration of C O in C O H bonds (e.g., glycosidic bonds). The peak at 1149 cm^{-1} corresponds to bending vibrational modes of C O, present in the pyranose ring, while the broad band between 1134 cm^{-1} and 983 cm^{-1} is a characteristic contribution of C OH bending [36]. While the broad band at around 2924.09 cm^{-1} (between 3000 cm^{-1}–2800 cm^{-1}) is attributed to the vibration of the methyl group –CH [49], the peaks between 3200 cm^{-1}–3600 cm^{-1} are attributed to OH stretching vibration [50]. The absorption of 1647.21 cm^{-1} was due to bound water [42].

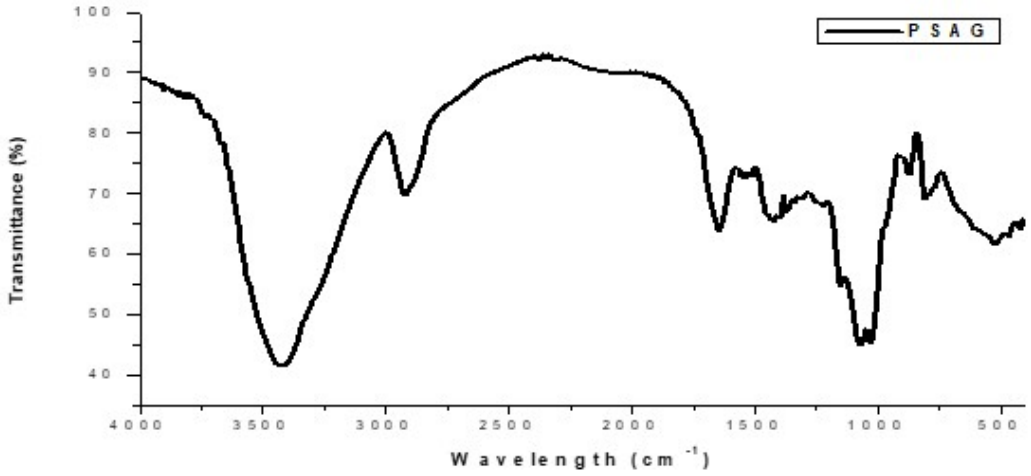

Figure 3. FT-IR spectra of PSAG (polysaccharides extracted from *Astragalus gyzensis* Bunge. seeds).

3.3.3. Thin Layer Chromatography (TLC)

The polysaccharides extract obtained from the seeds of *Astragalus gyzensis* Bunge. was hydrolyzed in an acid medium to obtain free monosaccharide units, which were characterized by the TLC technique. The first system showed the sample migrate with two spots: R_{f1}=0.43 and R_{f2}=0.54, like those obtained from the migration of the galactose and mannose patterns, respectively. The second system showed that it migrates with four similar spots: R_{f1}=0.18, R_{f2}=0.22, R_{f3}=0.45 and R_{f4}=0.531, as those obtained from the migration of galacturonic acid, glucuronic acid, galactose and mannose profiles, respectively. This is consistent with the results obtained previously for neutral sugar and uronic acid. Our results are similar to the result of TLC when the hydrolysate galactomannan from the *Senna tora* (Fabaceae) seeds showed two spots. The spots were identified as galactose and mannose by comparing their R_f values with standards of pure galactose and mannose [36]. Like the result of TLC of galactomannan from *Adenanthera avonine* L. (Fabaceae), it showed that it was comprised of mannose and galactose unities [13]. This result suggests that the constituent polysaccharides of PSAG are of a galactomannan type. It is well known that seeds of the genus Astragalus are a valuable source of galactomannans, which have been found in 16 species of this genus [24].

Table 2. The R_f values of the separated spots of PSAG (polysaccharides extracted from *Astragalus gyzensis* Bunge. seeds) and the pure standards (A. Gal : galacturonic acid, A. Glc: glucuronic acid, Ara: arabinose, Gal: galactose, Glc: glucose, Man: mannose and Rha: rhamnose) using system 1 and 2.

	A. Gal	**A. Glc**	**Ara**	**Gal**	**Glc**	**Man**	**Rha**
Standards (system1)	0.15	0.21	0.54	0.43	0.48	0.54	0.69
PSAG (system1)	/	/	/	0.43	/	0.54	/
Standards (system2)	0.18	0.22	0.537	0.45	0.51	0.531	0.64
PSAG (system2)	0.18	0.22	/	0.45	/	0.531	/

3.4. Evaluation of antihyperglycemic activity

In vitro antihyperglycemic effects of polysaccharides extracted from *A. gyzensis* Bunge. seeds was quantified and compared to acarbose using a positive control measuring the inhibition of α-amylase and α-glucosidase activity.

3.4.1. Inhibition of α-amylase activity

The inhibitory activity of PSAG extract on α-amylase was investigated in this study and the results shown in figure 4. From 0–1 mg/mL of PSAG and acarbose, there was a dose dependent increase in the percentage of inhibition. Then, from 1–2.5 mg/mL of PSAG and acarbose, there was a slight increase. From 2.5–5 mg/mL, PSAG and acarbose possessed certain stability. PSAG extract had a strong inhibitory effect on α-amylase activity with an IC_{50} value of 0.8±0.005 mg/mL, compared with acarbose as a positive control with an IC_{50} value of 0.295±0.006 mg/mL. The inhibitory effects of polysaccharides extracted from the plant seeds were considerably better against α-amylase. Two galactomannans fractions from *Alhagi maurorum* Medik. seeds showed IC_{50} values of 5.43 mg/mL and 6.81 mg/mL [34]. However, the inhibition of α-amylase activity by a polysaccharides fraction extracted from the seeds of *Plantago ciliata* Desf. showed an IC_{50} value of 3.60 mg/mL [18]. Another galactomannan from soybeans, *Glycine max* (L.) Merrill, is under investigation, as it possesses inhibitory activity against some pancreatic amylase associated with digestion of starches [51]. Two polysaccharides fractions isolated from wheat bran exhibited a competitive inhibition of α-amylase [16].

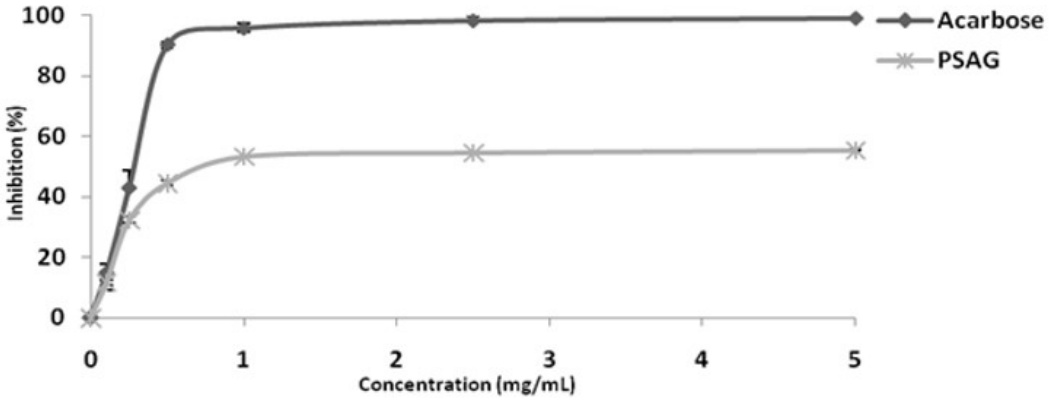

Figure 4. Inhibition of α-amylase activity using PSAG (polysaccharides extracted from *Astragalus gyzensis* Bunge) and acarbose.

3.4.2. Inhibition of α-glucosidase activity

α-Glucosidase is a key digestive enzyme that participates in the body's carbohydrate metabolism and cuts glucose from the non-reducing end of the polysaccharides by hydrolyzing the α-1,4-glycosidic bond. α-glucosidase inhibitors are an effective strategy in reducing post-prandial hyperglycemia [52]. The inhibitory activity of PSAG extract on α-glucosidase was investigated in this study and the results shown in figure 5. From 0–0.75 mg/mL of PSAG and acarbose, there was a dose dependent increase in the percentage of inhibition. Then, from 0.75–2.5 mg/mL of PSAG and acarbose, there was a small increase. From 2.5–5 mg/mL, PSAG and acarbose possessed certain stability.

The results of the inhibition of α-glucosidase activity showed that PSAG had a weak inhibitory effect on α-glucosidase with an inhibition value of 12.93% at 5 mg/ml respectively, compared with acarbose as a positive control with an inhibition value of 100% at 5 mg/ml. However, the inhibition of α-glucosidase activity by a polysaccharides fraction extracted from the seeds of *Plantago ciliata* Desf. showed a better IC_{50} value of 10 mg/mL [18]. Zhu et al. [53] reported a good inhibitory effect of α-glucosidase by a polysaccharides (APS) extract from dried Radix Astragalus. Two polysaccharides fractions that were isolated from wheat bran exhibited a mixed-type non-competitive inhibition of α-glucosidase [16]. The α-glucosidase inhibitory activities of polysaccharides were closely related to their monosaccharide compositions, molecular weights, and type of glycosidic linkages [54]. Further, *in vivo* experiments confirmed that galactomannan extracted from *Retama reatam* can reduce the glycemic index of starchy foods and inhibit the surge of postprandial blood glucose level [48].

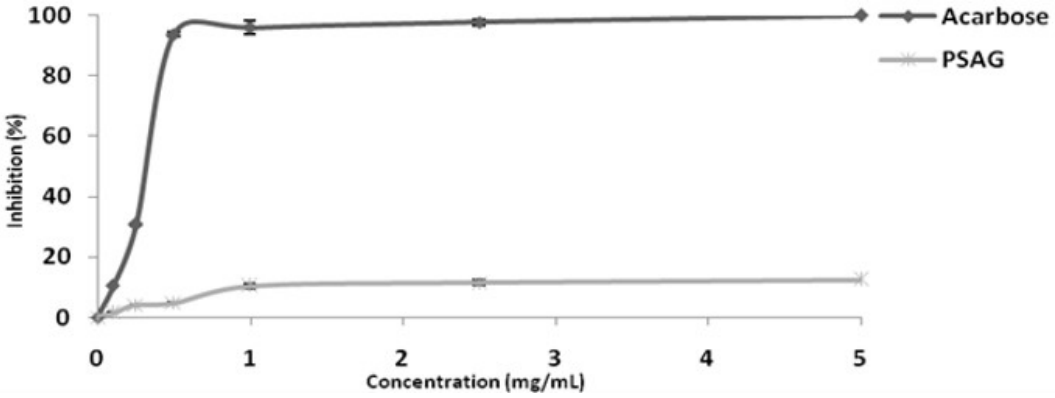

Figure 5. Inhibition of α-glucosidase activity using PSAG (polysaccharides extracted from *Astragalus gyzensis* Bunge seeds) and acarbose.

3.5. Evaluation of antioxidant activity by DPPH radical scavenging assay

Diabetes is closely associated with oxidative stress because hyperglycemia causes oxidative stress and produces free radicals, which can induce diabetic complications such as endothelial dysfunction and atherosclerosis [55]. The DPPH utilizes a scavenging mechanism causing hydrogen transfer from the antioxidant to hydrazyl DPP (radical) to convert it to hydrazine DPP. This is to avoid the presence of active free radicals, which can degenerate proteins, lipids and DNA in the human body or food, leading to degenerative diseases. Hydrogen transfer occurs through a possible reaction between the radical and the amine or amide groups present in the antioxidant [56]. In this context, the scavenging ability of PSAG on DPPH radical was investigated by comparison with ascorbic acid. PSAG possessed a weak antioxidant activity on DPPH radical with a high value of IC_{50}=1.339 mg/mL. This value is higher than that obtained by Boual et al. [42], a value of IC_{50}=33 µg/mL from a galactomannan extracted from *Astragalus armatus*. Furthermore, fenugreek galactomannan exhibits little antioxidant activity through lowered lipid peroxidation and elevated levels of antioxidant enzymes [57]. As inferred from FT-IR analysis, amide or amine groups are absent in the spectra of PSAG, therefore, it has low antioxidant activity. The biological activities of polysaccharides are correlated to their structure. The bond type as well as the number and position of branches present in the polymer chain strongly influence the three-dimensional arrangement, and in addition to the molecular size, these factors determine the behavior of the polysaccharides. Physical properties, such as solubility, viscosity, and gelation, can also influence biological activity as they can affect bioavailability. Some studies have proposed that polysaccharides have significantly different average molecular weights, however, fractions with similar monosaccharides compositions may display the same biological activity. Therefore, elucidating the molecular structures of polysaccharides present in medicinal plants is very important to predict their biological behaviour [44].

4. CONCLUSION

In this study, we reported for the first time the isolation, partial characterization and *in vitro* biological investigations of the water-soluble polysaccharides extracted from *Astragalus gyzensis* Bunge. seeds. The extraction method used gave a high yield. The chemical composition mainly consisted of neutral sugar. The partial characterization of the polysaccharides proposed they are of a galactomannans type by using TLC and FT-IR. The result showed an anti-diabetic effect through the inhibition of α-amylase, but a low inhibition of α-glucosidase and a low antioxidant effect, thus making it suitable for use in the supplemental dietary fiber of diabetic individuals. Further investigation on purification, total characterization, *in silico*, *in vitro* and *in vivo* studies will be carried out to understand the specific inhibitory mechanisms of antihyperglycemic activity of the polysaccharides extracted from the seeds of *Astragalus gyzensis* Bunge.

Authors' contributions: AT: conception and design, methodology development, data acquisition, analysis and data interpretation. ZB: revision of the manuscript, study supervision. MDOEH: project administration, methodology development, conception revision. LT and HB: laboratory administration. ZEAT, CEM, SA, IF, DLC, PD, CD, GP and PM: conception revision. All authors discussed the results and contributed to the final manuscript.

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