

Phytochemical Analysis and Evaluation of the Antioxidant, Anti-Inflammatory, Hemolytic, and Antibacterial Effects of *Astragalus gombo* (L.) Leaves

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Abstract

The purpose of this study was to determine the phytochemical content and biological activities of *Astragalus gombo* (endemic species). We conducted an HPLC analysis to identify the secondary metabolites. Antioxidant (DPPH and FRAP), anti-inflammatory, anti-hemolytic and antibacterial activities were evaluated. For the HPLC analysis, we obtained 65 peaks and identified six major bioactive compounds. The total concentration of polyphenols, flavonoids and condensed tannins varied, respectively, from 66.3 ± 0.9 mg GA eq/g, 34.31 ± 1.4 mg Q eq/g and 5.3 ± 2.7 mg Ca eq/g. In terms of antioxidant activity, the extract exhibited high inhibitory activity, equivalent to $IC_{50} = 62.81 \pm 0.01$ μ g/mL for DPPH and $IC_{50} = 19.37 \pm 0.04$ μ g/mL for FRAP. The anti-inflammatory activity was estimated to be high at 1615.8 ± 2.8 μ g/mL, the anti-hemolytic activity was weak and the antibacterial activity against the five strains under study was moderately strong. This study demonstrated that the aqueous extract of *A. gombo* from El Oued region has remarkable antioxidant, anti-inflammatory, and antibacterial activity.

Keywords: *Astragalus gombo*; phytochemical; biological activity; HPLC analysis.

1. Introduction

Medicinal plants are widely recognized for their therapeutic and nutritional benefits that help in the treatment of various illnesses and the development of new pharmaceuticals, according to a World Health Organization survey.^{1,2} Interest in the medicinal properties of plants, particularly their antioxidant, anti-inflammatory, and antibacterial effects, has notably increased. According to estimates by the World Health Organization, approximately 20,000 plant species are employed for medicinal purposes in 91 countries. The process of developing new pharmaceuticals involves several critical stages, including extraction, pharmacological screening, bioactive material isolation, characterization, toxicology, and clinical evalua-

tion.³ Polyphenol compounds, including flavonoids, tannins, and anthocyanins, have various biological activities and antioxidant properties, making them potential replacements for medical treatments.²

Algeria's diverse plant flora, with 3139 species across 150 botanical groups, includes 653 indigenous species. This has increased interest in medicinal plants with their antioxidant, anti-inflammatory and anti-bacterial capabilities significantly in recent years and among the therapeutic plants that we considered in our study is *Astragalus gombo*.⁴

Astragalus gombo is the most important genus of flowering plants and therefore of the family,^{5,6} comprising over 2,500 to 3,000 species.⁷ The species is widespread in Central Asia, South and North America, and North and

South Africa. The Algerian Sahara is also an indigenous to one of them, *Astragalus gombo*. It is a perennial plant, both common and endemic.⁸ *Astragalus gombo* thrives in well-drained, sandy loam soil with a pH range of 6.0 to 7.5. It is essential to avoid waterlogged conditions, as they can hinder root development and increase susceptibility to root diseases. This plant prefers moderate humidity levels, ideally between 40–60 %. Excessive humidity can promote fungal growth, whereas very low humidity might cause dehydration stress.⁶ In addition, optimal growth occurs at temperatures between 18 °C and 25 °C (64 °F to 77 °F). It can tolerate a slight frost, but prolonged exposure to temperatures below 10 °C or above 30 °C can negatively impact growth and yield. Further, *Astragalus gombo* requires full sunlight for at least 6–8 hours daily. Insufficient sunlight can lead to reduced photosynthetic activity and stunted growth.⁸ It is well used in the food industry, cosmetics and pharmacy. It is mainly used to treat diseases, among its active compounds are coumarins, alkaloids, tannins, phenolic acids, terpenes and flavonoids.⁹

Astragalus gombo is one of the astragalus plants found in Algerian flora. Several astragalus species are used in both traditional and modern medicine. Its leaves are used in traditional medicine to treat hemorrhoids, diabetes, leukemia and irregular menstruation, and the root is employed as an anti-stimulant.¹⁰

Considering the potential of this genus, it is important to reveal the phytochemical characteristics of *A. gombo*. The purpose of this work is to quantify the phenolic contents and identify the main phenolic compounds present in the crude extract of the leaf using HPLC analysis. The biological activity of the extract will be evaluated for antioxidant, anti-inflammatory, hemolytic, and antibacterial potentials for the first time in El Oued region (a north-east area in the Algerian Sahara).

2. Materials and Methods

2.1. Chemicals and Reagents

Sodium chloride (NaCl), Monobasic potassium phosphate (KH_2PO_4), Trichloroacetic acid (TCA), Butylated hydroxytoluene (BHT), Aluminum trichloride (AlCl_3) 2%, 2,2-diphenyl-1-picryl hydrazyl (DPPH), ascorbic acid, gallic acid, dimethyl sulfoxyl (DMSO), and ferric chloride (FeCl_3) were all obtained from Sigma-Aldrich (USA).

2.2. Preparation of Plant Material

In March 2023, during the *Astragalus gombo* flowering season, leaf portions of the plant were collected from the Southeast region of Algeria (specifically in the area of Hassi Khalifa, province of El-Oued). Professor Atef Chouikh (Faculty of Natural Science and Life, El Oued University) recognized the plant material. The aerial sec-

tion of the plant was washed with flowing water to remove dust and other extraneous objects. Then, it was powdered, dried, and kept for later use.

2.3. Preparation of Aqueous Extract

Approximately 10 grams of *Astragalus gombo* leaf powder were steeped in 100 mL of distilled water and left to stand at room temperature for 24 hours in darkness. Subsequently, filter paper was used to remove impurities. The material was extensively dried at 40 °C following extraction, according to Murugan and Parimelazhagan.¹¹ The extract was weighed and stored in a refrigerator at 4 °C for future study.

2.4. Phytochemical screening

Using the standard methods for phytochemical analysis (screening), the extract was examined for the presence of various compounds, including phenols, tannins (catechical and gallic tannins), alkaloids, steroids, saponins, flavonoids, and triterpenoids. A (+) indicates the existence of phytochemicals, whereas a (–) indicates their absence.¹²

2.5. Estimation of Total Phenolic Compounds

The total amount of phenolic was determined using the Folin-Ciocalteu method. To 1 mL of 10% Folin-Ciocalteu reagent, 0.2 mL of the aqueous extract of *A. gombo* was added. The addition of 800 L of saturated sodium carbonate (75 g/L) was made after 4 minutes. After 2 hours of incubation at room temperature, the absorbance was measured at 765 nm. To ensure that the results could be replicated, the tests were run three times.¹³ By using the linear calibration equation for gallic acid, the total phenolic content was computed as milligrams of gallic acid equivalent per gram of extract.

2.6. Estimation of Total Flavonoids

We used the aluminum chloride (AlCl_3) colorimetric method for determining the total flavonoid content of *A. gombo* extract,¹⁴ as follows: 1 mL of the AlCl_3 solution is mixed with 1 mL of the sample, and separately, with 1 mL of the standard. At 430 nm, the absorbance was measured, after 30 minutes against the prepared reagent blank. In order to determine the results, a linear calibration equation using quercetin as the standard was utilized. The results were represented as milligrams of quercetin per gram of extract.

2.7. Estimation of Condensed Tannin

The level of tannin in the extract was determined using spectrophotometry, according to Broadhurst and Jones¹⁵, catechin was used to make the calibration curve.

The sample was pipetted into an aluminum foil-wrapped tube along with 3.0 mL of newly prepared vanillin reagent (4% w/v vanillin in methanol), and the mixture was properly mixed before 1.5 mL of strong hydrochloric acid was added. After 15 minutes at 20 to 2 °C, the reaction's absorbance was assessed against water at 500 nm.

2. 8. HPLC Analysis

Phenolic compounds were qualitatively and quantitatively analyzed using a Shimadzu LC20 HPLC equipped with the universal injector (Hamilton 25l), an analytical column Shim-pack VP-ODSC18 (250 × 4.6 mm, 5 µm particle size), maintained at 25.0 °C and an injection volume of 20 µL. Chromatograms and UV spectra were collected using a Shimadzu UV-VIS detector SPD 20A. The mobile phase consisted of a gradient elution of a combination of acetonitrile and acetic acid (0.1%). The gradient program was initiated with 95% A and 5% B for the first 5 minutes, followed by a linear gradient to 50% A and 50% B from 5 to 25 minutes, further transitioning to 5% A and 95% B from 25 to 30 minutes, and concluded with re-equilibration at 95% A and 5% B from 30 to 35 minutes. The flow rate is maintained at 1.0 mL/min.

The sample preparation involves extracting phenolic compounds from the plant material using an aqueous solvent (ultra-pure distilled water). The resulting extract is then filtered through a 0.45 µm membrane filter to remove any particulate matter, and, if necessary, diluted with the mobile phase. The HPLC system is equipped with a UV-Vis detector set at 268 nm, a wavelength optimal for phenolic compounds based on their characteristic absorption.

Identification and quantification of chromatographic peaks were confirmed by comparison of the retention time (tR) of extract and standards.

2. 9. DPPH Free-Radical Scavenging Activity

1 mL of the DPPH• solution was mixed with 1 mL of each extract (or ascorbic acid as a control). To complete the reaction, the reaction mixture was stirred briefly and then kept at the room temperature for 30 minutes in the darkness, at 517 nm.¹⁶

2. 10.Reducing Power Assay (FRAP)

Oyaizu's methods were used to calculate the extract's reducing power. The sample was combined with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide water solution (2.5 mL, K₃[Fe(CN)₆]) at various concentrations (mg/mL) in distilled water. After aliquots of trichloroacetic acid (2.5 mL, 10% aqueous solution) were added, the mixture was incubated at 50 °C for 20 minutes before centrifuging for 10 minutes at 3000 rpm. The supernatant (2.5 mL) and filtered water were mixed with freshly prepared FeCl₃ (0.5 mL, 0.1%) solution (2.5 mL). The ab-

sorbance was measured at a wavelength of 700 nm. The use of ascorbic acid as a positive control was used.¹⁷

2. 11.Hemolytic Activity

The hemolysis experiment was conducted in accordance with the methodology outlined by Tlili and Benine, as detailed below.¹⁸ 5 mL of blood was centrifuged at 1000 rpm for 10 minutes at 40 °C in tubes containing 5.4 mg of EDTA to stop coagulation. The hemolytic assay was carried out on washed erythrocytes that were kept at 40 °C for 6 hours. 100 µL of test samples (containing an *A. gombo*) and 50 µL of erythrocyte suspension in 10 dilutions were employed. The positive and negative controls, 100 µL each of 1XPBS and 100 µL each of 1% SDS, were utilized. After that, the samples were incubated for 60 minutes in a water bath at 37 °C. 850 µL of XPB were added to the reaction mixture to bring the volume up to 1 mL. After centrifuging it for 3 minutes at 300 rpm, the hemolysis rate of the different extracts is calculated as a percentage (%) relative to the total hemolysis with a spectrophotometer at 540 nm, according to the following formula:

$$\text{Hemolysis Inhibition (\%)} = 100 - \left(\frac{DO_{\text{Sample}}}{DO_{\text{control}}} \times 100 \right) \quad (1)$$

2. 12. Anti-inflammatory Activity

The egg albumin denaturation inhibition method was used to investigate the anti-inflammatory potential of crude aqueous extract from *A. gombo*. The reaction mixture (5 mL) comprised 200 µL of fresh hen's egg albumin, 2.8 mL of phosphate buffer (pH 6.4), and 2 mL of various concentrations of the standard drug, Aspegic. 2 mL of distilled water was used instead of extract or Aspegic to prepare the control. Following a 15-minute incubation period at 37 °C in a water bath, the reaction mixtures underwent a 5-minute heating period at 70 °C. The reaction mixtures' absorbance was measured at 660 nm using a UV-visible spectrophotometer after cooling, with the buffer serving as the blank. Percentage of inhibition was calculated by using following equation¹⁹:

$$\text{Inhibition percentage} = \frac{DO_{\text{Control}} - DO_{\text{sample}}}{DO_{\text{control}}} \times 100 \quad (2)$$

2. 13.Antibacterial Activity

The agar well-diffusion method was used to evaluate the effectiveness of manufactured *A. gombo* against five hazardous bacteria. This included three Gram-negative bacteria (EC: *Escherichia coli* ATCC 8737; PA: *Pseudomonas aeruginosa* ATCC 9027; ST: *Salmonella typhimurium* ATCC 14028) and two Gram-positive bacteria (BS: *Bacillus subtilis* ATCC 6633 and LI: *Listeria innocua* CLIP 74915). The Petri plates were incubated at 37 °C for 24 hours. The inhibitory zone's diameter was measured in millimeters (mm). The antimicrobial tests were conducted in triplicate.²⁰

2. 14. Statistical Analysis

All of the experiments were performed in triplicate. The data were analyzed in Microsoft Excel and are presented as mean $\pm$ standard deviation ($n = 3$). Graphpad Prism 7 for Windows was used to calculate the IC_{50} and EC_{50} values. The analysis of variance (ANOVA) technique was used to statistically analyze the data and determine the significance level. The XLSTAT software was utilized for this purpose.

3. Results and Discussion

3. 1. Phytochemical Screening

Phytochemical test results in our study indicate the presence of secondary metabolites including alkaloids, tannins, flavonoids, terpenoids and polyphenols, in the *Astragalus gombo* extract (Table 1), albeit in varying concentrations.

Table 1. Phytochemical composition of the aqueous extract of *A. gombo*

Phytochemical composition		Observation
polyphenols		(+)
alkaloids	Mayer	(–)
	Wagner	(+)
Flavonoids		(+)
Tannins		(+)
terpenoids		(+)
Saponins		(–)

The results of the phytochemical tests reveal the richness of the extract from various active constituents. The bioactive properties of these substances include anti-oxidant, anti-inflammatory, and antibacterial effects. Results of phytochemical screening are consistent with those found by Benferdia et al.²¹ Hence, the existence of each secondary metabolite in *A. gombo* provides us with an evidence to support this plant's traditional use as a remedy for a variety of diseases.

3. 2. Quantification of Phytochemical Compounds

Total phenolic, flavonoid and condensed tannic compounds were expressed using the following equations based on the calibration curve: $Y = 0.0153x + 0.265$, $R^2 = 0.8835$ for phenolic compounds and $Y = 0.0096x + 0.0521$, $R^2 = 0.9371$ for flavonoid compounds and $Y = 0.0031x + 0.0301$, $R^2 = 0.9977$ for condensed tannin compounds (Table 2).

Table 2. Total content of phenols, flavonoids, condensed tannins.

Compounds	<i>A. gombo</i>
Polyphenols	66.3 $\pm$ 0.9
Flavonoids	34.3 $\pm$ 1.4
Tannins	5.3 $\pm$ 2.7

According to the results of the quantification of phytochemical compounds, the *A. gombo* plant contains high concentrations of flavonoid polyphenols and tannins. This may be due to the influence of climatic factors (high temperature, drought, exposure to sunlight and salinity), which stimulate the biosynthesis of these secondary metabolites.²² This proves the effectiveness of this plant and its high biological activity.

3. 3. HPLC Analysis

The HPLC results showed the presence of six compounds among nine reference compounds at the level of phenolic compounds on 65 peaks in the aqueous raw extract of *A. gombo*. The quercetin (9441.9 μ g/g) is the most abundant phenolic compound identified, while only trace amounts of other phenolic compounds including p-coumaric acid (113.1 μ g/g), caffeic acid (100.5 μ g/g), vanillic acid (63.5 μ g/g), vanillin (31.5 μ g/g), and rutin (12 μ g/g) were found in *A. gombo* (Table 3).

Table 3. Retention time and concentration of the phenolic compounds identified in the aqueous extract of *A. gombo*.

Retention time (min)	Compounds phenolic	Concentration (μ g/g extract)
5.29	Gallic acid	ND
13.39	Chlorogenic acid	ND
15.53	Vanillic acid	63.5
16.27	Caffeic acid	100.5
21.46	Vanillin	31.5
23.81	p-Coumaric acid	113.1
28.37	Rutin	12
34.79	Naringin	ND
45.05	Quercetin	9441.9

The qualitative analysis indicates the presence of quercetin, p-coumaric acid and caffeic acid in large amounts, suggesting that the plant has anti-inflammatory, antitumor and antioxidant effects,²³ and it has the ability to reduce the peroxidation of low-density lipoproteins (LDL) and the immune response.²⁴ This plant holds promise for applications in traditional medicine and drug development.

3. 4. Antioxidant Activity

The results of the DPPH assay and FRAP assay by the use of *Astragalus gombo* aqueous extract and different

standard antioxidants are summarized in Table 4. There are remarkable differences in the antioxidant capacity among the antioxidants tested; the antioxidant activity of *A. gombo* was much lower than that of the standard antioxidant with its IC₅₀ values for DPPH of 62.81±0.01 µg/mL, and the reducing power of FRAP was 19.37±0.04 µg/mL. However, ascorbic acid had the highest DPPH and reducing power activities with an IC₅₀ value of 18.79±0.05 µg/mL and 24.75±0.03 µg/mL, respectively. Additionally, statistical analysis demonstrated highly significant differences ($p < 0.001$) between the extract and the positive control (ascorbic acid). Both the DPPH and FRAP tests measure antioxidant capacity, but in different ways. The DPPH test focuses on the ability to neutralize a specific type of free radical, while the FRAP test evaluates the overall ability to reduce metal ions. Together, these tests provide a comprehensive view of the antioxidant capabilities of an *A. gombo*, showing both specific free radical neutralizing capacity and general reducing capacity.

Table 4. IC₅₀ in free-radical scavenging activity (DPPH) and Reducing Power Assay (FRAP) of the aqueous extract of *A. gombo*. a, b means with distinct letters in each column differ significantly ($p < 0.05$).

Extract/ standard	IC ₅₀ (µg/mL± SD) DPPH	EC ₅₀ (µg/mL± SD) FRAP
<i>A. gombo</i>	62.81±0.01 ^a	19.37±0.04 ^a
Ascorbic acid	18.79±0.05 ^b	24.75±0.03 ^b

The antioxidant activity findings were higher than those of ascorbic acid due to quercetin capacity to eliminate free radicals, as demonstrated by the HPLC analysis.²⁵

Moreover, P-coumaric acid and caffeic acid are phenolic compounds found in many plants, and they exhibit significant antioxidant properties due to their chemical structures, where both acids donate hydrogen atoms or electrons to neutralize free radicals, thereby preventing oxidative damage^{33,34}. Additionally, they can chelate transition metal ions such as iron, reducing their ability to catalyze the production of reactive oxygen species (ROS) through Fenton and Haber-Weiss reactions, thus limiting ROS generation.³⁵

3. 5. Hemolytic Activity

Weak hemolytic activity observed in general of crude extract of *A. gombo* was obtained at about 4.8–19.3% at 20–60 µg/mL, while SDS demonstrated the highest hemolytic activity when employed as a positive control (58.7–62.6%) and statistical analysis confirmed the significant difference ($p < 0.001$) between *A. gombo* and SDS (Figure 1).

The results of the hemolytic assay indicate that the extract exhibits a less significant hemolytic effect when in contact with human erythrocytes. This reduced hemolytic activity is attributed to the absence of saponins in the extract.²⁶ Clinical use of saponins is limited due to their hemolytic activity, which mediates toxicity in animals and humans. The mechanism of destruction of the erythrocyte membrane using saponins (hemolysis) is not yet fully elucidated,^{9,27} reported that saponin interacts with the sterols present in the membranes of erythrocytes and produces hemolytic reactions. This leads to rupture of the erythrocyte membrane, resulting in an increase in cell permeability and the loss of hemoglobin. Another mechanism involved in hemolysis has also been explored,²⁸ in which the

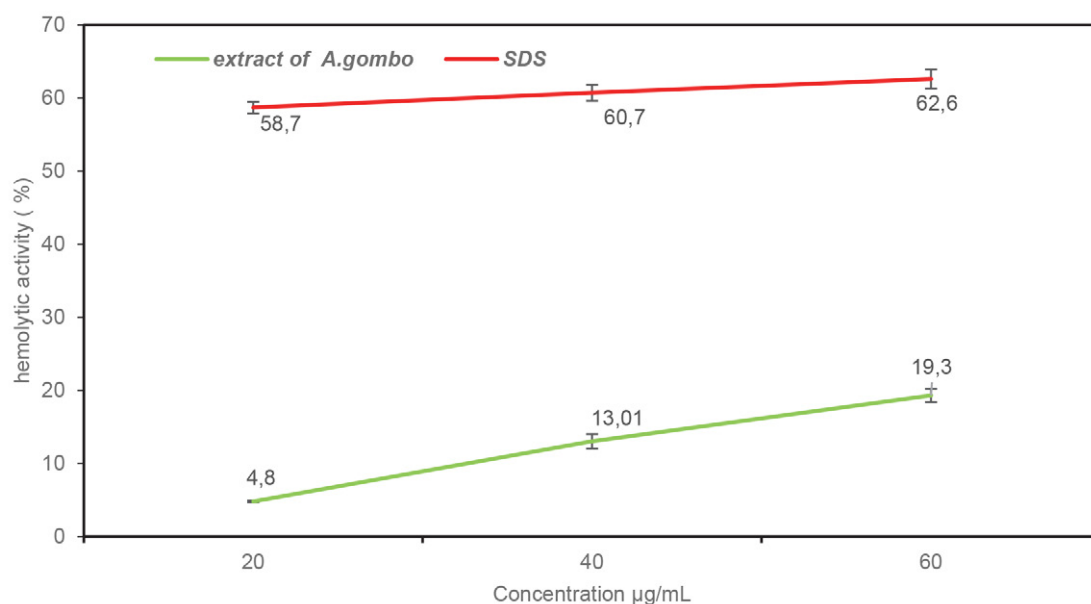


Figure 1. Hemolytic activity of *A. gombo* on red blood cells compared to 1% SDS.

extensive interaction between saponins and water channels, aquaporins, induces an increase in membrane permeability due to the entry of water molecules into the cells, causing the rupture of erythrocytes and the appearance of hemolysis.

3. 6. Anti-inflammatory Activity

The anti-inflammatory activity is very important ($IC_{50}=1615.81\pm2.8\text{ }\mu\text{g/mL}$). The percentage of inhibition of protein denaturation increased with increasing concentration (Figure 2). However, its anti-denaturation effects are generally lower compared to Aspegic.

Statistical analysis ($p < 0.001$) confirms a significant difference in anti-inflammatory activity between *A. gombo* and Aspegic.

Most biological proteins lose their efficacy when denatured. The ability of a substance to inhibit the denaturation of proteins implies its obvious potential for anti-inflammatory activity.

The findings show that the extract *A. gombo* has remarkable anti-inflammatory property. This activity may be attributed to the strong occurrence of polyphenolic compounds including alkaloids, flavonoids, tannins, steroids, and phenols.³³ The extract elements function as free radical inhibitors or scavengers by acting as primary oxidants and they are capable of controlling the production of autoantigens and inhibiting heat-induced albumin denaturation. Furthermore, p-coumaric acid, found abundantly in many plants, exhibits significant anti-inflammatory effects by inhibiting key enzymes and signaling pathways involved in

inflammation.^{34,36} It reduces the production of pro-inflammatory mediators like nitric oxide (NO), prostaglandins, and cytokines by downregulating the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Additionally, p-coumaric acid interferes with the activation of the nuclear factor-kappa B (NF- κ B) pathway, a critical regulator of inflammatory responses. By preventing NF- κ B activation, p-coumaric acid decreases the transcription of various inflammatory cytokines, thereby reducing overall inflammation in plant tissues.³⁶

Similarly, quercetin has well-known anti-inflammatory effects via multiple mechanisms. It inhibits the release of histamine and other inflammatory mediators from mast cells and basophils.³² Quercetin also downregulates the activity of COX-2 and lipoxygenase (LOX), leading to a reduction in the synthesis of pro-inflammatory prostaglandins and leukotrienes. Understanding these mechanisms highlights the importance of these compounds in plant defense and offers potential insights into their applications in medicinal contexts.²⁹

3. 7. Antibacterial Activity

The results presented in Table 5 clearly reveal a remarkable effect of the aqueous extract of *Astragalus gombo* leaves on the five strains studied. The extract exhibited notable antibacterial activity across all tested strains, with zones of inhibition varying from 8 to 11.3 mm. We also noted that in all the types of bacteria studied, the increase in the concentration of plant extract led to an increased zone of inhibition.

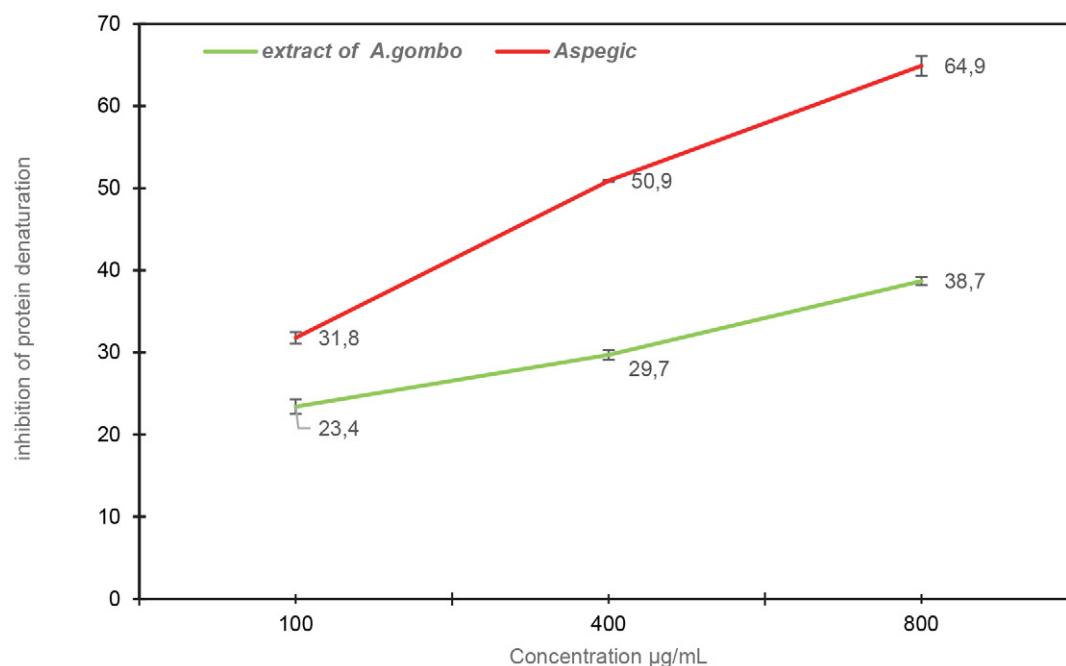


Figure 2. Anti-inflammatory activity of an aqueous extract of *A. gombo*.

Table 5. Result of the antibacterial activity of *A. gombo* extract on bacteria.

samples	Concentration ($\mu\text{g/mL}$)	Zone of inhibition (mm)				
		<i>Bacillus subtilis</i>	<i>Salmonella typhi</i>	<i>Listeria innocua</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
<i>A. gombo</i>	1000	8 $\pm$ 0.8	11.3 $\pm$ 0.5	10 $\pm$ 0.4	11 $\pm$ 0.1	10.5 $\pm$ 0.6
	750	8 $\pm$ 0.5	10 $\pm$ 0.3	10 $\pm$ 0.2	10 $\pm$ 0.1	9 $\pm$ 0.3
	500	8 $\pm$ 0.3	10 $\pm$ 0.7	9.6 $\pm$ 0.6	10 $\pm$ 0.1	8 $\pm$ 0.7
	250	8 $\pm$ 0.6	9.66 $\pm$ 0.8	8.7 $\pm$ 0.1	9.5 $\pm$ 0.1	8 $\pm$ 0.9
DMSO	–	6	6	6	6	6
Erythromycin	15	33	22	22	22	23
Ofloxacin	5	23	25	29	29	30

According to the results of the antibacterial assay, the wide range of antibacterial activity can be explained by the presence of a variety of active secondary metabolites in the extract.³⁰ This inhibitory action could be due to tannins and flavonoids, which have long been used as antimicrobial agents. In this study, it was proven that tannins exhibit antibacterial activity against Gram+ and Gram-negative microorganisms.⁷ Tannic acid impedes bacterial adhesion to surfaces and inhibits the absorption of sugars and amino acids, thereby restricting bacterial growth.³¹ Also, the ability of tannins to penetrate the bacterial cell wall and into the inner membrane interferes with cell metabolism, consequently leading cell death. Tannic acid works by blocking the NorA efflux pump, which is believed to be the primary mechanism behind its antibacterial effects.

The activity of flavonoids is due to their ability to combine with extracellular and soluble proteins, as well as with bacterial cell walls.³² Furthermore, P-coumaric acid exhibits significant antibacterial activity by disrupting bacterial cell membranes and interfering with cellular functions. It disrupts membrane integrity, leading to leakage of cellular contents and eventual cell death.³⁷ Additionally, p-coumaric acid inhibits bacterial enzyme systems involved in cell wall synthesis and metabolic processes, thereby impairing bacterial growth and survival.

Similarly, caffeic acid, another widely occurring phenolic compound in plants, demonstrates potent antibacterial effects through multiple mechanisms.³⁴ It disrupts bacterial cell membranes, leading to increased membrane permeability and leakage of intracellular components. Caffeic acid also interferes with bacterial DNA replication and protein synthesis, thereby further inhibiting bacterial growth and proliferation. Moreover, it enhances the plant's immune response by activating defense-related genes and pathways, contributing to its overall antibacterial efficacy.³⁵ Additionally, quercetin also interferes with bacterial enzyme systems crucial for energy production and metabolic processes, leading to impaired bacterial growth and survival.³⁷

4. Conclusions

In this study, we familiarized ourselves with the *A. gombo* plant and characterized its phytochemical and bio-

logical activities. The plant is rich in secondary metabolites, contains high concentrations of polyphenols and flavonoids. As for biological activity, it shows strong antioxidant activity through analysis of DPPH and FRAP, which opens up opportunities for protection and treatment of problems and diseases caused by oxidative stress. With regard to anti-inflammatory activity, it has shown significant activity, which allows it to be used as a treatment for infection. In addition, hemolytic activity was low and safely usable without causing the lysis of red blood cells. The antibacterial activity was remarkable. The *Astragalus gombo* plant could be used for its proven biological efficacy at different levels, which opens up promising prospects. It encourages its application in the medical field to treat a range of illnesses.

Conflict of interest

Authors declare no conflict of interest.

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Povzetek

Namen te študije je bil določiti vsebnost fitokemijskih snovi in biološko delovanje endemične vrste *Astragalus gombo*. Za identifikacijo sekundarnih metabolitov smo izvedli analizo HPLC. Ocenili smo antioksidativno (DPPH in FRAP), protivnetno, antihemolitično in antibakterijsko delovanje. Pri analizi HPLC smo dobili 65 vrhov in identificirali šest glavnih bioaktivnih spojin. Skupna koncentracija polifenolov, flavonoidov in kondenziranih taninov je znašala $66,3 \pm 0,9$ mg GA eq/g, $34,31 \pm 1,4$ mg Q eq/g in $5,3 \pm 2,7$ mg Ca eq/g. Kar zadeva antioksidativno aktivnost, je izvleček pokazal močno inhibitorno delovanje, in sicer $IC_{50} = 62,81 \pm 0,01$ µg/ml za DPPH in $IC_{50} = 19,37 \pm 0,04$ µg/ml za FRAP. Protivnetna aktivnost je bila ocenjena kot visoka ($1615,8 \pm 2,8$ µg/ml), antihemolitična aktivnost je bila šibka, antibakterijska aktivnost proti petim preučevanim sevom pa srednje močna. Ta študija je pokazala, da ima vodni izvleček *A. gombo* iz regije El Oued izjemno antioksidativno, protivnetno in antibakterijsko delovanje.



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