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**CHEMICAL AND BIOLOGICAL INVESTIGATION OF *ASTRAGALUS  
SIEBERI* FRUITS FROM NORTHERN BORDER REGION KSA**

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**ABSTRACT**

**Objective:** Valuable attention has been spent to genus *Astragalus* due to its biodiversity, phytoconstituents, economical and therapeutic benefits. The present study aims to investigate the chemical profile and biological activities of the Saudi population of *Astragalus sieberi* fruits growing in Northern Border Region.

**Materials and methods:** Biological activities including Cytotoxic, antimicrobial activities, and antioxidant have been investigated. The phytochemical profiling, determination of total phenolic content (TPC) and total Flavonoid content (TFC) in addition to HPLC tracing of common flavonoids and phenolic acids have been also determined.

**Results:** phytochemical screening revealed for the first time the presence of valuable phytoconstituents including phenolic compounds, flavonoids, triterpenes, alkaloids and saponins. The biological activity testing showed promising cytotoxic activities against all tested cancer cell

lines (HepG-2, HCT-116 and A-549), while no pronounced antimicrobial effects have been determined.

**Conclusion:** For the first time, *A. sieberi* fruits have been investigated for their biological activity and chemical constituents. This study showed valuable phytoconstituents and promising cytotoxic activities, further investigation is recommended for this valuable natural sources.

**Keywords:** *Astragalus sieberi* fruits, phytochemical profile, cytotoxic activities, antioxidant activity

## INTRODUCTION

Despite the great success and rapid development of anticancer drugs, determination of the proper treatment of various cancer diseases remains the major challenge for medical and paramedical researchers. In this concern, natural product chemists try with valuable success to share these efforts and present active natural products that help in achievement of this goal. Many natural sources (e.g. medicinal plants) produce valuable natural products that inhibits the cancer developments and / or treat the cancer disease either directly by inhibiting the cancer cell formation, activation of cell apoptosis, or indirectly through immunostimulation or targeting the carcinogens expression pathways. Polyphenolic compounds, flavonoids, polysaccharides and saponins have been reported to possess antioxidant, immunotimulation and anticancer activities through various pathways. Genus *Astragalus* is related to the fabaceous family and includes about 2500 species, [1]. The *Astragalus* plants are distributed worldwide

especially in the Mediterranean region [2]. Great no. of research articles have reported the medicinal, economic and biologic values of different phytoconstituents from *Astragalus* species [3-5]. several valuable effects (immune-stimulation, hepato-protection, analgesia, sedation and antiaging effects) have been reported to the roots of Asian species (*A. membranaceus*), this root is prescribed in traditional Chinese Medicines (TCM) to alleviates wide array of diseases [6-10]. Recent studies reported the cytoprotective and genoprotective effects of *Astragalus* derived natural products (*Astragalus* polysaccharides) against cytotoxicity and genotoxicity of formaldehyde [11-13]. *Astragalus* polysaccharides showed also a pronounced effect against sepsis-induced Acute kidney injury [14], immunomodulation [15], and hepatoprotection [16]. *Astragalus* polysaccharides have been reported to promotes the regeneration of intestinal stem cells through HIF-1 signalling pathway [17], ameliorates experimental

colitis [18]. Promising anti-inflammatory effects of *Astragalus* extracts and isolated compounds through different mechanisms including suppression of different pro-inflammatory mediators expression [19-27]. Furthermore, previous studies have also reported the cardioprotective effects [28-32], antioxidant [33], antitumor [29, 30, 31], antidiabetic [34-35], antidiabetic peripheral neuropathy [36], antidiabetic nephropathy [37-39] and antiaging [40] activities of *Astragalus* species. More than 130 Cycloartane triterpenoidal compounds have been isolated from *Astragalus* species [41], in addition to more than 20 oleanane-type triperpenes [41-42], more than 60 flavonoid derivatives [41], bioactive polysaccharides and other miscellaneous phytoconstituents [43-45] have been also reported for *Astragalus* species. our previous study of *Astragalus* species dealt with the chemical profiling and cytotoxic activities of the arial parts of three *Astragalus* species growing in the northern border of Saudi Arabia [46]. The present research study was adopted to evaluate specifically the cytotoxicity and antioxidant activity of *Astragalus sieberi* fruits together with recognition of its chemical profile.

## MATERIAL AND METHODS

### Plant Material and Phytochemical Screening

The plant sample of the present study was collected in March-April from Wadi Arar of the Northern region of Saudi Arabia (30° 55' 13" N, 41° 0' 3" E). Voucher specimen (ASF 20231) were kept in the herbarium of Pharmacognosy and alternative medicine Dept., College of Pharmacy, Northern Border University, Rafhaa, Saudi Arabia. The fruits were air dried completely in shade. After drying and grinding, the powder was extracted with 70% methanol till exhaustion; the extracts were dried under vacuum using rotatory evaporator at 45°C till dryness and kept in refrigerator.

Phytochemical screening of *Astragalus sieberi* fruits extract was carried out using the previous procedure [46] which tests the presence of related substances to the main phytochemical classes, including carbohydrates/glycosides, alkaloids, anthraquinones, flavonoids, saponins, sterols/triterpenes, proteins/aminoacids, tannins/polyphenolic compounds and cardiac glycosides.

### Determination of Total Flavonoid Content (TFC) and Total Polyphenolic Content (TPC)

Determination of TFCs was carried out using AlCl<sub>3</sub> colorimetric method [47], and the

determination of TPCs was carried out using Folin–Ciocalteu method [47] with minor changes. The results were obtained after spectrophotometric analysis using UV/Vis spectrophotometer (PD-303UV, APEL Ltd., Japan).

2 g of each powder was extracted three times with 100 ml of 70% hydromethanol solution using magnetic stirrer. The combined extract was concentrated to 20 ml each. The concentrated extract was defatted with petroleum ether, then divided into two equal portions. The first portion was diluted with distilled water to 200 ml for total flavonoid content assay, while the other portion was diluted with distilled water to 1000ml for total phenolic assay.

For determination of TFC, 1ml of each diluted sample (1/200 ml) was assayed colorimetrically using ALCL3 spectrophotometric assay and serial dilutions of rutin (quercetin-3-O-rutinoside) to obtain standard calibration curve. Briefly, 1 ml of diluted extract or standard solution of rutin (5-150 µg/ml) was mixed with 2 ml of dist. H<sub>2</sub>O and 300 µL of 5% sod. nitrite. After incubation for five minutes, 300 µL of 5% aluminum chloride was added. Then, 1 ml of sod. hydroxide (1 M conc.) was added and the total volume was adjusted to be 5 ml using dist. H<sub>2</sub>O. The absorbance at 510 nm was

recorded using APEL-spectrophotometer. Total flavonoid content was expressed as mg rutin equivalents (RE) per gram dry plant sample using a regression equation ( $y = 500.78x + 4.3799$ ) obtained from standard calibration curve of rutin samples. The determination of total flavonoid contents in the samples was carried out in triplicate and the results were averaged.

For determination of TPC, 1ml of each diluted sample (1/1000 ml) was assayed colorimetrically using Folin–Ciocalteu spectrophotometric assay and serial dilutions of tannic acid (deca-galloyl glucose). Briefly; 1 mL of standard tannic acid concentrations (12.5 - 125 µg/ml) or diluted sample solution and 1ml dist. H<sub>2</sub>O in addition to 1.0 ml of Folin–Ciocalteu reagent (10-fold diluted) were admixed thoroughly, 4 min later, then 1 ml of 10% sod. carbonate was added, after that, the mixture was allowed to stand for 1½ hrs. at room temperature. The absorbance was measured at 750 nm using APEL-spectrophotometer. The concentration of the total phenolics was calculated as mg of tannic acid equivalent (TAE) using a regression equation ( $y = 67.39x - 6.9995$ ) obtained from tannic acid calibration curve. The determination of total polyphenolic contents in the samples was carried out in triplicate and the results were averaged.

### HPLC Tracing of Flavonoid and Phenolic Acid Markers

For HPLC tracing of common flavonoids and phenolic acids of *A. sieberi* fruit extract, the defatted samples 10 microliters was injected into an HPLC using Software: Win Chrome Chromatography Version 1.3; adopted with UV/vis Detector (GBC); LC 1110 Pump (GBC); KROMASIL column 150 x 4.6 mm; Flow Rate: 0.8 ml/minute (for flavonoids) and 1.0 ml/minute (for phenolic acids); Detection: UV 356 nm (for flavonoids) and UV 280 nm (for phenolic acids); the eluent used was composed of [Acetonitrile/water/formic acid, (85:14:1)] (for flavonoids) and Methanol/water/tetrahydrofuran/acetic acid, (23:75:1:1) (for phenolic acids). Five common flavonoid markers (kaempferol, luteolin, quercetin-3-O-rutinoside (rutin), apigenin, and quercetin) and seven phenolic acids (gallic, chlorogenic, caffeic, coumaric, ferulic, cinnamic, and syringic acids) were used as reference compounds in this experiment. The presence of these compounds as well as their concentrations in the sample were traced using HPLC analytical experiment.

### Antioxidant Activity

The antioxidant activity of *Astragalus* fruits was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical

scavenging assay [48]. the experiment was repeated three times and the results were averaged. 0.004% w/v methanol solution of DPPH radical was prepared and stored at 9°-10°C in amber-colored bottle. A 40 µl of the sample was added to 3 ml of DPPH solution. Then the Absorbance was recorded at 515 nm using UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance was determined consequently with data being recorded at 1-minute intervals until the absorbance was stabilized. The absorbance of DPPH radical without antioxidant (control) and the reference compound (Ascorbic acid) was also measured. All determinations were measured three times and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:  $PI = \{(AC-AT)/(AC)\} \times 100$  ; Where: AC = Absorbance of the control at  $t = 0$  minute, AT = absorbance of the test sample + DPPH at  $t = 16$  minutes.

### Cytotoxic Activity

The total extract of the fruit sample was diluted with distilled water, defatted with n-hexane, and fractionated into two fractions; F1 (ethyl acetate fraction) and F2 (rest of the total extract). The obtained fractions of each sample were tested for cytotoxic activity using three mammalian cell lines: HepG-2 cells

(human hepatocellular cancer cell line), HCT-116 (colon carcinoma), and A-549 cells (human lung carcinoma) which were obtained from VACSERA Tissue Culture Unit Egypt. The procedure for cytotoxicity evaluation was applied using viability assay [46].

### Antimicrobial Activity

Antimicrobial activity tests for F1 and F2 fractions were carried out using well diffusion method [46] at antimicrobial activity unit in the Regional Center for Mycology and Biotechnology, Faculty of Science, Al-Azhar University, Cairo, Egypt, using six known microbial pathogens. These microorganisms were *Aspergillus fumigatus* (RCMB 002008), *Candida albicans* RCMB 005003 (1) ATCC 10231, *Staphylococcus aureus* (RCMB 010010), *Bacillus subtilis* RCMB 015 (1) NRRL B-543, *Proteus vulgaris* RCMB 004 (1) ATCC 13315 and *Escherichia coli* (RCMB 010052) ATCC 25955; the diffusion agar procedure was applied, Well diameter: 6.0 mm; sample volume: 100 µl; where Ketoconazole (100 µg/ml), Gentamycin (4 µg/ml) were used as positive control, the samples were tested at 10 mg/ml concentration.

## RESULTS

### Phytochemical Screening

The phytochemical screening test revealed the presence of carbohydrates / glycosides, sterols

/ triterpenes, flavonoids, tannins / phenolic compounds, and saponins, while it shows traces only of alkaloids / nitrogenous compounds and absence of anthraquinones and cardiac glycosides.

### Determination of TFC and TPC

The experiment for determination of TFC of *A. sieberi* fruit extract revealed the average concentration of the flavonoid content at 5.08 mg RE/g plant powder. Whereas the average concentration of TPC was 12.14 mg TAE/g plant powder.

### HPLC Tracing of Flavonoid and Phenolic Acid Markers

The results of HPLC tracing of flavonoid markers in *A. sieberi* fruit extract determined the average concentrations of the flavonoid markers as 25.4, 10.6, 21.3, 27.1, 13.5 µg/g plant powder for the common flavonoids kaempferol, luteolin, quercetin-3-O-rutinoside (rutin), apigenin, and quercetin respectively, whereas the common phenolic acids: gallic, chlorogenic, caffeic, coumaric, ferulic, cinnamic, and syringic acids have been detected at concentrations 2.61, 2.95, 0.61, 2.59, 3.42, 0.59, 2.59 µg/g plant powder respectively.

### Antioxidant Assay

The result of antioxidant activity assay determined the average IC<sub>50</sub> of *A. sieberi* fruit extract (the concentrations responsible for

50% DPPH scavenging activities) = 155.3 µg/ml. while IC<sub>50</sub> of the reference material (ascorbic acid) was 14.2 µg/ml.

### Cytotoxic Activity

The activity of the fractions, F1 (ethyl acetate fraction) and F2 (rest defatted methanol fraction), of *Astragalus sieberi* fruit extract against three human cell lines (HepG-2, HCT-116, and A-549 cells), showed promising results, with IC<sub>50</sub> 34.7, 29.6, and 47.9 µg/ml respectively for fraction F1 and 103.0, 45.9, and 56.1 µg/ml respectively for F2.

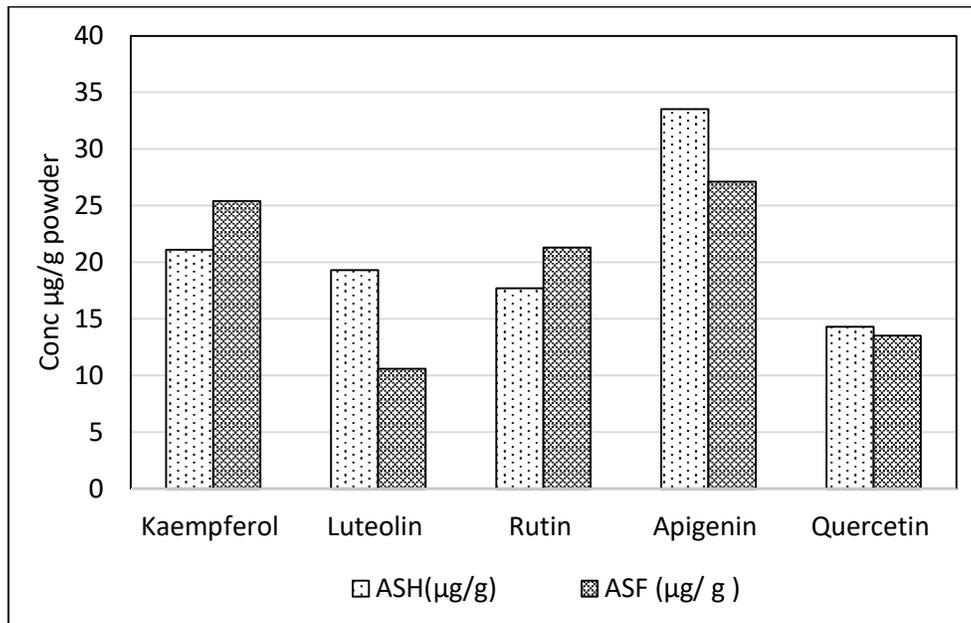
### Antimicrobial Activity

The preliminary antimicrobial activity testing results were expressed as zone of inhibition in mm beyond 6 mm well diameter. The experiment showed weak antimicrobial effects of both fractions against all microbial pathogens except *Escherichia coli* (RCMB 010052) showed 9 mm inhibition zone due to F1, while *Proteus vulgaris* RCMB 004 (1) ATCC 13315) showed 8 mm inhibition zone due to F2.

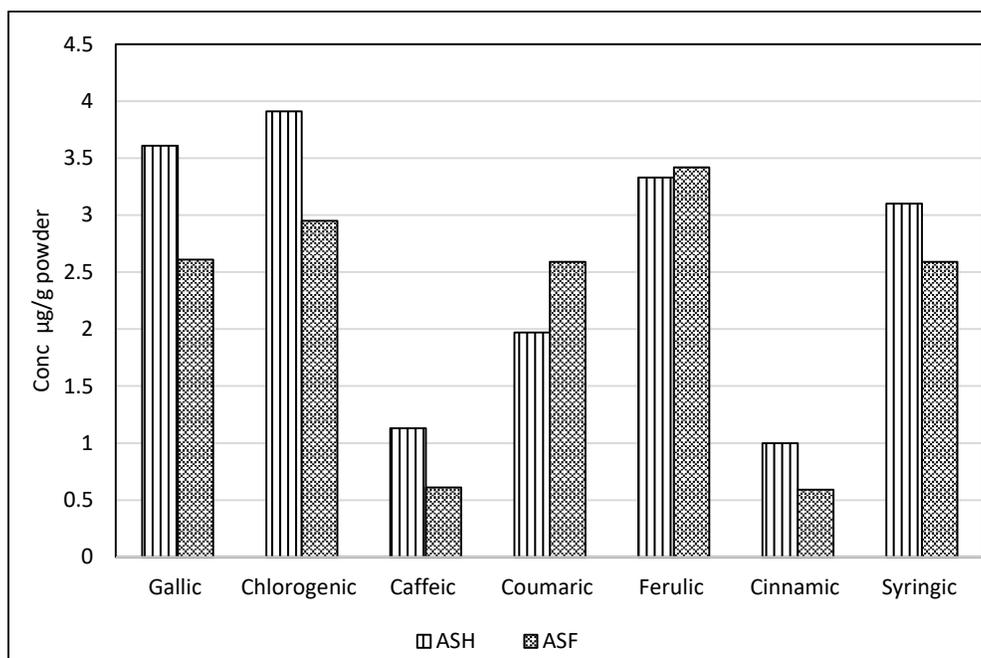
### DISCUSSION

Natural sources continue offer great benefits and support the human health from different ways, they submit the required trace elements, vitamins, fine chemicals and beneficial

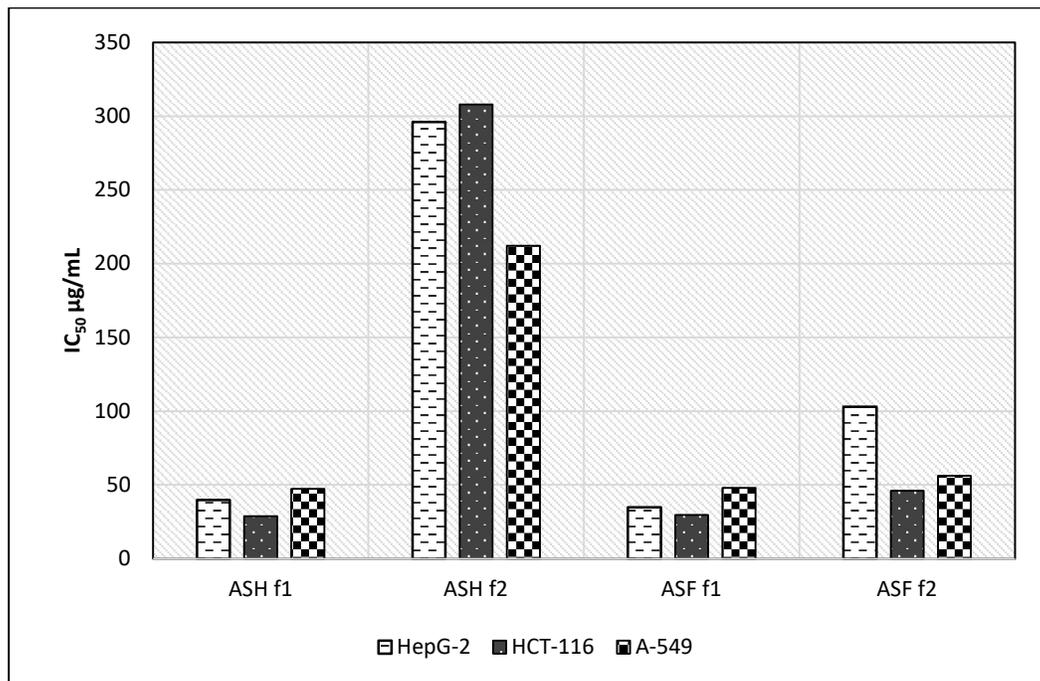
natural products, for treatment of specific diseases, compensate the suboptimal health conditions, counteract the mechanisms for health issues, and/or minimizes the health issue complications. The present research highlighted the chemical and biological profile of *A. sieberi* fruits and focused on the phytochemical compounds, HPLC tracing of common flavonoids and phenolic acids, determination of TFC, and TPC, in addition to antioxidant effect, antimicrobial activity against 6 microbial pathogens, and cytotoxic activity against three different Tumor cell lines. However, the average concentrations of the common flavonoids and phenolic acids, antioxidant activity and the cytotoxic activity results are different from those reported (Ashour, 2019) for the aerial part of the same Saudi population of *A. sieberi*. (See **Figures:1, 2, 3, 4**). It was found that the cytotoxicity of F1 is comparable to that of F1 fraction of aerial part of the same species (ASH f1), while fraction (ASF f2) showed cytotoxic activity stronger than that of (ASH f2) as seen in **Figure 3**. It may explain by the presence of bioactive phytoconstituents e.g. flavonoids, saponin and / or polysaccharide contents.



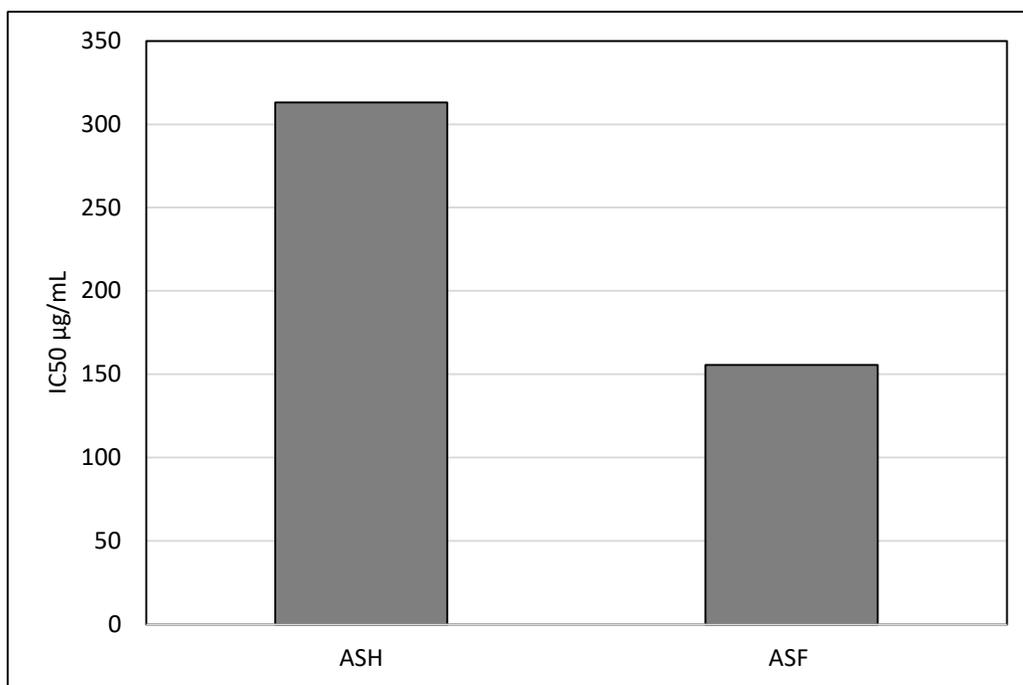
**Figure 1:** HPLC tracing of common flavonoids in *A. sieberi* fruit (ASF) compared with reported data [46] of *A. sieberi* aerial part (ASH)



**Figure 2:** HPLC tracing of common phenolic acids in *A. sieberi* fruit (ASF) compared with reported data [46] of *A. sieberi* aerial part (ASH)



**Figure 3:** Cytotoxic activity (IC<sub>50</sub>) of *A. sieberi* fruit extracts (ASF f1 & ASF f2) against three cancer cell lines compared with the data reported [46] for *A. sieberi* aerial part (ASH f1 & f2)



**Figure 4:** antioxidant activity determined as 50% DPPH scavenging concentration (IC<sub>50</sub>) of *A. sieberi* fruit total extract (ASF) compared with the data reported [46] for total extract of *A. sieberi* aerial part (ASH)

## CONCLUSION

The present study revealed, to the first time, the presence of a reasonable quantities of valuable phytoconstituents of *A. sieberi* fruits, and determined their antioxidant and cytotoxic activities. Further studies and experiments still on road in order to uncover the specific phytoconstituents especially saponins and polysaccharides which are common in genus *Astragalus*, and provide several therapeutic benefits.

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## Declaration of Conflicting Interests

The authors indicated no conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Statement of Informed Consent and Ethical Approval

Before beginning the study, the participants provided the necessary ethical clearances and informed consent.

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