

DOI: <https://dx.doi.org/10.21123/bsj.2022.6769>

## Phytochemical profile, Antioxidant, Enzyme inhibitory and acute toxicity activity of *Astragalus bruguieri*

Ahmed Aj Jabbar<sup>1\*</sup>  Kamaran Kaiani Abdulrahman<sup>2</sup>  Parween Abdulsamad<sup>3</sup>   
Sharoukh Mojarrad<sup>4</sup>  Güldal Mehmetcik<sup>5</sup>  Abdullah Sh Sardar<sup>4</sup> 

<sup>1</sup>Department of Medical Laboratory Technology, Erbil Technical Health and Medical College, Erbil Polytechnic University, 44001, Erbil, Iraq

<sup>2</sup>Chemistry Department, College of Science, Salahaddin University, 44001, Erbil, Iraq

<sup>3</sup>Chemistry Department, College of Education, Salahaddin University, 44001, Erbil, Iraq

<sup>4</sup>Biology Department, College of Education, Salahaddin University, 44001, Erbil, Iraq

<sup>5</sup>Department of Medical biochemistry, Faculty of Medicine, Cyprus International University, Mersin 10, Turkey

\*Corresponding author: [ahmed.abuljabbar@edu.epu.iq](mailto:ahmed.abuljabbar@edu.epu.iq)

E-mail addresses: [kamaran.abdulrahman@su.edu.krd](mailto:kamaran.abdulrahman@su.edu.krd), [parween.ismail@su.edu.krd](mailto:parween.ismail@su.edu.krd), [sharoukh.mojarrad@gmail.com](mailto:sharoukh.mojarrad@gmail.com), [gmehmetcik@ciu.edu.tr](mailto:gmehmetcik@ciu.edu.tr), [abdullah.sardar@su.edu.krd](mailto:abdullah.sardar@su.edu.krd)

Received 20/11/2021, Revised 20/3/2022, Accepted 22/3/2022, Published Online First 20/7/2022,  
Published 1/2/2023



This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/).

### Abstract:

The medicinal plants (*Astragalus* species) have been used traditionally as anti-inflammatory, antioxidant, and Anti-diabetics. The current research investigates the phytochemistry and some biological activity of methanol extract of different parts of *Astragalus bruguieri* Bioss., a wild medicinal plant grows on Safeen mountain, Erbil, Iraq. The methanol extracts of *A. bruguieri* were analyzed for total phenolic, flavonoid, and saponin contents. *In-vitro* antioxidant activity was analyzed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. Furthermore, the plant extracts were examined for *in-vitro* enzyme inhibitory activity and *in-vivo* sub-acute toxicity. The results have shown the highest total phenolic (28.83, 20.62 mg GAEs/g extracts) contents, in the leave and root extracts, respectively. While the highest total Flavonoid (50.08, 44.01 mg REs/g) contents, were found in the extracts of aerial parts and leaves, respectively. The total saponin was higher (25.33, 23.18 mg GAEs/g extracts) in the roots and aerial parts, respectively. *In-vitro* antioxidant measurement by (DPPH) assay showed leaves as superior part in this activity (42.19mg TE/g extract), while antioxidant evaluation by (ABTS) assay indicated roots as the most active part (86.90mg TE/g extract). The  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activity were found as 0.45-0.67, and 1.2-1.8 mmol ACAEs/g, respectively. The oral acute toxicity test indicated the safety of 600mg/kg dosage of different parts of *A. bruguieri* on albino rats without behavioral abnormality or mortality. The current study is considered as the first report on the *A. bruguieri* as a possible new source of biocompatible material for many industrial products.

**Keywords:** Acute toxicity, Antioxidant, *Astragalus bruguieri*, Enzyme inhibitory, Phytochemistry.

### Introduction:

Herbal-based medicine gained more popularity as curative agents for various health problems because of the drawbacks related to synthetic chemical compounds. In contrast, phytochemical compounds that are secondary metabolites of plants show multiple pharmacological activities with their safer advantages than chemically based drugs<sup>1, 2</sup>. The interest in herbal medicine has increased after finding the pathogenesis route of diseases such as

diabetes and conditions related to oxidative stress. A condition that will develop due to the disability of the body's antioxidant defense system (including innate elements superoxide dismutase, catalase, and hydro peroxidase and acquired antioxidants from the plant) to neutralize the excess amount of reactive oxygen species (ROS) such as superoxide, singled oxygen, and H<sub>2</sub>O<sub>2</sub> produced by cell metabolism. Realizing these facts has motivated scientists to search for pharmacologically active

antioxidants to help the antioxidant defense system fight various diseases<sup>3-5</sup>. The ROS can stimulate oxidative stress and cell apoptosis, which may lead to a series of health conditions like chronic inflammatory proliferative diseases if they were not treated<sup>6</sup>. Synthetic chemicals have been used in controlling oxidative stress and cell apoptosis such as butylated hydroxy anisole (BHA) used as blockage of ROS production in cerebral glioma cells and as a food preservative<sup>6</sup>. However, scientists have warned consumers about the aftermath of these synthetic antioxidants on human health because of their carcinogenic effect on human genes<sup>7</sup>. Thus, a recent plethora of works has shown interest in searching for natural antioxidant and anti-proliferative agents to replace chemical synthetics<sup>8,9</sup>.

Diabetes is considered a series of health problems related to islet  $\beta$  cell dysfunction, glucose immobilization, and lipid metabolism. Diabetes is usually classified into two types based on the insulin production in patients. Approximately 90 % of all diabetes patients falls under type II diabetes and if they were not treated, they will face serious health condition and organ failure<sup>10</sup>. Medical pathways have been progressed in dealing with diabetes in recent years. One of these new approaches is stabilizing postprandial hyperglycemia immediately after meals<sup>11</sup>. This stabilization can be achieved by controlling glucose release into the circulation by inhibiting enzymes rolling in carbohydrate digestion<sup>12</sup>. Food carbohydrates are digested into oligosaccharides by  $\alpha$ -Amylase and then into monosaccharides by  $\alpha$ -glucosidase in the small intestine<sup>13</sup>. Blood glucose levels can be prevented from rising through inhibiting enzymes involved in carbohydrate metabolism<sup>14</sup>. The current synthetic chemicals seem to be effective as hypoglycemic agents, however, many of these chemical drugs need upgrading for better treating outcomes<sup>15</sup>. For instance, metformin is a synthetic drug that can effectively adjust glucose metabolism; however, researchers have shown its side effects on the functionality of islet cells<sup>16</sup>. As an alternative for synthetic anti-diabetics, searching for  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors in natural sources like plants, become a continuous mission by scientists<sup>2,17</sup>.

*Astragalus* L. is the largest vascular plant with nearly 2900 species in the Fabaceae family. The traditional use of *Astragalus* species as a remedy root back to more than two millenniums<sup>18</sup>. *Astragalus* comprises a major part in the curative usage of Chinese folk medicine, alongside other Asian countries, particularly, Iran, Pakistan, and

Korea. The *Astragalus* species was traditionally used for curing different health problems, including hypertension, stomach pain, laxatives, kidney disease, and diabetes<sup>19</sup>. The previous studies on the *Astragalus* species and their phytochemicals showed a significant exhibition of the biological activities by this plant such as antioxidant, anti-inflammatory<sup>20</sup>, immunostimulant<sup>21</sup>, enzyme inhibitory<sup>22</sup>, anti-tumor<sup>23</sup>, and anti-diabetes<sup>24</sup>. The phytochemicals namely polysaccharide, polyphenolic, and saponins were mainly correlated with the various bioactivities of *Astragalus* species. Such plant metabolites could be beneficial or toxic to humans<sup>25</sup>. The same is true with synthetic drugs which may be curative in a certain amount and hazardous at a certain level<sup>26</sup>. To guarantee the safe usage of natural products, certain quality measurements must be on targeted herbs before approving as a natural medicinal agent<sup>27</sup>. In today's drug industry, the development of more than one-quarter of drugs becomes expensive due to their toxicity studies<sup>28</sup>. Acute toxicity and sub-acute toxicity are regular tests used by scientists to check the safety of natural or synthetic compounds. The toxicity test is also considered as the borderline to determine the Lethal dosage to kill 50% of animals (LD<sub>50</sub>), the downside of targeted compounds after single-dose administration within a certain period<sup>29</sup>. The administration is usually made through the oral cavity of laboratory animals (rats or mice) to assess the median lethal dose (LD<sub>50</sub>) for a specific biocompatible material or plant extract<sup>30</sup>. The current study is inspired by the traditional usage of *Astragalus* species and is considered as the first record of the chemical composition and biological activities of *A. bruguieri*.

## Materials and Methods:

### Plant collection

In May 2020, the whole part of *A. bruguieri* was gathered from Safeen mountain/Shaqalawa in Erbil, Iraq (Latitude: 36°18'08.2"N, Longitude: 44°25'18.9E) (Fig. 1). The authentication was completed by botanist Prof. Dr. Abdullah Sh. Sardar and the plant details were deposited from the Education Salahaddin University Herbarium (ESUH), Erbil, Iraq. (voucher no. 7841).



Figure 1. The general appearance of *A. bruguieri*

### Sample preparation

The plant parts, roots, stems, leaves, and aerial portions of *A. bruguieri* were air-dried, and (500 g) was obtained from each. Then they were macerated in 500 mL of methanol for 24 hours. The filtration was performed by using Whatman grade 1 paper. After that, the methanol was separated by using a rotary evaporator. The resulting extracts were kept at 4°C until they were examined<sup>31</sup>.

### Phytochemical composition

The total phenolic, flavonoids, and saponins were measured for the roots, stems, leaves, and aerial parts of *A. bruguieri* by using spectrophotometer accordingly with the previous studies<sup>32, 33</sup>.

### Biological activity

The antioxidant activity of the methanolic extracts of different parts of *A. bruguieri* by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays were measured as previously described<sup>4</sup>. The inhibitory activity on  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity of different parts of *A. bruguieri* was estimated by using spectrophotometer as previously explained<sup>17</sup>.

### 7-day repeated-dose oral toxicity Experiment in rats

A group of rats was chosen at random and kept in their cages for at least 5 days in animal house of Educational College, Salahaddin University, where the study performed. Before

dosing, the animals were kept without food for the night but had access to water. The rats were individually marked to allow for acclimation to the laboratory settings.

A total of 15 rats were chosen randomly and assigned into five groups (3 rats in each group). The control group (G1) had free access to food and water with no supplementations, while the treated groups (G2, G3, G4, and G5) received 1 dose per 600 mg/kg bw/day extracts of roots, stems, leaves, and aerial parts, respectively for 7 consecutive days (600mg considered as the standard dosage for the safety test of the plant)<sup>25</sup>. Food was provided after 1-2 hours of dosing. Observation of animals began immediately for the first 30 minutes and then following the oral dose. The record continued for 7 days every 8 hours. Clinical symptoms of toxicity, such as intake of food and water, convulsion, the overall behavior, and death of treated animals, were noted for seven days<sup>26, 34</sup>.

## Results and Discussion:

### Chemical profile

The extraction yield of various parts of *A. bruguieri* extracts was between 7.26 to 14.43%. The leaves extraction yield was the highest followed by the aerial parts, stems, and roots, respectively (Table 1). In the current study, total phenolic and flavonoid values were significantly different between the extracts, and it ranged from 12.96±0.37 to 28.83±0.58mg GAEs/g extract and 10.68±0.13 to 50.8±0.61mg REs/g extract, respectively.

The data shown in Table 1 indicate that the total phenolics was significantly higher in leaves 28.83 mg/g than that of 20.62, 17.85, 12.96 mg/g for roots, aerial parts, and stems, respectively. The total flavonoid was higher in aerial parts 50.8 mg/g than that of 44.01, 11.39, 10.68 mg/g for leaves, stems, and roots, respectively. The total saponin was higher in roots 25.33 mg/g than that of 23.11, 21.47, 13.38 mg/g for aerial parts, leaves, and stems, respectively.

Table 1. Extraction yield, total phenolic and total flavonoid contents of methanolic extracts of different parts of *A. bruguieri*.

Assays	Roots	Stems	Leaves	Aerial parts
Yields (%)	7.26	7.57	14.43	12.68
Total phenolic (mg GAEs/g extract)	20.62±0.26 <sup>b</sup>	12.96±0.37 <sup>d</sup>	28.83±0.58 <sup>a</sup>	17.85±0.45 <sup>c</sup>
Total flavonoids (mg REs/g extract)	10.68±0.13 <sup>c</sup>	11.39±0.19 <sup>c</sup>	44.01±0.86 <sup>b</sup>	50.8±0.61 <sup>a</sup>
Saponins (mg GAEs extract)	25.33±0.47 <sup>a</sup>	13.38±0.15 <sup>d</sup>	21.47±0.18 <sup>c</sup>	23.11±0.89 <sup>b</sup>

-The variety of subscripts in the same rows show the variances between plant parts by Tukey's test at  $p < 0.05$ . GAEs, REs, and: gallic acid, rutin equivalents, respectively. Data represented as mean±standard deviation (n=5).

Total phenolic estimation is considered a reliable method to estimate the phenolic contents in plant extracts. The phenolic compounds as secondary metabolites have been reported repeatedly as antioxidant materials against various free radicals<sup>4</sup>. The results of the chemical profiling of *A. bruguieri* showed a significant difference in the phytochemical contents of different plant parts. Similarly, Platikanov et al. reported variances in the phenolic concentration of various parts of *Astragalus* spp. In the current study, the leaves and roots were superior in terms of total phenolic content and the aerial parts were superior in terms of total flavonoid<sup>35</sup>. Similarly, previous phytochemical studies on *A. glycyphyllos* by Butkute et al, have found increased levels of total phenolic (25.99 and 23.71 mg GAE/g) and total flavonoids (21.00 and 16.71 mg RE/g) contents in leaves and flowers, respectively<sup>36</sup>. Furthermore, a chemical study on *A. Gombiformis* reported the lowest phenolic (3.340–9.194 mg GAE/g DW) and flavonoid (0.767–3.133 mg CE/g DW) contents in roots and stems, respectively<sup>37</sup>. Accordingly, the current study shows roots and stems as the poorest parts in terms of total phenol and total flavonoids.

According to our literature search, data on the phytochemical were not published elsewhere. But previous research studies have correlated the phenolic contents of *Astragalus* species with its higher bioactivity, viz. antioxidant<sup>4, 14, 17, 32</sup>. The phenolic compounds are also linked with improving defense mechanisms through reversing ROS formation, increasing cell survival, and decreasing nuclear damages and microorganism attacks<sup>38-41</sup>.

Flavonoids are considered secondary metabolites of the plants known as potent antioxidant agents<sup>42</sup>. The flavonoids are also used as a flavoring and food coloring agent<sup>43</sup>. The presence of hydroxyl group flavonoids is thought to be the reason behind their ability to scavenge free radicals. Saponins are another chemical that is found in significant amounts in different parts of *A. bruguieri* that is confirmed as a main chemical compound isolated from *Astragalus* species. Saponins, namely Cycloartane- and oleanane-type glycosides were isolated from *Astragalus* species and reported to have different biological activity, immune-stimulating<sup>21</sup>, cytotoxicity<sup>8</sup>, and anti-inflammatory activity<sup>20</sup>. The literature search did not show any previous study on the chemical profile of *A. bruguieri* and thus, the current work is considered as the first investigation of the phytochemistry of this species.

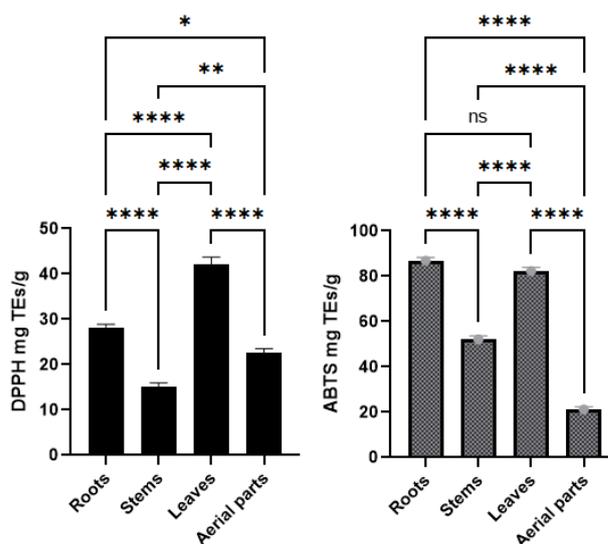
### Antioxidant activity of methanolic extracts *A. bruguieri*

In the current study, DPPH radical scavenging of different parts of *A. bruguieri* ranged between 15.07±0.89 and 42.19±1.5mg TE/g extract, and it varied significantly ( $p < 0.05$ ) between the plant extracts. The antioxidant activity of leaves was higher 42.19±1.5 TE/g than that of 28.07±0.81, 22.53±1.0, and 15.07±0.89 TE/g extract for roots, aerial parts, and stems, respectively (Fig. 2). The ABTS scavenging activity of roots was higher (86.90±1.4mg TE/g extract) than that of 82.3±1.67, 52.14±1.54, and 21.23±1.19 TE/g extract for leaves, stems, and aerial parts, respectively (Table 2).

**Table 2. The antioxidant activity of *A. bruguieri* by DPPH and ABTS radical scavenging.**

Plant organs	Assay	
	DPPH radical(mg TE/g extract)	ABTS radical cation (mg TE/g extract)
Roots	28.07±0.81 <sup>c</sup>	86.90±1.4 <sup>b</sup>
Stems	15.07±0.89 <sup>d</sup>	52.14±1.54 <sup>c</sup>
Leaves	42.19±1.5 <sup>b</sup>	82.3±1.67 <sup>b</sup>
Aerial Parts	22.53±1.0 <sup>d</sup>	21.23±1.19 <sup>d</sup>
Significance	*	*

- The variety of subscripts in the same columns show the variances between different parts by Tukey's test at  $p < 0.05$ . TE: trolox equivalents. Significance: \*( $p < 0.05$ ). Data represented as mean±standard deviation(n=5).



**Figure 2. Antioxidant activity of different parts of *A. bruguieri*.**

DPPH reagent has been depended upon as the reliable reagent to estimate the antioxidant activity of any biocompatible<sup>1, 3, 6</sup>. Natural antioxidants are considered safer than synthetic chemicals in preventing oxidative damage by neutralizing and breaking the free radical chains, thus reducing

health problems resulting from oxidative degradations. While, synthetic antioxidants, such as butylated hydroxytoluene (BHT), have been correlated with many drawbacks, including liver damage and carcinogenesis. Therefore, to replace this synthetic antioxidant and avoid its side effects, natural sources have been extensively studied<sup>44</sup>.

The current study showed significant antioxidant activity exhibited by the different parts of *A. Bruguieri*. A literature search on the free radical scavenging of *A. Bruguieri* has not been reported elsewhere, but previous studies on the several *Astragalus* species reported significant antioxidant activity brought by those plants<sup>36, 37</sup>. In the last decades, researchers have linked phenolic compounds, viz. simple phenolic, phenolic acids, anthocyanin, and flavonoids of several plants with the plants, antioxidant potentials, which include free radicals scavenging, and reducing power activity<sup>45-47</sup>. Earlier studies also reported phenolic compounds as reducing agents, hydrogen givers, singlet oxygen inhibitors, and effective metal chelators because of their redox properties<sup>5, 14</sup>. The leaves and roots of *A. bruguieri* were superior in terms of antioxidant activity by both DPPH and ABTS assays, which could be correlated with phenolic, saponin, and flavonoid contents. Previous antioxidant studies on *A. Membranaceus* has correlated its increased antioxidant potentials of roots extracts with its total

polyphenol contents<sup>48</sup>. Furthermore, a previous study reported potent antioxidant activity by flavonoids isolated from *Astragalus mongholicus* Bunge, a potential adjuvant of the atherosclerosis profile and possible reducer of cardiovascular disease<sup>49</sup>. In addition, the antiradical study on *A. acmophyllus*, *A. talasseus*, *A. microcephalus*, and *A. gammifer* reported significant antioxidant potentials exhibited by their extracts and have linked this action with their increased total phenolic and flavonoid contents<sup>50</sup>. The antioxidant activity of different parts of *A. bruguieri* was found very compatible with their chemical profiles presented in Table 1, as leaves showed the highest antioxidant activity because of their higher phenolic and flavonoid contents.

### Enzyme inhibitory activity of *A. bruguieri* against selected enzymes

Data results from Table 3 show roots as the richest part in terms of the  $\alpha$ -Amylase inhibitory with the value of 0.51 ACEs /g extract followed by 0.49, 0.48, 0.46 ACEs/g extract for leaves, Aerial parts, and Stems, respectively. The  $\alpha$ -Glucosidase inhibitory activity was higher in leaves (18.27 ACEs/g extract) followed by 12.95, 3.99, and 3.21 ACEs/g extract for Aerial parts, stems, and roots, respectively.

**Table 3. Enzyme inhibitory activity of *A. bruguieri* against selected enzymes.**

Assay	Roots	Stems	Leaves	Aerial parts
$\alpha$ -Amylase inhibition (mmol ACEs/g extract)	0.51±0.01 <sup>a</sup>	0.46±0.01 <sup>b</sup>	0.49±0.008 <sup>ab</sup>	0.48±0.008 <sup>ab</sup>
$\alpha$ -Glucosidase inhibition (mmol ACEs/g extract)	3.21±0.06 <sup>c</sup>	3.99±0.08 <sup>c</sup>	18.27±0.05 <sup>a</sup>	12.95±0.03 <sup>b</sup>

- The variety of subscripts in the same rows show the variances between different parts by Tukey's test at  $p < 0.05$ . kojic acid, and acarbose equivalents. Data represented as mean±standard deviation (n=5).

There is a renewed interest in the natural inhibitors from plant-based medicines to modulate the physiological effects of enzymes linked with several pathologies such as diabetes<sup>15</sup>. The inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase which is involved in the hydrolysis of sugars *in-vivo* has been an important strategy for the management of diabetes thereby lowering postprandial glucose levels. Inhibitors of  $\alpha$ -glucosidase delay the breaking down of carbohydrates in the gut and decrease postprandial blood glucose peak in diabetic patients<sup>16</sup>.

In the past few decades several synthetic chemicals have been innovated as curative agents for diabetic Mellitus, however, none of which seemed to be free of drawbacks, thus WHO proposed the research and investigate the alternative medicines to control diabetics, plant-based  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitors seems to be promising for controlling such disease with fewer

side effects than synthetic drugs<sup>12</sup>. The present study exposed a moderate enzyme inhibitory effect of different parts of *A. bruguieri* on  $\alpha$ -amylase and a strong inhibitory effect on  $\alpha$ -glucosidase and considered as the first report on the enzyme inhibitory activity of *A. bruguieri* and can be linked with its phenolic, flavinoid, saponin contents. The literature search did not show any previous reports in that regard, however many studies have reported the antidiabetic effect of phytochemicals like phenolic, flavonoid, and saponin and linked this action with their stimulating effect insulin production<sup>17, 22</sup>. The previous study also showed antidiabetic activity of *Astragalus* polysaccharides in the diabetic mice and linked this activity with the chemical's capability to increase serum insulin levels and restore islet cell function, stimulating the protein expression in the pancreas and liver of drug-induced diabetic mice<sup>33</sup>. Similarly, a previous study has correlated the antidiabetic role of *Astragalus*

*ponticus* with increased percentage contents of its polysaccharides, saponins, and flavonoids<sup>17</sup>. The above data can be considered as a reliable source to explain the enzyme inhibitory activity of *A. bruguieri*.

### The effect of the extracts on acute oral toxicity studies

The results of the acute toxicity test for 7 days, indicated that the consumed nutrient and liquid by all rat groups were equal with no changes

in their body weight. It is suggested that the normal metabolism of lipids, carbohydrates, and proteins in the animal's body because food and water are vital to the physiology of the animal's body<sup>51</sup>. Parameters like eyes, face consistency, respiration, sleep, and urination (color) were normal during the study. Other parameters like aggressiveness, itching, coma, convulsion, and tremors were absent even after the experimental period of acute oral toxicity of methanolic extracts of different parts of *A. bruguieri* (Table 4).

**Table 4. Effect of *A. bruguieri* different extracts on behavior of rats in acute toxicity studies.**

Parameters	G1	Dosage 600mg/kg			
		G2	G3	G4	G5
Feed and water intake	N	N	N	N	N
Coma	A	A	A	A	A
Convulsion and tremors	A	A	A	A	A
Eyes	N	N	N	N	N
Faces consistency	N	N	N	N	N
Fur and skin	N	N	N	N	N
Itching	A	A	A	A	A
Respiration	N	N	N	N	N
Sleep	N	N	N	N	N
Urination (color)	N	N	N	N	N
Aggressiveness	A	A	A	A	A
Mortality	A	A	A	A	A

Key. A- Absent; P-Present; N- Normal; ↑- Increase. G1-Control rats with no supplementation; G2, G3, G4, and G5 are rats receiving one dose of 600mg/kg extracts of roots, stems, leaves, and aerial parts, respectively.

The acute toxicity test for the methanolic extract 600mg/kg of different parts of *Astragalus bruguieri* administered by rats resulted in the absence of physiological changes or rat mortality. Thus, the oral LD<sub>50</sub> of the extracts could be suggested as higher than 600 mg/kg.

The purpose of testing the safety of any biocompatible, which is claimed traditionally as a medicinal agent, is to investigate its nature and determine its side effect for the potentiality of using it as a natural medicine in repeated doses<sup>52</sup>. A literature study did not find any acute toxicity record of *Astragalus bruguieri*, however, the acute toxicity test of *Astragalus membranaceus* showed the safety of up to 1200 mg/kg bw/day of this plant on Wister rats<sup>52,53</sup>. The outcomes of the current experiment could be considered as starting line for more detailed experiments.

### Conclusion:

The current study shows the exhibition of antioxidant and enzyme inhibitory activity by the roots, stems, leaves, and aerial parts of *A. bruguieri* with roots and leaves exerting the highest activity, which may be correlated to their higher phenolic, saponins, and flavonoid contents. The acute toxicity test for different organ extracts of *A. bruguieri* on rats shown the safety of this plant as the rats have

not experienced any abnormalities in their behavior or appearance. Future research is needed to identify the active compounds and determine the mechanism of action responsible for their biological activities.

### Authors' declaration:

- Conflicts of Interest: None.
- Ethical Clearance: The project was approved by the local ethical committee in Erbil Polytechnic University.

### Authors' contributions statement:

A. A. J. has conceptualized, designed, and wrote the article. K. K. A. has analyzed the data. P. A. has participated in the writing process. Sh. M. and G. M. have analyzed the data results, and A. Sh. S. has identified and authenticated the plant species. All authors have participated equally in reviewing and the finalizing manuscript.

### References:

1. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. Discovery and resupply of pharmacologically active plant derived natural products: a review. Biotechnol Adv. 2015;

- 33(8): 1582–614. <https://doi.org/10.1016/j.biotechadv.2015.08.001>
2. Jabbar AA. *Onosma mutabilis*: Phytochemical composition, antioxidant, cytotoxicity, and acute oral toxicity. *Food Sci Nutr* 2021; 9(10): 5755–5764. <https://doi.org/10.1002/fsn3.2544>
  3. Al-Muwaly KY, Al-Flayeh KA, Ali A. Antioxidant and free radical scavenging effects of Iraqi sumac (*Rhus coriaria* L). *Baghdad Sci J*. 2013; 10(3): 921–33. <https://doi.org/10.21123/bsj.2013.10.3.921-933>
  4. Taqi RA. Phenolic Content and Antioxidant, Antibacterial Activities of Ethanolic Extract from Lemon Balm and Oregano Plants. *Baghdad Sci J*. 2014; 11(1): 103–110. <https://doi.org/10.21123/bsj.2014.11.1.103-110>
  5. AL-Muhammadi QN. Physiological study to investigate the activity of an aqueous extract of *Cinnamomum cassiabark* on the blood glucose levels in healthy and diabetic rats induced by streptozotocin (stz). *Baghdad Sci J*. 2016; 13(4): 681-693. <https://doi.org/10.21123/bsj.2016.13.4.0681>
  6. Hwang GH, Jeon YJ, Han HJ, et al. Protective effect of butylated hydroxyanisole against hydrogen peroxide-induced apoptosis in primary cultured mouse hepatocytes. *J Vet Sci*. 2015;16(1):17-23. <https://doi.org/doi:10.4142/jvs.2015.16.1.17>
  7. Sasaki YF, Kawaguchi S, Kamaya A, Ohshita M, Kabasawa K, Iwama K, et al. The comet assay with 8 mouse organs: results with 39 currently used food additives. *Mutat Res*. 2002; 519(1–2): 103–19 . [https://doi.org/10.1016/S1383-5718\(02\)00128-6](https://doi.org/10.1016/S1383-5718(02)00128-6)
  8. Bao W-R, Li Z-P, Zhang Q-W, Li L-F, Liu H-B, Ma D-L, et al. Astragalus Polysaccharide RAP Selectively Attenuates Paclitaxel-Induced Cytotoxicity Toward RAW 264.7 Cells by Reversing Cell Cycle Arrest and Apoptosis. *Front Pharmacol*. 2019; 9: 1580. <https://doi: 10.3389/fphar.2018.01580>
  9. Graziani V, Esposito A, Scognamiglio M, Chambery A, Russo R, Ciardiello F, et al. Spectroscopic Characterization and Cytotoxicity Assessment towards Human Colon Cancer Cell Lines of Acylated Cycloartane Glycosides from *Astragalus boeticus* L. *Molecules*. 2019; 24(9) 1725-1542. <https://doi.org/10.3390/molecules24091725>.
  10. Clemens KK, O'Regan N, Rhee JJ. Diabetes Management in Older Adults with Chronic Kidney Disease. *Curr Diab Rep*. 2019; 19(3): 11-29. <https://doi.org/10.1007/s11892-019-1128-3>
  11. Takahashi M, Ozaki M, Kang M-I, Sasaki H, Fukazawa M, Iwakami T, et al. Effects of Meal Timing on Postprandial Glucose Metabolism and Blood Metabolites in Healthy Adults. *Nutrients*. 2018 Nov; 10(11): 1763-1774. <https://doi.org/10.3390/nu10111763>.
  12. Telagari M, Hullatti K. In-vitro  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activity of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. extracts and fractions. *Indian J Pharmacol*. 2015; 47(4): 425–9. <https://doi.org/10.4103/0253-7613.161270>
  13. Kotowaroo MI, Mahomoodally MF, Gurib-Fakim A, Subratty AH. Screening of traditional antidiabetic medicinal plants of Mauritius for possible alpha-amylase inhibitory effects in vitro. *Phytother Res*. 2006; 20(3): 228–31. <https://doi.org/10.1002/ptr.1839>.
  14. Funke I, Melzig MF. Effect of different phenolic compounds on alpha-amylase activity: screening by microplate-reader based kinetic assay. *Pharmazie*. 2005; 60(10): 796–7 .
  15. Al-Chalabi NS, Al-Sawaf RN. Effect of Polyherbs-Mixture Composed of *Nigella sativa*, *Trigonella foenum-graceum*, *Cyperus rotundus* and *Teucrium polium* on the Levels of Malondialdehyde and Glutathione for Diabetic Patients Type II. *Baghdad Sci J*. 2013; 10(3): 854–65. <https://doi.org/10.21123/bsj.2013.10.3.854-865>
  16. Herman WH, Kalyani RR, Wexler DJ, Matthews DR, Inzucchi SE. Response to Comment on American Diabetes Association. Approaches to Glycemic Treatment. Sec. 7. In *Standards of Medical Care in Diabetes—2016*. *Diabetes Care* 2016; 39 (Suppl. 1): S52–S59. *Diabetes Care*. 2016 Jun 1; 39(6): e88-9. <https://doi.org/10.2337/dci16-0003>
  17. Abduljabbar, A. A., & Abdoulrahman, K. K. Onion (*Allium Cepa*) and Garlic (*Allium Sativa* L.) Oil effects on Blood Glucose Levels and Body Weight of Local Quails in Erbil Province. *Zanco J. of Pure and Applied Sc*. 2018; 30(5): 158–167. <https://doi.org/10.21271/zjpas.30.5.14>
  18. Knyazev MS. Review of the genus *Astragalus* section *Helmia* (Fabaceae). *Nov Sist Vyss Rastenii*. 2019; (50): 120–31 . <https://doi.org/10.31111/novitates/2019.50.120>
  19. Shahrajabian Mh. A Review of *Astragalus* Species as Foodstuffs, Dietary Supplements, A Traditional Chinese Medicine and A Part of Modern Pharmaceutical Science. *Appl Ecol Environ Res*. 2019; 17 (6):13371-13382. [https://doi.org/10.15666/aeer/1706\\_1337113382](https://doi.org/10.15666/aeer/1706_1337113382)
  20. Sevimli-Gür C, Onbaşlar İ, Atilla P, Çakar N, Deliloğlu-Gürhan İ, Bedir E. Wound healing effects of cycloartane-type triterpenes isolated from *Astragalus* species. *Planta Med*. 2009; 75(09): PA57 .
  21. Huang L, Yao Y, Li J, Zhang S, Li W, Dong N, et al. The effect of Astragaloside IV on immune function of regulatory T cell mediated by high mobility group box 1 protein in vitro. *Fitoterapia*. 2012; 83(8): 1514–22 . <https://doi.org/10.1055/s-0029-1234382>
  22. Zengin G, Ceylan R, Guler O G, Carradori S, Uysal S, Aktumsek A. Enzyme inhibitory effect and antioxidant properties of *Astragalus lagurus* extracts. *Curr Enzym Inhib*. 2016; 12(6): 177–82. <https://doi.org/10.2174/1573408012666160127231058>
  23. Li H, Zhou X, Wu M, Deng M, Wang C, Hou J, et al. The cytotoxicity and protective effects of *Astragalus membranaceus* extracts and butylated hydroxyanisole on hydroxyl radical-induced apoptosis in fish erythrocytes. *Anim Nutr*. 2016; 2(4): 376–82. <https://doi.org/10.1016/j.aninu.2016.08.004>

- 24 .Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. *J Ethnopharmacol.* 2004; 92(1): 1–21 . <https://doi.org/10.1016/j.jep.2003.12.031>
- 25 .Tchamadeu MC, Dzeufiet PD, Nana P, Nougá CK, Tsofack FN, Allard J, et al. Acute and sub-chronic oral toxicity studies of an aqueous stem bark extract of *Pterocarpus soyauxii* Taub (Papilionaceae) in rodents. *J Ethnopharmacol.* 2011 Jan 27;133(2):329–35. <https://doi.org/10.1016/j.jep.2010.09.035>.
- 26 .Sharif HB, Mukhtar MD, Mustapha Y, Baba G, Lawal AO. Acute and Subchronic Toxicity Profile of *Euphorbia pulcherrima* Methanol Extract on Wistar Albino Rats. *Adv Pharm.* 2015; 2015: 1–9 .
- 27 da Silva Moreira S, Tamashiro LK, Jorge BC, da Silva Balin P, Heredia-Vieira SC, de Almeida GL, et al. Toxicological safety evaluation in acute and 28-day studies of aqueous extract from *Serjania marginata* Casar. (Sapindaceae) leaves in rats. *J Ethnopharmacol.* 2019; 231: 197–204 . <https://doi.org/10.1016/j.jep.2018.11.024>.
28. Guengerich FP. Mechanisms of drug toxicity and relevance to pharmaceutical development. *Drug Metab Pharmacokinet.* 2011; 26(1): 3–14. <https://doi.org/10.2133/dmpk.dmpk-10-rv-062>
29. Brígido HPC, Varela ELP, Gomes ARQ, Bastos MLC, de Oliveira Feitosa A, do Rosário Marinho AM, et al. Evaluation of acute and subacute toxicity of ethanolic extract and fraction of alkaloids from bark of *Aspidosperma nitidum* in mice. *Sci Rep.* 2021 ;11(1): 18283. <https://doi.org/10.1038/s41598-021-97637-1>
- 30 .Morris-Schaffer K, McCoy MJ. A Review of the LD50 and Its Current Role in Hazard Communication. *ACS Chem Heal Saf.* 2021; 28(1): 25–33. <https://doi.org/10.1021/acs.chas.0c00096>
31. Chauhan CK, Joshi MJ, Vaidya AD. Growth inhibition of struvite crystals in the presence of herbal extract *Commiphora wightii*. *J Mater Sci Mater Med.* 2009 Dec; 20(1): 85-92. <https://doi.org/10.1007/s10856-008-3489-z>
32. Ahmed Aj.Jabbar, Fuad O. Abdullah, Kamaran K. Abdulrahman, Yaseen Galali, A. S. S.. *Papaver Decaisnei* : GC-MS Alkaloids Profiling , in Vitro Antioxidant , and Anticancer Activity. *Research Square,* 1–18. <https://doi.org/10.21203/rs.3.rs-1207324/v2>.
- 33 .Ani emmanuel, Adekunle AA, Aboluwade JB, Ibrahim O. EX-SITU Characterization of *Luffa aegyptiaca* in Lagos State, Nigeria. *Baghdad Sci J.* 2020; 17(3 Suppl.): 946-952. [https://doi.org/10.21123/bsj.2020.17.3\(Suppl.\).0946](https://doi.org/10.21123/bsj.2020.17.3(Suppl.).0946)
- 34 .Wang W, Dong Z, Zhang J, Zhou X, Wei X, Cheng F, et al. Acute and Subacute Toxicity Assessment of Oxyclozanide in Wistar Rats. *Front Vet Sci.* 2019; 6: 294. <https://doi.org/10.3389/fvets.2019.00294>
35. Platikanov S, Nikolov S, Pavlova D, Evstatieva L, Popov S. Volatiles from four *Astragalus* species: phenological changes and their chemotaxonomical application. *Z Naturforsch C.* 2005; 60(7–8): 591–9 .
- 36 .Butkutė B, Dagilytė A, Benetis R, Padarauskas A, Cesevičienė J, Olšauskaitė V, et al. Mineral and phytochemical profiles and antioxidant activity of herbal material from two temperate *Astragalus* species. *Biomed Res Int.* ID 6318630. <https://doi.org/10.1155/2018/6318630>
- 37 .Teyeb H, Houta O, Najjaa H, Lamari A, Neffati M, Douki W, et al. Biological and chemical study of *Astragalus gombiformis*. *Z Naturforsch C.* 2012; 67(7–8): 367–74 . <https://doi.org/10.1515/znc-2012-7-803>
- 38 .Świątek M, Lu Y-C, Konefał R, Ferreira LP, Cruz MM, Ma Y-H, et al. Scavenging of reactive oxygen species by phenolic compound-modified maghemite nanoparticles. *Beilstein J Nanotechnol.* 2019; 10: 1073–88. <https://doi.org/10.3762/bjnano.10.108>
39. Jabbar AA, Saeed CH, Abdulaziz SM, Mahmood BJ. Chemical Differentiation and Antimicrobial Potential of Four *Brassica napus* L Seed Oils. *Iraqi J Sci.* 2021 Dec; 30: 4597-613. <https://doi.org/10.24996/ijs.2021.62.12.1>
40. Abudoulrahman KK, Mustafa MA, Abduljabbar AA. The Effect of Heat Stress on Oxidative Stress and Antioxidant Status in Local Quail Hens Supplemented with Onion and Garlic Oils. *Tikrit J Agric Sci.* 2019 Apr 8; 19(1): 103-10. DOI: <http://dx.doi.org/10.25130/tjas.v19i1.356>
41. Jabbar AA. Gastroprotective and Immuno-Supportive Role of *Alcea kurdica* against Stress Induced Lesion in Japanese Quails. *Baghdad Sci J* 2022; 19(4):716–724. <https://doi.org/10.21123/bsj.2022.19.4.0716>
- 42 .Jabbar, A. A., Abdullah, F. O., Abdulrahman, K. K., Galali, Y., & Sardar, A. S. GC-MS Analysis of Bioactive Compounds in Methanolic Extracts of *Papaver decaisnei* and Determination of Its Antioxidants and Anticancer Activities. *J. of Food Quality.* 2022; 2022: 1405157. <https://doi.org/10.1155/2022/1405157>
43. .Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. *J Nutr Sci.* 2016; 5: e47 .
- 44 .Paponov IA, Budnyk V, Paponov M, Teale W, Palme K. Butylated Hydroxytoluene (BHT) Inhibits PIN1 Exocytosis from BFA Compartments in Arabidopsis Roots. *Front Plant Sci.* 2020; 11: 393. <https://doi.org/10.3389/fpls.2020.00393>
45. Dehghani N, Afsharmanesh M, Salarmoini M, Ebrahimnejad H, Bitaraf A. Effect of pennyroyal, savory and thyme essential oils on Japanese quail physiology. *Heliyon.* 2018; 4(10): e00881. <https://doi.org/10.1016/j.heliyon.2018.e00881>
46. .Brglez M E, Knez H M, Škerget M, Knez Ž, Bren U. Polyphenols: Extraction Methods, Antioxidative Action, Bioavailability and Anticarcinogenic Effects. *Molecules.* 2016 Jul; 21(7): 901. <https://doi.org/10.3390/molecules21070901>
47. Shahidi F, Yeo J. Bioactivities of Phenolics by Focusing on Suppression of Chronic Diseases: A Review. *Int J Mol Sci.* 2018; 19(6): 1573.
48. Auyeung KK, Han Q-B, Ko JK. *Astragalus membranaceus*: A Review of its Protection Against Inflammation and Gastrointestinal Cancers. *Am J*

- Chin Med. 2016; 44(1): 1–22.  
<https://doi.org/10.1142/S0192415X16500014>
49. Wang D, Zhuang Y, Tian Y, Thomas GN, Ying M, Tomlinson B. Study of the Effects of Total Flavonoids of Astragalus on Atherosclerosis Formation and Potential Mechanisms. *Oxid Med Cell Longev.* 2012 Jan 29; 2012: 1-10  
<https://doi.org/10.1155/2012/282383>
50. Albayrak S, Kaya O. Antioxidant and antimicrobial activities of four Astragalus species growing wild in Turkey. *Turkish J. Biochem.* 2018 Aug 1; 43(4): 425-34. <https://doi.org/10.1515/tjb-2017-0241>
51. Taj S, Irm M, Jin M, Yuan Y, Andriamialinirina HJT, Zhou Q. Effects of Dietary Carbohydrate to Lipid Ratios on Growth Performance, Muscle Fatty Acid Composition, and Intermediary Metabolism in Juvenile Black Seabream (*Acanthopagrus schlegelii*). *Front Physiol.* 2020; 11: 507.  
<https://doi.org/10.3389/fphys.2020.00507>
52. Murbach TS, Glávits R, Endres JR, Hirka G, Vértesi A, Béres E, et al. Toxicological Evaluation of a Mixture of Astragalus membranaceus and Panax notoginseng Root Extracts (InnoSlim®). *J Toxicol.* 2019;2019: 5723851.  
<https://doi.org/10.1155/2019/5723851>
53. Song J, Lee D, Min B, Bae JS, Chang GT, Kim H. Safety evaluation of Astragalus extract mixture HT042 and its constituent herbs in Sprague-Dawley rats. *Phytomedicine.* 2017;32:59-67.  
<https://doi.org/10.1016/j.phymed.2017.03.005>

## الملف الكيميائي النباتي، مضادات الأكسدة، مثبط الإنزيم ونشاط السمية الحادة من *Astragalus bruguieri*

احمد عبد الجلال عبد الجبار<sup>1</sup>      كامة ران كياني عبدالرحمان<sup>2</sup>      بروين عبدالصمد<sup>3</sup>      شاروخ مجرد<sup>4</sup>  
كولدل محمدجيك<sup>5</sup>      عبدالله شكور سردار<sup>4</sup>

- <sup>1</sup> قسم تقنيات المختبرات الطبية، كلية أربيل للتقنيات الصحية، جامعة بوليتكنيك أربيل، أربيل، 44001، العراق.
- <sup>2</sup> قسم الكيمياء، كلية العلوم، جامعة صلاح الدين – أربيل، 44001، العراق.
- <sup>3</sup> قسم الكيمياء، كلية التربية، جامعة صلاح الدين – أربيل، 44001، العراق.
- <sup>4</sup> قسم العلوم الحياتية، كلية التربية، جامعة صلاح الدين – أربيل، 44001، العراق.
- <sup>5</sup> قسم الكيمياء الحيوية الطبية، كلية الطب، جامعة قبرص الدولية، مرسين 10، تركيا.

### الخلاصة:

تم استخدام النباتات الطبية (أنواع استراغالوس) تقليدياً كمضاد للالتهابات ومضاد للأكسدة ومضاد لمرض السكر. يبحث البحث الحالي في الكيمياء النباتية وبعض النشاط البيولوجي لمستخلص الميثانول لأجزاء مختلفة من *Astragalus bruguieri* Bios، وهو نبات طبي بري ينمو في جبل سفين، أربيل، العراق. تم تحليل المستخلصات الميثانولية للنبات *A. bruguieri* لمعرفة محتويات الفينول والفلافونويد والصابونين. تم تحليل نشاط مضادات الأكسدة في المختبر بواسطة مقاييسات DPPH-2،2-diphenyl-1-picrylhydrazyl و azino-2،2 bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). علاوة على ذلك، تم فحص المستخلصات النباتية لمعرفة النشاط المثبط للإنزيم المختبري والسمية شبه الحادة في الجسم الحي. أظهرت النتائج أعلى محتوى إجمالي من الفينول (28.83، 20.62 مجم GAEs / جم) في الأوراق والجزء الهوائية في الأوراق والجزور على التوالي. بينما وجد أعلى محتوى إجمالي من الفلافونويد (50.08، 44.01 مجم REs / جم) في الأجزاء الهوائية والأوراق على التوالي. كان إجمالي الصابونين أكثر انتشاراً (25.33، 23.18 مجم من مستخلص GAEs / جم) في الجذور والأجزاء الهوائية، على التوالي. أظهر قياس نشاط مضادات الأكسدة في المختبر بواسطة مقاييسات DPPH) أن الأوراق كجزء متفوق في هذا النشاط (42.19 mg مستخلص / g TEs)، بينما أشار تقييم مضادات الأكسدة بواسطة مقاييسات ABTS) إلى أن الجذور هي الجزء الأكثر نشاطاً (86.90 mg TEs / مستخلص ز). تم العثور على  $\alpha$ -glucosidase و  $\alpha$ -amylase لتكون 0.67-0.45 و 1.8-1.2 مللي مول ACAEs / جم على التوالي. أشار اختبار السمية الفموية الحادة إلى سلامة جرعة 600 مجم / كجم لأجزاء مختلفة من *A. bruguieri* على الجرذان البيضاء دون حدوث خلل سلوكي أو الموت. تعتبر الدراسة الحالية أول تقرير عن *A. bruguieri* كمصدر جديد محتمل للمواد المتوافقة حيويًا للعديد من المنتجات الصناعية.

**الكلمات المفتاحية:** السمية الحادة، مضادات الأكسدة، استراغالوس بروغيري، مثبطات الإنزيم، كيمياء النبات