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

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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 Bios., 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 leaf and root extracts, respectively. While the highest total Flavonoid (50.08, 44.01 mg REs/g) contents, were found in the 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 TEs/g extract), while antioxidant evaluation by (ABTS) assay indicated roots as the most active part (86.90mg TEs/g extract). The α-glucosidase and α-amylase inhibitory activity were found as 0.45-0.67, and 1.2-1.8 mmol CAEs/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. 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, singed oxygen, and H2O2 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. 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. 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. However, scientists have warned consumers about the aftermath of these synthetic antioxidants on human health because of their carcinogenic effect on human genes. Thus, a recent plethora of works has shown interest in searching for natural antioxidant and anti-proliferative agents to replace chemical synthetics.

Diabetes is considered a series of health problems related to islet β 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. Medical pathways have been progressed in dealing with diabetes in recent years. One of these new approaches is stabilizing postprandial hyperglycemia immediately after meals. This stabilization can be achieved by controlling glucose release into the circulation by inhibiting enzymes rolling in carbohydrate digestion. Food carbohydrates are digested into oligosaccharides by α-Amylase and then into monosaccharides by α-glucosidase in the small intestine. Blood glucose levels can be prevented from rising through inhibiting enzymes involved in carbohydrate metabolism. The current synthetic chemicals seem to be effective as hypoglycemic agents, however, many of these chemical drugs need upgrading for better treating outcomes. 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. An alternative for synthetic anti-diabetics, searching for α-amylase and α-glucosidase inhibitors in natural sources like plants, become a continuous mission by scientists.

*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 millennia. *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. 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, immunostimulant, enzyme inhibitory, anti-tumor, and anti-diabetes. 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. The same is true with synthetic drugs which may be curative in a certain amount and hazardous at a certain level. To guarantee the safe usage of natural products, certain quality measurements must be on targeted herbs before approving as a natural medicinal agent. In today’s drug industry, the development of more than one-quarter of drugs becomes expensive due to their toxicity studies. 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 (LD50), the downside of targeted compounds after single-dose administration within a certain period. The administration is usually made through the oral cavity of laboratory animals (rats or mice) to assess the median lethal dose (LD50) for a specific biocompatible material or plant extract. 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/Shaqlawa in Erbil, Iraq (Latitude: 36°18'08.2"N, Longitude: 44°25'18.9"E) (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).
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 suplementations, 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) \textsuperscript{25}. 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 \textsuperscript{26, 34}.

### Results and Discussion:

#### Chemical profile

The extraction yield of various parts of \textit{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±0.58 mg GAEs/g extract and 10.68±0.13 to 50.8±0.61 mg 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 \textit{A. bruguieri}.

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

- 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. The results of the chemical profiling of A. bruguieri showed a significant difference in the phytochemical contents of different plant parts. Similarly, Platikkanov 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. Similarly, previous phytochemical studies on A. glycyphylllos 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. 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. 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. The phenolic compounds are also linked with improving defense mechanisms through reversing ROS formation, increasing cell survival, and decreasing nuclear damages and microorganism attacks.

Flavonoids are considered secondary metabolites of the plants known as potent antioxidant agents. The flavonoids are also used as a flavoring and food coloring agent. 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 and 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, cytotoxicity, and anti-inflammatory activity. 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 activity of different parts of A. bruguieri ranged between 15.07±0.89 and 42.19±1.5mg TEs/g extract, and it varied significantly (p< 0.05) between the plant extracts. The antioxidant activity of leaves was higher 42.19±1.5 TEs/g than that of 28.07±0.81, 22.53±1.0, and 15.07±0.89 TEs/g extract for roots, aerial parts, and stems, respectively (Fig. 2). The ABTS scavenging activity of roots was higher (86.90±1.4mg TEs/g extract) than that of 82.3±1.67, 52.14±1.54, and 21.23±1.19 TEs/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.

<table>
<thead>
<tr>
<th>Plant organs</th>
<th>DPPH radical (mg TEs/g extract)</th>
<th>ABTS radical cation (mg TEs/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roots</td>
<td>28.07±0.81</td>
<td>86.90±1.4</td>
</tr>
<tr>
<td>Stems</td>
<td>15.07±0.89</td>
<td>52.14±1.54</td>
</tr>
<tr>
<td>Leaves</td>
<td>42.19±1.5</td>
<td>82.3±1.67</td>
</tr>
<tr>
<td>Aerial Parts</td>
<td>22.53±1.0</td>
<td>21.23±1.19</td>
</tr>
</tbody>
</table>

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. 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.

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. 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. Earlier studies also reported phenolic compounds as reducing agents, hydrogen givers, singlet oxygen inhibitors, and effective metal chelators because of their redox properties. 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. Furthermore, a previous study reported potent antioxidant activity by flavonoids isolated from *Astragalus mongholicus* Bunge, a potential adjunct of the atherosclerosis profile and possible reducer of cardiovascular disease. 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. 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 α-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 α-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>
<thead>
<tr>
<th>Assay</th>
<th>Roots</th>
<th>Stems</th>
<th>Leaves</th>
<th>Aerial parts</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Amylase inhibition (mmol ACEs/g extract)</td>
<td>0.51±0.01a</td>
<td>0.46±0.01b</td>
<td>0.49±0.008ab</td>
<td>0.48±0.008ab</td>
</tr>
<tr>
<td>α-Glucosidase inhibition (mmol ACEs/g extract)</td>
<td>3.21±0.06a</td>
<td>3.99±0.08c</td>
<td>18.27±0.05a</td>
<td>12.95±0.03b</td>
</tr>
</tbody>
</table>

* 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. The inhibition of α-amylase and α-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 α-glucosidase delay the breaking down of carbohydrates in the gut and decrease postprandial blood glucose peak in diabetic patients.

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 α-glucosidase and α-amylase inhibitors seems to be promising for controlling such disease with fewer side effects than synthetic drugs. The present study exposed a moderate enzyme inhibitory effect of different parts of *A. bruguieri* on α-amylase and a strong inhibitory effect on α-glucosidase and considered as the first report on the enzyme inhibitory activity of *A. bruguieri* and can be linked with its phenolic, flavonoid, 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. 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. Similarly, a previous study has correlated the antidiabetic role of *Astragalus*
ponticus with increased percentage contents of its polysaccharides, saponins, and flavonoids. 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. 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>
<thead>
<tr>
<th>Parameters</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed and water intake</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Coma</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Convulsion and tremors</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Eyes</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Faces consistency</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Fur and skin</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Itching</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Respiration</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<td>N</td>
</tr>
<tr>
<td>Sleep</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Urination (color)</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
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<tr>
<td>Aggressiveness</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
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<tr>
<td>Mortality</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>

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 LD₅₀ 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. 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. 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:


Astragalus bruguieri، مضادات الأكسدة، مثبط الإنزيم ونشاط السمية الحادة من

الخلاصة:
تم استخدام النباتات الطبية (أنواع استراغالوس) تقليديًا كمضاد للالتهابات ومضاد للأكسدة ومضاد لمرضى السكر. يبحث البحث


الملف الكيميائي النباتي، مضادات الأكسدة، مثبط الإنزيم ونشاط السمية الحادة من

A. bruguieri

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أربيل. تعتبر الدراسة الحالية أول تقرير عن

A. bruguieri

والأوراق على التوالي. كان إجمالي الصابونين أكثر انتشارًا (GAEs) بين الأوراق والأجزاء الهوائية، وفي الأوراق أعلي (GAEs) في الأجزاء الهوائية (34.18 مجم). كانت مضادات الأكسدة أكثر انتشارًا (ABTS) في الأوراق على التوالي.

أ. درش، ن. ش Neighboric و. العمال. نقص الأنزيمات الباردة في مصادر مختلفة من

A. bruguieri

للعديد من المنتجات الصناعية.

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