




Comparative Assessment of Antimicrobial Activity of Methanolic Extracts of Five Species of *Astragalus* (Fabaceae) Collected from Southeast of Iran, Kerman

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Abstract

Background: The antimicrobial properties of certain plants against five microorganisms have been investigated and compared.

Objectives: In this research, we collected, dried, and identified five perennial (chamaephyte) species of *Astragalus* (Fabaceae) from a mountainous region (Takht-e-Sartashtak) with an average elevation of 3600 meters in southeastern Iran. The species include *Astragalus cemerinus*, *Astragalus dactylocarpus*, *Astragalus glaucacanthus*, *Astragalus rhodosemius*, and *Astragalus siliquosus*.

Methods: The methanolic extracts of these plant species were tested to evaluate their antimicrobial activity against five pathogenic microorganisms, including one fungus (*Candida albicans*), two gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), and two gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*). The tests were conducted using Mueller Hinton Agar culture media and the agar-well diffusion method.

Results: The antimicrobial activity of these five species was compared. It was found that the plant species displayed different antimicrobial activities. *Astragalus glaucacanthus* was active against four of the five microorganisms and was the only plant active against *Escherichia coli*. *Astragalus siliquosus* showed no activity against any of the microorganisms.

Conclusions: Most of the antimicrobial activity was observed with *Astragalus rhodosemius* against *Staphylococcus aureus*. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) of the plant extracts were almost similar.

Keywords: *Astragalus*, Methanolic Extract, Antimicrobial Activity, Medicinal Plants, Iran

1. Background

Many microorganisms cause diseases that are commonly treated with antibiotics. However, it is important to note that the effective period of each antibiotic is limited, after which microorganisms can develop resistance, rendering the antibiotic ineffective. Therefore, microbiologists are always searching for new antimicrobial agents. One approach to finding new drugs is to explore the natural sources of these drugs, such as plants. It is known that certain plants were used as remedies before microbes were discovered (1). Human beings have always utilized plants and their products as drugs since the beginning of human

civilization. Medicinal herbs have significant economic value worldwide (2). Additionally, plants are much cheaper and more accessible than existing drugs. There are many herbs that could be used as drug sources, but they remain unknown. Among the many plant species, only a few percent have been studied phytochemically, with most still unexplored (3). Iran's vegetation is very diverse, and for many years, botanists have been working to collect and identify plants in different parts of Iran, studying them phytochemically and pharmacologically.

Within the legume family, there is a large genus called *Astragalus* L., which is dispersed across a wide

range of temperate areas worldwide, primarily in North America, Europe, and Asia. About 2000 species have been reported, with 372 in North America and 133 in Europe (4). *Astragalus* has shown a variety of potential pharmaceutical applications in treating immunodeficiency syndromes, cancer, and regulating heart and kidney functions. The roots of *Astragalus* are used to improve immune function and increase stamina strength. *Astragalus* is also used to treat respiratory diseases and strengthen respiratory health (5). Other traditional therapies use *Astragalus* to treat sweating, persistent injuries, paralysis, and swelling (6).

2. Objectives

In this research, we collected, dried, and identified five species of *Astragalus* from mountainous areas in some parts of southeastern Iran in Kerman Province. The methanolic extracts of these species were then prepared, tested, and compared with each other using the antibiogram test for antimicrobial activity.

3. Methods

3.1. Collection of Plant Material

Fresh plants from the mountainous region (Takht-e-Sartashtak) of southeastern Iran in Kerman Province, with an average height of 3600 meters, were collected, dried, and identified in the spring and summer of 2017 and 2018. The whole parts of dried plants were used for extraction.

3.2. Preparation of Plant Extracts

For the extraction of the dried plants, 10 g of each powdered plant was mixed with 50 mL of methanol solvent. The mixture was then placed on a shaker for 3 days to isolate the methanol-soluble compounds from the plant and dissolve them in the solvent. The mixture was then filtered with filter paper, and the extract was left to dry. The resulting powder was ready for antimicrobial work (7).

3.3. Tested Microbes

The Knowledge Base company of Dana Gene Researchers, Kerman, Iran, provided the standard microbial species. The bacterial species tested included one fungus, *Candida albicans* (PTCC:5027), two gram-

negative bacteria, *Pseudomonas aeruginosa* (PTCC:1074) and *Escherichia coli* (PTCC:1330), and two gram-positive bacteria, *Staphylococcus aureus* (PTCC:1112) and *Bacillus cereus* (PTCC:1015) (8).

3.4. Evaluation of Antimicrobial Activity

First, the dried extract of the plant was used to prepare different dilutions (mg/mL). Then, 0.05 g of the dry extract of the plant was weighed with a digital scale and dissolved in 1 mL of Dimethyl sulfoxide (DMSO) solvent to obtain an extract with a dilution of 50 mg/mL. Further dilutions were similarly prepared. The Mueller Hinton Agar culture medium, manufactured by Merck in Germany, was used to evaluate the effect of the plant extract on selected microorganisms. After preparing the culture medium, 0.5 McFarland of microorganisms was prepared in sterile physiological serum, and 0.2 μ L of the suspension was poured onto the culture medium under aseptic conditions. The microorganism was then cultured on Mueller Hinton Agar using a glass spreader to achieve complete uniformity. In the next phase, wells were created in each culture medium at regular intervals, and the 50 mg/mL extract dilution was poured into the wells (9). The amount of extract in each well was 20 μ L, added using a sampler. The plates were incubated at 30°C for 24 hours to test and check the growth inhibition zone.

It should be noted that a well containing negative control DMSO was used, and for a positive control, a commercially available antibiotic disk was employed (10).

3.5. Characterization of Minimum Fungicidal Concentration, Minimum Bactericidal Concentration, and Minimum Inhibitory Concentration

To determine the Minimum Fungicidal Concentration (MFC), minimum bactericidal concentration (MBC), and minimum inhibitory concentration (MIC), we used the broth dilution method (9). First, the preparation of the standard solution was done as follows: 0.5 mL of 1% dehydrated barium chloride solution was added to a 100 mL dish, and the volume was increased to 100 mL with 1% sulfuric acid. The resulting barium sulfate solution is called the 0.5 McFarland standard opacity solution. To obtain a dilution of 50 mg/mL of the extract, 50 mg of plant extract powder was poured into 1 mL of solvent. In each

of the 5 test tubes, 1 mL of the extract solution was poured, and 1 mL of the standard opacity solution was added to each tube. The results were considered after incubating the tubes for 18 hours. The last tube without microorganism growth or opacity was considered the MIC, and the previous tube was usually the MBC or MFC. As a rule, the contents of the MBC and MFC tubes should not show any growth on the culture medium (11).

4. Results

The methanolic extracts of five *Astragalus* species were examined for their antimicrobial activity against one fungus, two gram-negative bacteria, and two gram-positive bacteria using Mueller Hinton Agar culture medium and the agar-well diffusion method.

The results showed that the methanolic extracts of four of these plant species were active against at least three microorganisms. One species, *Astragalus siliquosus*, did not show any antimicrobial activity. Table 1 presents the results of the antimicrobial activity of the five plants against the five microorganisms mentioned.

It was found that the plant species displayed different antimicrobial activities. *Astragalus glaucacanthus* was active against four of the five microorganisms and was the only plant active against *Escherichia coli*. *Astragalus siliquosus* did not show any antimicrobial activity against any of the microorganisms. The greatest antimicrobial activity was observed with *Astragalus rhodosemius* against *Staphylococcus aureus*. The values of the diameter of the growth inhibition zones for the five species are shown in Table 1. The MIC, MBC, and MFC of the plant extracts were approximately similar (Table 2). The total values of MIC, MBC, and MFC of the plant extracts are shown in Table 2.

5. Discussion

So far, many studies have been conducted worldwide to investigate the medicinal and antimicrobial properties of different species of *Astragalus*. In most cases, these studies have shown positive effects on various diseases as well as their antimicrobial activity. Many *Astragalus* species are used as medicinal plants; for example, one of the most widely used and studied species is *Astragalus membranaceus*, which is officially listed in the Chinese Pharmacopoeia, like the species

Astragalus mongholicus (*Astragalus membranaceus* var. *mongholicus*) and *Astragalus complanatus*. Some medicines are made from extracts of these species. The pharmacological activity of *Astragalus* is attributed to two chemical compounds: Polysaccharides and saponins. Most of the potential therapeutic effects include liver protection, immune stimulation, and antiviral activity (12).

The effect of extracts from some wild Egyptian *Astragalus* species was studied for their hypoglycemic effects on alloxanized diabetic rats (13). The saponin astramembrannin I in *Astragalus* prevented the increase in vascular permeability caused by serotonin and histamine and decreased edema in rats (14). The antimutagenic activity of *Astragalus membranaceus* aqueous extract on aflatoxin B₁-induced mutagenesis, using *Salmonella typhimurium* as a test strain and rat liver ultra-supernatant as the activating system, has been demonstrated (15). There are also positive results for using this medicinal plant as a chemopreventive agent for cancer in China. Some medicinal herbs from China are known to stimulate sperm motility (16), and *Astragalus membranaceus* aqueous extract was found to be active in this regard. However, it is noteworthy that some constituents of *Astragalus* species can negatively affect male reproduction (17). Therefore, *Astragalus* species are a group of medicinal plants whose active ingredients are primarily saponins and polysaccharides (12).

The aqueous root extracts of some *Astragalus* species are used to promote wound healing and treat leukemia in folk medicine in Turkey (18, 19). Many compounds found in the polysaccharide- and saponin-rich roots of *Astragalus* species have effects on the treatment of cancer and the effective functioning of the immune system (12, 19-21). *Astragalus* is one of the largest herbal genera, with many species. In Iran, this genus also has several species that can be used effectively by collecting, identifying, and evaluating their properties and developing new drugs for treating diseases.

Previous research has shown that *Astragalus* is a traditional medicinal plant used in many countries, including China, for treating various diseases, including anemia, diabetes, digestive diseases, nervous and respiratory system diseases, and some types of cancer. Its role in preventing bone loss and its anti-aging properties have also been proven (22).

Table 1. Antimicrobial Activity of Five Plants Against Five Microorganisms, Including one Fungus, *Candida albicans* (PTCC:5027), Two Gram-Negative Bacteria, *Pseudomonas aeruginosa* (PTCC:1074) and *Escherichia coli* (PTCC:1330), and Two Gram-Positive Bacteria, *Staphylococcus aureus* (PTCC:1112) and *Bacillus cereus* (PTCC:1015)^a

Plants Name	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
<i>Astragalus cemerinus</i> Beck	-	13	13	11	-
<i>Astragalus dactylocarpus</i> Boiss.	-	13	10	-	10
<i>Astragalus glaucacanthos</i> Fisch.	10	12	12	12	-
<i>Astragalus rhodosemius</i> Boiss. and Hausskn.	-	14	10	11	-
<i>Astragalus siliquosus</i> Boiss.	-	-	-	-	-

^a Zone of growth inhibition (mm, ±1).**Table 2.** Minimum Inhibitory Concentration, Minimum Bactericidal Concentration, and Minimum Fungicidal Concentration of five Plant Species Against Five Microorganisms^a

Plants Name	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>		<i>Bacillus cereus</i>		<i>Pseudomonas aeruginosa</i>		<i>Candida albicans</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
<i>Astragalus cemerinus</i> Beck	-	-	50	100	50	100	50	100	-	-
<i>Astragalus dactylocarpus</i> Boiss.	-	-	50	50	50	100	-	-	50	100
<i>Astragalus glaucacanthos</i> Fisch.	50	100	50	100	100	100	50	100	-	-
<i>Astragalus rhodosemius</i> Boiss. and Hausskn.	-	-	50	100	50	50	50	100	-	-
<i>Astragalus siliquosus</i> Boiss.	-	-	-	-	-	-	-	-	-	-

Abbreviations: MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; MIC, minimum inhibitory concentration.

^a MIC, MBC and MFC of plants extracts against 5 microorganisms (mg/mL, ±1).

Despite the proven antimicrobial activity of many plants, these plants are still not widely used in medical science. Therefore, pharmacists and doctors are working to enhance the status of these plants in medical science (23).

Footnotes

Authors' Contribution: All authors: Specimen and plant collection, performing antimicrobial tests, check out the results, manuscript draft preparation, Supervising the study, manuscript revision, Supervising the study, manuscript revision.

Conflict of Interests Statement: There is no Conflict of Interest.

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