Cytotoxic and antibacterial activities of leaf extracts of *Astragalus gombiformis* Pomel (Fabaceae) growing wild in Tunisia

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**Abstract:** Many species of the genus *Astragalus* have been long used in folk medicine because of their biological properties. The aim of this study was to investigate the antibacterial and cytotoxic activities of extracts from the leaves of wild *Astragalus gombiformis* Pomel. Antibacterial activity was assayed against several common human pathogenic bacterial strains using the paper disk-agar diffusion method. Cytotoxicity was measured against human lung carcinoma cells using the colorimetric MTT assay. The strongest cytotoxic activity against the human A549 lung epithelial carcinoma cell line was detected for dichloromethane extracts at IC₅₀ = 85 ± 21.7 μg/mL. The best incubation time for this extract was 48 h. Most of the tested extracts were active against the tested bacterial strains. The methanol extract was found to be the most active against *Pseudomonas aeruginosa* and *Salmonella typhimurium*. This study is the first report on the biological activity of leaf extracts of *Astragalus gombiformis* with respect to antibacterial activity as well as cytotoxicity.

**Key words:** *Astragalus gombiformis*, antibacterial effect, cytotoxicity

**Introduction**

In recent decades, many studies have been carried out on different plant species to discover compounds of possible interest for medicinal application against various diseases, such as cancer, oxidative stress, and fungal, viral, and bacterial infections. Among these studies, several have focused on the biological and phytochemical properties of different species of *Astragalus*, the largest genus of the family Fabaceae and, with over 2500 species, probably the largest genus of flowering plants (1). Several species of this genus are used in folk medicine due to their hepatoprotective, antioxidative biological activities and their antiviral properties (2). In Turkish folk medicine, the roots of *Astragalus* species are used for the treatment of leukemia and for the healing of wounds (3). Furthermore, *A. mongholicus* Bunge and *A. membranaceus* Bunge are among the most popular Chinese medicines and are used for a variety of diseases, including as an adjunctive in cancer chemotherapy (4-6). Some *Astragalus* products, such as gum tragacanth, are widely in use in the preparation of pharmaceuticals and as thickening agents in certain foods (7). Some products may even have applications in controlling cancer cells (8). Among the different compounds used from *Astragalus*, saponins extracted from *A. corniculatus* M.Bieb. have an antineoplastic effect against myeloid
Graffi tumors in hamsters (9). Antibacterial activity has also been reported for some Astragalus species, such as *A. gymnolobus* Fisch. and *A. brachystachys* DC. (10,11). In addition, bioactive saponins have been extracted from *A. suberi* (12). Gođevac et al. investigated the antioxidant activity of methanol extract from the aerial part of *A. glycyphyllus* L. (13). Another study showed that an ethanol extract of *A. membranaceus* roots may inhibit the growth of Trypanosoma cruzi (14).

Several species of Astragalus are reported from the Tunisian flora, including *A. gombiformis* Pomel, which grows in desert regions. In these areas, the plants normally synthesize several metabolites in order to adapt to drought stress and are therefore a promising source of active molecules. *A. gombiformis* is traditionally used against the bites of snakes and scorpions (15) and it is probable that this species contains active products that act against scorpion envenomation. Given the great interest in novel natural compounds able to fight against cancer and many other diseases, we undertook the present study as a primary biological investigation of the cytotoxic and antibacterial effects of leaf extracts from wild *A. gombiformis*.

**Materials and methods**

**Plant collection**

Leaves of wild *A. gombiformis* were collected from Bir Soltane (33°28'10"N, 009°23'50"E; 107 m above sea level) in southern Tunisia. The samples were air-dried at room temperature and protected from direct sunlight. They were then powdered and stored until use.

**Preparation of leaf extracts**

**Organic solvent extracts**

Powdered leaves (25 g) were extracted successively using 3 solvents with increasing polarity: petroleum ether, dichloromethane, and methanol. After the evaporation of these solvents, 3 residues were obtained.

**Alkaloid extraction**

The basic fraction of alkaloid extract was prepared according to the method of Rujjanawanate et al. (16), with slight modifications. Powdered leaves (25 g) were macerated in 300 mL of 85% methanol at ambient temperature. After filtration, the methanol extract was concentrated under reduced pressure and acidified with 0.5 M H₂SO₄. The resulting extract was washed 3 times with chloroform. The aqueous acidic fraction was then made basic with ammonia (pH 10) and extracted with chloroform until the aqueous layer was free of alkaloids. Finally, the chloroform was evaporated and the basic fraction of alkaloids was obtained.

**Extract dissolution**

All residues were dissolved in dimethyl sulfoxide (DMSO) and filter-sterilized through a 0.2-μm Millipore filter. They were then diluted to the desired concentrations with culture medium for the cytotoxicity tests and with distilled water for the antibacterial assays. The final DMSO concentration was not above 0.1%.

**Cytotoxic activity**

**Cell cultures**

The human A549 lung epithelial carcinoma cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in HAM F12 medium (Eurobio, Les Ulis, France) containing 10% (v/v) fetal calf serum, 2 mM glutamine, and antibiotics (200 U/L of penicillin and 50 mg/L of streptomycin). It was maintained at 37 °C in a humidified 5% CO₂ atmosphere.

**Cell viability assay**

Cytotoxicity was measured using an MTT test with slight modifications (17). Cells were seeded at 5 × 10³ cells/well in 200 μL of growth medium and incubated at 37 °C for 24 h for the cells to adhere. The microplates were treated by extracts and incubated for 3 different lengths of time (24, 48, and 72 h); then 10 μL of MTT was added to each well (5 mg/mL) and the incubation was continued for 2 h. After this, 100 μL of DMSO was added to each well. The absorbance (A) was measured at 570 nm by a Multiskan Ascent (Ascent Software version 2.6) microplate reader. This assay was conducted in triplicate as a cell viability index. The percentages of cell growth were calculated as follow: Cell growth (%) = \[ \frac{A (\text{sample})}{A (\text{control})} \] × 100. Cytotoxicity was expressed as the concentration of extract inhibiting cell growth by 50% (IC₅₀).
Antibacterial activity

**Bacterial strains**

Seven bacterial strains were used: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* CIP 106510, *Salmonella typhimurium* NRRLB 4420, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* ATCC 19115, and *Bacillus subtilis* ATCC 168. The microorganisms were stored on Mueller-Hinton agar (Bio-Rad) at 4 °C. The nutrient broth (Bio-Rad) and the Mueller-Hinton agar were used, respectively, for growing and diluting the microorganism suspensions for the antibacterial assays.

**Antibacterial assays**

Antibacterial activity was tested by the paper disk-agar diffusion method (18). Pure bacterial strains were suspended in molten nutrient agar and the optical density was adjusted to 0.5 at 570 nm (Jenway 6405 UV/Vis spectrophotometer). Mueller-Hinton agar plates (90 mm) were inoculated with this bacterial suspension and Whatman paper disks (6 mm in diameter) were deposited. Each disk was impregnated with 15 μL of extract or diluted DMSO for negative control. The plates were then incubated at 37 °C for 24 h. Antimicrobial activity was evaluated by measuring the diameter of the clean inhibitory zone around each disk, including its diameter. An absence of inhibition was expressed using the value of 0 mm. Gentamicin (15 μg) was used as the positive control. Each extract was tested in duplicate at 6 different concentrations (0.125 to 50 mg/mL).

Results and discussion

A biological screening of *A. gombiformis* extracts is presented for the first time in the present study. In the framework of research on natural active compounds, we tested the antibacterial and cytotoxic activities of 4 different extracts of *A. gombiformis* leaves.

**Cytotoxic activity**

The cytotoxicity of extracts against the human A549 lung epithelial carcinoma cell line was studied at 3 different times of incubation and with concentrations not exceeding 200 μg/mL. The IC50 values are summarized in Table 1. An incubation time of 48 h seems to be the best based on cytotoxicity assay results; the variation of extract activity is illustrated in the Figure.

![Figure. Variation of cell proliferation inhibition on the function of extract concentrations after 48 h of incubation.](image)

<table>
<thead>
<tr>
<th>Extract</th>
<th>Yield (%)</th>
<th>Incubation time</th>
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<td>Alkaloids (basic fraction)</td>
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Table 1. Cytotoxicity of leaf extracts from Astragalus gombiformis as evaluated by MTT assay and expressed in IC50 values (μg/mL, mean ± SD).
Cytotoxic and antibacterial activities of leaf extracts of *Astragalus gombiformis* Pomel (Fabaceae) growing wild in Tunisia

The strongest cytotoxic activity was found for the dichloromethane extract with an IC$_{50}$ of 85 ± 21.7 μg/mL after 48 h of incubation. Among other *Astragalus* species that have been investigated for cytotoxic activities in previous studies, volatile fractions of *A. corniculatus* showed an antiproliferative effect against various tumor cell lines, with IC$_{50}$ values varying from 24 to 287 μg/mL after 48 h of incubation (19). Al-Fatimi et al. (20) investigated the cytotoxicity of many species and showed that *Plicosepalus curviflorus* (Benth. ex Oliv.) Tiegh. was the most active, with an IC$_{50}$ of 5 μg/mL. Species studied by Mena-Rejon et al. (21) showed an IC$_{50}$ of between 13 and 98 μg/mL against different carcinoma cell lines.

In general, the activity of extracts can be related to an active compound or to the synergic action of several compounds. For *Astragalus* species, the toxicity may be due to swainsonine and its derivatives, which are widely distributed in these species (2). In fact, Sun et al. (22) showed swainsonine to inhibit the growth of the human gastric carcinoma SGC-7901 cell completely at a concentration of 6.2 μg/mL and an IC$_{50}$ value of 0.84 μg/mL at 24 h. Na et al. (23) showed that *A. membranaceus* can be used as a chemotherapy adjuvant in the treatment of gastric cancer. Recently, Li et al. (24) also showed that *Astragalus* polysaccharides display strong antitumor activity.

**Antibacterial activity**

The antibacterial activities of extracts were evaluated by the diameter of the inhibition zone around the disk; these diameters are reported in Table 2. For the interpretation of antibacterial assay results, we adopted the following scale of measurement: zone of inhibition of >15 mm as strongly inhibitory, 10-15 mm as moderately inhibitory, and <10 mm as not inhibitory, according to Carović-Stanko et al. (25). Thus, many of the tested extracts were found to be active against different bacteria. The dichloromethane extract was not active against *S. aureus* or *E. coli* at any of the tested concentrations. The methanol extract was also inactive against *E. coli* and *B. subtilis* at 50 mg/mL. With the exception of *S. typhimurium*, the alkaloids fraction was moderately active against the tested bacteria. It showed a low inhibition zone against *S. epidermidis* (diameter of 10 mm) at a low concentration (125 μg/mL). Extracted alkaloids were also active against *S. aureus* and *L. monocytogenes* at 750 μg/mL. In the present study, *S. typhimurium* was found to be susceptible to the methanol extract at 500 μg/mL. This is particularly significant because *S. typhimurium* is responsible for several kinds of salmonellosis and is resistant to many antibiotics such as chloramphenicol (26).

Petroleum ether extract was active against *S. epidermidis* at 250 μg/mL and against other tested bacterial strains at concentrations above 1000 μg/mL. With regard to *P. aeruginosa*, the most activity was detected for the methanolic extract at 750 μg/mL, corresponding to 11.25 μg/disk. Other *Astragalus* species, such as *A. ponticus*, *A. microcephalus* Willd., *A. erinaceus* Fisch., and *A. argyroides* Beck, have also been tested for antibacterial activities in previous studies. At 300 μg/disk, methanolic extracts of the aerial parts of these species were found to be inactive against *S. epidermidis*, *E. coli*, *P. aeruginosa*, and *B. subtilis* (27). In a study by Türker et al. (11), aqueous, methanolic, and ethanolic extracts of *A. gymnolobus* Fisch. showed inhibition zones against *Aeromonas hydrophila* with diameters of 7.75, 8.50, and 7.25 mm, respectively. On the other hand, these 3 extracts were found to be inactive against *Yersinia ruckeri*, *Streptococcus agalactiae*, *Lactococcus garvieae*, and *Enterococcus faecalis*. Another example concerning active compounds from *Astragalus* species was provided by Jassbi et al., who indicated that isolates from *A. brachystachys* showed antibacterial activity against *Bacillus subtilis*. Disks charged with 40, 80, 120, 160, and 200 μg of sclareol showed 8-, 9-, 10-, 11-, and 18-mm inhibition zone diameters, respectively (10).

**Conclusion**

The leaves of *A. gombiformis* are biologically active and show a high cytotoxic effect. In particular, the dichloromethane extract of the leaves of this species exhibits the highest cytotoxic activity. *A. gombiformis* leaf extracts were also shown to have high antimicrobial activity against several bacterial strains.
Acknowledgements

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Table 2. The antibacterial activity of *Astragalus gombiformis* leaf extracts as expressed by inhibition zone diameter (mm).

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<th>Extract</th>
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<th>S. epidermidis</th>
<th>P. aeruginosa</th>
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*C = extract concentration

References


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