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Research Article

Some biological activities of ethanol extract of aerial parts of *Gentiana olivieri*

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ABSTRACT

In recent years, due to the possible side effects of synthetic drugs, people have turned to natural drugs in combating diseases. Plants are important natural resources with these properties. In our study, some biological activities of the aerial parts of *Gentiana olivieri* were determined. In this context, the plant was extracted with ethanol in a Soxhlet apparatus. The antioxidant activity of the plant was measured with Rel Assay TAS and TOS kits. Its antimicrobial activity was determined by the agar dilution method. Determination of total phenolic content was determined using the Folin-Ciocalteu reagent. Total flavonoid quantification was performed using aluminum chloride assay. As a result of the analyzes, the TAS value of the ethanol extract of the aerial parts of *Gentiana olivieri* was determined as 7.775±0.114 mmol/L, the TOS value as 12.252±0.094 µmol/L and the OSI value as 0.158±0.002. In addition, total phenolic content was measured as 104.92±1.40 mg/g, total flavonoid content as 73.83±1.29 mg/g. In addition, it was determined that the plant extract was effective against standard bacterial and fungal strains at extract concentrations in the range of 50-200 µg/mL. As a result, it was determined that *Gentiana olivieri* could be an important natural source in terms of antioxidant and antimicrobial activity.

Keywords: Afat, Gentianaceae, *Gentiana olivieri*, Medicinal Plants, Traditional Medicine

1. INTRODUCTION

Many plant species have been widely used in traditional medicine since ancient times.[1-3] In addition to plant species, mushrooms and animals are also used in traditional medicine. Plants are used in traditional medicine practices by obtaining different products such as tea, direct consumption, essential oil, and extract.[4] They are also important foods thanks to the vitamins, minerals, and nutritional elements they contain.[5] In addition to their nutritional properties, plants exhibit very important activities in terms of medicine.[6]

Many studies have reported that plants exhibit many biological activities such as antiallergic, digestive, anti-inflammatory, immune system strengthening, antioxidant, antimicrobial, anticancer, DNA protective, and painkiller.[7-16] In this context, the determination of biological activities of plants is very important in order to reveal their medical potential. In our study, total antioxidant and total oxidant status, antimicrobial activity and total phenolic and flavonoid contents of *Gentiana olivieri* Griseb. were determined.

Gentiana olivieri (Gentianaceae) is a flowering plant with an upright stem and 15-30 cm in height. The basal leaves are oblanceolate and can grow up to 15 cm. The flowers can grow up to 3 cm. The flowers are blue, usually with white throats and three to ten in terminal clusters. It is seen from late spring to summer. It is distributed at 350-2300 m in Turkey, Iran, Iraq and nearby. It grows in meadows on limestone, marl or clay in its habitats. *G. olivieri* has been used in traditional medicine practices for centuries thanks to the secoiridoid, flavonoid and alkaloids it contains. It is known as "Afat" in Turkey, "Agher" and "Bangera" in Pakistan. It is known to be used as an antidiabetic, sedative, digestive and antianemic in Turkish folk medicine. In the Republic of Uzbekistan, it is widely preferred in the treatment of diarrhea, cold, stomach ache and indigestion.[17-19]

2. EXPERIMENTAL

2.1. Materials

Plant samples were collected from Duhok (Iraq). Aerial parts of the plant were dried in a laboratory environment that was dry, free from humidity and direct sunlight. After drying, 30 g of aerial parts of the plant were weighed and powdered. Then, it was extracted with 250 mL of ethanol in a Soxhlet apparatus for approximately 6 hours. After the extraction process, ethanol was evaporated using a Buchi R100 Rotary Evaporator at 40 °C. The final product, crude extracts, was stored at +4 °C until the experiment was performed.

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2.2. Methods

2.2.1. Total Antioxidant Oxidant status

Total antioxidant and total oxidant statuses were determined using ethanol extracts of aerial parts of the plant. Rel Assay TAS and TOS kits were used for this purpose. Analyses were performed by following the kit manufacturer's protocol. Calibration of TAS tests was performed with trolox. Calibration of TOS tests was performed with hydrogen peroxide. TAS values were expressed as mmol/L, TOS values as µmol/L.[20,21] Oxidative stress index was determined by proportioning TOS and TAS values by equating their units and taking the percentage at the last stage.[22]

2.2.2. Antimicrobial Tests

Antimicrobial activities of ethanol extract of aerial parts of the plant against bacterial and fungal strains were determined by agar dilution test. The bacterial strains for which activity test was performed were *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606. The fungal strains for which activity test was performed were *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 and *C. glabrata* ATCC 90030. The plant extract was prepared at concentrations of 12.5, 25, 50, 100, 200, 400 and 800 µg/mL. These concentrations were then used to determine the lowest concentration that inhibited the growth of microorganisms. Bacterial strains were pre-cultured in Muller Hinton Broth medium, fungal strains in RPMI 1640 Broth medium.[23-26]

2.2.3. Total Phenolic, Flavonoid and Protein tests

1 mg/mL stock solutions were created using distilled water from the ethanol extract of the aerial parts of the plant. Then, 250 µL of the solution was added to 1 mL of Folin-Ciocalteu reagent (1:9, v/v) and mixed. Then, 0.75 mL of 1% Na_2CO_3 was added to this solution and incubated for 2 hours (at room temperature). Finally, the reading was performed at 760 nm. Total phenolic content was expressed as mg/g according to the calibration curve of the gallic acid standard solution.[27]

Total flavonoid content of ethanol extract of aerial parts of the plant was determined by aluminum chloride test.[28] 0.5 mL plant extract, 0.1 mL $Al(NO_3)_3$ (%10), 0.1 mL NH_4CH_3COO (1M), 4.3 mL methanol and 0.5 mL Quercetin were mixed to form a solution. This solution was incubated for 40 min. Then, measurements were made at 415 nm. Total flavonoid contents were expressed as mg/g.

3. RESULTS and DISCUSSION

3 .1. Antioxidant activity

Free radicals are oxidant compounds produced as a result of routine metabolic activities. The decreasing levels of these compounds can be tolerated.[29] However, as their levels increase due to environmental effects, cellular damage may occur.[30] In this direction, the antioxidant defense system works to suppress oxidant compounds. However, high levels of oxidant compounds may suppress the antioxidant defense system. In this case, oxidative stress occurs.[31] Serious diseases such as cancer, diabetes, obesity, cardiological disorders, Alzheimer's, and Parkinson's may be observed as a result of oxidative stress. Supplemental antioxidants can be used to suppress these effects of oxidative stress.[32-34] Plants are important antioxidant sources.[35] In this study, antioxidant potentials of ethanol extracts of aerial parts of *Gentiana olivieri* samples collected from Iraq were determined. The findings are shown in Table 1.

In this study, TAS, TOS and OSI values of ethanol extracts of aerial parts of *Gentiana olivieri* samples collected from Iraq were determined. The antioxidant potential of *Gentiana olivieri* has not been reported in the literature using Rel Assay kits. It was detected for the first time in our study. The antioxidant potential of *Gentiana olivieri* has been reported in the literature using different methods.[36] In our study, the antioxidant potential of *Gentiana olivieri* was determined using Rel Assay kits. TAS, TOS and OSI values of different plant species have been reported in the literature using Rel Assay kits. In this context, the TAS value of *Salvia absconditiflora* was determined as 7.350 mmol/L, TOS value as 8.501 µmol/L and OSI value as 0.116.[37] The TAS value of *Lepidium spinosum* was determined as 4.550 mmol/L, TOS value as 12.610 µmol/L and OSI value as 0.277.[38] The TAS value of *Alcea kurdica* was determined as 3.298 mmol/L, TOS value as 8.312 µmol/L and OSI value as 0.252.[39] The TAS value of *Satureja hortensis* was determined as 5.403 mmol/L, TOS value as 3.537 µmol/L and OSI value as 0.065.[40] The TAS value of *Adiantum capillus-veneris* was determined as 3.086 mmol/L, TOS value as 21.532 µmol/L and OSI value as 0.698.[41] The TAS value of *Glycyrrhiza glabra* was determined as 8.770 mmol/L, TOS value as 14.590 µmol/L and OSI value as 0.16.7.[42] The TAS value of *Datura stramonium* was determined as 7.559 mmol/L, TOS value as 10.711 µmol/L and OSI value as 0.142.[43] The TAS value of *Viola odorata* was determined as 6.752 mmol/L, TOS value as 7.886 µmol/L and OSI value as 0.117.[44] Compared to these studies, the TAS value of *Gentiana olivieri* used in our study was determined to be higher than the TAS values of *Salvia absconditiflora*, *Lepidium spinosum*, *Alcea kurdica*, *Satureja hortensis*, *Adiantum capillus-veneris*, *Datura stramonium* and *Viola odorata*, and lower than the TAS value of *Glycyrrhiza glabra*. The TAS value is an indicator of the entirety of antioxidant compounds produced in natural products.[45] It was observed that the TAS value of *Gentiana olivieri* used in our study was high. The TOS value is an indicator of the totality of oxidant compounds produced in natural products.[45] The TOS value of *Gentiana olivieri* used in our study was determined to be lower than *Lepidium spinosum*, *Adiantum capillus-veneris* and *Glycyrrhiza glabra*, and

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higher than *Salvia absconditiflora*, *Alcea kurdica*, *Satureja hortensis*, *Datura stramonium* and *Viola odorata*. In this context, it was determined that *Gentiana olivieri* used in our study had normal levels of TOS values. The OSI value shows how much endogenous oxidant compounds in natural products are suppressed by endogenous antioxidant compounds. In this context, it is recommended not to consume natural products with high OSI values.[45] The OSI value of *Gentiana olivieri* used in our study was determined to be higher than *Salvia absconditiflora*, *Satureja hortensis*, *Datura stramonium* and *Viola odorata*, but lower than *Lepidium spinosum*, *Alcea kurdica*, *Adiantum capillus-veneris* and *Glycyrrhiza glabra*. As a result, it was determined that *Gentiana olivieri* has antioxidant potential.

3.2. Total phenolic and total flavonoid contents

Plants contain many bioactive compounds. Thanks to these bioactive compounds, they have different biological activities.[46] In our study, the total phenolic content (TPC) and total flavonoid content (TFC) of *Gentiana olivieri* were determined. The findings are shown in **Table 1**. According to the findings obtained at the end of the study, the total phenolic content of *Gentiana olivieri* was determined as 104.92±1.40 mg/g and the total flavonoid content was determined as 73.83±1.29 mg/g. In the literature, the total phenolic contents of dichloromethane, ethylacetate and methanol extracts of aerial parts of *Gentiana olivieri* were reported as 63.9-130.9 mg/g and the total flavonoid contents as 15.4-49.6 mg/g.[35] Compared to this study, ethanol extracts of aerial parts of *Gentiana olivieri* were used in our study and it was determined that the total phenolic content showed similar results, but the total flavonoid content was higher. It is thought that this difference in results is due to the difference in the solvent used in the extraction of the plant and the differences in the regions where it was collected.

Table 1. Antioxidant and Oxidant Status of *Gentiana olivieri* ethanol extract.

Samples	TAS	TOS	OSI	TPC	TFC
Gentiana olivieri	7.775±0.114	12.252±0.094	0.158±0.002	104.92±1.40	73.83±1.29

* Values are presented as mean±SD

3.2. Antimicrobial activity

Today, there is an increase in the number of diseases caused by microorganisms. Accordingly, antimicrobial drugs used to combat microorganisms are insufficient.[47] The main reasons for this are the unconscious use of antibiotics. As a result of unconscious use of antibiotics, the number of resistant microorganisms increases and the fight becomes difficult.[48,49] In this context, researchers are turning to the discovery of new antimicrobial drugs. Plants are important natural products in terms of antimicrobial sources.[50] In our study, the effects of *Gentiana olivieri* against standard bacterial and fungal strains were investigated. The findings are shown in Table 2. As a result of our analyses, the antimicrobial activity of *Gentiana olivieri* against bacterial and fungal strains was determined. Ethanol extract of the aerial parts of the plant was used and it was effective against bacterial strains at concentrations ranging from 50 to 200 µg/mL and against fungal strains at a concentration of 100 µg/mL. In our study, it was found that *Gentiana olivieri* extract was effective against standard *S. aureus*, *S. aureus* MRSA, *C. glabrata*, *C. albicans*, *C. krusei* and *P. aeruginosa* at a concentration of 100 µg/mL. It was also found to be effective against *E. faecalis* and *E. coli* at a concentration of 50 µg/mL and against *A. baumannii* at a concentration of 200 µg/mL. In the literature, it has been reported that *Gentiana lutea* is effective against *Bacillus subtilis*, *Listeria monocytogenes*, *Micrococcus flavus*, *M. luteus*, *Proteus mirabilis*, *Sarcina lutea*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus faecalis*, *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *P. tolaasii*, *Salmonella typhimurium*, *S. enteritidis* and *Candida albicans* at different concentrations.[51] In a different study, *Gentiana asclepiadea* was reported to be effective against *Escherichia coli*, *Micrococcus lysodeikticus*, *Candida albicans*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Staphylococcus aureus*.[52] In a different study, *Gentiana cruciata* was reported to be effective against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*.[53] In our study, *Gentiana olivieri* was found to be effective against test bacteria and fungi. As a result, it was determined that *Gentiana olivieri* has antimicrobial potential.

Table 2. Antimicrobial activity of *Gentiana olivieri* ethanol extract

	S. aureus	***S. aureus* MRSA**	***E. faecalis***	***E. coli***	***P. aeruginosa***	***A. baumannii***	***C. glabrata***	***C. albicans***	***C. krusei***
Gentiana olivieri	100	100	50	50	100	200	100	100	100

*50, 100 and 200 µg/mL are the lowest concentrations that inhibit the growth of microorganisms.

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4.CONCLUSION

Today, many plant species are used in traditional medicine. Determining the biological activities of plants is very important. In our study, some biological activities of *Gentiana olivieri* were determined. In this context, it was seen that the antioxidant potential of the plant was high. In addition, it was determined that the total phenolic and total flavonoid contents of the plant were at normal levels. Moreover, *Gentiana olivieri* had high antimicrobial activity, especially against *E. faecalis* and *E. coli*. As a result, *Gentiana olivieri* could be an important antioxidant and antimicrobial source in pharmacological designs.

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None

Conflict of Interest

I declare that there is no a conflict of interest with any person, institute, company, etc.

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