Sigma Journal of Engineering and Natural Sciences
Web page info: https://sigma.yildiz.edu.tr
DOI: 10.14744/sigma.2024.00006

Research Article

Phenolic composition, total antioxidant, antiradical and antimicrobial potential of endemic *Glaucium Alakirensis*

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ARTICLE INFO

Article history
Received: 10 December 2021
Accepted: 08 March 2022

Keywords:
Antimicrobial; Antiradical; Antioxidant; Medicinal Plants; Phenolic

ABSTRACT

Plants have been used by many communities for different purposes such as food, shelter, medicine. The present study aimed to determine phenolic content and biological potential of *Glaucium alakirensis* Aykurt, K. Yıldız & A. Özçandır, which is endemic to Antalya (Turkey). Phenolic composition of the plant was analyzed with HPLC device, while antiradical activity was determined by DPPH method. Antimicrobial tests were conducted with agar dilution method against standard bacteria and fungus strains. Total antioxidant and oxidant status were determined using Rel Assay kits. As a result of HPLC analysis, the presence of catechin, chlorogenic acid, hydroxybenzoic acid, quercetin and gallic acid was determined. Plant extracts were effective against microorganisms at concentrations of 50-200 µg/mL. It was determined that the plant has high antiradical activity. It is also thought to be used as a natural source for relieving oxidative stress.

Cite this article as: Özcandır A, Mohammed FS, Mustafa Sevindik M, Aykurt C, Selamoğlu Z, Akgül H. Phenolic composition, total antioxidant, antiradical and antimicrobial potential of endemic *Glaucium Alakirensis*. Sigma J Eng Nat Sci 2024;42(1):42–48.

INTRODUCTION

Herbal medicine, phyto-nutritional products or nutraceuticals are used in the treatment of diseases in different regions in the world [1,2]. Plants are quite rich in various secondary metabolites such as phenols or tannins [3,4]. These compounds exhibit strong protective biological activities in living organisms [5,6]. Previous studies reported that plants have anti-proliferative, antimicrobial, antioxidant, anti-fungal, anti-cancer, anti-inflammatory, antidiabetic, antimutagenic, antithrombotic, liver protective and estrogenic effects [7-13].

Glaucium Miller (1754) is a distinct genus due to its two stigmas, bicornute and stipitate, and its acrid, non-milky sap among the Papaveraceae family genera. This genus, indigenous in Europe, Central and Southwest Asia, includes

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This paper was recommended for publication in revised form by Regional Editor Banu Mansuroglu

about 25 species [14,15]. There are about 17 species in Iran [16]. In Turkey, there are 11 taxa, including 6 endemic ones, and Turkey is the second richest country in this plant [17-19]. *Glaucium alakirensis* was discovered at Alakır Valley (Kumluca, Antalya) in 2017, and published. Phenological studies reported that the plant flowers during May and June, and bears mature fruits during June and August [20]. *G. alakirenis*, which has only two populations in the habitat range and is represented by fewer than 50 mature individuals, was listed as CR D in IUCN (2012) red list for endangered species [20]. The present study aimed to determine DPPH free radical scavenging activity, antimicrobial activity and oxidative stress status of *G. alakirenis*, as well as conducting phenolic content analyzes on the species. The objective of the present study was to reveal the potential protective properties of *G. alakirenis* species, which was not previously researched for biological activities.

MATERIALS AND METHODS

The plant material tested in the study was collected in Alakır valley (Kumluca, Antalya) (Figure 1). Plant herbarium samples were stored in Akdeniz University Herbarium (AKDU). Plant specimens were dried under adequate laboratory conditions. The samples were then powdered by a mechanical grinder. 30 g powder material was weighed and then extracted with ethanol in Soxhlet extractor.

Determination of Phenolic Content

Phenolic compounds in the plant were scanned with the SHIMADZU system HPLC device [21]. The presence of Gallic acid, Catechin, Epicatechin, Cinnamic acid, Syringic acid, Chlorogenic acid, Quercetin, Caffeic acid, Coumaric acid, Benzoic acid, t-phenolic, Hesperidin, Rosmarinic acid, Hydroxybenzoic acid and Sinapic acid was scanned in the HPLC device. The volume for the injection process was set to 20 µL. 0.8 mL was set as the flow rate. A: 3% acetic acid and B: methanol were used as mobile phase. Chromatographic separation was performed on an Agilent Eclipse XDB-C18 column (250x4.6 mm id 5 µm) at 30 °C.

Antiradical Activity

The antiradical activity in the plant sample was determined using DPPH method (DPPH: 1,1-diphenyl-2-picrylhydrazyl (Sigma, Aldrich)). Stock solutions that included 1 mg/mL extract were prepared with dimethyl sulfoxide (DMSO). 50µL of the solution was added to 160µL of 0.039% DPPH. The final solution samples were incubated for 30 minutes at room temperature in darkness. The sample absorbance values were determined by spectrophotometer at 517 nm following the incubation [22]. Caffeic acid and rosmarinic acid were used as reference antioxidants. Antiradical activity rates were calculated with the following formula.: Scavenging activity (%) = [(ADPPH-ASample) / ADPPH)] x100.

Antimicrobial Activity Tests

The effects of the plant sample on bacteria and fungi were determined by agar dilution method. The bacteria for which the plant extract was tested (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) were cultured in Muller Hinton Broth (Merck) medium. The fungi for which the plant extracts were tested (*Candida albicans* ATCC 10231 and *C. tropicalis* ATCC 13803) were cultured in RPMI 1640 broth medium. Test extracts were adjusted with DMSO at 6.25-800 µg/mL concentrations. Fluconazole was used as a standard for fungi, while Ampicillin and Ciprofloxacin were used as standards for bacteria. The concentrations of plant extracts that inhibit proliferation were determined as the minimum inhibitory concentration (MIC) [23-25].

Determınatıon of TAS, TOS and OSI

The antioxidant (TAS) and oxidant (TOS) potentials of the plant sample were determined using Rel Assay commercial kits (Assay Kit Rel Diagnostics, Turkey). Trolox was used as calibrator in antioxidant tests. Hydrogen peroxide was used as calibrator in oxidant tests [26,27]. The units of TOS and TAS values were equated and proportioned, then

Figure 1. *Glaucium alakirensis* Aykurt, K. Yıldız & A. Ozcandır.

the percentages were taken and the oxidative stress index (OSI) value was obtained [28].

RESULTS AND DISCUSSION

Phenolic Content

Phenolic compounds are organic acids composed of one or more hydroxyl groups attached to one or more aromatic rings and play a significant role in human health due to their pharmacological effects although they possess no nutritional properties [29,30]. The phenolic compounds in *G. alakirensis* extracts utilized in the study were analyzed with HPLC and the results are presented in Table 1.

The analysis findings demonstrated that phenolic compounds, gallic acid, chlorogenic acid, catechin, quercetin and 4-hydroxybenzoic acid were found in the plant. Gallic acid has been reported to have many biological activities. These have been reported to have antimicrobial, antioxidant, antidepressant, antidiabetic, cardioprotective effects [31-39]. It has been reported that chlorogenic acid has anticancer, antidiabetic, antioxidant, anti-inflammatory and hepatoprotective properties [40,41]. Quercetin, epicatechin and catechin have been reported to have important biological effects such as antioxidant, antimicrobial and anti-inflammatory [42-46]. It was reported that hydroxy benzoic acid has antimicrobial, antimutagenic, antiviral, anti-atherogenic, anti-inflammatory, hypoglycemic and antioxidant effects [47]. In the present study, it was determined that *G. alakirensis* might be a natural antioxidant source in healthcare due to the presence of above-mentioned phenolic compounds.

Antiradical Activity

Oxidative stress occurs when excessive amounts of reactive oxygen and/or nitrogen species exceed the endogenous antioxidative capacity of cells that stimulate the oxidation of macromolecules such as proteins, enzymes, lipids and DNA [48-50]. Antioxidant compounds play a vital role in the physical defense system against free radicals, which are harmful by-products of normal aerobic cellular respiration [51]. In cases where endogenous antioxidant content is insufficient, the adverse effects of oxidative stress can be reduced with supplemental antioxidant intake. Thus, antioxidant activity tests are important for the determination of new antioxidant sources. In the present study, the DPPH radical scavenging activity of *G. alakirensis* was determined and the findings are presented in Table 2.

It was determined that the DPPH free radical scavenging percentage of the plant sample ethanol extracts increased with the increase in extract concentration. It was observed that *G. alakirensis* demonstrated a higher antioxidant activity when compared to caffeic acid and rosmarinic acid, which were used as the standards in the study. No previous studies were conducted on the *G. alakirensis* plant. However, several studies were conducted on *Glaucium* genus plants. In studies conducted on *G. contortuplicatum* Boiss., *G. elegans* Fisch. & C. A. Mey, *G. fimbrilligerum* Boiss. and *G. flavum* Crantz, it was reported that these plants can be used as antioxidant sources [52,53]. In the present study, it was determined that the DPPH free radical scavenging rate of *G. alakirensis* ethanol extracts was high. As reported in similar studies on different species in the literature, the present study results demonstrated that *G. alakirensis* species can be used as a strong natural antioxidant source as well.

TAS, TOS and OSI

TAS (mmol/L), TOS (μmol/L) and OSI values for *G. alakirensis* ethanol extract were determined with Rel Assay commercial kits and presented in Table 3.

Table 3. TAS, TOS and OSI values of *G. alakirensis*

	TAS	TOS	OSI
G. alakirensis	3.496±0.121	2.204±0.259	0.063±0.009

*Values are presented as mean±S.D.

Table 1. Phenolic contents of *G. alakirensis*

Gallic acid	Catechin	Clorogenic acid	Hydroxy benzoic acid	Quercetin
5.57 ppm	39.08 ppm	32.95 ppm	28.37 ppm	0.73 ppm

Table 2. DPPH free radical scavenging activity of *G. alakirensis* (% inhibition)

	100 (%)	75 (%)	50 (%)	25 (%)
Caffeic acid	54.47±0.05	38.39±0.66	21.34±0.66	8.62±0.91
Rosmarinic acid	61.92±0.15	35.09±7.96	7.00±0.41	6.03±0.15
G. alakirensis	80.98±3.91	74.19±3.70	50.56±7.96	29.51±4.97

*Values are presented as mean±S.D.

The analyses conducted in the context of the present study, it was determined that the TAS value of *G. alakirensis* was 3.496±0.121 mmol/L, the TOS value was 2.204±0.259 μmol/L and the OSI value was 0.063±0.009. Although there was no previous study on *G. alakirensis*, various TAS values were reported on different plants. It was reported that TAS value of *Salvia sclarea* L. was 4.40 mmol/L [54]. It was determined that the TAS value of *G. alakirensis*, used in the present study, was lower than the values determined for *S. sclarea* plant in the obove-mentioned study. Furthermore, it was reported that the TAS value of *Mentha longifolia* (L.) HUDSON *subsp. longifolia* (L.) HUDSON was 3.628 mmol/L, the TOS value was 4.046 μmol/L and the OSI value was 0.112[55]. TAS value of *Datura stramonium* L. was determined as 7.559 mmol/L, TOS value was 10.711 μmol/L, and OSI value was 0.142 [56]. TAS value of *Marrubium globosum* Montbret & Aucher ex Benth. was determined as 7.677 mmol/L, TOS value as 12.387 μmol/L and OSI value as 0.162 [57]. TAS value of *Galium aparine* L. was determined as 5.147 mmol/L, TOS value as 12.387 μmol/L and OSI value as 0.162 [58]. TAS value of *Ferulago platycarpa* Boiss. & Balansa was determined as 5.688 mmol/L, TOS value as 15.552 μmol/L and OSI value as 0.273 [11]. TAS value of *Adiantum capillus-veneris* L. was determined as 3.086 mmol/L, TOS value as 21.532 μmol/L and OSI value as 0.698 [59].

The TAS value is an indicator of the whole of the endogenous antioxidant compounds in the plant [60]. TAS value of *G. alakirensis* was determined higher than *A. capillus-veneris* and lower than *M. longifolia* subsp. *longifolia, D. stramonium, M. globosum, G. aparine* and *F. platycarpa*. TAS value of *G. alakirensis* was found to be at normal levels. In this context, it is thought that the plant may be a natural antioxidant source. TOS value shows the whole of the oxidant compounds produced as a result of environmental factors and metabolic activities of the plant. The OSI value is an indicator of how much the oxidant compounds produced within the plant are suppressed by the antioxidant defense system [60]. The OSI and TOS values of *G. alakirensis* were determined to be lower than that of *A. capillus-veneris, M. longifolia* subsp. *longifolia, D. stramonium, M. globosum, G. aparine* and *F. platycarpa*. Due to the low levels of oxidative compounds, which are produced as a result of environmental and metabolic effects, in the plant, it was considered that the region where the plant was collected was a more suitable region for the growth of the plant.

Antimicrobial Activity

The antimicrobial activity was determined with *G. alakirensis* ethanol extract and the findings are presented in Table 4.

The negative and adverse effects of synthetic drugs administered against antibiotic-resistant microorganisms and other microorganisms led individuals to prefer natural resources [61,62]. The identification, safe and active use of new natural resources is very important in the fight against these microorganisms. Antimicrobial activity tests demonstrated that *G. alakirensis* ethanol extracts exhibited different levels of antimicrobial activity against the tested microorganisms in all administered doses. It was determined that the plant extract was effective against *E. coli*, *C. albicans* and *C. tropicalis* at 50 μg/mL concentration. It was also observed that 100 μg/mL extract concentrate was effective on *S. aureus* and *E. faecalis*. Furthermore, it was determined that the extract exhibited antimicrobial activity against *P. aeruginosa* at 200 μg/mL concentration. Although there are no previous studies where biological activity alalyses were conducted on *G. alakirensis*, in other studies conducted on other species in the same genus, it was determined that *G. grandiflorum* Boiss. A. Huet, *G. oxylobum* Boiss. & Buhse, and *G. paucilobum* species methanol and chloroform extracts were active against *Staphylococcus aureus, Streptococcus sanguis, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [63]. It was reported that *G. grandiflorum* Boiss. & A.Huet. var. *grandiflorum* methanol extract was active against *Candida krusei* [64]. It was reported that *G. elegans* methanol extracts was active against *S. aureus, E. coli, Salmonella enteritidis, Bacillus anthracis* and *Proteus* sp. [65]. *G. flavum* was reported to exhibit activities against *Geotricum candidum, S. aureus, Bacillus subtilis, E. coli, Salmonella typhimurium* and *K. pneumoniae* [66]. Furthermore, it was demonstrated that the alkaloid fraction of *G. vitellinum* Boiss. & Buhse species was active against *S. typhimurium* [67]. In addition to the above-mentioned studies, the analyses conducted within the context of the present study demonstrated that

Table 4. Antimicrobial activity of *G. alakirensis*

	S. aureus **(μg/mL)**	***E. faecalis*** **(μg/mL)**	***E. coli*** **(μg/mL)**	***P. aeruginosa*** **(μg/mL)**	***C. albicans*** **(μg/mL)**	***C. tropicalis*** **(μg/mL)**
G. alakirensis	100	100	50	200	50	50
Flukonazole	-	-	-	-	1.56	3.12
Ampicillin	3.12	1.56	3.12	-	-	-
Ciprofloxacin	0.78	0.78	1.56	3.12	-	-

*200, 100 ve 50 (μg/mL) extract concentrates affecting microorganisms

the endemic *G. alakirensis* ethanol extracts were active against gram-positive, gram-negative and fungal strains. It is suggested that the tested plant species could be used as a natural antimicrobial source, similar to the species mentioned-above.

CONCLUSION

In the present study, phenolic compound profile, antioxidant and antimicrobial activities, TAS, TOS and OSI values of *G. alakirensis*, an endemic plant prevalent in Antalya province, Turkey, were determined. Analysis findings demonstrated that gallic acid, chlorogenic acid, catechin, quercetin and hydroxybenzoic acid were present in the plant structure. It was also found that the plant species demonstrated high antioxidant potential. It was observed that the plant sample could be a natural antimicrobial source against the tested microorganisms, in addition to its antioxidant potential. It was determined that the *G. alakirensis* could be ultimately used as a potential pharmacological agent as a result of the analyses conducted for the first time in the present study.

AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

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