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Research Article

Multitarget biological activities and RP-HPLC-Based phytochemical profiling of *Glaucium leiocarpum* Boiss.

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Abstract: This study presents a comprehensive evaluation of the phenolic composition and biological activities of methanolic extracts obtained from the aerial (GLM) and root (GLRM) parts of *Glaucium leiocarpum*. Phenolic profiling was conducted using reversed-phase high-performance liquid chromatography (RP-HPLC), revealing catechin hydrate, 3-hydroxybenzoic acid, syringic acid, hesperidin, chlorogenic acid, caffeic acid, and trans-cinnamic acid as major constituents in GLM. In contrast, GLRM exhibited a more diverse phenolic profile, including epicatechin, benzoic acid, quercetin, gallic acid, and rosmarinic acid. Antioxidant activities were evaluated using DPPH and FRAP assays, with GLRM demonstrating stronger radical scavenging capacity (IC_{50}: 36.96 µg/mL) and higher ferric reducing power (173.20±0.68 mg TE/g extract). GLRM also contained significantly higher levels of total phenolics (142.81±2.87 mg GAE/g) and flavonoids (76.67±0.72 mg QE/g). Antimicrobial assays demonstrated that only GLRM exerted inhibitory effects against *Staphylococcus aureus* (8.7±0.57 mm), *Enterococcus faecalis* (7.7±0.57 mm), and *Streptococcus pneumoniae* (15.0±0.0 mm). Cytotoxicity studies, assessed by the XTT assay, revealed potent antiproliferative effects against HT-29 colorectal cancer cells (IC_{50}: 3.59 µg/mL for GLM, 2.29 µg/mL for GLRM) and moderate activity against A549 lung carcinoma cells (IC_{50}: 36.01 µg/mL for GLM, 21.99 µg/mL for GLRM). Furthermore, GLRM exhibited strong anticholinesterase activity, with IC_{50} values of 2.85±0.27 µg/mL for acetylcholinesterase (AChE) and 2.47±0.07 µg/mL for butyrylcholinesterase (BChE). Overall, these findings underscore *G. leiocarpum,* particularly its root extract, as a valuable source of bioactive secondary metabolites, owing to its rich polyphenolic profile and notable antioxidant, antimicrobial, cytotoxic, and cholinesterase inhibitory activities.

INTRODUCTION

Medicinal plants have been widely used for thousands of years in traditional medicine as natural therapeutic agents and dietary supplements. The bioactive compounds they contain have become integral components of modern pharmaceutical applications. In addition to protecting plants against environmental stressors and pathogens, these metabolites exhibit a broad spectrum of biological activities, including antioxidant, antimicrobial, enzyme inhibitory, and anticancer effects. These phytochemicals play a significant role in the prevention and

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management of various human diseases (Mariangela, 2017; Saxena *et al.*, 2013; Thebti *et al.*, 2023; Torunoğlu & Akdağ, 2025; Toul *et al.*, 2025).

Many chronic diseases are closely associated with oxidative stress caused by the overproduction of free radicals, reactive oxygen, and nitrogen species that are natural byproducts of aerobic metabolism. While free radicals play essential roles in normal physiological processes, their excessive accumulation can cause cellular damage and contribute to the onset and progression of numerous pathological conditions, including cancer, Alzheimer's, Parkinson's, inflammatory disorders, and diabetes (Manoharan *et al.*, 2016; Ahmadinejad *et al.*, 2017; Nwozo *et al.*, 2023; Zhang *et al.*, 2022).

Plants are rich sources of antioxidant compounds that help mitigate the harmful effects of free radicals. These antioxidant agents are predominantly synthesized as secondary metabolites, with phenolic compounds representing the most abundant class, accounting for approximately 45% of all secondary phytochemicals. Phenolics and flavonoids are the primary constituents recognized for their potent antioxidant activity. Their chemical structures enable them to scavenge free radicals effectively and inhibit oxidative damage. Numerous studies have demonstrated that these plant-derived antioxidants can play a significant role in preventing or reducing the risk of chronic diseases such as diabetes, cancer, cardiovascular disorders, and neurodegenerative conditions (Hassanpour & Doroudi, 2023; Kopustinskiene, *et al.*, 2020; Nwozo *et al.*, 2023; Sauceda *et al.*, 2017; Sun & Shahrajabian, 2023; Tavsan & Kayali, 2019, Zhang *et al.*, 2022).

Glaucium is a widely distributed genus within the family *Papaveraceae*, comprising approximately 28 species worldwide, with a significant concentration in Southwest Asia, particularly in Iran, Türkiye, and other Middle Eastern countries. Species of the *Glaucium* genus have traditionally been utilized for both culinary and medicinal purposes. Various plant parts, including the green tissues, seeds, and petals, are incorporated into the diet. For instance, young leaves are consumed either roasted or raw in salads, and a traditional beverage known as "poppy sherbet" is prepared from the petals (Saraç *et al.*, 2018). Medically, the aerial parts and seeds have been used as infusions or decoctions for their laxative, antidiabetic, and dermatological effects (Akaberi *et al.*, 2021). Extracts of *G. corniculatum* have been applied topically to treat conditions such as headaches, eye disorders (e.g., blepharitis), edema, burns, and varicose veins. Traditional uses also include treatments for joint inflammation, excessive lacrimation, and ulcerative eye conditions. Similarly, *G. flavum* has been used in decoction form for alleviating sciatica and liver-related ailments and is noted for its diuretic, expectorant, and sedative effects. Moreover, it has traditionally been used to treat bronchitis, colds, and insomnia (Al-Qura'n, 2015; Kocanci *et al.*, 2017; Saraç *et al.*, 2018).

Pharmacologically, several *Glaucium* species, including *G. acutidentatum*, *G. corniculatum*, *G. flavum*, *G. grandiflorum*, *G. oxylobum*, *G. paucilobum*, and *G. vitellinum*, have been reported to exhibit a broad range of biological activities. These include acetylcholinesterase (AChE) inhibitory, analgesic, antinociceptive, neuroprotective, and anticonvulsant effects, particularly on the nervous system. Additionally, antifungal, antibacterial, antioxidant, antidiabetic, and antitumor properties have been documented for various species within this genus (Akaberi *et al.*, 2021; Altın & Köksal, 2024; Arafa *et al.*, 2016; Bektaş *et al.*, 2018; Bournine *et al.*, 2013; Cushnie & Lamb, 2005; Morteza-Semnani *et al.*, 2005; Ozsoy *et al.*, 2018).

Although many *Glaucium* species have been extensively studied for their various pharmacological activities, a comprehensive investigation into the phytochemical composition and biological potential of *G. leiocarpum* has not been conducted. This significant gap in the literature highlights the originality and scientific value of the current study.

This study provides the first comprehensive evaluation of *G. leiocarpum*, offering a detailed characterization of its phenolic constituents alongside a broad assessment of its pharmacological properties, including antioxidant, antimicrobial, cytotoxic, and anticholinesterase activities. These findings provide a foundational framework for future

phytochemical and biomedical research on this previously overlooked species, highlighting its potential as a novel source of bioactive compounds.

METHOD

Materials

2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Folin & Ciocalteu's phenol reagent, quercetin, ethylenediaminetetraacetic acid (EDTA), trihydroxymethylaminomethane (Tris), sodium citrate, 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), acetylthiocholineiodide (AChI), and acetylcholinesterase (AChE), which were used in the studies, were obtained from Sigma-Aldrich (Germany).2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) was obtained from Biological Industries (BI, USA).

Plant Sample Collection

The *G. leiocarpum* plant samples used in the study were collected in July 2022 from the Erzincan Binali Yıldırım University campus (39°48′22″ N, 39°22′24″ E, 1329 m) during the flowering stage. The plant species identification was made by Prof. Dr. Ali Kandemir (Erzincan Binali Yıldırım University, Faculty of Science, Department of Biology). The collected plants were stored in the EBYU herbarium (ALTIN S.1).

Extraction Process

The aerial and root parts of *G. leiocarpum* were separated and dried in the shade at room temperature under cool and dry conditions to prevent degradation from direct sunlight. Once fully dried, the plant materials were ground into a fine powder using a laboratory grinder. A total of 10 grams from each part (aerial and root) was extracted with 100 mL of methanol. The extraction was performed in an ultrasonic bath for 45 minutes and repeated three times under the same conditions to ensure thorough extraction. After extraction, the mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated by removing the solvent using a rotary evaporator at 40 °C (Azwanida, 2015). The extraction yield was calculated as the weight of the dried extract divided by the weight of the dried plant material, expressed as a percentage (w/w). The yields were found to be 41.4% for the aerial part (GLM) and 37.2% for the root part (GLRM). Concentrated extracts were stored in dark bottles at +4°C until analysis and biological activity studies were performed.

Assessment of Phenolic Compounds by RP-HPLC Analyses

The phenolic composition of *G. leiocarpum* extracts was identified and quantified using high-performance liquid chromatography (HPLC) equipped with a diode array detector (DAD) (Agilent Technologies, 1290 Infinity and 1260 Infinity models, Santa Clara, CA, USA). Chromatographic separation was performed on a C18 Inertsil ODS-3 analytical column (4.6 × 250 mm, 5 µm particle size; Tokyo, Japan), with the column temperature maintained at 25°C. A gradient elution system was applied using mobile phase A (3% acetic acid in deionized water) and mobile phase B (methanol), following the method described by Kavaz Yüksel *et al.* (2021). The flow rate was set at 0.8 mL/min, and the injection volume was 20 µL. Each compound was analyzed in triplicate, and the results were expressed quantitatively (Dikici *et al.*, 2024).

Antioxidant Activity

Free radical scavenging activity

The free radical scavenging activity of *G. leiocarpum* extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, based on the method described by Blois (1958) with minor modifications. Stock solutions (1 mg/mL) were prepared, and various concentrations (20, 40, 80, 120, 160, 240, 320, and 400 µg/mL) were tested. Each sample was adjusted to a final volume of 3 mL with methanol, then 1 mL of 0.26 mM DPPH solution was added. The reaction mixtures were vortexed and incubated in the dark at 25 °C for 30 minutes. Absorbance was measured at 517 nm using an ELISA spectrophotometer (BioTek, USA). The radical

scavenging activity was expressed as a percentage of inhibition (%I), calculated using the following formula:

$$\%I = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

where A_{blank} is the absorbance of the control (containing all reagents except the test sample), and A_{sample} is the absorbance of the test extract. The IC_{50} values (concentration of extract required to inhibit 50% of DPPH radicals) were determined from the dose-response curve plotting % inhibition against extract concentration. Trolox was used as the reference antioxidant. All measurements were performed in triplicate to ensure reproducibility (Köksal *et al.*, 2025).

Iron reduction power activity

The ferric reducing antioxidant power (FRAP) of the methanolic extracts obtained from the aerial and root parts of *G. leiocarpum* was determined based on the method of Oyaizu (1986), with slight modifications. Extracts were initially prepared at a concentration of 1 mg/mL. For the assay, 100 µL of each extract was mixed with 1.15 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] in test tubes. The mixtures were incubated at 50 °C for 20 minutes to allow reduction reactions to occur. Following incubation, 1.25 mL of 10% trichloroacetic acid and 0.25 mL of 0.1% ferric chloride ($FeCl_3$) were added to the tubes. The absorbance of the final solution was recorded at 700 nm using an ELISA spectrophotometer (BioTek, USA). The reducing capacities of the extracts were expressed as µg trolox equivalents per gram of extract (µg TE/g) (Oyaizu, 1986; Altın & Köksal, 2024).

Determination of total phenolic content

The total phenolic content (TPC) of the extracts was determined spectrophotometrically using the Folin–Ciocalteu colorimetric method. Briefly, 100 µL of each extract (1 mg/mL) was transferred into test tubes, followed by the addition of 4.5 mL of distilled water. Subsequently, 100 µL of Folin–Ciocalteu reagent was added, and the mixture was allowed to stand at room temperature for 10 minutes. After this period, 300 µL of 2% sodium carbonate (Na_2CO_3) solution was introduced into the reaction mixture. The samples were then vortexed and incubated at room temperature for 120 minutes to allow color development. Absorbance was measured at 760 nm using a spectrophotometer (BioTek, USA). A standard calibration curve was prepared using various concentrations of gallic acid, and results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract) (Slinkard & Singleton, 1977; Köksal *et al.*, 2025).

Determination of total flavonoid content

The total flavonoid content (TFC) of the plant extracts was determined using the aluminum chloride ($AlCl_3$) colorimetric method, as described by Chang *et al*. (2002), with minor modifications (Chang *et al.*, 2002). This method is based on the formation of a colored complex between flavonoid compounds and $AlCl_3$, the intensity of which correlates with flavonoid concentration. For the assay, 100 µL of each extract (1 mg/mL in methanol) was transferred to test tubes and brought to a final volume of 4.8 mL with methanol. Subsequently, 100 µL of 1 M ammonium acetate (NH_4CH_3COO) and 100 µL of 10% $AlCl_3$ solution were added. The mixture was vortexed briefly and then incubated at room temperature for 45 minutes. After incubation, absorbance was measured at 415 nm using an ELISA spectrophotometer (BioTek, USA). A standard calibration curve was constructed using various concentrations of quercetin, and the results were expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract) (Altın & Köksal, 2024).

Antimicrobial Activity Assay

Preparation of bacterial isolates

The antimicrobial activities of methanolic extracts derived from the aerial and root parts of *G. leiocarpum* were evaluated against a panel of standard microbial strains. The tested microorganisms included Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853,

Escherichia coli ATCC 25922, colistin-resistant *Escherichia coli* ATCC 19846, and *Klebsiella pneumoniae* ATCC 700603; Gram-positive bacteria *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 45616, and *Staphylococcus aureus* ATCC 25922; as well as the fungal strain *Candida albicans* (clinical isolate). These strains were selected to assess the broad-spectrum antimicrobial potential of the extracts.

Determination of antimicrobial activity: Disc diffusion method

The antimicrobial activity of the plant extracts was assessed using the Kirby-Bauer disc diffusion method, following the guidelines and standards established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Bauer *et al*., 1966).

Bacterial suspensions were evenly spread onto Mueller-Hinton agar plates (BioMérieux, France) using sterile cotton swabs. For *S. pneumoniae*, Mueller-Hinton Fastidious (MH-F) agar supplemented with 5% defibrinated horse blood and 20 mg/L β-NAD was used to support optimal growth. 20 µL of 200 mg/mL extracts prepared with sterile water was added to sterile blank discs (6 mm diameter, Bioanalyse, Türkiye). After drying, the extract-loaded discs were carefully placed on the agar surface using sterile techniques. The plates were incubated at 37°C for 16–18 hours. Following incubation, the diameters of inhibition zones around the discs were measured in millimeters. All assays were performed in triplicate to ensure reproducibility. As positive controls, vancomycin (5 µg/disc, Bioanalyse, Cat No. ASD09410) and erythromycin (15 µg/disc, Bioanalyse, Cat No. ASD03700) were used against Gram-positive bacteria; amikacin (30 µg/disc, Bioanalyse, Cat No. ASD00100) was used for Gram-negative bacteria; and cycloheximide (10 mg/mL) was employed for *C. albicans*. Sterile distilled water served as the negative control (Altın *et al.*, 2023).

Cell Culture Studies

Cell culture

Human cancer cell lines, including lung carcinoma A549 and colorectal adenocarcinoma HT-29, were obtained from the American Type Culture Collection (ATCC, USA). All culture media were supplemented with 10% fetal bovine serum (FBS), 10 mM sodium pyruvate, and a penicillin-streptomycin antibiotic mixture before use. Cell culture procedures were conducted under sterile conditions in a Class II biosafety cabinet. Cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Cytotoxicity assay

The cytotoxic effects of methanolic extracts from the root and aerial parts of *G. leiocarpum* were evaluated against human lung cancer (A549) and colorectal cancer (HT-29) cell lines using the XTT assay (Dikici *et al.*, 2024; Yeniçeri *et al.*, 2024; Köksal *et al.*, 2025). Briefly, 200 µL of cell suspensions (7.5×10^3 cells/well) were seeded into 96-well plates and incubated for 24 hours at 37 °C in a humidified atmosphere containing 5% CO_2. Stock solutions of the plant extracts were prepared in complete medium containing 0.2% DMSO, and serial dilutions were made to obtain the desired concentrations. Cells were then treated with extract concentrations ranging from 1 to 250 µg/mL and incubated for an additional 24 hours under the same conditions. Following treatment, the medium was removed and replaced with 150 µL of fresh medium and 50 µL of XTT reagent. After 5 hours of incubation, absorbance was measured at 490 nm using a microplate reader (BioTek, USA). Cytotoxicity was evaluated by determining the IC_{50} values, defined as the concentration of extract required to inhibit 50% of cell viability. Each treatment was performed in duplicate, and each concentration was tested in three independent experiments ($n = 6$). Medium containing 0.2% DMSO served as the negative control, while 5-fluorouracil (5-FU) was used as the positive control.

Enzyme Inhibition Studies

The anticholinesterase activities of the aerial and root extracts of *G. leiocarpum* were evaluated using the spectrophotometric method described by Ellman *et al.* (1961). Acetylthiocholine

iodide (AChI) and butyrylthiocholine iodide (BChI) were used as substrates for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) assays, respectively. Tacrine was included as a positive control. The extracts and tacrine were diluted in deionized water to the desired concentrations. The reaction mixture consisted of 100 µL of 1 M phosphate buffer (pH 7.8), 10 µL of the sample solution, 50 µL of 10 mM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), and 10 µL of enzyme solution (0.385 U/mL for AChE and 0.880 U/mL for BChE). The enzymatic reaction was initiated by adding 50 µL of the corresponding substrate (AChI or BChI). The formation of the yellow 5-thio-2-nitrobenzoic acid product was monitored spectrophotometrically at 412 nm. Enzyme inhibition was quantified based on IC_{50} values, which represent the concentration of extract required to inhibit 50% of the enzymatic activity. The results were expressed relative to tacrine, which served as the positive control (Altın & Köksal, 2024).

Statistical Analysis

Statistical analyses for the biological activity studies were performed using GraphPad Prism software (version 7.0; GraphPad Software, La Jolla, CA, USA). One-way ANOVA and Student's t-test were applied to assess differences among the samples. All experiments were conducted in triplicate (n = 3), and the results were expressed as mean ± standard deviation (SD). A $p < 0.05$ was considered statistically significant.

RESULTS

RP-HPLC Analysis of Phenolic Constituents

The phenolic profiles of methanol extracts derived from the aerial parts (GLM) and roots (GLRM) of *G. leiocarpum* were comprehensively analyzed using reversed-phase high-performance liquid chromatography (RP-HPLC) against sixteen reference standards. Representative chromatograms are provided in Figures S1 and S2, while quantitative data are summarized in Table 1, and the chemical structures of the identified secondary metabolites are shown in Figure 1.

Table 1. *Phenolic profiles of Glaucium leiocarpum extracts by RP-HPLC*

No	Phenolic compound	Correlation (r^2)	λ (nm)	RT (min.)	GLM (ppm)	GLRM (ppm)
1-	3-Hydroxy benzoic acid	0.99928	287	22.545	90.255	20.968
2	4-Hydroxy benzoic acid	0.99994	278	17.647	N.D.	N.D.
3	Benzoic acid	0.99986	278	47.629	N.D.	27.015
4	Catechin hydrate	0.99906	278	11.499	183.960	212.257
5	Chlorogenic acid	0.99970	330	16.239	6.696	9.902
6	Caffeic acid	0.99892	330	21.476	1.462	1.095
7	Epicatechin	0.99879	278	20.169	N.D.	36.226
8	Gallic acid	0.99966	278	5.912	0.409	4.665
9	Hesperidin	0.99705	287	65.989	11.196	4.681
10	p-Coumaric acid	0.99982	330	33.597	N.D.	0.435
11	Quercetin	0.99962	254	76.313	N.D.	5.120
12	Rosmarinic acid	0.99907	330	70.655	N.D.	3.086
13	Sinapic acid	0.99925	330	38.264	0.234	0.472
14	Syringic acid	0.99839	278	22.628	24.877	6.636
15	t-Cinnamic acid	0.99998	278	75.207	1.069	0.527
16	t-Ferrulic acid	0.99993	330	37.202	0.101	0.188

Pure standard solutions at concentrations of 1, 5, 10, 20, 50, 70, and 100 ppm were read to create the calibration curve.
RT=Retention Time,
ppm=mg/kg-mg/L, N.D.=Not Detected
GLM: *Glaucium leiocarpum* methanol extract
GLRM: *Glaucium leiocarpum* root methanol extract

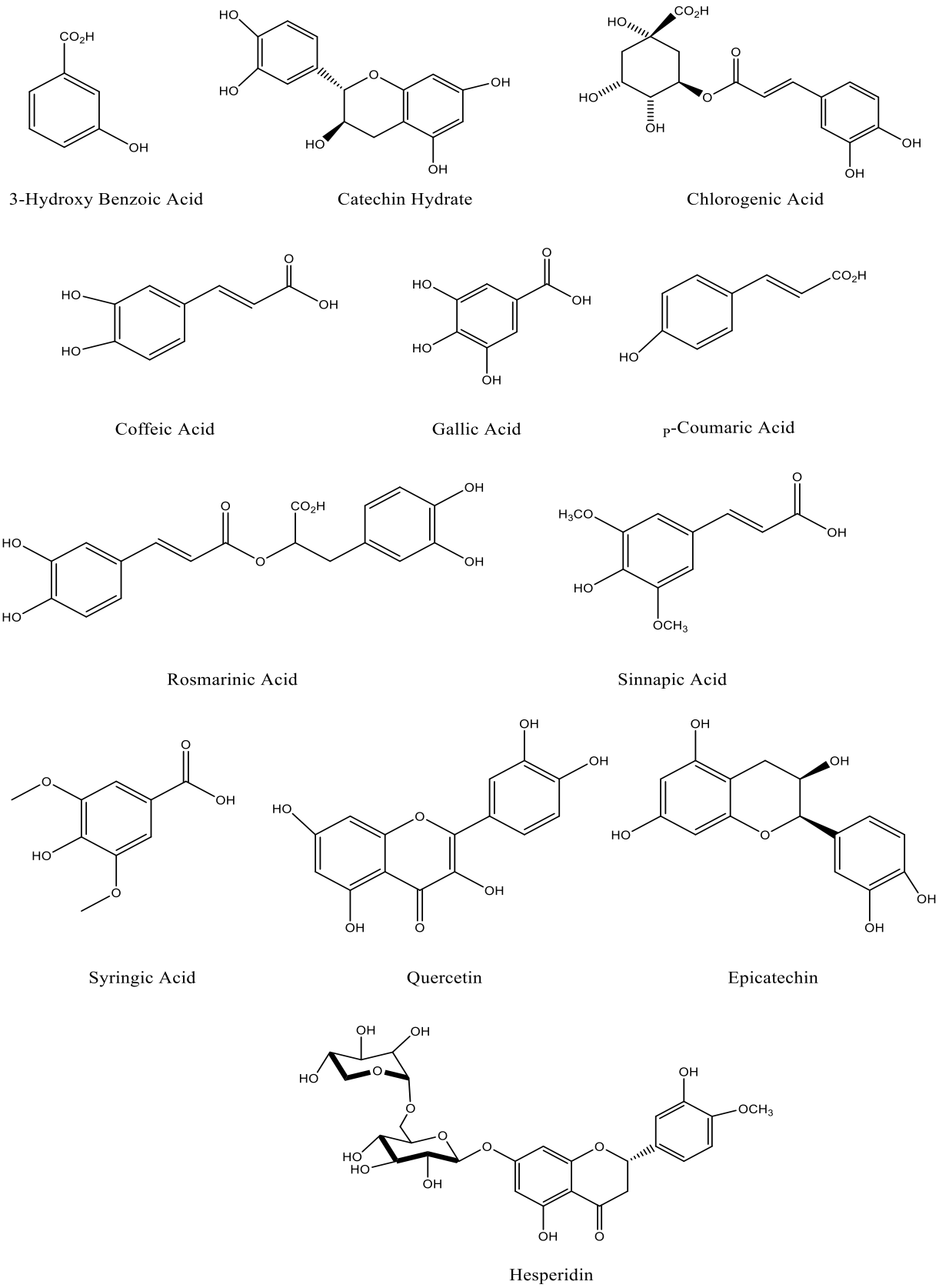


Figure 1. *Comprehensive characterization of bioactive secondary metabolites in G. leiocarpum methanol extract (GLM) and G. leiocarpum root methanol extract (GLRM) extracts.*

RP-HPLC analysis of the GLM extract revealed the presence of 10 phenolic compounds, with catechin hydrate being the predominant constituent at 183.960 ppm, followed by 3-hydroxybenzoic acid (90.225 ppm), syringic acid (24.877 ppm), hesperidin (11.196 ppm), chlorogenic acid (6.696 ppm), caffeic acid (1.462 ppm), and t-cinnamic acid (1.069 ppm).

Minor components detected in lower concentrations included gallic acid (0.409 ppm), cinnamic acid (0.234 ppm), and t-ferulic acid (0.101 ppm). In contrast, the GLRM extract demonstrated a more complex phenolic composition, with 15 compounds identified. The major constituents were catechin hydrate (212.257 ppm) and epicatechin (36.226 ppm), followed by benzoic acid (27.015 ppm), 3-hydroxybenzoic acid (20.968 ppm), chlorogenic acid (9.902 ppm), syringic acid (6.636 ppm), quercetin (5.120 ppm), hesperidin (4.681 ppm), and gallic acid (4.665 ppm). Additionally, rosmarinic acid (3.086 ppm), caffeic acid (1.095 ppm), t-cinnamic acid (0.527 ppm), cinnamic acid (0.472 ppm), p-coumaric acid (0.435 ppm), and t-ferulic acid (0.188 ppm) were also present in lower amounts. These results highlight the richer and more diverse phenolic profile of the root extract compared to the aerial parts.

Antioxidant Activity

The antioxidant potential of *G. leiocarpum* root (GLRM) and aerial (GLM) extracts was assessed using DPPH radical scavenging and iron-reducing power assays, while total phenolic and flavonoid contents were quantified via the Folin-Ciocalteu and $AlCl_3$ colorimetric methods, respectively. The data obtained from the study are tabulated in Table 2, with corresponding graphical trends depicted in Figure 2. As can be seen, the IC_{50} of the GLRM extract was determined as 36.96 µg mL^{-1}, while the IC_{50} of the GLM extract was determined as 44.73 µg mL^{-1} in terms of DPPH scavenging activity. These findings indicate that the root extract possesses superior antioxidant efficacy, as reflected by its lower IC_{50} value.

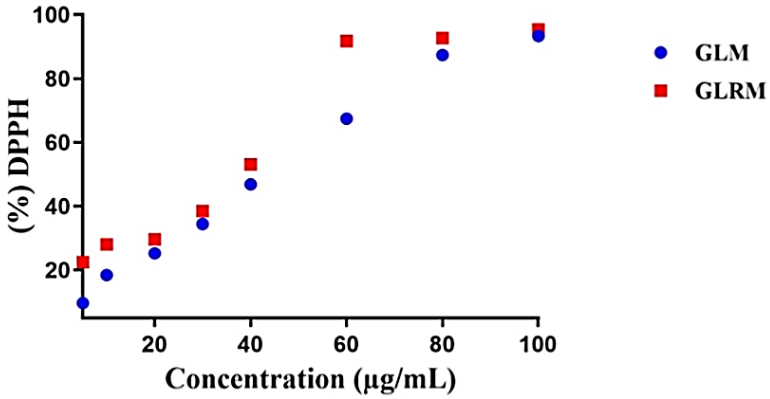


Figure 2. *DPPH radical scavenging activity of G. leiocarpum methanol extract (GLM) and G. leiocarpum root methanol extract (GLRM).*

Consistent with the DPPH radical scavenging assay, the GLRM extract demonstrated markedly higher iron-reducing power compared to the GLM extract, with values of 173.20 ± 0.68 mg Trolox equivalents (TE) per gram of extract and 154.50 ± 0.79 mg TE/g, respectively. This enhanced reducing capacity further underscores the potent antioxidant potential of the root extract.

In the quantitative assessment of total phenolic compounds, the GLRM extract exhibited significantly elevated levels, measuring 142.81 ± 2.87 mg gallic acid equivalents (GAE) per gram of extract, which was nearly double that of the aerial extract, recorded at 72.44 ± 0.44 mg GAE/g. The higher phenolic content in the root extract likely contributes to its superior free radical scavenging and reducing abilities. Similarly, the total flavonoid content followed the same trend, with the GLRM extract containing 76.67 ± 0.72 mg quercetin equivalents (QE) per gram, substantially exceeding the 14.29 ± 0.29 mg QE/g detected in the aerial part extract. Given that flavonoids are well-known for their antioxidant properties, these findings highlight the root extract as a richer source of bioactive flavonoids, potentially explaining its enhanced biological activity observed in this study.

Table 2. *Total phenolic, flavonoid contents, and antioxidant capacities of G. leiocarpum extracts.*

Samples	DPPH• scavenging lC_{50} (µg mL^{-1})	Total phenolics mg GAE g^{-1} extract	Total flavonoids mg QE g^{-1} extract	Reducing power mg TE g^{-1} extract
GLRM	36.96±0.98	142.81±2.87	76.67±0.72	173.20±0.68
GLM	44.73±0.99	72.44 ±0.44	14.29±0.29	154.50±0.79
Trolox	11.95±0.15			

GAE: Gallic acid equivalent
QE: Quercetin equivalent
TE: Trolox equivalent
GLM: *Glaucium leiocarpum* methanol extract
GLRM: *Glaucium leiocarpum* root methanol extract

Antimicrobial Activity

The antimicrobial potential of methanol extracts obtained from both the aerial and root parts of *G. leiocarpum* was systematically evaluated using the Kirby-Bauer disc diffusion assay. This method, widely recognized for its reliability in assessing antimicrobial efficacy, was employed against a panel of clinically relevant microbial strains. The tested microorganisms included seven bacterial species: *P. aeruginosa*, *E. coli*, *K. pneumoniae*, colistin-resistant *E. coli*, *E. faecalis*, *S. pneumoniae*, and *S. aureus*. Additionally, the antifungal activity was assessed against the opportunistic yeast *C. albicans*. These strains represent a diverse spectrum of Gram-negative and Gram-positive bacteria as well as fungal pathogens, which are often implicated in human infections and are of significant clinical concern due to their resistance profiles. According to the results presented in Table 3, the methanolic aerial extract (GLM) of *G. leiocarpum* did not show any antimicrobial activity against the tested bacterial and fungal strains. In contrast, the root extract (GLRM) showed inhibition against three Gram-positive bacteria, *S. aureus*, *E. faecalis*, and *S. pneumoniae*. With GLRM extract at a concentration of 200 mg mL^{-1}, inhibition zones of 8.7±0.57 mm, 7.7±0.57 mm, and 15.0±0.0 mm were obtained on these bacteria, respectively. These values are lower when compared to the inhibition zones formed by the positive controls, erythromycin (15 µg) and vancomycin (5 µg) discs used under the same test conditions (*S. aureus*: 21.3±0.57 mm, *E. faecalis*: 14.3±0.57 mm, *S. pneumoniae*: 25.3±0.57 mm). This reveals that the GLRM extract exhibits a significant, albeit weaker, antimicrobial activity compared to the positive controls and exhibits a higher level of activity, especially against *S. pneumoniae*. The antimicrobial activity was limited only to Gram-positive bacteria; no inhibition was observed against Gram-negative species and *C. albicans*. This demonstrates that the GLRM extract has a selective antimicrobial spectrum and suggests that the effective compounds may be more concentrated in the root part or only found there. These results show that the bioactive compounds contained in the root extract can be a potential source for the development of natural antimicrobial agents by isolating them at an advanced level.

Table 3. *Antimicrobial activities of G. leiocarpum extracts (mean ± SD, n = 3).*

	Zone Diameter of Standard Strains (mm ± SD)		
Samples	*S. aureus*	*E. faecalis*	*S. pneumoniae*
GLM	-	-	-
GLRM	8.7±0.57	7.7±0.57	15.0±0.0
E (15 µg)	21.3±0.57	nt	nt
VA (5 µg)	nt	14.3±0.57	25.3±0.57

GLM: G. leiocarpum methanol extract; GLRM; G. leiocarpum root methanol extract; E: Erythromycin, VA: Vancomycin, -: no inhibition, nt: Not tested

Antiproliferative Activity

The cytotoxic effects of methanol extracts derived from the roots (GLRM) and aerial parts (GLM) of *G. leiocarpum* were assessed using the XTT assay against human lung carcinoma (A549) and colorectal adenocarcinoma (HT-29) cell lines. The results are summarized in Table

4. For the A549 cell line, the half-maximal inhibitory concentration (IC_{50}) values were determined as 36.01±2.00 µg/mL for GLM and 21.99±0.04 µg/mL for GLRM. In comparison, the reference chemotherapeutic agent, Cisplatin, exhibited a significantly lower IC_{50} value of 1.34±1.00 µg/mL, reflecting its high potency. Although both plant extracts demonstrated weaker cytotoxicity than Cisplatin, the root extract (GLRM) showed notably greater efficacy than the aerial part extract (GLM), as evidenced by its lower IC_{50} value.

In contrast, against the HT-29 colorectal cancer cell line, both *G. leiocarpum* extracts showed significantly increased cytotoxic activity. The IC_{50} values for GLM and GLRM were 3.59 ± 1.00 µg/mL and 2.29 ± 0.99 µg/mL, respectively, both markedly lower than the IC_{50} of the standard chemotherapeutic agent 5-Fluorouracil (5-FU, 23.2 ± 1.00 µg/mL). These results indicate that *G. leiocarpum* extracts, particularly the root extract, exert potent antiproliferative effects and even outperform 5-FU in this in vitro model. Interestingly, similar observations have been reported in the literature, suggesting that the enhanced cytotoxicity of crude plant extracts may arise from the number, quantity, and diversity of bioactive molecules they contain, which can act synergistically or additively to produce stronger effects than single-agent drugs (Vaou *et al*., 2022). Such interactions among phytochemicals may explain the high potency observed and highlight the need for further investigation into the mechanisms underlying these effects and the therapeutic potential of *G. leiocarpum*.

Table 4. *IC_{50} (µg/mL) values of G. leiocarpum extracts on cancer cell lines.*

Samples	A549	HT-29
GLM	36.01±2.00^{c}	3.59±1.00^{b}
GLRM	21.99±0.04^{b}	2.29±0.99^{a}
5-FU	-	23.2±1.00^{c}
Carboplatin	1.34±1.00^{a}	-

5-FU: Fluorouracil, GLM: *G. leiocarpum methanol extract*, GLRM: *G. leiocarpum root methanol extract*, A549: human lung cancer cells, HT-29: human colon cancer cells.
Note: Values with different superscripts (a–c) within the same column are significantly different ($p < 0.05$).

Anticholinesterase activity

In this study, anticholinesterase activities of *G. leiocarpum* extracts were determined by the Ellman method, and the results are expressed as IC_{50} values. The results are presented in Figure 3 and Table 5. The reliability of the results was confirmed by using tacrine, a widely used positive control. IC_{50} values of tacrine were obtained for AChE of 3.19±0.06 µg/mL and for BChE of 2.54±0.10 µg/mL. According to the experimental results, GLRM extract showed the lowest IC_{50} values for both enzymes (AChE: 2.85±0.27 µg/mL; BChE: 2.47±0.07 µg/mL, $^{**}p<0.05$), indicating its high inhibitory activity against cholinesterase enzymes. GLM extract exhibited a lower inhibition profile with an IC_{50} value of 4.65±0.43 µg/mL ($^{***}p<0.05$) for AChE and 4.47±0.14 µg/mL ($^{****}p<0.05$) for BChE. Remarkably, the GLRM extract exhibited equivalent or even stronger inhibitory activity on AChE and BChE compared to the positive control, tacrine. Specifically, for BChE inhibition, GLRM extract had a lower IC_{50} than tacrine (2.47±0.07 vs. 2.54±0.10 µg/mL), indicating that the bioactive compounds present in the root extract may be potent and potentially pharmaceutically important natural cholinesterase inhibitors. In this context, *G. leiocarpum*, particularly its root, could be considered a potential source of natural inhibitors for use in cholinesterase-related neurodegenerative diseases, particularly Alzheimer's disease.

Table 5. *Anticholinesterase activity values of G. leiocarpum extracts (IC_{50}; µg/mL).*

	IC_{50} (µg/mL)			
Inhibitor	AChE	R^2	BChE	R^2
GLM	4.65±0.43^{c}	0.9318	4.47±0.14^{b}	0.9142
GLRM	2.85±0.27^{a}	0.9756	2.47±0.07^{a}	0.9743
Tacrine	3.19±0.06^{b}	0.9896	2.54±0.10^{a}	0.9878

GLM: *G. leiocarpum methanol extract*
GLRM: *G. leiocarpum root methanol extract*
Note: Values with different superscripts (a–c) within the same column are significantly different ($p < 0.05$).

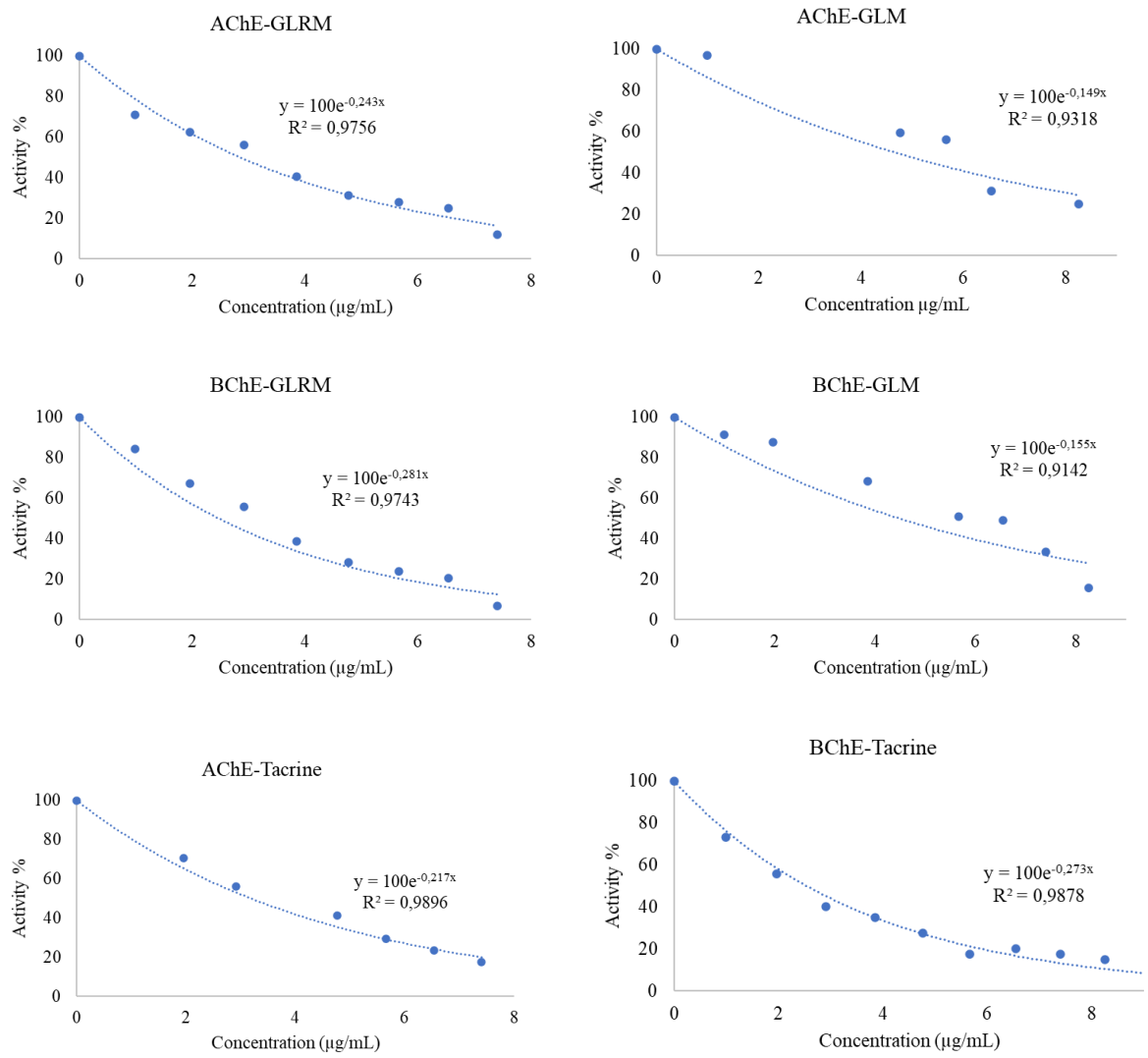


Figure 3. *Cholinesterase inhibitory effects of G. leiocarpum methanol extract (GLM) and G. leiocarpum root methanol extract (GLRM), and tacrine.*

DISCUSSION and CONCLUSION

Glaucium species are a significant plant group with potential pharmacological applications due to their diverse bioactive properties. Various studies have investigated their antioxidant, antimicrobial, anticancer, and anticholinesterase activities, highlighting their relevance in therapeutic research (Altın & Köksal, 2024; Kocanci *et al*., 2017; Kalantari & Entezari, 2020; Özcandır *et al*., 2024; Ozsoy *et al*., 2018). In this context, the present study provides the first comprehensive evaluation of the phytochemical profile and biological activities of *Glaucium leiocarpum.* RP-HPLC analysis demonstrated that both aerial and root extracts contain several phenolic constituents commonly reported in other *Glaucium* species, including catechin, chlorogenic acid, gallic acid, quercetin, caffeic acid, and syringic acid. The presence of these shared metabolites suggests a relatively consistent phenolic profile within the genus, which may contribute to the biological activities previously attributed to *Glaucium* species (Gümrükçüoğlu, 2025; Özcandır *et al*., 2024; Yagi *et al*., 2024).

The root extract was found to have a higher total phenolic content (142.81 ± 2.87 mg GAE/g) and total flavonoid content (76.67 ± 0.72 mg QE/g) compared to the above-ground parts. Such differences are generally associated with tissue-specific metabolic roles; roots often function as important sites for the accumulation of defense-related secondary metabolites, which may explain the stronger biological activities observed in the root extract. The antioxidant activity

results support this phytochemical distribution. The GLRM extract exhibited stronger radical scavenging activity (DPPH IC_{50} = 36.96 µg/mL) and higher ferric-reducing power (173.20 ± 0.68 mg TE/g extract) compared with the aerial extract. Previous findings on *G. leiocarpum* also demonstrate that phytochemical composition and antioxidant activity vary considerably depending on the plant part and extraction solvent. Adıgüzelli *et al.* (2024) investigated ethyl acetate, methanol, and water extracts prepared from the leaves, branches, and roots of *G. leiocarpum*. In their study, the water extracts exhibited the highest DPPH radical scavenging activities among the tested solvents, with IC_{50} values of 70.56 ± 0.98, 163.69 ± 0.51, and 132.54 ± 0.75 µg/mL for the leaves, branches, and roots, respectively. Similarly, the highest FRAP activities were observed in the water extracts, with values ranging from 48.49 ± 0.58 to 62.53 ± 1.04 mg TE/g extract. The highest total phenolic contents were reported for the leaf water extract (63.97 ± 1.69 mg GAE/g extract), branch methanol extract (42.12 ± 1.41 mg GAE/g extract), and root methanol extract (45.75 ± 0.71 mg GAE/g extract), whereas the methanol extracts showed the highest total flavonoid contents, reaching 43.62 ± 3.88, 11.43 ± 2.44, and 28.48 ± 3.80 mg QE/g extract for the leaves, branches, and roots, respectively. Compared with these findings, the GLRM extract in the present study exhibited substantially higher total phenolic and flavonoid contents, as well as stronger DPPH radical scavenging activity and ferric-reducing capacity. These differences may be attributed to variations in extraction conditions, plant material, geographical origin, and the tissue-specific distribution of secondary metabolites. Comparable studies on other *Glaucium* species have also reported markedly lower antioxidant capacities. For instance, Gümrükçüoğlu (2025) reported a total phenolic content of 13.84 ± 0.46 mg GAE/g and a FRAP value of 10.98 ± 0.22 µmol Fe^{2+}/g for *G. flavum*, while Yagi *et al*. (2024) reported a FRAP value of 33.20 ± 0.27 mg TE/g for *G. acutidentatum*. These values, together with the findings of Adıgüzelli *et al*. (2024), are considerably lower than those obtained for the GLRM extract, indicating a comparatively higher antioxidant potential in *G. leiocarpum*, particularly its root fraction.

The antimicrobial activity observed in this study further supports the bioactive potential of the species. The GLRM extract exhibited inhibitory effects against *Staphylococcus aureus* (8.7 ± 0.57 mm), *Enterococcus faecalis* (7.7 ± 0.57 mm), and *Streptococcus pneumoniae* (15.0 ± 0.0 mm), while the aerial extract showed no activity. Previous studies reported inhibition zones of 12 mm against *S. aureus* for *G. alakirensis* (Delik *et al*., 2024) and 17.6 mm for alkaloid extracts of *G. vitellinum* (Zhang *et al*., 2022). Although the activity of *G. leiocarpum* against *S. aureus* is comparatively lower, the pronounced inhibition observed against *S. pneumoniae* suggests a distinct antibacterial spectrum. The antiproliferative activity of the extracts against HT-29 and A549 cancer cell lines revealed strong cytotoxic potential, particularly for the root extract (HT-29 IC_{50} = 2.29 µg/mL; A549 IC_{50} = 21.99 µg/mL). These values are significantly more effective than those reported for other *Glaucium* species. For example, Haji *et al.* (2013) reported IC_{50} values of 22.32 ± 1.34 µg/mL for *G. flavum* alkaloid extract and 29.81 ± 1.05 µg/mL for *G. grandiflorum*, while Kocanci *et al*. (2017) reported much higher values (979–1166 µg/mL) for methanolic extracts of *G. acutidentatum* and *G. corniculatum*. These comparisons indicate that *G. leiocarpum*, particularly its root extract, exhibits relatively strong antiproliferative activity among the studied *Glaucium* species.

The cholinesterase inhibitory activity further highlights the pharmacological relevance of the species. The GLRM extract showed IC_{50} values of 2.85 ± 0.27 µg/mL for AChE and 2.47 ± 0.07 µg/mL for BChE, which are comparable to those reported for other *Glaucium* species, such as *G. cappadocicum* (0.55–4.05 µg/mL for AChE and 0.65–4.15 µg/mL for BChE) (Altın & Köksal, 2024). These findings suggest that *G. leiocarpum* may possess significant cholinesterase inhibitor potential within its genus range. The biological activities observed in this study are thought to be related to the combined and possibly synergistic effects of phenolic compounds, including catechin, quercetin, and gallic acid, identified in the extracts.

Despite these promising results, several limitations should be considered. The use of crude extracts does not allow the identification of individual active compounds, and possible synergistic or antagonistic interactions remain unclear. Additionally, the lack of non-cancerous cell lines limits the evaluation of selectivity. Environmental factors may also influence phytochemical composition, as plant material was collected from a single location. In conclusion, this study provides the first integrated phytochemical and biological evaluation of *Glaucium leiocarpum*. The root extract, characterized by higher phenolic and flavonoid content, exhibited stronger antioxidant, antimicrobial, antiproliferative, and cholinesterase inhibitory activities compared with the aerial extract. The results suggest that these effects are driven by the combined action of phenolic constituents rather than single compounds. Overall, *G. leiocarpum* and its root in particular can be considered a promising natural source of bioactive metabolites for further pharmacological research.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Data Availability Statement

Research data is available and can be sent via email upon request.

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Authorship Contribution Statement

S.A.: Writing–original draft, Writing–review and editing, Conceptualization, Data curation, Formal Analysis, Methodology, Investigation, Resources, Visualization, Project administration.

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APPENDICES

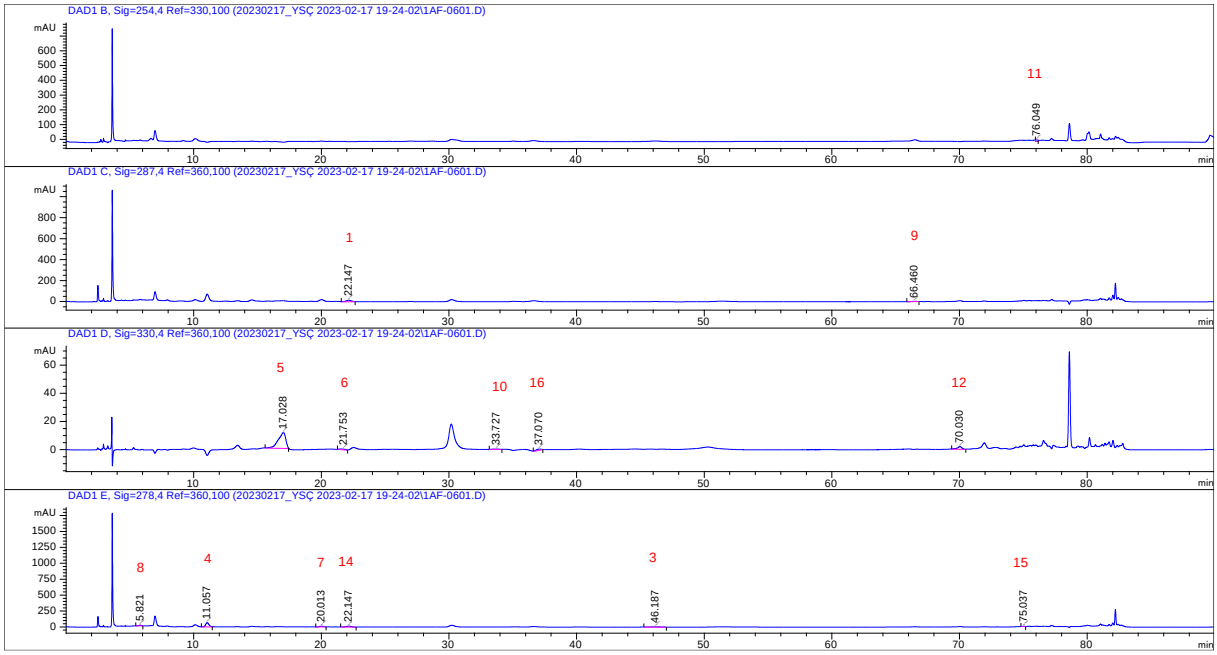


Figure S1. RP-HPLC chromatogram of the methanolic extract of *G. leiocarpum* root.

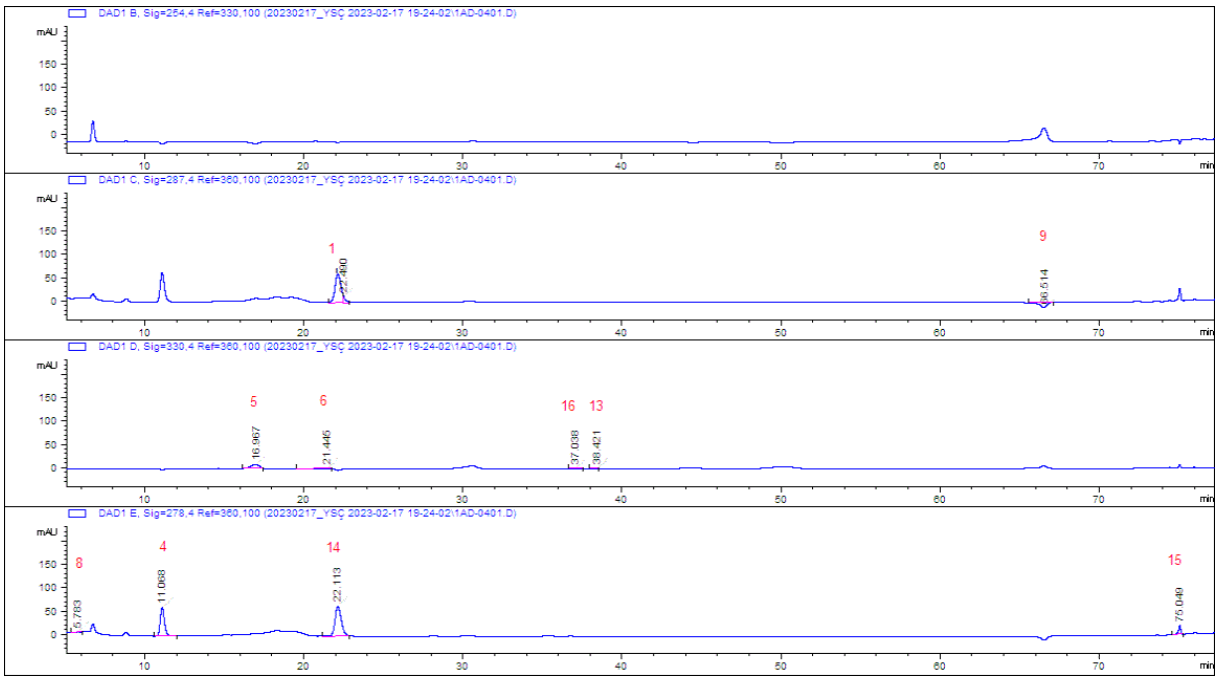


Figure S2. RP-HPLC chromatogram of the methanolic extract of *G. leiocarpum* aerial parts.