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Comparative Analysis of the Antioxidant, Enzyme Inhibitory, and Antimicrobial Properties of *Glaucium leiocarpum* Extracts Obtained by Different Extraction Methods

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ABSTRACT
The genus *Glaucium* Mill. (Papaveraceae), comprising approximately 30 taxa worldwide and represented by 12 taxa in Türkiye, remains underexplored in terms of its bioactive potential. In this study, the antioxidant, enzyme inhibitory, and antimicrobial properties of *Glaucium leiocarpum* Boiss. extracts were comparatively evaluated to generate novel insights into this neglected species. The aerial parts of *G. leiocarpum* were collected from Denizli, Türkiye, and extracted using two solvents, 80% methanol or ethanol, through either ultrasonic-assisted or orbital-shaker methods. Antioxidant capacity was assessed via the ABTS and CUPRAC assays, while tyrosinase, α-amylase, and α-glucosidase inhibition tests were performed to determine enzyme inhibitory activity. Antimicrobial efficacy was evaluated against Gram-positive, Gram-negative, and fungal strains by disk diffusion and microdilution techniques. The orbital methanolic extract showed the highest ABTS radical-scavenging activity (152.67±2.75 mg TEs/g), whereas the ultrasonic methanolic extract exhibited the strongest CUPRAC reducing power (168.64 ± 7.42 mg TEs/g). Tyrosinase inhibition was most pronounced in ultrasonic and orbital ethanol extracts, while α-glucosidase inhibition was markedly stronger in ethanol-based extracts compared with their methanol counterparts. All extracts displayed weak antimicrobial activity with inhibition zones of 8–9 mm and MIC values ranging from 512 to 1024 µg/mL, with *Pseudomonas aeruginosa* being the most susceptible. This investigation represents the first systematic report on the antioxidant, enzyme inhibitory, and antimicrobial activities of *G. leiocarpum*. Although antimicrobial effects were limited relative to positive controls, the strong antioxidant and selective enzyme inhibitory activities emphasize its potential as a source of novel phytochemicals, warranting further phytochemical characterization and pharmacological exploration.

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Farklı Ekstraksiyon Yöntemleriyle Elde Edilen *Glaucium leiocarpum* Ekstraktlarının Antioksidan, Enzim İnhibitörü ve Antimikrobiyal Özelliklerinin Karşılaştırmalı Analizi

ÖZET
Glaucium Mill. (Papaveraceae) cinsi dünya genelinde yaklaşık 30 takson ile temsil edilmekte olup Türkiye'de 12 taksonla yer almaktadır ve biyolojik etkinlik potansiyeli açısından hâlen yeterince araştırılmamıştır. Bu çalışmada, ihmal edilmiş bir tür olan *Glaucium leiocarpum* Boiss. ekstraktlarının antioksidan, enzim inhibitörü ve antimikrobiyal özellikleri karşılaştırmalı olarak değerlendirilerek bu bitki hakkında yeni veriler sunulması amaçlanmıştır. *G. leiocarpum*'un toprak üstü kısımları Denizli (Türkiye) bölgesinden toplanmış ve %80 metanol veya etanol kullanılarak ultrason destekli ya da orbital çalkalayıcı yöntemleriyle ekstrakte edilmiştir. Antioksidan kapasite ABTS ve CUPRAC testleriyle belirlenmiş, tirozinaz, α-amilaz ve α-glukozidaz inhibisyon testleri enzim inhibitör aktivitesini değerlendirmek için uygulanmıştır. Antimikrobiyal

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etkinlik ise Gram pozitif, Gram negatif ve fungal suşlara karşı disk difüzyon ve mikrodilüsyon yöntemleriyle test edilmiştir. Orbital metanolik ekstrakt en yüksek ABTS radikal süpürücü aktivitesini (152.67±2.75 mg TEs/g) gösterirken ultrasonik metanolik ekstrakt en güçlü CUPRAC indirgeme gücünü (168.64±7.42 mg TEs/g) sergilemiştir. Tirozinaz inhibisyonu ultrasonik ve orbital etanol ekstraktlarında en belirgin şekilde gözlenmiş, α-glukozidaz inhibisyonu ise metanol bazlılara kıyasla etanol bazlı ekstraktlarda belirgin olarak daha yüksek bulunmuştur. Tüm ekstraktlar 8–9 mm inhibisyon zonları ve 512–1024 µg/mL aralığında MIC değerleri ile zayıf antimikrobiyal aktivite sergilemiş, *Pseudomonas aeruginosa* en duyarlı bakteri olmuştur. Bu çalışma *G. leiocarpum*'un antioksidan, enzim inhibitörü ve antimikrobiyal aktiviteleri üzerine yapılmış ilk sistematik araştırmadır. Antimikrobiyal etkinlikler pozitif kontrollere göre sınırlı olsa da, güçlü antioksidan ve seçici enzim inhibitörü aktiviteleri bu türün yeni fitokimyasallar açısından potansiyel bir kaynak olduğunu vurgulamaktadır.

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INTRODUCTION

Reactive oxygen species (ROS), as by-products of cellular oxygen metabolism, play key roles in maintaining redox homeostasis, regulating gene expression, signal transduction, and supporting immune responses. However, their excessive accumulation can lead to oxidative stress, causing cellular damage and contributing to the pathogenesis of chronic inflammation, neurodegenerative conditions, and cardiovascular diseases (He et al., 2017). Antioxidants—compounds that neutralize or prevent oxidative damage—are therefore essential for preserving cellular integrity. Some wild plant species have even demonstrated stronger antioxidant activities than synthetic analogs (Alper & Özay, 2022).

Tyrosinase, a copper-containing enzyme, catalyzes the oxidation of monophenols to o-quinones and plays a critical role in melanin biosynthesis (Sugumaran & Barek, 2016). While melanin provides photoprotection and antioxidant defense, its overproduction—triggered by genetic or environmental factors—may cause hyperpigmentation disorders, including melasma and solar lentigines (Trivedi et al., 2017). Although synthetic tyrosinase inhibitors such as hydroquinone and kojic acid are widely used in dermatology, they are associated with adverse effects like skin irritation and potential carcinogenicity (Pillaiyar et al., 2017). This has led to increasing interest in plant-derived tyrosinase inhibitors with dual antioxidant and depigmenting properties. In this context, phytochemical-rich medicinal plants serve as valuable sources of multifunctional agents for dermatological and cosmetic applications (Ribeiro et al., 2015).

Moreover, the global rise in chronic diseases—driven by factors such as sedentary lifestyles and poor nutrition—has intensified the need for safer therapeutic alternatives. Diabetes mellitus (DM), characterized by elevated blood glucose levels due to impaired insulin secretion or action, is among the most prevalent of these diseases, with its incidence expected to increase significantly in the coming years (WHO, 2018). While synthetic drugs such as sulfonylureas and thiazolidinediones are commonly used, their side effects limit long-term use (Rahimi-Madiseh et al., 2016). Thus, interest in antidiabetic plant-based therapies has grown, as numerous plant species have demonstrated promising α-amylase- and α-glucosidase-inhibitory activity (Haouari & Rosado, 2019; Ashagrie et al., 2025).

Another global health threat is the emergence of antibiotic-resistant microbial strains due to the widespread and often inappropriate use of antibiotics (Hughes & Andersson, 2017). This has renewed scientific interest in exploring plant-based antimicrobial agents and elucidating the therapeutic potential of bioactive phytochemicals in combating microbial infections (Vaou et al., 2021).

Medicinal plants have long been central to traditional healthcare systems, and their therapeutic effects have been the subject of numerous studies (Shahrajabian et al., 2020). Their relatively low toxicity, cost-effectiveness, accessibility, and pharmacological versatility make them attractive candidates for drug discovery (Salm et al., 2023). Nevertheless, the biological activities of many species remain underexplored. One such species is *Glaucium*

leiocarpum Boiss., a member of the Papaveraceae family. Türkiye ranks second in the world for *the diversity of Glaucium species*. In the country, there are 9 species and 12 taxa, of which 4 are endemic (Güner et al., 2012). *G. leiocarpum*, commonly known in Turkish as 'gâvur haşhaşı' or 'boynuzlu gelincik,' is traditionally recognized for its antitussive and wound-healing properties (Ertuğ, 2000; Deniz et al., 2010) and is known to contain alkaloids (Aynur, 1999). However, the phytochemical and pharmacological characterization of *G. leiocarpum* remains limited in the scientific literature.

To the best of our knowledge, this is the first study to evaluate the antioxidant, enzyme-inhibitory (α-amylase, α-glucosidase, and tyrosinase), and antimicrobial properties of *G. leiocarpum* extracts. Moreover, both solvent type (methanol vs. ethanol) and extraction method (ultrasonic-assisted vs. orbital shaker) were compared, representing an additional novel aspect of the study. The findings are expected to contribute to a deeper understanding of this underutilized species and its potential applications in developing multifunctional plant-based therapeutics.

MATERIAL and METHOD

Plant Material and Extraction

The aerial parts of *G. leiocarpum* were collected during the flowering period in May 2021 from Yatağan Village, Denizli, Türkiye. After taxonomic identification by Asst. Prof. Cennet Özay, the plant material (Voucher no: CO1900) was air-dried in the shade and ground into a fine powder. A 10 g portion of the powdered sample was extracted with 100 mL of 80% methanol or 80% ethanol (plant-to-solvent ratio: 1:10, w/v) using either orbital shaking or an ultrasonic bath for 2 hours at room temperature in the dark. Extracts were filtered, pooled, and evaporated at 40 °C using a rotary evaporator, then concentrated under reduced pressure and lyophilized. Three parallel extractions were performed for each method, and dry extracts were stored at -20 °C until needed. The dried extracts were weighed to determine the percent yield (Ozay and Mammadov, 2022). The percentage yield was calculated using the formula W1/W0 × 100. Where W1 is the final weight of the extract, and W0 is the initial weight of the sample.

The following abbreviations were used for the extracts: GLUM (ultrasonic methanol extract of *G. leiocarpum*), GLUE (ultrasonic ethanolic extract), GLOM (orbital methanolic extract), and GLOE (orbital ethanolic extract).

Antioxidant Activity

ABTS assay

The 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+}$) radical cation was produced in situ by reacting a 7 mM ABTS solution with 2.45 mM potassium persulfate and incubating the mixture in the dark at room temperature for 12-16 h. The resulting solution was diluted with methanol to an absorbance of 0.700 ± 0.02 at 734 nm. Sample solution (1 mL) was then mixed with $ABTS^{+}$ solution (2 mL), and after 30 min of incubation at room temperature, absorbance was measured at 734 nm. Results were expressed as mg trolox equivalents per gram of extract (mg TEs/g extract) (Ozay, 2023).

CUPRAC assay

Cupric ion reducing activity (CUPRAC) was determined by adding sample solution (0.5 mL) to a premixed reaction mixture containing $CuCl_2$ (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM), and NH_4Ac buffer (1 mL, 1 M, pH 7.0). A blank was prepared similarly, omitting $CuCl_2$. After 30 min of incubation at room temperature, absorbance was measured at 450 nm. Results were expressed as mg trolox equivalents per gram of extract (mg TEs/g extract) (Melucci et al., 2018).

Skin-Related Enzyme Inhibition

Tyrosinase Inhibition Assay

The assay was carried out by mixing 25 µL of sample solution with 40 µL of tyrosinase enzyme and 100 µL of phosphate buffer (pH 6.8) in a 96-well microplate, then incubating at 25 °C for 15 minutes. The reaction was initiated by adding 40 µL of L-DOPA. A blank was prepared by omitting the enzyme. After a further 10-minute incubation at room temperature, absorbance was measured at 492 nm. Results were expressed as milligrams of kojic acid equivalents per gram of extract (mg KAE/g extract) (Zengin et al., 2017).

Anti-diabetic Activity

α-Amylase Inhibition Assay

The α-amylase inhibitory activity was evaluated by mixing the sample with α-amylase in phosphate buffer (pH 6.9) in a 96-well microplate, followed by incubation at 37°C for 10 minutes. The reaction was initiated by adding

starch solution and further incubated for 10 minutes. It was then stopped with HCl, followed by the addition of iodine-potassium iodide reagent. Absorbance was measured at 630 nm, and the activity was expressed in acarbose equivalents (mmol ACAEs/g extract), with the blank (without enzyme) absorbance subtracted (Zengin, 2016).

α-Glucosidase Inhibition Assay

In this assay, the sample was mixed with α-glucosidase, glutathione, and 4-N-trophenyl-α-D-glucopyranoside (PNPG) substrate in phosphate buffer (pH 6.8), and incubated at 37°C for 15 minutes. The reaction was terminated with sodium carbonate, and absorbance was measured at 400 nm. The inhibitory activity was calculated by subtracting the blank (without enzyme) and expressed as mmol ACAEs/g extract (Zengin, 2016).

Antimicrobial Activity

To determine the antibacterial and antifungal activities of different compounds, microbial strains obtained from the Refik Saydam National Culture Collection (RSCC) and the American Type Culture Collection (ATCC) were used. Gram-positive bacterial strains included *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Bacillus subtilis* RSCC 02021; Gram-negative bacterial strains included *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella enterica* RSCC 04059; and yeast strains included *Candida albicans* ATCC 90028, *Candida parapsilosis* RSCC 04057, and *Candida krusei.* ATCC 6258. Prior to revival, all strains were stored at –80°C in brain heart infusion broth containing 10% glycerol. Before the experiments, the bacterial and fungal strains were reactivated on tryptic soy agar.

Disk Diffusion Assay

The antimicrobial activity of the extracts was evaluated using the disk diffusion method (Başpınar et al., 2018). Bacterial strains were cultured on Mueller-Hinton agar (MHA) and fungal strains on Sabouraud dextrose agar (SDA). After reactivation, the microorganisms were suspended in physiological saline and adjusted to a 0.5 McFarland standard using a densitometer. The suspensions were then spread onto the agar plates, and 6 mm sterile blank disks were placed on the surface. Each disk was loaded with 10 µL of extract solution (200 µg/disk). Following incubation at 37°C for 16-20 hours for bacteria and 48 hours for fungi, the diameters of inhibition zones were measured. Results were expressed as mean ± standard deviation. Gentamicin and fluconazole were used as reference drugs. Quality control interpretations were based on EUCAST guidelines (EUCAST, 2017; EUCAST, 2018).

Microdilution Method

The minimum inhibitory concentration (MIC) of the extracts was investigated using the microdilution method (Kotmakçı et al., 2015). Serial dilutions of the extracts were prepared in 96-well plates using broth media to adjust concentrations between 1 and 2048 µg/mL. Microplates were incubated at 37°C for 16-20 hours for bacterial strains and at 48 °C for 48 hours for fungal strains. The lowest concentration that inhibited microbial growth was defined as the MIC. Gentamicin and fluconazole were used as reference molecules in the assays. The quality control ranges of the results were assessed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017; EUCAST, 2018).

Statistical Analysis

Statistical analysis was performed using SPSS (v22.0). One-way ANOVA followed by Tukey's HSD post hoc test was used to assess differences among extract types. Results are expressed as mean ± standard deviation, with significance set at $P < 0.05$.

RESULTS

The antioxidant properties of four different *G. leiocarpum* extracts were evaluated using ABTS and CUPRAC assays. According to the ABTS results, the highest radical-scavenging capacity was observed in the GLOM extract (152.67 ± 2.75 mg TEs/g), which was significantly higher than those of the other extracts ($P < 0.05$). In the CUPRAC assay, the GLUM extract exhibited the strongest reducing power (168.64 ± 7.42 mg TEs/g). In both assays, the GLUE extract exhibited significantly lower antioxidant capacity than the others. Regarding extraction yield, the highest percentages were obtained from the GLUE (19.98 ± 1.02%) and GLOM (19.55 ± 0.09%) extracts (Table 1).

The extracts were assessed for their inhibitory effects on tyrosinase, α-amylase, and α-glucosidase enzymes. GLUE (50.65 ± 0.33 mg KAEs/g) and GLOE (48.02 ± 0.97 mg KAEs/g) demonstrated the highest tyrosinase inhibitory activity, showing a statistically significant difference from GLUM and GLOM ($P < 0.05$). No significant differences

were found among the extracts in α-amylase inhibition. For α-glucosidase inhibition, GLUE (3.75 ± 0.04 mmol ACAEs/g) and GLOE (3.47 ± 0.12 mmol ACAEs/g) were the most active extracts, with significantly stronger effects compared to GLOM and GLUM. These results are summarized in Table 2.

Disk diffusion assays revealed average inhibition zone diameters of 8-9 mm for the extracts against the tested bacterial strains. These values were considerably lower than those observed for the positive control gentamicin (18-20 mm). In the MIC assays, all extracts exhibited relatively weak antimicrobial activity, with MIC values generally ranging from 512 to 1024 µg/mL. The only exception was the GLOE extract, which showed a lower MIC value of 256 µg/mL against *E. faecalis*. These findings suggest that the antimicrobial potential of the tested extracts is limited (Tables 3 and 4).

Table 1. Antioxidant activities and extraction yields of *G. leiocarpum* extracts (mean ± SD)
Çizelge 1. *G. leiocarpum* ekstraktlarının antioksidan aktiviteleri ve ekstraksiyon verimleri (ort ± SS).

Antioxidant activity assays	GLUM	GLUE	GLOM	GLOE
ABTS assay (mg TEs/g)	134.44 ± 9.89^{b}	73.56 ± 3.85^{c}	152.67 ± 2.75^{a}	131.88 ± 3.97^{b}
CUPRAC assay (mg TEs/g)	168.64 ± 7.42^{a}	87.57 ± 1.18^{d}	134.98 ± 8.15^{c}	142.61 ± 5.00^{b}
Extraction yield (%)	9.05 ± 0.08^{c}	19.98 ± 1.02^{a}	19.55 ± 0.09^{a}	13.05 ± 1.01^{b}

TEs: Trolox equivalents
In each row, different letters indicate significant difference ($P < 0.05$).

Table 2. Enzyme inhibitory properties of *G. leiocarpum* extracts (mean ± SD).
Çizelge 2. *G. leiocarpum* ekstraktlarının enzim inhibisyon özellikleri (ort ± SS).

Enzyme inhibition assays	GLUM	GLUE	GLOM	GLOE
Tyrosinase inhibition (mg KAEs/g)	43.81 ± 0.93^{b}	50.65 ± 0.33^{a}	44.35 ± 1.21^{b}	48.02 ± 0.97^{a}
Amylase inhibition (mmol ACAEs/g)	0.33 ± 0.01^{a}	0.32 ± 0.01^{a}	0.32 ± 0.02^{a}	0.34 ± 0.01^{a}
Glucosidase inhibition (mmol ACAEs/g)	0.13 ± 0.03^{c}	3.75 ± 0.04^{a}	0.18 ± 0.03^{b}	3.47 ± 0.12^{a}

KAEs: kojic acid equivalents; *ACAEs:* acarbose equivalents.
In each row, different letters indicate significant difference ($P < 0.05$).

Table 3. Mean inhibition zone diameter values of *G. leiocarpum* Extracts (mm)
Çizelge 3. *G. leiocarpum* ekstraktlarının ortalama inhibisyon zon çapı değerleri (mm)

Strains	GLUM	GLUE	GLOM	GLOE
		Gram (+) bacteria		
Staphylococcus aureus	8.00	8.00	8.00	8.00
Enterococcus faecalis	-	-	-	-
Bacillus subtilis	8.00	8.00	-	-
		Gram (–) bacteria		
Escherichia coli	8.00	8.00	-	8.00
Pseudomonas aeruginosa	9.00	8.00	9.00	8.00
Salmonella enterica	8.00	8.00	8.00	8.00
Gentamicin (positive control)*	19.00	20.00	18.00	20.00
		Fungi		
Candida albicans	-	-	-	-
Candida parapsilosis	-	-	-	-
Candida krusei	-	-	-	-
Fluconazole (positive control)**	23.00	24.00	22.00	23.00

(-): Zone diameter could not be determined; *: Gentamicin inhibition zone represents the average diameter observed across all tested bacterial strains; **: Fluconazole inhibition zone represents the average diameter observed across all tested fungal strains

Table 4. Minimum inhibitory concentration values of *G. leiocarpum* extracts (µg/mL)
Çizelge 4. *G. leiocarpum* ekstraktlarının minimum inhibitif konsantrasyon (MİK) değerleri (µg/mL)

Strains	GLUM	GLUE	GLOM	GLOE
Gram (+) bacteria				
Staphylococcus aureus	1024	1024	1024	1024
Enterococcus faecalis	512	512	512	256
Bacillus subtilis	512	512	512	512
Gram (–) bacteria				
Escherichia coli	1024	1024	1024	1024
Pseudomonas aeruginosa	512	512	512	512
Salmonella enterica	1024	1024	1024	1024
Gentamicin (positive control)*	0.8	1	0.7	1
Fungi				
Candida albicans	1024	1024	1024	1024
Candida parapsilosis	1024	1024	1024	1024
Candida krusei	1024	1024	1024	1024
Fluconazole (positive control)**	1.5	2	1.5	2

*: MIC value represents the average concentration observed across all tested bacterial strains,
**: MIC value represents the average concentration observed across all tested fungal strains.

DISCUSSION

The present study provides the first assessment of the antioxidant, enzyme-inhibitory, and antimicrobial activities of G. leiocarpum extracts obtained using different extraction techniques and solvents. By applying both ultrasonic bath and orbital shaker-assisted methods using ethanol and methanol, the impact of extraction conditions on bioactivity was compared. The findings reveal that the extraction method plays a critical role in determining the phytochemical yield and corresponding biological activities. The results not only contribute to the phytopharmacological understanding of *G. leiocarpum*, a relatively underexplored species, but also highlight its potential as a source of bioactive metabolites.

The *genus Glaucium* has a long history of use in traditional medicine, where its seeds and aerial parts have been used for various purposes, including as a laxative, an antidiabetic agent, and in the treatment of dermatological conditions (Akaberi et al., 2021; Elbermawi et al., 2018). In modern pharmacological studies, *Glaucium* species have demonstrated a wide range of bioactivities, including antimicrobial, anti-inflammatory, antioxidant, hypoglycemic, cytotoxic, anticancer, and anticholinesterase effects (Arafa et al., 2016; Bozkurt et al., 2022; Özcandir et al., 2024; Altın & Köksal, 2024; (Yagi et al., 2024).

There are no available reports in the literature regarding the antioxidant activity of *G. leiocarpum*. However, antioxidant activity studies have been conducted on certain other *Glaucium* species. For instance, a study on *G. grandiflorum* evaluated the antioxidant potential of its ethyl acetate and methanol extracts, demonstrating that the species can scavenge free radicals and inhibit lipid peroxidation (Önder et al., 2020). The methanol extract obtained via ultrasonic-assisted extraction in the present study exhibited notably elevated antioxidant capacity, as evidenced by both ABTS radical-scavenging and CUPRAC reducing-power assays (Table 1), highlighting the efficiency of this method in concentrating bioactive compounds. These findings suggest that both solvent polarity and ultrasonic-assisted extraction play crucial roles in enhancing the yield of antioxidant constituents in *G. leiocarpum*. The genus Glaucium, a member of the Papaveraceae family, is well recognized for its abundance of alkaloids—particularly aporphine-type structures—which are associated with a broad spectrum of pharmacological effects (Akaberi et al., 2021; Arafa et al., 2016). The strong antioxidant responses observed in this study may thus be partially attributed to the presence of such alkaloid constituents.

Although the methanol extract obtained via ultrasonic treatment (GLUM) exhibited the lowest extraction yield among the tested samples, it demonstrated higher antioxidant activity in both the ABTS radical-scavenging and CUPRAC reducing-power assays. This discrepancy may be attributed to the selective extraction and enrichment of potent antioxidant constituents under ultrasonic cavitation. Similar observations have been reported in the literature, where ultrasound-assisted extraction yielded extracts with higher bioactivity despite lower mass recovery, owing to enhanced disruption of plant tissues and improved solubilization of bioactive molecules (Chemat et al., 2017).

In contrast to the antioxidant capacity findings, ethanol-based extracts of *G. leiocarpum* (GLUE and GLOE) demonstrated superior enzyme-inhibitory potential against the tested targets (Table 2). GLUE exhibited the most

pronounced tyrosinase and α-glucosidase inhibition, while GLOE was the most effective against α-amylase. These findings suggest that ethanol, as a moderately polar solvent, may facilitate the selective extraction of enzyme-inhibitory phytochemicals, particularly alkaloids—bioactive constituents known for their inhibitory activity against neuroactive enzymes (Bozkurt et al., 2022). Additionally, ultrasonic-assisted extraction enhanced this effect, as the cavitation and mechanical forces generated by ultrasound contribute to efficient cell wall rupture, thus promoting the release and availability of intracellular secondary metabolites that interact with enzyme targets.

It is noteworthy that this study is the first to report the inhibitory potential of *G. leiocarpum* extracts against tyrosinase, α-glucosidase, and α-amylase. Although these results are based on *in vitro* assays, they offer promising insights into the plant's potential for modulating melanogenesis and glycemic control—indicating its value as a candidate for antidiabetic and dermatological applications.

When compared to recent studies on other *Glaucium* species, such as the work of Yagi et al. (2024), in which *G. acutidentatum m*ethanol extracts (prepared via homogenizer-assisted and maceration methods) exhibited anti-tyrosinase activities of 25.15 and 26.79 mg KAE/g, respectively, the values reported in the current study are markedly higher. This suggests that *G. leiocarpum*, particularly in its ultrasonic ethanol extract (GLUE), possesses a richer, more bioactive alkaloid and phenolic profile that enhances its enzyme-inhibitory capacity, surpassing even that of other closely related species.

Regarding antimicrobial activity, all *G. leiocarpum* extracts exhibited weak inhibition, with inhibition zones of 8–9 mm and MIC values of 512–1024 µg/mL, which were markedly higher than those of standard antibiotics (Tables 3 and 4). This effect was consistent across both Gram-positive and Gram-negative strains, although *P. aeruginosa* displayed slightly greater susceptibility. When interpreted according to the established criteria for plant extracts—≤8 mm as weak, 9–12 mm as moderate, and ≥13 mm as strong antimicrobial activity—the observed values correspond to weak, with some cases approaching borderline moderate inhibition (Doughari et al., 2007).

Comparable findings have been reported for other species of the genus *Glaucium*. For instance, in the study by Hakemi vala et al (2017), methanol extracts and alkaloid fractions of *G. vitellinum* showed no inhibition zones against the tested microorganisms in the disc diffusion assay. In MIC evaluations, the methanol extracts were ineffective against Gram-negative strains, whereas the alkaloid fraction of *G. vitellinum* demonstrated activity solely against *Salmonella typhimurium*. These findings further highlight the variability in antimicrobial efficacy among *Glaucium* species and suggest a selective spectrum of activity, potentially influenced by the chemical composition of the extracts.

Additionally, Morteza-Semnani et al (2005) observed that methanol extracts from three *Glaucium* species were selectively active against Gram-negative bacteria, while chloroform extracts demonstrated broader efficacy across all tested strains. These differences across studies are likely attributable to variations in extraction methods and solvent polarity, which strongly influence the chemical profile and, consequently, the antimicrobial efficacy of plant extracts.

The observed differences across biological assays underscore the complex and variable nature of plant secondary metabolism. The biological activities of plant extracts are strongly influenced by the types and quantities of secondary metabolites, which, in turn, are shaped by numerous factors, including geographical origin, environmental conditions, soil composition, altitude, and plant developmental stage (Jain et al., 2019; Özay & Pehlivan, 2024). This complexity necessitates a strategic approach in extraction design to selectively enrich compounds of interest for specific pharmacological applications.

CONCLUSION

This study provides the first comparative evaluation of the antioxidant, enzyme-inhibitory, and antimicrobial activities of Glaucium leiocarpum extracts obtained using different extraction techniques and solvents. The results demonstrated that both the extraction solvent and method significantly influenced the biological responses. Methanol-based extracts generally exhibited higher antioxidant activity, while ethanol-based ultrasonic extracts were more effective in inhibiting enzymes such as tyrosinase and α-glucosidase. However, antimicrobial activity was limited across all extracts, with only *Pseudomonas aeruginosa* showing moderate susceptibility.

Based on the antioxidant capacity and enzyme inhibition results obtained in this study, *G. leiocarpum* extracts demonstrate notable potential as natural bioactive sources for anti-diabetic formulations, anti-hyperpigmentation skincare products, and food preservation systems requiring oxidative stability. However, further studies involving compound isolation, fraction purification, and cell-based mechanistic investigations are needed to substantiate and clarify these potential applications.

Despite these promising findings, certain limitations should be acknowledged. The study focused solely on *in vitro* bioactivity assessments, and the phytochemical composition of the extracts—particularly alkaloid content—was not characterized. Furthermore, the antimicrobial tests were limited in scope, and broader microbial strain screening is necessary to draw more conclusive results.

These initial findings highlight the potential of *G. leiocarpum* as a source of bioactive compounds, particularly antioxidants and selective enzyme inhibitors. To advance its pharmaceutical relevance, future studies should aim to perform LC-MS/MS-based phytochemical profiling, especially targeting alkaloids and other secondary metabolites, and assess cytotoxicity and in vivo effects. Such investigations will contribute to a deeper understanding of the underlying mechanisms of action and help unlock the therapeutic potential of this underexplored species.

Author Contributions

Conception: CÖ - Design: CÖ;İÖ;GZ - Supervision: GZ - Materials: CÖ;İÖ;GZ - Data Collection and/or Processing: CÖ;İÖ;GZ - Analysis and/or Interpretation: CÖ;İÖ;GZ - Literature: CÖ;İÖ;GZ - Review: CÖ;İÖ;GZ - Writing: CÖ;İÖ;GZ - Critical Review: CÖ;GZ

Conflict of Interest

The authors declare no conflicts of interest.

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