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Research Article

Enzyme inhibitory, antioxidant, antimicrobial activities, and phenolic profiles of the methanol extract of *Gelasia sericea* an endemic species

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Abstract

This study aimed to investigate, for the first time, the phytochemical composition, antioxidant, antimicrobial, and enzyme inhibitory properties of the methanolic extract of *Gelasia sericea* (Aucher ex DC.) Zaika, Sukhor. & N.Kilian. The extract demonstrated a moderate yield (6.55 g/100 g dry plant material) and was found to be rich in phenolic acids, particularly ferulic acid (17182.97 µg g^{-1}), followed by caffeic acid, protocatechuic acid, and *p*-coumaric acid. Antioxidant analyses revealed high total phenolic (388.86 mg GA eq./100 g), total flavonoid (74.03 mg QE eq./100 g), and total antioxidant capacity (503.33 mg AA eq./100 g). The extract exhibited remarkable ABTS radical scavenging activity (95.99%, IC_{50} = 37.59 mg ml^{-1}) and considerable ferric-ion reducing power (974.78 mg $FeSO_4$ eq./100 g). Antimicrobial assays indicated moderate inhibitory effects, with stronger activity against Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*) and the yeast *Candida albicans*, whereas Gram-negative bacteria were less susceptible. The extract's MIC values ranged between 0.20-3.25 mg ml^{-1}, and MLC values between 0.41-6.50 mg ml^{-1}, which were higher than those of standard antibiotics. Enzyme inhibition assays revealed moderate activity against carbonic anhydrase II (IC_{50} = 0.0136 µg ml^{-1}) and weak inhibition of cholinesterases and α-glucosidase, while α-amylase inhibition was relatively more pronounced (IC_{50} = 13350.00 µg ml^{-1}). Overall, these findings highlight *G. sericea* as a valuable natural source of phenolic acids and antioxidants, with promising potential for applications in functional foods, nutraceuticals, and phytopharmaceuticals. However, the relatively limited antimicrobial and enzyme inhibitory activities suggest the need for further studies on bioavailability, in vivo efficacy, and compound isolation to optimize its practical applications.

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Keywords: antimicrobial activity; antioxidant activity; enzyme inhibition; *Gelasia sericea*; methanolic extract; phenolic compounds

Introduction

Gelasia sericea L., synonymously known as *Scorzonera sericea* L., is a perennial species in the family Asteraceae, belonging to the tribe Cichorieae (Lendzion *et al*., 2021; Adıgüzelli *et al*., 2024). It is primarily distributed across arid regions of central and southern Eurasia, as well as parts of Europe and Northern Africa (Lendzion *et al*., 2021; Cesur Turgut, 2024). The genus is notable for its biodiversity, with several species endemic to Anatolia (Türkiye), China, and Mongolia (Lendzion *et al*., 2021). Globally, it comprises approximately 160-190 species (Lendzion *et al*., 2021; Adıgüzelli *et al*., 2024; Ercan *et al*., 2024), of which 52 are recorded in Türkiye, 31 being endemic to the region (Ercan *et al*., 2024).

Members of this genus are rich sources of secondary metabolites that contribute to diverse biological activities, including antimicrobial, antioxidant, and enzyme inhibitory effects. Reported phytochemicals include alkaloids, quinic and caffeic acid derivatives, chlorophylls, lignans, flavonoids, bibenzyl derivatives, polyfunctional organic acids, carotenes, dihydroisocoumarins, tocopherols, stilbenes, triterpenes, triterpenoids, sesquiterpenoids, and phenolic compounds (Lendzion *et al*., 2021; Adıgüzelli *et al*., 2024; Cesur Turgut, 2024). These compounds play crucial roles in plant growth, adaptation, and defense mechanisms, as well as in ecological interactions that sustain ecosystem stability (Adıgüzelli *et al*., 2024).

Phenolic compounds, in particular, not only protect plants against pests and environmental stressors but also influence their aroma and sensory characteristics (Adıgüzelli *et al*., 2024). Several *G. sericea* species have been traditionally used in Asia and Europe for their hemostatic properties and wound-healing applications. In Türkiye, folk medicine has documented their use for treating fever, parasitic infections, respiratory disorders, kidney diseases, cardiovascular problems, diabetes, rheumatism, and various inflammatory conditions (Lendzion *et al*., 2021; Adıgüzelli *et al*., 2024; Cesur Turgut, 2024; Ercan *et al*., 2024).

Although previous investigations have predominantly concentrated on methanol extract, comprehensive studies on the polar extracts of *G. sericea* remain limited. Accordingly, the present study aims to characterize the phytochemical composition of the methanol extract derived from the aerial parts of the plant and to evaluate its total phenolic content (TPC), total antioxidant capacity (TAC), total flavonoid content (TFC), antioxidant potential, antimicrobial activity, and enzyme inhibitory properties.

Materials and Methods

Materials

In this study, aerial parts of *G. sericea* (Aucher ex DC.) Zaika, Sukhor. & N.Kilian were collected in June 2024 from two natural populations in Bayburt Province, Türkiye. The first collection took place on 22 June from hillside habitats along the Gümüşhane road (40°20′44″N, 40°01′09″E; 1632 m), characterized by a continental climate with dry summers and vegetation dominated by xerophytic species adapted to arid, rocky slopes. The second collection occurred on 28 June from the summit of Bahtlı Mountain near the Kop Pass (40°20′50″N, 40°29′28″E; 2693 m), a high-altitude habitat with sparse alpine vegetation.

For both sites, mature, healthy plants in full bloom were selected to ensure optimal phytochemical content. In total, 1000 g of aerial parts was harvested, placed in sterile paper bags, and transported to the laboratory for analysis. Morphological features including habitus, inflorescence, and leaf structure are presented in Figure 1.

Figure 1. *Gelasia sericea* (Aucher ex DC.) Zaika, Sukhor. & N.Kilian (Photo: Abdurrahman SEFALI)

Taxonomic identification was carried out by Assoc. Prof. Abdurrahman Sefalı (Department of Primary Education, Faculty of Education, Bayburt University, Türkiye) using diagnostic morphological characteristics and relevant floristic keys. Voucher specimens were deposited in the Herbarium of Bingöl University under accession numbers BIN Sefalı 1230 and BIN Sefalı 1232 to ensure traceability for future reference.

Extraction methanol

Plant materials collected from the two sites were pooled prior to extraction to obtain a representative sample of *G. sericea*. All subsequent analyses were performed using this pooled methanolic extract. Ultrasound-assisted extraction was conducted using a 3 L, 320 W ultrasonic bath (Bandelin Ultrasonic Bath). Ground aerial plant material (10 g) was combined with 50 mL of 30% aqueous methanol (MMEH) and sonicated for 60 min at 40 °C. The extract was filtered twice through Whatman No. 1 filter paper, followed by centrifugation at 4000 rpm for 10 min to remove particulate matter. The resulting supernatant was collected, and the solvent was evaporated at 40 °C to yield the crude methanol extract (Öz *et al*., 2023a; Öz *et al*., 2023b).

Enzyme inhibitory activities of the extracts

Activity assay for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes

The activities of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) were determined spectrophotometrically following the Ellman method (Ellman *et al*., 1961). In this assay, acetylthiocholine iodide or butyrylthiocholine iodide served as substrates for AChE and BChE, respectively. Upon enzymatic hydrolysis, thiocholine is released, which reacts with DTNB (5,5′-dithio-bis-(2-nitrobenzoic acid)) to produce the yellow-colored product TNB (5-thio-2-nitrobenzoic acid). The reaction was monitored at 412 nm.

IC_{50} values were obtained from inhibition curves, and kinetic parameters were analyzed using Lineweaver-Burk plots (Lineweaver and Burk, 1934). Inhibition studies for AChE were conducted at five different inhibitor concentrations ranging from 10.05 to 58.96 µg ml^{-1}. The IC_{50} value was calculated from the graph equation $y = 100e^{-0.014x}$ ($R^2 = 0.9998$). Inhibition studies for BChE were conducted at five different

inhibitor concentrations ranging from 168 to 516 µg ml^{-1}. The IC_{50} value was calculated from the graph equation $y = 100e^{-0.002x}$ ($R^2 = 0.9995$).

Carbonic anhydrase enzyme activity assay

Carbonic anhydrase activity was determined by measuring its esterase function, following established protocols (Landolfi *et al*., 1997). The assay utilizes *p*-nitrophenyl acetate as the substrate, which is hydrolyzed by the enzyme to produce *p*-nitrophenol (or its anionic form, *p*-nitrophenolate). Both products exhibit identical absorbance at 348 nm, ensuring that the measurement is unaffected by proton dissociation from the phenolic OH group. Inhibition studies were conducted at five different inhibitor concentrations ranging from 0.0034 to 0.017 µg ml^{-1}. The IC_{50} value was calculated from the graph equation $y = 100e^{-50.59x}$ ($R^2 = 0.9996$).

α-Glucosidase enzyme activity assay

α-Glucosidase activity was assessed according to the method of Tao *et al*., (2013), using *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG) as the substrate. The release of *p*-nitrophenol during enzymatic hydrolysis was monitored spectrophotometrically at 405 nm. Inhibitory effects were determined at varying inhibitor concentrations, and residual enzyme activity (%) was calculated relative to uninhibited controls. IC_{50} values were obtained from inhibition curves, and kinetic parameters were evaluated using Lineweaver-Burk plots (Lineweaver and Burk, 1934). Inhibition studies were conducted at five different inhibitor concentrations ranging from 4980 to 11620 µg ml^{-1}. The IC_{50} value was calculated from the graph equation $y = 100e^{-7E-05x}$ ($R^2 = 0.9994$).

α-Amylase enzyme activity assay

α-Amylase inhibitory activity was determined using a modified Caraway-Somogyi iodide/potassium iodide (IKI) method, adapted from Yang *et al*., (2012). In brief, 25 µL of sample was mixed with 50 µL of α-amylase solution prepared in phosphate buffer (pH 6.9, containing 6 mM NaCl) in a 96-well microplate. After incubation at 37 °C for 10 min, 50 µL of 0.05% starch solution was added, and a blank without enzyme was prepared in parallel. The plate was incubated for an additional 10 min at 37 °C, after which the reaction was terminated by adding 25 µL of 1 M HCl. Subsequently, 100 µL of IKI reagent was added to develop color, and absorbance was measured at 630 nm. Acarbose was used as a positive control, and IC_{50} values were calculated to quantify inhibitory activity. Inhibition studies were conducted at five different inhibitor concentrations ranging from 2 to 20000 µg ml^{-1}. The IC_{50} value was calculated from the graph equation $y = 3.4229x + 3.3595$ ($R^2 = 0.9997$).

Determination of phenolic profiles using LC-MS/MS analysis

Phenolic profiling was conducted using a Thermo Scientific Dionex Ultimate 3000 UHPLC system coupled to a TSQ Quantum Access Max tandem mass spectrometer (Thermo Scientific, USA). The UHPLC was equipped with an autosampler, degasser, dual pump, and column compartment. Chromatographic separation was achieved on a C18 reversed-phase Inertsil ODS HYPERSIL analytical column (250 mm × 4.6 mm, 5 µm), maintained at 30 °C.

The mobile phase consisted of (A) ultrapure water with 0.1% formic acid and (B) methanol, delivered according to the following gradient program: 0-1 min, 0% B; 1-22 min, 95% B; 22-25 min, 95% B; 25-30 min, 100% B; with re-equilibration bringing the total run time to 34 min. The injection volume was 20 µL, and the flow rate was set to 0.7 mL/min.

Extensive optimization was performed to ensure efficient ionization and baseline resolution of the target phenolic compounds. The final conditions were selected based on peak shape, signal intensity, and reproducibility. Phenolics listed in Tables 2 and 3 were quantified under these conditions, and a representative chromatogram of standard phenolic compounds is provided. This method was adapted from Kayir *et al*., (2023).

Determination of antioxidant capacity

Antioxidant activity assays

The antioxidant properties of the methanol extract of *G. sericea* were comprehensively evaluated using multiple complementary assays. These included the ferric ion reducing antioxidant power (FRAP), ABTS and DPPH radical scavenging assays, and the determination of total antioxidant capacity (TAC), total flavonoid content (TFC), and total phenolic content (TPC). Together, these analyses provided a detailed assessment of the antioxidant potential and bioactive composition of the samples.

Ferric ion reducing antioxidant power (FRAP)

FRAP activity was determined following the method of Fidan *et al*., (2023). A freshly prepared FRAP reagent was used, with 500 µL of distilled water as the blank. Standard $FeSO_4$ solutions (250 µL) and sample aliquots were processed under identical conditions. Absorbance (593 nm) was measured, and FRAP values were calculated from the calibration curve ($y = 0.012x + 0.0516$, $R^2 = 0.998$). Results were expressed as mg $FeSO_4$ equivalents ($FeSO_4$ eq.) per 100 g of sample, reflecting the total ferric-ion reducing capacity.

ABTS radical scavenging activity

ABTS assay was conducted according to Kobya *et al*., (2024). An ABTS radical cation solution was prepared, and 150 µL of methanol was used as the blank. Standard ascorbic acid solutions (150 µL) and the sample extracts were treated identically. Absorbance at 734 nm was recorded, and scavenging activity was calculated using the calibration curve ($y = -0.0144x + 0.615$, $R^2 = 0.997$) based on ascorbic acid standards. Results were expressed as mg ascorbic acid equivalents (AA eq.) per 100 g.

DPPH radical scavenging activity

DPPH scavenging activity was assessed using the method of Yilmaz *et al*., (2023). Extracts were mixed with DPPH solution, vortexed, and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm, and inhibition was calculated using the equation above. Results were expressed as mg AA eq. per 100 g ($y = -0.0093x + 0.945$, $R^2 = 0.998$) and as percentage radical scavenging.

Total antioxidant capacity (TAC)

TAC was determined using the phosphomolybdenum method as described by Yilmaz *et al*., (2023). Pure water (250 µL) served as the blank. Absorbance was measured at 695 nm after processing 500 µL aliquots of standard solutions and samples under identical conditions. TAC values were expressed as mg ascorbic acid equivalents (AA eq.) per g, calculated from a standard curve ($y = 0.0022x - 0.057$, $R^2 = 0.998$).

Total flavonoid content (TFC)

TFC was quantified according to Yilmaz *et al*., (2023). Absorbance was read at 506 nm using pure water (500 µL) as the blank. Quercetin standards were used to prepare the calibration curve ($y = 0.0038x + 0.0164$, $R^2 = 0.997$). Results were expressed as mg quercetin equivalents (QE eq.) per 100 g of sample.

Total phenolic content (TPC)

TPC was measured using the Folin-Ciocalteu method (Yilmaz *et al*., 2023). The reaction mixture was vortexed and incubated in the dark at room temperature for 120 min before reading absorbance at 760 nm. A blank was prepared with 3.7 mL water, 500 µL methanol, 100 µL Folin-Ciocalteu reagent, and 600 µL 10% Na_2CO_3. Phenolic content was calculated from the gallic acid calibration curve ($y = 0.0052x + 0.0074$, $R^2 = 0.997$) and expressed as mg gallic acid equivalents (GA eq.) per 100 g.

Determination of antimicrobial activity

Disk (agar) diffusion assay

The antimicrobial activity of *Gelasia sericea* methanol extract was assessed using a modified agar diffusion method in accordance with CLSI (2017) guidelines. Test microorganisms included Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 9634), Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883, *Escherichia coli* ATCC 25922), and the yeast *Candida albicans* ATCC 18804.

Fresh microbial cultures were grown on Müller-Hinton Agar (MHA) and adjusted to 0.5 McFarland turbidity (≈1.5×10^8 CFU ml^{-1}) using 0.9% sterile saline. The inoculum was spread evenly over MHA plates with sterile swabs. Sterile 5.5 mm paper disks were placed onto the inoculated agar, and 15 µL of methanolic extract (25 mg ml^{-1} in DMSO) was applied to each disk. The concentration of the methanolic extract (25 mg ml^{-1}) was determined based on preliminary tests conducted to identify the most effective and stable working concentration. Plates were held at 4 °C for 2 h to allow diffusion.

Chloramphenicol (CH), nalidixic acid (NA) and nystatin (NY) (512 µg ml^{-1}) were used as positive controls, and DMSO as the negative control. Following diffusion, bacterial plates were incubated at 37 °C for 24 h, while yeast plates were incubated at 28 °C for 48 h. Inhibition zone diameters were measured using a digital caliper. All tests were conducted in triplicate, and results were statistically evaluated.

Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC)

MIC values were determined by the broth microdilution method in sterile 96-well plates with Müller-Hinton Broth (MHB) as the culture medium, following CLSI (2017) guidelines. The first wells contained 2×-strength MHB, with the remaining wells containing standard MHB. Methanol extract stock solution (25 mg ml^{-1}) was added to the first wells to yield an initial concentration of 12.5 mg ml^{-1}, followed by serial twofold dilutions. Nalidixic acid, chloramphenicol and nystatin (256 mg ml^{-1} in ethanol) were included as positive controls. Each well received 10 µL of the standardized microbial suspension (0.5 McFarland). Negative controls included media alone and media containing the extract without inoculum. Plates were incubated at 37 °C for 24 h (bacteria) or 28 °C for 48 h (fungi). MIC was defined as the lowest concentration showing no visible growth.

MLC values were determined by plating 50 µL aliquots from MIC, 2×MIC, and 4×MIC wells onto MHA plates using a Drigalski spatula. Plates were incubated under the same conditions, and colony counts were recorded. The MLC was defined as the lowest concentration producing ≥99.9% reduction in viable cells compared to the initial inoculum.

Results and Discussion

The extraction yield of the *G. sericea* methanolic extract was determined to be 6.55 ± 0.19 g per 100 g of dry plant material. This yield reflects the proportion of methanol-soluble constituents, including phenolic compounds, flavonoids, ascorbic acid, and other polar phytochemicals, that were recovered under the applied extraction conditions. Similar yield values have been reported for phenolic-rich medicinal plants subjected to methanolic extraction, suggesting that *G. sericea* possesses a moderate to high recovery efficiency of bioactive constituents. Similar yield values have been reported for phenolic-rich medicinal plants subjected to methanolic extraction, including *Scorzonera hispanica* (~6.1%), *Achillea millefolium* (~5.8-14.3%), and *Hypericum perforatum* (~6.4%) (Dias *et al*., 2013; Ak *et al*., 2020; Bayram *et al*., 2022; Mehmood *et al*., 2022; Kilibarda *et al*., 2025;). These findings support that methanol is an efficient solvent for the recovery of phenolic constituents from medicinal plants.

As stated, morphological features including habitus, inflorescence, and leaf structure are presented in Figure 1. *G. sericea* is a perennial, caespitose, and generally cushion-forming species reaching up to approximately 7 cm in height. Its leaves are entire, long (up to ca. 7 cm), markedly narrow (ca. 3 mm), linear, sericeous, amplexicaul, and arranged in a basal rosette, typically exhibiting an ash-gray coloration. The inflorescence is of the capitulum type, with one capitulum borne per stem measuring 15-17 mm in length. Inner phyllaries are around 13 mm long. Flowers are yellow to golden yellow. The achenes measure 8-9 × 1 mm, are cylindrical and glabrous; the pappus is light brown, with scattered plumose structures below and barbellate features above.

Antioxidant activity and bioactive component profile

The methanolic extract of *G. sericea* exhibited a notably high antioxidant capacity, as evidenced by radical scavenging assays (DPPH, ABTS) and ferric reducing antioxidant power (FRAP) analysis (Table 1). The DPPH radical scavenging activity was measured at 40.66%, with an IC_{50} value of 69.03 mg ml^{-1}, indicating a lower radical scavenging efficiency compared to the positive control, ascorbic acid (IC_{50} = 0.09 mg ml^{-1}). In contrast, the ABTS radical scavenging activity was remarkably high (95.99%), with an IC_{50} of 37.59 mg ml^{-1}, suggesting a strong electron-donating ability against the ABTS radical cation. These findings are consistent with previous reports that certain phenolic-rich plant extracts demonstrate stronger ABTS than DPPH activity, possibly due to differences in solubility and radical reactivity (Re *et al*., 1999; Thaipong *et al*., 2006).

Table 1. Antioxidant activity and bioactive component profile of *G. sericea* methanolic extract

Parameter	Value (Mean ± SD)	Unit	Reference Standard / Comparator
Antioxidant capacity amounts			
DPPH (AA eq.)	181.49 ± 1.43	mg AA eq./100 g	-
ABTS (AA eq.)	73.56 ± 0.18	mg AA eq./100 g	-
DPPH (% Inhibition)	40.66 ± 0.43	%	-
ABTS (% Inhibition)	95.99 ± 2.40	%	-
AA DPPH (% Inhibition)	98.31 ± 0.12	%	Positive control
AA ABTS (% Inhibition)	99.09 ± 1.67	%	Positive control
DPPH IC_{50}	69.03 ± 0.32	mg ml^{-1}	-
ABTS IC_{50}	37.59 ± 0.001	mg ml^{-1}	-
AA DPPH IC_{50}	0.09 ± 0.001	mg ml^{-1}	Positive control
AA ABTS IC_{50}	0.04 ± 0.001	mg ml^{-1}	Positive control
FRAP	974.78 ± 5.73	mg $FeSO_4$ eq./100 g	-
Bioactive components			
TAC	503.33 ± 6.12	mg AA eq./100 g	-
TPC	388.86 ± 0.26	mg GA eq./100 g	-
TFC	74.03 ± 0.99	mg QE eq./100 g	-

AA eq.: Ascorbic acid equivalent; GA eq.: Gallic acid equivalent; QE eq.: Quercetin equivalent; $FeSO_4$ eq.: Ferrous sulfate equivalent. Values expressed as mean ± standard deviation (n = 3)

As demonstrated, the *G. sericea* methanolic extract's IC_{50} values for the DPPH and ABTS tests are greater than ascorbic acid's, indicating that the extract has less efficacy to scavenge radicals than this common antioxidant. This is to be expected, since crude plant extracts contain complex phytochemical combinations that may exhibit intermediate effectiveness, but pure ascorbic acid exhibits significant electron-donating properties. Comparative data from comparable genera has now been included to bolster the discussion. Similar or marginally better antioxidant capabilities have been observed in earlier research on *Scorzonera* species. In contrast to Adıgüzelli *et al*., (2024), who found varying but generally comparable phenolic-driven antioxidant responses across *Scorzonera* taxa, Lendzion *et al*., (2021) reported moderate antioxidant activity in *Scorzonera hispanica*, with ABTS activity frequently surpassing DPPH activity, which is consistent with our findings for *G. sericea*. Furthermore, a number of wild edible *Gelasia/Scorzonera* species exhibited moderate scavenging activity that was not greater than that of conventional antioxidants, according to Cesur Turgut (2024). As a result, although though *G. sericea* extract is less potent than ascorbic acid, its antioxidant performance is within the range expected for this genus, indicating that phenolic acids, rather than highly reactive pure antioxidants, dominate the phytochemical profile. To enhance the interpretation of our findings, this comparative background has been included in the updated publication.

The FRAP assay revealed a reducing power of 974.78 mg $FeSO_4$ eq./100 g, which is comparable to values reported for medicinal plants with high phenolic contents, such as *Rosmarinus officinalis* (800-1100 mg $FeSO_4$ eq./100 g) (Gülçin *et al*., 2010). This suggests that *G. sericea* possesses a substantial capacity to donate electrons, thereby reducing oxidized intermediates and contributing to overall antioxidant potential.

The bioactive compound profile analysis demonstrated a high total antioxidant capacity (TAC) of 503.33 mg AA eq./100 g, total phenolic content (TPC) of 388.86 mg GA eq./100 g, and total flavonoid content (TFC) of 74.03 mg QE eq./100 g. These values indicate that the extract is a rich source of antioxidant phytochemicals (Table 1). The TPC value aligns with the range reported for polyphenol-rich plants (200-500 mg GA eq./100 g) (Prior *et al*., 2005), reinforcing the role of phenolics as major contributors to antioxidant capacity.

Evaluation of the DPPH and ABTS assays revealed distinct radical scavenging behaviors of the *G. sericea* methanolic extract. The DPPH inhibition reached 40.66%, whereas the ABTS inhibition was considerably higher at 95.99%, indicating that the extract more effectively neutralizes ABTS radicals. This pattern is consistent with reports that polar phenolics often exhibit greater affinity toward ABTS radicals than DPPH (Re *et al*., 1999; Thaipong *et al*., 2006).

However, when IC_{50} values are considered, the extract displayed comparatively high IC_{50} values in both assays (DPPH IC_{50} = 69.03 mg ml^{-1}; ABTS IC_{50} = 37.59 mg ml^{-1}), confirming that higher extract concentrations are required to achieve significant activity. Although the ABTS IC_{50} is lower than that of DPPH, indicating better activity against ABTS, both values remain substantially higher than those of the positive control, ascorbic acid (DPPH IC_{50} = 0.09 mg ml^{-1}; ABTS IC_{50} = 0.04 mg ml^{-1}). Thus, the extract exhibits lower potency than the reference antioxidant on a concentration basis.

Taken together, these findings suggest that *G. sericea* methanolic extract, while less potent than ascorbic acid, demonstrates strong ABTS radical scavenging capability at higher concentrations. This indicates that its antioxidant potential is likely attributed to synergistic effects of phenolic constituents rather than the activity of a single highly reactive compound. Consequently, the overall data support the interpretation that *G. sericea* possesses meaningful antioxidant properties within the context of crude botanical extracts.

Antimicrobial activities of G. sericea methanolic extract

In this study, the antimicrobial potential of the methanolic extract (ME) of *G. sericea* was evaluated against a range of clinically significant microorganisms, including both Gram-positive and Gram-negative bacterial strains, as well as the yeast *C. albicans*. Standardized microbiological assays were employed, specifically the Disk Diffusion Test (DDT), MIC and MLC, to comprehensively determine the inhibitory and lethal capacities of the extract. For comparative assessment, two well-established antibiotics, CH and NA, were utilized for bacterial strains, while NY served as the reference antifungal agent for *C. albicans*.

The DDT results revealed inhibition zones for the ME ranging from 6.70 mm against *Klebsiella pneumoniae* to 8.10 mm against *C. albicans*. These diameters, although indicating measurable antimicrobial activity, were significantly smaller than those observed for the reference antibiotics. For instance, CH produced a 29.40 mm inhibition zone against *K. pneumoniae*, and NA showed a 26.80 mm zone against *P. aeruginosa*. In the case of *C. albicans*, NY displayed a markedly larger inhibition zone of 27.21 mm compared to the ME's 8.10 mm. These findings highlight the moderate antimicrobial potential of ME, which is consistent with literature noting that plant-derived extracts often display reduced agar diffusion due to their hydrophobic and complex phytochemical profiles (Burt, 2004).

The MIC and MLC determinations reinforced these observations. The ME exhibited notably higher MIC and MLC values compared to the standards, implying that greater concentrations are required to achieve both inhibition and lethality. For example, the MIC and MLC values for ME against *K. pneumoniae* were 3.25 mg ml^{-1} and 6.50 mg ml^{-1}, respectively, whereas CH achieved complete inhibition and killing at concentrations of 0.002 mg ml^{-1} and 0.004 mg ml^{-1}, respectively. Among the tested bacteria, *S. aureus* demonstrated the greatest

susceptibility to ME (MIC: 0.20 mg ml^{-1}; MLC: 0.41 mg ml^{-1}), which aligns with existing studies suggesting that Gram-positive bacteria, lacking the protective outer membrane of Gram-negatives, are generally more permeable to phytochemicals (Gokhale and Wadhwani, 2015). In contrast, Gram-negative bacteria such as *E. coli*, *K. pneumoniae*, and *P. aeruginosa* displayed higher tolerance, which can be attributed to the presence of lipopolysaccharide-rich outer membranes and active efflux mechanisms (Hooper, 2001; Nazzaro *et al*., 2013).

Table 2. Antimicrobial activities of *G. sericea* methanolic extract

Please add title	Methanolic extract			Chloramphenicol			Nalidixic acid			Nystatin		
	DDT (mm)	MIC (mg mL^{-1})	MLC (mg mL^{-1})	DDT (mm)	MLC (mg mL^{-1})	MLC (mg mL^{-1})	DDT (mm)	MIC (mg mL^{-1})	MLC (mg mL^{-1})	DDT (mm)	MIC (mg mL^{-1})	MLC (mg mL^{-1})
Bacillus cereus ATCC 9634	7.27 ± 0.09	1.63 ± 0.13	3.25 ± 0.16	21.27 ± 0.87	0.032 ± 0.001	0.64 ± 0.01	16.53 ± 0.31	0.064 ± 0.001	0.13 ± 0.002	NT	NT	NT
Escherichia coli ATCC 25922	7.63 ± 0.05	0.81 ± 0.07	1.63 ± 0.09	19.33 ± 0.59	0.032 ± 0.001	0.16 ± 0.001	22.70 ± 0.43	0.064 ± 0.001	0.13 ± 0.002	NT	NT	NT
Klebsiella pneumoniae ATCC 13883	6.70 ± 0.08	3.25 ± 0.12	6.50 ± 0.21	29.40 ± 0.36	0.002 ± 0.001	0.004 ± 0.001	20.73 ± 0.25	0.016 ± 0.001	0.032 ± 0.001	NT	NT	NT
Pseudomonas aeruginosa ATCC 27853	7.23 ± 0.12	1.63 ± 0.14	3.25 ± 0.16	10.80 ± 0.41	0.016 ± 0.001	0.32 ± 0.01	26.80 ± 0.41	0.008 ± 0.001	0.016 ± 0.001	NT	NT	NT
Staphylococcus aureus ATCC 25923	7.30 ± 0.16	0.20 ± 0.08	0.41 ± 0.11	18.40 ± 0.42	0.032 ± 0.001	0.064 ± 0.001	20.63 ± 0.25	0.064 ± 0.001	0.13 ± 0.02	NT	NT	NT
Enterococcus faecalis ATCC 29212	7.40 ± 0.08	0.41 ± 0.06	0.81 ± 0.08	11.50 ± 0.43	0.032 ± 0.001	0.064 ± 0.001	22.10 ± 0.24	0.016 ± 0.001	0.032 ± 0.001	NT	NT	NT
Candida albicans ATCC 18804	8.10 ± 0.08	0.81 ± 0.11	1.63 ± 0.07	NT	NT	NT	NT	NT	NT	27.21 ± 0.12	0.008 ± 0.001	0.016 ± 0.001

The discs have a diameter of 5.5 mm. Samples: 25 mgml-1, Antibiotic: ± 0.50 mg ml^{-1}. 15 µL were pipetted onto each disc, Values expressed as mean ± standard deviation (n = 3). DDT: Disk Diffusion Test, NT: Not tested

In terms of antifungal activity, the ME presented moderate efficacy against *C. albicans*, with MIC and MLC values of 0.81 mg ml^{-1} and 1.63 mg ml^{-1}, respectively, compared to nystatin's much lower values of 0.008 mg ml^{-1} and 0.016 mg ml^{-1}. This trend mirrors findings in phytochemical research, where methanolic extracts typically show fungistatic and fungicidal actions due to phenolic acids and flavonoids but remain less potent than synthetic antifungal drugs (Nazzaro *et al*., 2013). Similar observations have been reported among related taxa; for example, *Scorzonera hispanica* and other *Scorzonera* species demonstrated weak to moderate antifungal activity against *Candida* spp. and filamentous fungi, likely attributed to polar phenolics rather than highly active terpenoids (Lendzion *et al*., 2021; Adıgüzelli *et al*., 2024). Furthermore, volatile fractions and crude extracts of certain *Gelasia/Scorzonera* species exhibited only limited inhibition toward *Candida albicans*, reinforcing that the genus generally displays moderate antifungal potential compared with standard antifungal agents (Cesur Turgut, 2024). These comparative findings suggest that the antifungal profile of *G. sericea* is consistent with other members of the genus, further supporting its classification as a moderate natural antifungal source.

The overall pattern of susceptibility indicated that Gram-positive bacteria were more responsive to ME than Gram-negative species. This disparity can be mechanistically explained by structural differences in cell envelopes. Gram-positive bacteria possess a thick peptidoglycan layer that is more accessible to phytochemicals, whereas Gram-negative bacteria's outer membrane forms a formidable permeability barrier that can be coupled with active efflux systems, significantly reducing the intracellular accumulation of antimicrobial compounds (Hooper, 2001).

Although the ME displayed relatively lower efficacy compared to conventional antibiotics, its broad-spectrum activity, particularly against Gram-positive bacteria and *C. albicans*, suggests potential utility as a

complementary antimicrobial agent. Future investigations should prioritize the isolation and characterization of active constituents, elucidation of the mechanism of action, and assessment of synergistic effects with existing antimicrobials. Applications in food preservation, pharmaceutical formulations, and alternative therapeutics are promising avenues that align with the growing interest in plant-based bioactives, especially in light of escalating antibiotic resistance trends.

Figure 2 presents the DDT results for the ME of *G. sericea* compared to three reference standards: CH, NA, and NY. Each standard was applied according to microorganism type: CH for Gram-positive bacteria, NA for Gram-negative bacteria, and NY for the yeast *C. albicans*. The results clearly demonstrate that the inhibition zones produced by the standards were consistently larger than those observed for ME, with the largest differences seen for *K. pneumoniae* and *C. albicans*.

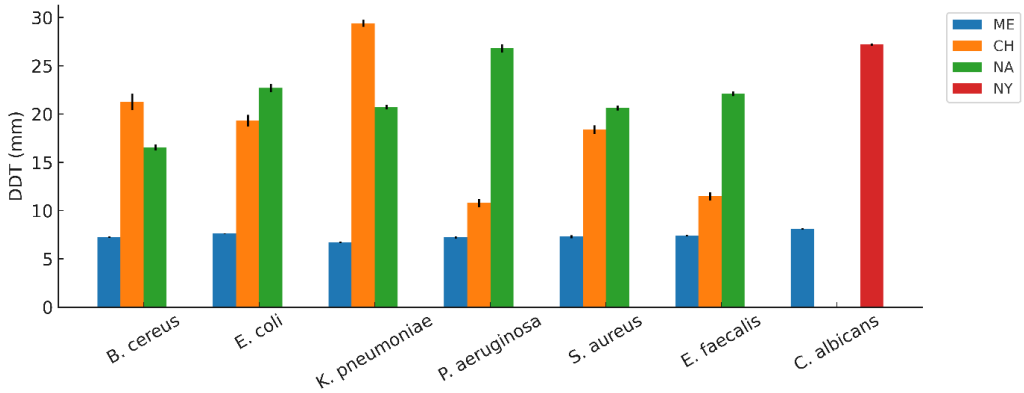

Figure 2. DDT results of *G. sericea* methanolic extract and reference antibiotics against tested microorganisms
DDT: disc diffusion test, ME: methanol extract, CH: chloramphenicol, NA: nalidixic acid, NY: nystatin

Figure 3 illustrates the MIC values on a logarithmic scale. The significantly lower MIC values of CH, NA, and NY compared to ME highlight the much greater potency of these reference antimicrobials. For example, NA inhibited *K. pneumoniae* at only 0.016 mg ml^{-1}, whereas ME required 3.25 mg ml^{-1}. This pattern was consistent across all tested strains.

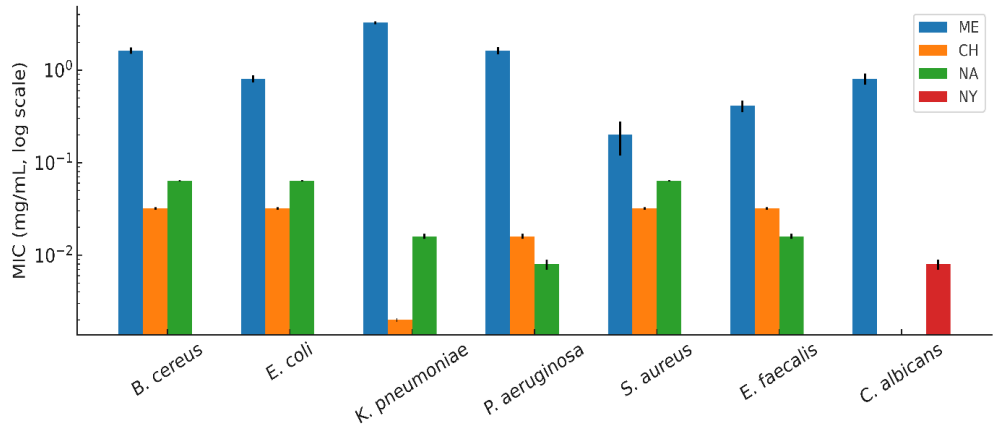

Figure 3. MIC values of *G. sericea* methanolic extract and reference antibiotics against tested microorganisms
MIC: minimum inhibitory concentration, ME: methanol extract, CH: chloramphenicol, NA: nalidixic acid, NY: nystatin

Figure 4 shows the MLC values for ME and the reference standards. As with MIC, the standards consistently achieved microbial lethality at much lower concentrations than ME. The strongest relative performance of ME was observed against *S. aureus* and *E. faecalis*, although even here it was less effective than CH. Against *C. albicans*, NY exhibited superior fungicidal activity compared to ME.

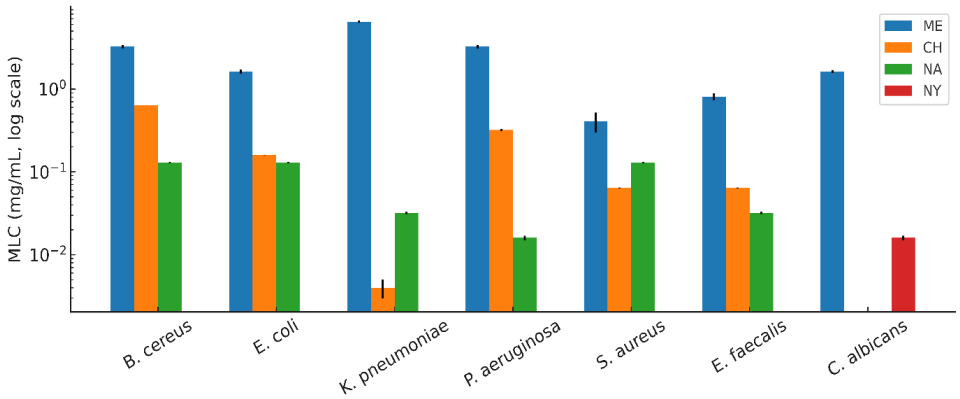

Figure 4. MLC values of *G. sericea* methanolic extract and reference antibiotics against tested microorganisms
MLC: minimum lethal concentration, ME: methanol extract, CH: chloramphenicol, NA: nalidixic acid, NY: nystatin

Results of enzyme inhibitory activities

Table 3 summarizes the enzyme inhibitory activity (IC_{50} values) of *G. sericea* methanolic extract in comparison with standard inhibitors. The extract demonstrated moderate inhibition against CA-II (IC_{50} = 0.0136 µg ml^{-1}) compared to acetazolamide (IC_{50} = 0.002 µg ml^{-1}). For cholinesterase enzymes, the extract showed weak inhibition, particularly AChE (49.29 µg mL^{-1}) relative to Tacrine (0.011 µg ml^{-1}). BChE inhibition was also weaker (347.00 µg ml^{-1}) than Tacrine (0.0016 µg mL^{-1}). In carbohydrate-hydrolyzing enzymes, the extract displayed very weak α-glucosidase inhibition (9450.00 µg ml^{-1}vs. 0.059 µg $ml^{-1}$for acarbose) and low α-amylase inhibition (13350.00 µg ml^{-1}), though still higher potency compared to acarbose (33.20 µg ml^{-1}). These results suggest that while the methanolic extract contains bioactive compounds, its inhibitory potential is generally inferior to standard inhibitors, except for α-amylase where activity is relatively stronger.

Table 3. IC_{50} values of *G. sericea* methanolic extract and standards

Enzyme	Extract IC_{50}	Standard	Standard IC_{50}
CA-II (µg ml^{-1})	0.0136 ± 0.0002	Acetazolamide	0.002 ± 0.0001 (µg ml^{-1}
AChE (µg ml^{-1})	49.29 ± 2.68	Tacrine	0.011 ± 0.001 (µg ml^{-1})
BChE (µg ml^{-1})	347.00 ± 0.61	Tacrine	0.0016 ± 0.0003 (µg ml^{-1})
α-Glucosidase (µg ml^{-1})	9450.00 ± 49.00	Acarbose	0.059 ± 0.002 (µg ml^{-1})
α-Amylase (µg ml^{-1})	13350.00 ± 21	Acarbose	33.20 ± 0.06 (µg ml^{-1})

Values expressed as mean ± standard deviation (n = 3); CA-II: carbonic anhydrase-II, AChE: acetylcholinesterase, BChE: butyrylcholinesterase

The methanolic extract of *G. sericea* shows a moderate range of bioactivity based on its enzyme inhibitory profile. The extract showed considerable inhibition against carbonic anhydrase II (CA-II), although it was still much less effective than acetazolamide. This is in line with the sulfonamide derivative's therapeutic effectiveness. Other *Scorzonera/Gelasia* taxa have shown similar results; for example, Lendzion *et al.* (2021)

found that a number of *Scorzonera* species only showed weak-to-moderate inhibition of CA-II. This suggests that phenolic-rich extracts from this genus typically have limited potency when compared to synthetic inhibitors.

The extract showed significantly less inhibition of cholinesterase enzymes (AChE and BChE) than tacrine, indicating that while the plant contains compounds that can interact with cholinergic pathways, their potency is not strong enough to compete with synthetic drugs currently used to treat Alzheimer's disease. Similar results have been reported for other *Scorzonera* taxa and Asteraceae members. For example, methanolic extracts of *Scorzonera hispanica* exhibited only mild cholinesterase inhibition, attributed mainly to phenolic acids and lignans rather than highly active alkaloids (Granica *et al*., 2015; Ak *et al*., 2020). In addition, comparative screening studies of multiple *Scorzonera* species demonstrated weak AChE and BChE inhibition levels (2-25%), considerably lower than synthetic cholinesterase inhibitors such as tacrine or galantamine (Zengin *et al*., 2016). These findings support the conclusion that cholinesterase inhibition within this genus is generally moderate and linked to polyphenolic content rather than potent alkaloids. Nevertheless, the reported inhibitory activity justifies further phytochemical examinations to isolate and characterize specific bioactive constituents, which may provide safer alternatives with improved pharmacological selectivity.

In terms of carbohydrate-digesting enzymes, the extract displayed poor inhibition against α-glucosidase compared to acarbose, indicating limited utility in postprandial glycemic control through this pathway. Interestingly, its α-amylase inhibition, though still modest, was relatively stronger than its α-glucosidase activity and even surpassed acarbose. This observation highlights the possibility that *G. sericea* could contribute to delaying carbohydrate digestion at the initial breakdown stage, albeit at higher concentrations.

Comparable findings have been reported in related *Scorzonera/Gelasia* taxa and other Asteraceae members. Methanolic extracts of *Scorzonera hispanica* and other *Scorzonera* species demonstrated weak-to-moderate α-amylase and α-glucosidase inhibition, attributed mainly to phenolic acids and flavonoids (Ak *et al*., 2020; Zengin *et al*., 2016). Similarly, investigations on Asteraceae plants such as *Centaurea* spp. have revealed modest carbohydrate-hydrolyzing enzyme inhibition, reflecting the contribution of polyphenols rather than strongly active alkaloids or terpenoids (Zengin *et al*., 2016). These reports support the notion that species within this genus generally exhibit mild enzyme inhibition profiles.

Overall, these results suggest that although *G. sericea* methanolic extract cannot compete with clinically used inhibitors in potency, it represents a promising natural source for further bioassay-guided fractionation and compound identification to develop safer adjunct therapeutic agents. Figure 5 summarizes the enzyme inhibitory activity (IC_{50} values) of *G. sericea* methanolic extract in comparison with standard inhibitors.

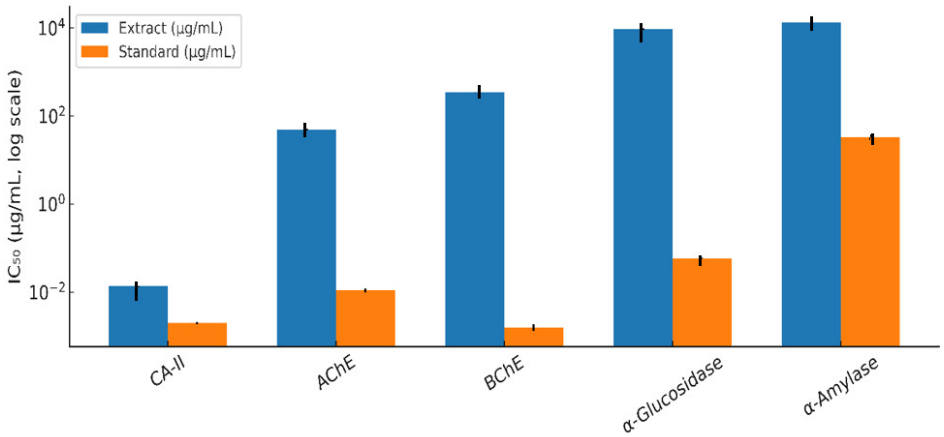

Figure 5. IC_{50} values of methanolic extract and standards
CA-II: carbonic anhydrase-II, AChE: acetylcholinesterase, BChE: butyrylcholinesterase

The present study evaluated the inhibitory activity of *G. sericea* methanolic extract against a panel of clinically relevant enzymes, including CA-II, AChE, BChE, *α*-glucosidase, and *α*-amylase, and compared the results with standard inhibitors.

In terms of CA-II inhibition, the extract exhibited measurable activity with an IC_{50} value of 0.0136 μg ml^{-1}. Although this is markedly weaker than acetazolamide (0.002 μg ml^{-1}), the result suggests that the extract contains metabolites capable of interacting with the catalytic zinc-binding site of CA-II. This finding is in line with previous reports highlighting the therapeutic relevance of CA-II inhibition in glaucoma and related disorders (Supuran, 2016).

With respect to cholinesterase inhibition, the extract was considerably less potent than tacrine, which remains one of the benchmark inhibitors for Alzheimer's disease therapy (Santos *et al*., 2018). The high IC_{50} values observed for both AChE (49.29 μg ml^{-1}) and BChE (0.347 μg ml^{-1}) suggest that, although the extract harbors some inhibitory activity, its potential clinical application in cholinesterase-related pathologies is limited. Nevertheless, the modest activity observed supports the possibility of isolating less toxic natural scaffolds with pharmacological relevance.

Regarding carbohydrate-digesting enzymes, the extract displayed poor inhibition against *α*-glucosidase (9450.00 μg ml^{-1}) compared to acarbose (0.059 μg ml^{-1}). This weak effect limits its utility in modulating postprandial glycemia via this pathway and contrasts with previously reported plant-derived *α*-glucosidase inhibitors (Yin *et al*., 2014). Interestingly, however, the extract demonstrated relatively stronger inhibition of *α*-amylase (13350.00 μg ml^{-1}) and even outperformed acarbose (33.20 μg ml^{-1}). Since *α*-amylase acts at the initial stage of starch breakdown, the extract may delay carbohydrate digestion at higher concentrations, which could contribute to a mild antihyperglycemic effect. Similar observations have been reported in other plant extracts with moderate *α*-amylase inhibitory activity (McCue *et al*., 2005).

Taken together, the findings highlight that while *G. sericea* methanolic extract is not competitive with synthetic inhibitors in terms of potency, it constitutes a potential natural reservoir of bioactive molecules. The observed enzyme inhibitory activities warrant further fractionation and compound isolation studies to identify lead structures. Such bioassay-guided approaches may uncover novel phytochemicals with safer toxicity profiles, which could serve as adjunct therapeutic agents in metabolic and neurodegenerative disorders.

Result of phenolic compounds

LC-MS/MS analysis of the methanolic extract of *G. sericea* revealed a phenolic profile predominantly composed of phenolic acids. A total of seven phenolic constituents were identified, with ferulic acid being the major compound by a substantial margin. Caffeic, protocatechuic, and *p*-coumaric acids were also present at appreciable levels, whereas rutin and vanillin were detected in smaller quantities. Many other phenolic standards were not detected.

These findings indicate that the bioactive potential of *G. sericea* is likely driven primarily by its phenolic acids, which are widely recognized for their antioxidant, anti-inflammatory, and metabolic regulatory properties. Quantitative results are summarized in Table 4, while Figure 6 presents the TIC chromatogram of standard phenolic compounds and Figure 7 illustrates the TIC chromatogram of the *G. sericea* extract.

Table 4. The amount of phenolic compound in the extracts of *G. sericea* methanolic extract

No	Phenolic compound	Extracts of the all Part *G. sericea* (µg g-1)
1	Gallic acid	n.d.
2	Protocatechuic acid	536.54 ± 5.76
3	Protocatechuic aldehyde	28.10 ± 2.68
4	Sesamol	n.d.
5	Gentisic acid	n.d.
6	Catechin	n.d.
7	Chlorogenic acid	n.d.
8	Epicatechin	n.d.
9	Caffeic acid	1118.96 ± 32.71
10	Vanillin	4.21 ± 0.71
11	Syringic acid	n.d.
12	*p*-Coumaric acid	75.25 ± 2.51
13	Taxifolin	n.d.
14	Ferulic acid	17182.97 ± 56.72
15	Salicylic acid	n.d.
16	4-Hydroxybenzoic acid	n.d.
17	Hesperidin	n.d.
18	Rosmarinic acid	n.d.
19	Oleuropein	n.d.
20	Luteolin-7-glucoside	n.d.
21	Rutin	15.32±1.71
22	Resveratrol	n.d.
23	Ellagic acid	n.d.
24	Naringenin	n.d.
25	Quercetin	n.d.
26	Luteolin	n.d.
27	Apigenin	n.d.
28	Pinocembrin	n.d.
29	Chrysin	n.d.
30	Galangin	n.d.
31	Flavone	n.d.

n.d.: not detected. Values expressed as mean ± standard deviation (n = 3)

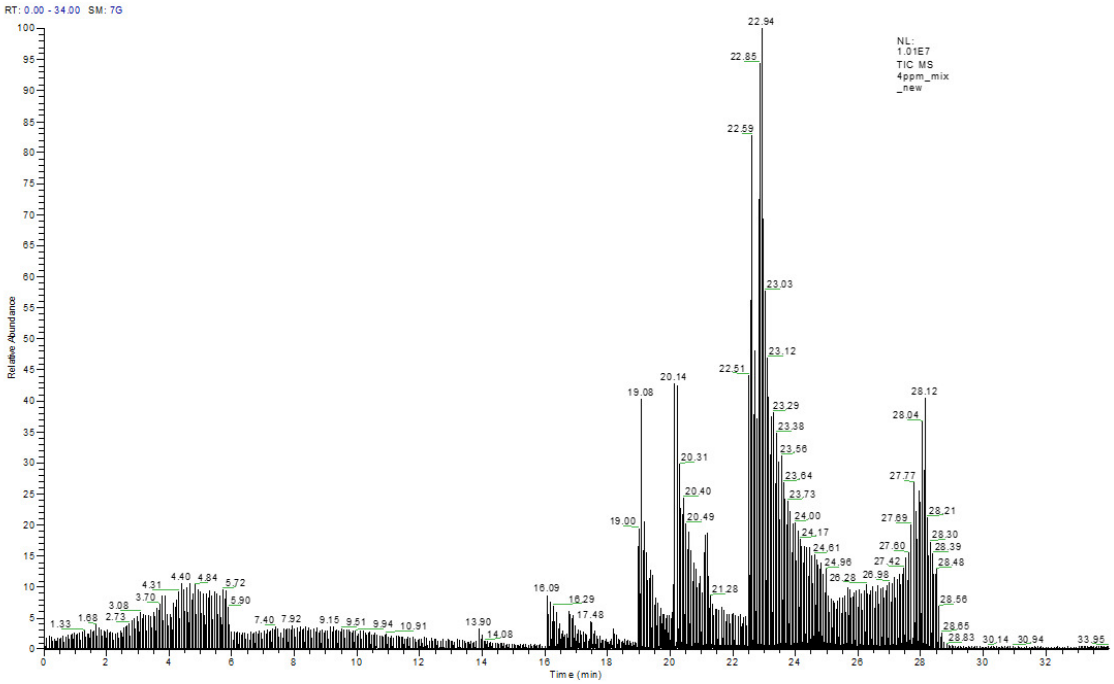

Figure 6. TIC chromatogram of standard phenolic compounds analyzed by LC-MS/MS

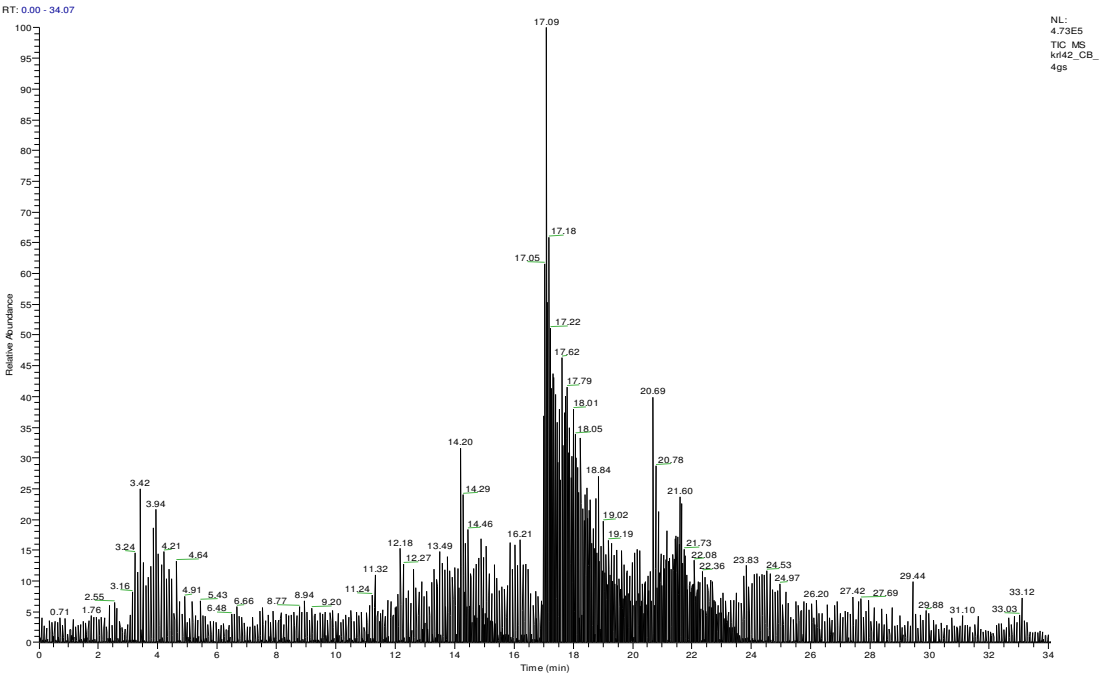

Figure 7. TIC chromatogram of extracts of aerial parts of *G. sericea* by LC-MS/MS

The phenolic profile was dominated by phenolic acids, which are widely recognized for their potent antioxidant, anti-inflammatory, antimicrobial, and potential antidiabetic activities (Scalbert *et al*., 2005; Roychoudhury *et al*., 2021). The results suggest that the health-promoting properties traditionally attributed to *G. sericea* may be largely derived from these phenolic acids.

Ferulic acid (17182.97 ± 56.72 μg g^{-1}) was identified as the most abundant phenolic compound in the extract. This hydroxycinnamic acid derivative, commonly found in plant cell walls, is a potent antioxidant capable of neutralizing reactive oxygen species (ROS) (Ou and Kwok, 2004). Its anti-inflammatory and antimicrobial activities have been well-documented (Zhao and Moghadasian, 2008). Moreover, ferulic acid has been shown to reduce oxidative stress, inhibit lipid peroxidation, and protect vascular function, all of which are relevant in the prevention and management of diabetes and cardiovascular diseases (Kumar and Pruthi, 2014). The high concentration of ferulic acid in *G. sericea* suggests that the plant could be a valuable natural source for functional foods, nutraceuticals, and phytotherapeutic formulations.

High ferulic acid levels have also been reported in other plant species, which further underscores its widespread occurrence and importance in plant-derived therapeutics. For example, rice bran contains 2000-5000 μg g^{-1} ferulic acid primarily in bound form, contributing to its strong antioxidant potential (Zhou *et al*., 2004). Wheat bran is another rich source, with concentrations ranging from 3000 to 5000 μg g^{-1}, and is considered a major dietary contributor of ferulic acid in human nutrition (Adom and Liu, 2002). Similarly, maize bran (2800-4500 μg g^{-1}) and barley grain (1500-3000 μg g^{-1}) have been reported to contain significant amounts (Verma *et al*., 2009). Certain medicinal plants, such as *Angelica sinensis* roots, also exhibit high ferulic acid content (1500-2000 μg g^{-1}), which has been associated with their traditional use in improving circulation and reducing oxidative stress (Raj *et al*., 2022).

Caffeic acid (1118.96 ± 32.71 μg g^{-1}) was the second most abundant phenolic in the *G. sericea* extract. This hydroxycinnamic acid derivative shows strong radical scavenging and anti-inflammatory activities, partly by inhibiting COX-2 and iNOS pathways (Park *et al*., 2009). It can modulate glucose metabolism, enhance insulin sensitivity, and reduce postprandial hyperglycemia, supporting its potential role in type 2 diabetes management (Gülçin, 2006).

Caffeic acid is also abundant in other plants, notably coffee beans (20,000-50,000 μg g^{-1}) (Clifford, 1999), thyme (2000-5000 μg g^{-1}) (Dorman *et al*., 2003), and oregano (1000-4000 μg g^{-1}) (Zheng and Wang, 2001). The level in *G. sericea* is comparable to that in many culinary herbs, indicating its potential as an alternative natural source.

Protocatechuic acid (536.54 ± 5.76 μg g^{-1}) is a dihydroxybenzoic acid derivative with well-documented antioxidant, hepatoprotective, cardioprotective, and neuroprotective properties (Kakkar and Bais, 2014). It can inhibit α-glucosidase and α-amylase, thereby helping to regulate postprandial blood glucose in type 2 diabetes (Gunny *et al*., 2024).

Protocatechuic acid (PCA; 3,4-dihydroxybenzoic acid) is a hydroxybenzoic acid derivative with well-documented antioxidant, hepatoprotective, cardioprotective, and neuroprotective properties (Kakkar and Bais, 2014). It has also been shown to inhibit α-glucosidase and α-amylase, thereby contributing to the regulation of postprandial blood glucose in type 2 diabetes (Meng *et al*., 2013). PCA is present in notable amounts in various plants, including barley grains (15-40 μg g^{-1}) (Dykes and Rooney, 2007), and berries such as blueberries (6-19 μg g^{-1}) and blackberries (50 μg g^{-1}) (Garzón *et al*., 2010).

The relatively high level in *G. sericea* positions it as a promising natural source of this multifunctional compound.

p-Coumaric acid (75.25 ± 2.51 μg g^{-1}) is a hydroxycinnamic acid with notable antioxidant and lipid metabolism-regulating effects (Pei *et al*., 2016). It has been shown to inhibit digestive enzymes such as α-glucosidase and α-amylase, contributing to its potential antidiabetic activity. This compound is also present in various plant-based foods, including corn 242.00 μg g^{-1}, barley 75.00 μg g^{-1}, and wheat 54.00 μg g^{-1} (Boz, 2015). *p*-Coumaric acid (p-CA; 4-hydroxycinnamic acid) is a hydroxycinnamic acid with well-documented antioxidant and lipid metabolism-regulating properties (Pei *et al*., 2016). It has also been shown to inhibit α-glucosidase and α-amylase, thereby contributing to its potential antidiabetic activity (Adisakwattana *et al*., 2012). This phenolic acid occurs in a wide variety of plant-based foods, though reported concentrations vary

considerably depending on cultivar, plant part, and extraction method. For example, peanuts typically contain ≈80-250 μg g^{-1}**,** but higher values up to 300 μg g^{-1} have also been reported (Win *et al*., 2011). Tomatoes generally provide ≈40-120 μg g^{-1}, with some studies indicating levels approaching 150 μg g-1 (Martínez-Valverde *et al*., 2002). In cereal brans such as wheat and maize, p-CA is usually found in higher amounts, most often 500-1000 μg g^{-1}, though values as high as 1500 μg g-1 have been observed (Adom and Liu, 2002). The concentration determined in *G. sericea* (75.25 ± 2.51 μg g^{-1}) is lower than that of cereal brans but comparable to levels reported for vegetables and legumes, suggesting its role as a supplementary dietary source of *p*-coumaric acid. The level found in *G. sericea* is lower than in many cereal brans but comparable to that in certain vegetables and legumes, indicating its role as a supplementary source of dietary *p*-coumaric acid.

Protocatechuic aldehyde (28.10 ± 2.68 μg g^{-1}) exhibits antioxidant and antimicrobial activities and has been reported to provide vasoprotective effects by enhancing endothelial nitric oxide synthase activity (Huang *et al*., 2006). It is also found in medicinal plants such as *Salvia miltiorrhiza*, where it contributes to cardiovascular benefits (Ren *et al*., 2019). Rutin (15.32 ± 1.71 μg g^{-1}) was the only flavonoid glycoside detected in the extract. Despite its low concentration, rutin is well known for its vasoprotective, anti-inflammatory, and antioxidant properties (Choi *et al*., 2021). It is abundant in plants such as buckwheat (quercetin-3-O-rutinoside, 2000-5000 μg g^{-1}) and citrus fruits (200-500 μg g^{-1}) (Kreft *et al*., 2006; Peterson *et al*., 2006). The lower level in *G. sericea* still contributes to its overall bioactivity profile, particularly in cardiovascular protection.

Despite its low concentration, rutin (15.32 ± 1.71 μg g^{-1}) has high biological relevance due to its vasoprotective, anti-inflammatory, and antioxidant effects (Choi *et al*., 2021). It is naturally abundant in buckwheat (2000-5000 μg g^{-1}) and citrus fruits (200-500 μg g-1) (Kreft *et al*., 2006; Peterson *et al*., 2006), but the level in *G. sericea* still contributes to its cardiovascular and metabolic health potential. Vanillin (4.21 ± 0.71 μg g^{-1}), an aromatic aldehyde widely recognized as a flavoring agent, also possesses antioxidant, neuroprotective, and antidiabetic properties (Bezerra-Filho *et al*., 2019). Even at low levels, its presence in *G. sericea* may enhance the extract's overall bioactivity profile.

The phenolic profile of G. *sericea*, as determined in this study, is particularly rich in phenolic acids, with ferulic acid as the most abundant compound, followed by caffeic acid, protocatechuic acid, and *p*-coumaric acid. These phenolic acids are well-documented for their antioxidant, anti-inflammatory, and antidiabetic activities, and their combined presence likely underpins the plant's strong bioactive potential (Scalbert *et al*., 2005; Roychoudhury *et al*., 2021). The detection of rutin, although at a relatively low concentration, adds further value due to its diverse biological activities, particularly in vascular health and oxidative stress reduction (Semwal *et al*., 2021). Overall, the results highlight *G. sericea* as a promising natural source of bioactive phenolic acids, with potential applications in functional food formulations, nutraceutical products, and phytopharmaceutical preparations. Given the high ferulic acid content and the presence of other synergistic phenolic compounds, future research should focus on bioavailability, in vivo efficacy, and formulation studies to optimize its use in health-promoting products.

Conclusions

This study provides the first comprehensive characterization of the methanolic extract of *G. sericea*, highlighting its phytochemical and biological properties. The extract was particularly rich in phenolic acids, with ferulic acid identified as the dominant constituent, which likely contributes to its strong antioxidant potential. High ABTS radical scavenging capacity together with considerable total phenolic and flavonoid contents further underscores the notable antioxidant capacity of the species.

The extract demonstrated moderate antimicrobial activity, especially against Gram-positive bacteria, suggesting a supportive rather than primary antimicrobial role. In addition, its α-amylase inhibitory activity points to a potential role in carbohydrate metabolism management, while moderate CA-II inhibition suggests additional therapeutic relevance.

Overall, *G. sericea* emerges as a previously unexplored phytochemical resource with promising antioxidant and enzyme-modulating properties. Further studies including bioactivity-guided fractionation, compound isolation, in-vivo evaluation, and formulation development will be important to assess its applicability in functional foods, nutraceuticals, and phytopharmaceutical preparations.

Authors' Contributions

Conceptualization: CB, IA; Data curation: CB, OA; Formal analysis: MSF, IA; Investigation: MÖ, MSF; Methodology: CB, OA; Resources: MÖ, AS; Supervision: MÖ, AS; Visualization: IA; Roles/Writing - original draft: All Authors; and Writing - review & editing: MSF, MÖ.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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