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RESEARCH ARTICLE OPEN ACCESS

Detection and quantification of roemerine in wild *Papaver rhoeas* and *Papaver glaucum* from Sulaymaniyah, Iraq, and comparison of their extracts' total antioxidant activity

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Abstract
Objective: This study aimed to detect, tentatively identify, and quantify the aporphine alkaloid roemerine in wild *Papaver rhoeas* L. and *Papaver glaucum* Boiss. and Hausskn. from northern Iraq, and to compare the total antioxidant activity of their purified extracts.
Methods: Whole plants were collected from Sulaymaniyah in April 2023. Methanolic extracts were subjected to alkaloid purification and analysed by chemical tests, GC-MS, TLC, and HPLC. Roemerine was quantified via a validated HPLC method. The total antioxidant capacity of extracts, standardised to an equivalent roemerine content, was evaluated using the phosphomolybdenum assay.
Results: Chemical tests confirmed alkaloids in both species. GC-MS analysis tentatively identified roemerine as a major component in *P. rhoeas* (26.4% relative abundance) and *P. glaucum* (14.7%). TLC (Rf ~0.6) and HPLC supported its presence. HPLC quantification found a higher concentration of roemerine in *P. rhoeas* (2.25 ± 0.11 mg/g dry weight) than in *P. glaucum* (1.49 ± 0.09 mg/g). The phosphomolybdenum assay indicated a higher, though not statistically significant (p=0.18), antioxidant activity in *P. glaucum* (0.43 ± 0.05 AAE/g) compared to *P. rhoeas* (0.14 ± 0.03 AAE/g).
Conclusion: Roemerine was tentatively identified and quantified for the first time in wild Iraqi *P. rhoeas* and *P. glaucum*, with a higher concentration found in *P. rhoeas*. The differential antioxidant activity, despite standardised roemerine content, suggests a significant contribution from other phytoconstituents, particularly in *P. glaucum*.

Keywords: Aporphine alkaloid, *Papaver*, Phytochemistry, Natural antioxidants, HPLC validation

Plain English Summary

We studied two wild poppy species, *Papaver rhoeas* (common poppy) and *Papaver glaucum* (tulip poppy), collected from the Sulaymaniyah region in northern Iraq. Our goal was to find and measure a specific compound called roemerine, which is a type of alkaloid with potential biological activities. Using laboratory techniques, we confirmed the presence of roemerine in both plants, finding a higher amount in the common poppy. We then tested the antioxidant strength of purified extracts from both species. Interestingly, while the tulip poppy extract had less roemerine, it showed a greater antioxidant capacity, indicating that other natural compounds in this species are likely responsible for this effect. This is the first report of roemerine in these poppies from Iraq.

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Introduction

The use of medicinal plants is deeply rooted in human history, forming the basis of traditional therapeutic systems in ancient civilisations from India and China to Egypt and Mesopotamia (1). The Papaveraceae family, a prolific source of bioactive phytochemicals, comprises over 700 species, including the genus *Papaver*, which is distributed across temperate regions, the Middle East, and notably, Iraq (2). In Iraq, the genus *Papaver* is represented by numerous species, thriving particularly in the northern provinces such as Sulaymaniyah and Erbil (3, 4). *Papaver* species are reservoirs of diverse secondary metabolites, including a vast array of alkaloids; over 170 distinct structures have been documented (5). These alkaloids underpin both traditional remedies and modern pharmaceuticals, offering analgesic, sedative, antiviral, and antitussive properties (6, 7, 8). Among these is roemerine, an aporphine-type alkaloid (Figure 1) previously isolated from species like *Annona senegalensis* and *Nelumbo nucifera*, and reported in some *Papaver* species such as *P. decaisnei* from Iraq (9, 10, 11). Roemerine exhibits promising biological activities, including antifungal and antibacterial effects against pathogens like *Candida albicans* and methicillin-resistant *Staphylococcus aureus* (MRSA) (12, 13).

While previous studies have explored the alkaloid profiles of *Papaver rhoeas* and *Papaver glaucum* in other regions (14, 15), the specific presence and quantity of roemerine in wild populations from Iraq remain uninvestigated. Furthermore, the relationship between specific alkaloid content and overall bioactivity, such as antioxidant potential, is often complex and underexplored.

This study therefore aimed to: (i) detect and tentatively identify roemerine in wild *P. rhoeas* and *P. glaucum* from Sulaymaniyah, Iraq, using a multi-method approach (chemical tests, GC-MS, TLC, HPLC); (ii) quantify roemerine content via a validated HPLC method; and (iii) compare the total antioxidant activity of extracts standardised for roemerine content using the phosphomolybdenum assay. This work contributes to the phytochemical profiling of Iraqi flora and elucidates the bioactivity potential of these common yet understudied species.

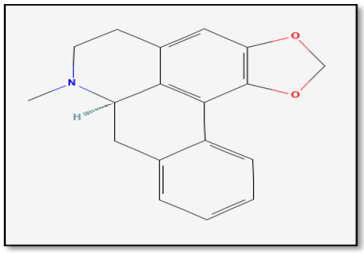


Figure 1: Chemical structure of roemerine

Materials and Methods

Harvesting and Taxonomic Identification

Whole plant specimens (aerial parts) of *Papaver rhoeas* L. and *Papaver glaucum* Boiss. and Hausskn. were collected from wild populations in the Sulaymaniyah Governorate, Iraqi Kurdistan, in April 2023. Collection sites are detailed in Table 1. Approximately 20-25 individual plants per species were pooled to form a representative sample. Taxonomic identification was performed by a qualified botanist through direct morphological comparison with authenticated voucher specimens housed in the National Herbarium of Iraq. High-resolution photographic vouchers are archived by the corresponding author. Plants were shade-dried at ambient temperature, ground to a coarse powder, and stored in airtight containers.

Table 1: Collection sites for *Papaver* species in Sulaymaniyah, Iraq.

Species	Area	Geographic Coordinates
Papaver rhoeas	Dukan Road, Sulaymaniyah	35.901900 N, 44.979689 E
Papaver glaucum	Byara Road, Halabja	35.628698 N, 45.518526 E

Extraction and Alkaloid Purification

The extraction protocol aimed to isolate total alkaloids (Figure 2). Powdered plant material (20 g dry weight per species) was macerated in 300 mL of 80% aqueous methanol with magnetic stirring for 48 hours at room temperature. The mixture was filtered, and the marc was re-extracted once with fresh solvent. Combined filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator. The resultant crude extract was acidified with 100 mL of 5% v/v hydrochloric acid (HCl) and defatted by partitioning twice with 100 mL petroleum ether. The acidic aqueous layer was basified to pH 9-10

using 32% ammonium hydroxide, precipitating the alkaloids. These were then extracted into chloroform (3 × 100 mL). The combined chloroform layers were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness to yield the purified alkaloidal extract. The extraction yield was calculated as (mass of dry extract/mass of dry plant material) × 100%.

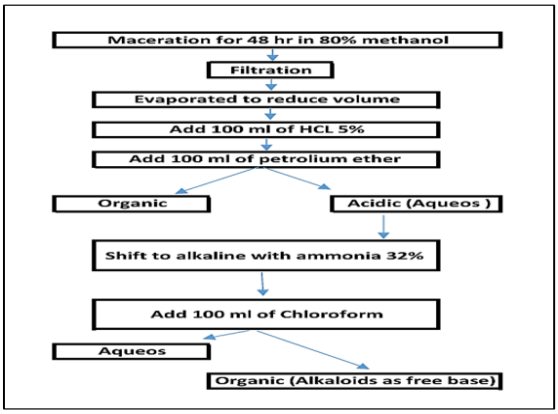


Figure 2: Schematic workflow for the extraction and purification of alkaloids from *Papaver* species

Preliminary Phytochemical Screening

The methanolic extracts were tested for the presence of alkaloids using standard precipitation reagents:

Mayer's Test: Formation of a creamy white precipitate.

Wagner's Test: Formation of a reddish-brown precipitate.

Dragendorff's Test: Formation of an orange-red precipitate.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The initial methanolic extracts were analysed using an Agilent 7890B GC system coupled with a 5977B MSD (Mass Selective Detector). Separation was achieved on an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed from 100°C (hold 2 min) to 300°C at 10°C/min, with a final hold time of 10 min. Helium was the carrier gas at a constant flow of 1.0 mL/min. The injector temperature was 250°C, and a 1 µL sample was injected in splitless mode. Mass spectra were obtained in electron impact (EI) mode at 70 eV, scanning from *m/z* 40 to 600. Compound identification was performed tentatively by comparing the mass spectra with the NIST 2020 library. A match factor >80% was considered a good tentative identification.

Thin Layer Chromatography (TLC)

TLC was performed on pre-coated silica gel 60 F_{254} aluminium plates. The purified alkaloid extracts and an authentic roemerine standard were spotted and developed in a saturated chamber using the mobile phase Ethyl Acetate: Methanol: Ammonia (85:10:5, v/v/v). After development, plates were air-dried and visualised under UV light at 254 nm and 365 nm.

High Performance Liquid Chromatography (HPLC) Analysis and Quantification

Instrumentation and Conditions

Analysis was performed on a Shimadzu HPLC system (LC-20AD pump, SPD-M20A diode array detector, SIL-20AC autosampler) at the Iraqi Ministry of Science and Technology. Separation was achieved on a reversed-phase C18 column (4.6 mm × 250 mm, 5 µm particle size) at ambient temperature. The mobile phase was an isocratic mixture of methanol and water (78:22, v/v) at a flow rate of 1.0 mL/min. Detection was at 288 nm. The injection volume was 20 µL.

Quantification Method and Validation Parameters

A stock solution of roemerine standard (ChromaDex, USA) was prepared in methanol. A six-point calibration curve (1, 2, 5, 10, 20, 50 µg/mL) was constructed by plotting peak area against concentration. The linear regression equation was y = [Slope]x + [Intercept], with a correlation coefficient (R^2) > 0.999. The limit of detection (LOD) and limit of quantification (LOQ) were determined as 3.3σ/S and 10σ/S, respectively, where σ is the standard deviation of the response, and S is the slope of the calibration curve. System suitability was verified by analysing six replicates of the standard; parameters included retention time (RT) repeatability (%RSD), theoretical plates (N > 2000), and tailing factor (T < 1.5).

Sample Preparation and Calculation

Precisely weighed aliquots of the dry, purified alkaloid extracts were dissolved in methanol to a

known concentration. After filtration (0.45 µm), samples were injected in triplicate. The roemerine concentration in the sample solution (C_S, µg/mL) was calculated from the calibration equation. The final content in the dry plant material (mg/g) was calculated as follows:

$$\text{Roemerine (mg/g dry plant)} = \frac{(C_s \times V \times D)}{(W \times 1000)}$$

Where:
C_s = Concentration from HPLC (µg/mL)
V = Final volume of sample solution (mL)
D = Dilution factor (if any)
W = Mass of dry plant material used to prepare the extract (g)
1000 = Conversion from µg to mg.

Total Antioxidant Capacity by Phosphomolybdenum Assay

The total antioxidant capacity (TAC) was determined by the green phosphomolybdenum complex formation method (16, 17), with modifications. An ascorbic acid (AA) standard curve (0-100 µg/mL) was prepared. The purified alkaloid extracts were prepared at a concentration calculated to contain an equivalent of 2 mg/mL of roemerine, based on the HPLC quantification results. An aliquot (0.1 mL) of this standardised extract or standard was mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate). The mixture was incubated at 95°C for 90 min, cooled to room temperature, and the absorbance was measured at 695 nm against a blank. TAC was expressed as mg Ascorbic Acid Equivalent per gram of extract (AAE/g). All assays were performed in triplicate on three independently prepared extracts (n=3 biological replicates).

Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism version 10.0.2. An unpaired two-tailed Student's t-test was used to compare the mean roemerine content and antioxidant activity between the two species. A p-value < 0.05 was considered statistically significant.

Results

Preliminary Phytochemical Screening and Extraction Yield

The methanolic extracts of both *P. rhoeas* and *P. glaucum* gave positive results for all three alkaloid detection tests (Mayer's, Wagner's, and Dragendorff's), confirming the presence of alkaloids. The extraction yield for the purified alkaloid fraction was 1.8% (w/w) for *P. rhoeas* and 1.5% for *P. glaucum*.

GC-MS Analysis

The GC-MS chromatograms of the methanolic extracts revealed complex phytochemical profiles (Figures 3 & 4). A peak at a retention time (RT) of 27.88 min was tentatively identified as roemerine based on library matching (NIST, 2020; match score, 88%). The relative abundance of this peak, expressed as a percentage of the total ion chromatogram area, was 26.4% in *P. rhoeas* and 14.7% in *P. glaucum* (Table 2). This indicates roemerine is a major component, especially in *P. rhoeas*. The full GC-MS compound list is presented in Supplementary Table 1.

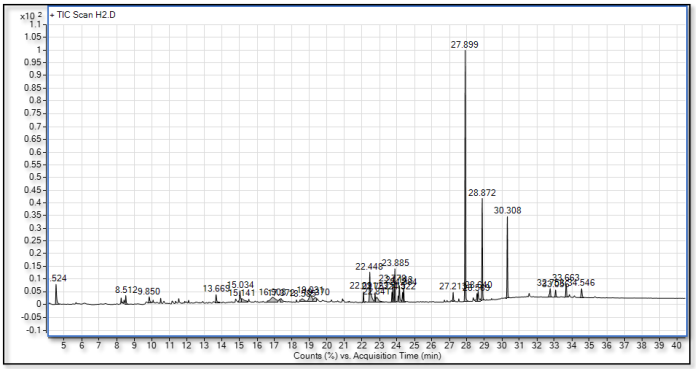


Figure 3: Representative GC-MS total ion chromatogram (TIC) of the methanolic extract of *Papaver rhoeas*. The peak tentatively identified as roemerine is indicated (RT 27.88 min).

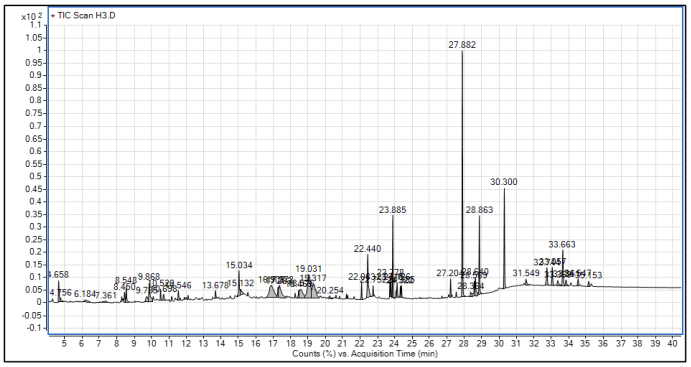


Figure 4: Mass spectrum of the peak at RT 27.88 min from *P. rhoeas* extract, with a library match suggesting roemerine.

Table 2: Key compounds tentatively identified by GC-MS in *Papaver* species extracts

RT (min)	Tentative Identification	Library Match (%)	Relative Area %	Major Fragments (*m/z*)	
			P. rhoeas	P. glaucum	
27.88	Roemerine	88	26.4	14.69	279, 264, 236, 221
22.44	n-Hexadecanoic acid (Palmitic acid)	94	7.98	5.36	256, 213, 129
33.66	β-Sitosterol	91	1.96	3.1	414, 396, 329
23.78	Methyl linolenate	89	2.64	2.11	292, 236, 150

Thin Layer Chromatography (TLC)

TLC analysis using the optimised mobile system showed a distinct band in both *P. rhoeas* and *P. glaucum* extracts that co-migrated with the authentic roemerine standard (Rf ≈ 0.6), providing supporting evidence for its presence (Figure 5). Visualisation under UV at 254 nm showed strong quenching for this band.

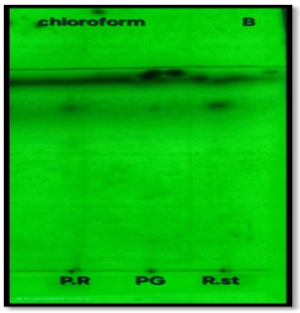


Figure 5: TLC profile. Lanes: 1- Roemerine standard, 2- *P. rhoeas* extract (PR), 3- *P. glaucum* extract (PG). Mobile phase: Ethyl Acetate: Methanol: Ammonia (85:10:5). Visualised under UV 254 nm. The Rf value for the roemerine band is approximately 0.6

HPLC Quantification of Roemerine

HPLC analysis provided clear separation and detection of roemerine. The standard eluted at 3.01 min (Figure 6). The calibration curve was linear over the range 1-50 µg/mL (y = [Slope]x + [Intercept], R^2 = 0.9997). LOD and LOQ were 0.3 µg/mL and 0.9 µg/mL, respectively. Representative chromatograms of the samples (Figure 7) showed peaks corresponding to the roemerine standard. Quantification results (Table 3) demonstrated that *P. rhoeas* contained a significantly higher ($p < 0.01$) concentration of roemerine (2.25 ± 0.11 mg/g dry weight) compared to *P. glaucum* (1.49 ± 0.09 mg/g).

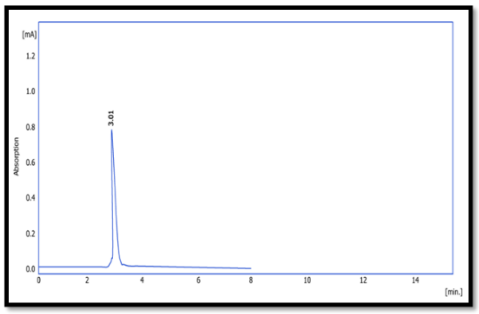


Figure 6: HPLC chromatogram of the roemerine standard (10 µg/mL). RT = 3.01 min

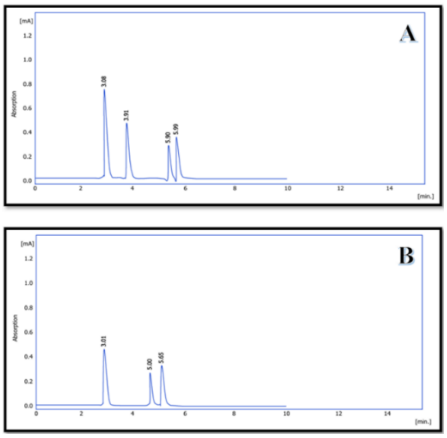


Figure 7: Overlaid HPLC chromatograms: (A) Roemerine standard, (B) *P. rhoeas* extract, (C) *P. glaucum* extract.
The arrow indicates the roemerine peak

Table 3: HPLC quantification of roemerine in purified extracts of *Papaver* species

Sample	Retention Time (min)	Mean Peak Area (mAU·s)	Concentration in Extract (µg/mL)*	Roemerine Content (mg/g dry plant)*
P. rhoeas	3.00 ± 0.02	114521 ± 5800	45.1 ± 2.3	2.25 ± 0.11
P. glaucum	3.01 ± 0.02	75841 ± 4600	29.9 ± 1.8	1.49 ± 0.09

Values are mean ± SD, n=3 independent injections from three extract preparations

Total Antioxidant Capacity

The phosphomolybdenum assay measured the total reducing capacity of the extracts. When standardized to contain an equivalent concentration of roemerine (2 mg/mL), the extract from *P. glaucum* exhibited a higher antioxidant activity (0.43 ± 0.05 mg AAE/g) than that from *P. rhoeas* (0.14 ± 0.03 mg AAE/g) (Figure 8). However, this difference did not reach statistical significance at the 95% confidence level (p = 0.18, t-test).

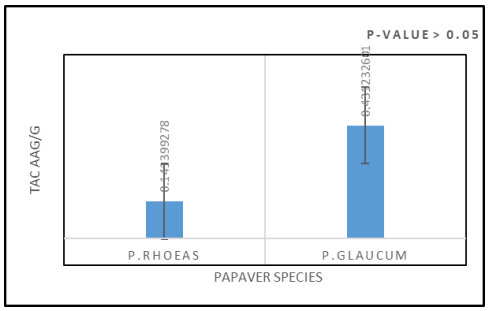


Figure 8: Total antioxidant capacity of *P. rhoeas* (PR) and *P. glaucum* (PG) extracts, standardised to equivalent roemerine content
Data are mean ± SD (n=3 biological replicates, assayed in triplicate). AAE = Ascorbic Acid Equivalent. The difference between means was not statistically significant (p = 0.18)

Discussion

This study provides the first report on the tentative identification and quantification of the aporphine alkaloid roemerine in wild *P. rhoeas* and *P. glaucum* from Iraq. Our multi-methodological approach reinforces the finding: positive alkaloid tests, tentative GC-MS identification, supportive TLC migration, and confirmatory HPLC retention time matching and quantification.

The GC-MS analysis revealed roemerine as a major component, particularly in *P. rhoeas* (26.4% relative abundance). This aligns with findings from Ajabduljabbar et al. (9), who reported roemerine as a dominant alkaloid in another Iraqi species, *P. decaisnei*. The lower relative abundance in *P. glaucum* (14.7%) is consistent with interspecific variation in alkaloid profiles within the genus *Papaver* (14, 15). It is crucial to note that our identification remains tentative, pending orthogonal confirmation via NMR or LC-MS/MS, a common limitation in non-targeted GC-MS studies of complex plant extracts.

HPLC quantification provided more precise and absolute measurements, confirming the trend suggested by GC-MS. The significantly higher roemerine content in *P. rhoeas* (2.25 mg/g) compared to *P. glaucum* (1.49 mg/g) suggests that *P. rhoeas* may be a richer source for this specific compound. These values fall within the range reported for roemerine in other plant sources (10, 11), highlighting the potential of these Iraqi poppies.

The most intriguing finding relates to the antioxidant activity. Despite standardising the test solutions to an equivalent roemerine content, the extract from *P. glaucum* demonstrated a notably higher, though not statistically significant, total antioxidant capacity. This strongly implies that the overall antioxidant potential of these extracts is not solely dictated by roemerine content. GC-MS data (Table 2) offer a plausible explanation: *P. glaucum* contained higher relative levels of compounds like β-sitosterol and shared significant amounts of phenolic compounds (e.g., 2-methoxy-4-vinylphenol) and polyunsaturated fatty acid derivatives (e.g., methyl linolenate), all known for their antioxidant properties (18, 19). The presence of various polysaccharide precursors, as indicated in the GC-MS profile, may also contribute, as polysaccharides from plants can exhibit antioxidant effects (20). This synergistic or additive effect of multiple phytochemicals likely accounts for the observed bioactivity, underscoring the complexity of plant extract pharmacology.

Our findings on antioxidant activity partially support and extend previous work. Butnariu et al. reviewed the general bioactive potential of *Papaver* species, while studies on specific species like *P. rhoeas* have shown variable antioxidant results depending on the assay and extract type (21). Our direct comparison of two species under standardised conditions clarifies that chemoprofile differences significantly impact bioactivity, even for a shared major alkaloid.

Study limitations

Several limitations should be considered when interpreting the findings of this study. Firstly, the identification of roemerine by GC-MS remains tentative, as orthogonal structural confirmation through techniques such as NMR spectroscopy or LC-MS/MS was not performed. Secondly, while the HPLC method was validated for linearity, sensitivity (LOD and LOQ), and system suitability, it did not include a full assessment of accuracy via recovery experiments using a spiked plant matrix. Thirdly, the evaluation of antioxidant activity was confined to a single method, the phosphomolybdenum assay; the inclusion of additional, complementary assays (e.g., DPPH, FRAP, ABTS) would provide a more robust and comprehensive understanding of the extracts' antioxidant potential. Finally, this study utilised pooled plant samples to obtain representative extracts, meaning that natural variation in roemerine content and bioactivity among individual plants was not quantified and remains an area for future investigation.

Conclusion

This study successfully tentatively identified and quantified roemerine in wild *Papaver rhoeas* and *Papaver glaucum* from Sulaymaniyah, Iraq. *P. rhoeas* was found to be a richer source of this aporphine alkaloid. Furthermore, the total antioxidant capacity, assessed by the phosphomolybdenum assay, was not directly proportional to the roemerine content, with *P. glaucum* extract showing higher activity. This suggests a substantial contribution from other antioxidant phytoconstituents present in the extracts. These findings contribute valuable data to the phytochemical atlas of Iraqi flora and highlight the importance of comprehensive metabolite profiling when evaluating the bioactivity of plant species. Future work should focus on the isolation and orthogonal structural confirmation of roemerine from these sources, and the investigation of individual and synergistic bioactivities of the other major compounds detected.

List of Abbreviations

AAE: Ascorbic Acid Equivalent
GC-MS: Gas Chromatography-Mass Spectrometry

HPLC: High-Performance Liquid Chromatography
LOD: Limit of Detection
LOQ: Limit of Quantification
RT: Retention Time
TLC: Thin Layer Chromatography

Declarations

Ethics Approval and Consent to Participate

Collections were made from non-protected, wild populations in publicly accessible areas. Under Iraqi regulations concerning the collection of common wild plants for non-commercial academic research, no specific permits were required. Plant identification was verified by comparison with reference specimens.

Data Availability

All data generated or analysed during this study are included in this published article and its supplementary information files. The raw datasets (GC-MS files, HPLC chromatograms, calibration data) are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions

AHA: Conceptualisation, Methodology, Investigation, Writing – Original Draft.
AUMN: Formal analysis, Data Curation, Visualisation, Writing – Review & Editing.
ASA: Resources, Validation, Supervision, Project administration.
All authors have read and agreed to the submitted version of the manuscript.

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