PLANT SCIENCE TODAY
Vol 13(2): 1-8
https://doi.org/10.14719/pst.13624

eISSN 2348-1900

RESEARCH ARTICLE

Isolation and spectroscopic characterisation of scopoletin from the chloroform fraction of the whole plant of Iraqi *Rhanterium epapposum* Oliv.

Dina Abdullah Eida* & Enas Jawad Kadhim

Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad, Baghdad 10001, Iraq

*Correspondence email - Dina.Ayada2200@copharm.uobaghdad.edu.iq

Received: 11 January 2026; Accepted: 28 February 2026; Available online: Version 1.0: 31 March 2026; Version 2.0: 17 April 2026

Cite this article: Dina AE, Enas JK. Isolation and spectroscopic characterisation of scopoletin from the chloroform fraction of the whole plant of Iraqi *Rhanterium epapposum* Oliv. Plant Science Today. 2026; 13(2): 1-8. https://doi.org/10.14719/pst.13624

Abstract

Rhanterium epapposum Oliv. is a desert plant from the family Asteraceae, widely distributed in Iraq and traditionally utilised for treating skin infections and gastrointestinal disorders, as well as for its insecticidal properties. However, its phytochemical composition, particularly with respect to coumarin constituents, remains insufficiently explored. The present study aimed to isolate and identify a major coumarin compound from the whole plant of Iraqi *R. epapposum*. The coarse powdered plant material was defatted with *n*-hexane, extracted by cold maceration with 85 % aqueous methanol and subjected to successive solvent fractionation. The chloroform fraction was selected for further purification based on its phytochemical profile. Thin-layer chromatography revealed a prominent fluorescent spot indicative of a coumarin compound and high-performance liquid chromatography confirmed its presence. The compound was isolated using preparative layer chromatography and obtained as a single major constituent (coded DA10) with a yield of 0.013 %. Structural identification was achieved through combined chromatographic and spectroscopic analyses, including ultraviolet–visible (UV–Vis) spectroscopy, high-performance liquid chromatography (HPLC) spiking, Fourier transform infrared (FTIR) spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and gas chromatography-mass spectrometry (GC-MS), which showed excellent agreement with an authenticated scopoletin standard. The isolated compound DA10 was identified as scopoletin. This study provides the first confirmed isolation and comprehensive characterisation of scopoletin from *R. epapposum* collected in Iraq. These findings contribute to the phytochemical profiling of the Iraqi chemotype of the species and provide a scientific basis for future investigations on the biological activities and pharmacological potential of the isolated coumarin and other fractions of the plant.

Keywords: chloroform fraction; coumarin; high-performance liquid chromatography; preparative layer chromatography; *Rhanterium epapposum*; scopoletin

Introduction

Rhanterium epapposum Oliv. is a desert plant belonging to the family Asteraceae (1). It is commonly known as "Al-Arfaj". Morphologically, it is a perennial dwarf shrub with a richly branched, pale stem, growing up to 70 cm tall. The plant is characterised by tiny, narrow leaves and yellow flowers approximately 1.5 cm in diameter, which are cupped in a soft-spiny involucre (2). It is the sole species of the genus *Rhanterium* found in the Middle East and represents the genus in Saudi Arabia. This species is geographically widespread across North and Western Africa, as well as in Afro-Asian countries. Its distribution includes the northern border region of Saudi Arabia, Kuwait, the northeastern parts of the United Arab Emirates, Iraq, Iran and Sudan (3). It plays an important role ecologically and economically as essential forage for livestock grazing by sheep and camels in the desert and it is also utilised as a fuel source by Bedouins (4).

Traditionally, *R. epapposum* has been used in folk medicine to treat skin infections and gastrointestinal disturbances and it is also employed as an insecticide in rural areas of Asia and Africa and has antioxidant properties (5). In previous studies, Phytochemical investigations of the aerial parts of *R. epapposum* have revealed the presence of essential oils, flavonoids, alkaloids, saponins, tannins, sterols, steroids, triterpenes and phenolic compounds (1, 4, 6, 7).

Furthermore, previous studies have demonstrated that extracts and essential oils obtained from the aerial parts exhibit diverse pharmacological activities, including antimicrobial, anticholinesterase, insecticidal (4), antioxidant, anti-inflammatory (1), antifungal and anticancer effects (8). These biological activities have been attributed to various bioactive constituents such as monoterpenes, phenolic compounds, fatty acids, flavonoids, tannins and phytosterols. Collectively, these findings highlight the significant pharmacological potential of *R. epapposum* and justify further investigation of its bioactive constituents.

However, coumarin compounds particularly scopoletin have not been previously isolated from the chloroform fraction of Iraqi *R. epapposum* and their detailed spectroscopic characterisation remains insufficiently explored. Scopoletin is a biologically active coumarin derivative widely distributed in plants and has attracted

considerable interest due to its pharmacological relevance (1).

Therefore, the present study aimed to isolate and identify compound DA10 from the chloroform-soluble fraction of Iraqi *R. epapposum*, focusing on compounds that may have been overlooked in previous investigations. The compound DA10 was characterised using ultraviolet–visible (UV–Vis) spectroscopy, high-performance liquid chromatography (HPLC) spiking, Fourier transform infrared (FTIR) spectroscopy, proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and gas chromatography-mass spectrometry (GC-MS) analyses. The results confirm that compound DA10 was identified as scopoletin and is present in the chloroform fraction of the plant.

Materials and Methods

Plant collection

In April 2024, whole plants of *R. epapposum* were collected from the desert plain region of Najaf City, Iraq, which is characterised by deep sandy soil and arid climatic conditions typical of the species. The plant material was taxonomically identified and authenticated at the Department of Biology, College of Science, University of Baghdad, Iraq. An official plant identification certificate confirming the species and family was issued by the department; however, voucher specimen deposition and numbering were not available due to institutional limitations. The freshly collected plant material was washed thoroughly, shade-dried and ground into a coarse powder.

Extraction and fractionation

A total of 300 g of the powdered plant material was defatted with *n*-hexane (1:3 w/v) for three days at room temperature (25 °C) to remove lipophilic constituents. The defatted residue (287.9 g) was subsequently macerated with 3 L of 85 % aqueous methanol (1:10 w/v) for 3 consecutive days at room temperature (25 °C) in a dark place (protected from light), with intermittent shaking (9). The extract was filtered and re-macerated twice under the same conditions. The combined filtrates were concentrated under reduced pressure to yield 17.25 g of crude methanolic extract. The dried extract was suspended in 100 mL of distilled water and sequentially fractionated using solvents of different polarity: petroleum ether, chloroform, ethyl acetate and *n*-butanol (10). Each fraction was dried over anhydrous sodium sulfate, filtered, evaporated and weighed. The yields of the fractions were as follows: petroleum ether (0.71 g), chloroform (1.45 g), ethyl acetate (1.25 g) and *n*-butanol (3.4 g).

Preliminary observations indicated that the chloroform fraction exhibited the highest intensity of coumarin-like fluorescence under UV light. Therefore, this fraction underwent detailed phytochemical evaluation. Thin layer chromatography (TLC) profiling was first performed to compare its major fluorescent spot with the scopoletin reference standard. In addition, HPLC analysis was carried out to confirm the retention time of the major peak, supporting the hypothesis that the target compound (DA10) was scopoletin. Based on these findings, the chloroform fraction was subjected to preparative layer chromatography (PLC) for isolation of the fluorescent compound DA10, which was subsequently purified and submitted to full structural elucidation using TLC, UV–Vis, HPLC spiking, FTIR, ^{1}H- NMR and GC-MS.

Identification by thin-layer chromatography

The chloroform fraction of Iraqi *R. epapposum* was subjected to TLC for the qualitative detection of the coumarin compound DA10 (scopoletin). This was carried out using pre-coated silica gel GF_{254} plates as the stationary phase (11). The chloroform fraction was developed in a mobile phase composed of hexane: ethyl acetate: methanol (7:3:1 v/v/v) (12). After development, the TLC plate was examined under UV light at 365 nm and the retardation factor (Rf) values were measured for both the DA10 compound and the scopoletin standard using Eqn. 1 (13).

$$R_f = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by the solvent}} \quad (1)$$

High-performance liquid chromatography (HPLC) of the chloroform fraction

High-performance liquid chromatography was performed to identify the presence of coumarin compound in the chloroform fraction of *R. epapposum*. Reverse-phase HPLC was performed on a SYKAM liquid chromatograph (Germany) equipped with a binary pump, vacuum degasser, autosampler, diode-array detector (DAD) and a thermostatted column compartment. Identification was achieved on an ODS C18 analytical column (5 µm, 250 mm × 4.6 mm) using an isocratic mobile phase of methanol: distilled water (80:20 v/v). The column temperature was set at 40 °C, the flow rate was maintained at 1 mL/min and the injection volume was 100 µL of the 1 mg/mL sample solution. A relatively high injection volume (100 µL) was employed to improve detection sensitivity for identification of scopoletin in the chloroform fraction. The chromatographic peak showed no distortion or overloading, indicating that the injected volume was compatible with the column capacity. Detection was performed at 245 nm using a UV detector. Identification of the coumarin compound was confirmed by comparing the retention times of the detected peaks with those of the authenticated reference standard analysed under the same chromatographic conditions (14).

Isolation by preparative layer chromatography

The chloroform fraction was subjected to PLC using silica gel plates (1 mm thickness). The mobile phase consisted of hexane: ethyl acetate: methanol (70:30:10 v/v/v). Bands were visualised under UV light at 365 nm and the major fluorescent band corresponding to scopoletin was carefully scraped, eluted, filtered and evaporated to obtain the purified compound DA10. The amount and percentage yield of DA10 were calculated based on the dry weight of the chloroform fraction using Eqn. 2 (15).

$$\text{Yield (\%)} = \frac{\text{Weight of solvent free compound (g)} \times 100}{\text{Dried extract weight}} \quad (2)$$

Characterisation of the isolated compound DA10

The isolated compound DA10 was subjected to chromatographic and spectroscopic analyses. Thin-layer chromatography was used to evaluate the purity of the isolated compound DA10 and the developed plate was examined under UV light at 365 nm. Ultraviolet–visible spectroscopy was employed to record the absorption maxima (λ_max) of DA10 (16). Spiking HPLC analysis was performed by co-analysing the isolated compound DA10 with an authenticated standard under identical chromatographic

conditions (17). Fourier-transform infrared spectroscopy was carried out to examine the functional groups of DA10 in the mid-infrared region (4000–400 cm^{-1}) (18). Proton nuclear magnetic resonance spectroscopy was conducted for structural analysis of the isolated compound DA10 (19). The GC-MS analysis of the isolated compound DA10 was performed using an Agilent 7820A gas chromatograph coupled with a 5977E mass spectrometer, operating under electron ionisation (EI) mode at 70 eV, with a mass scan range of 25–1000 m/z.

Results and Discussion

The chloroform fraction of *R. epapposum* revealed the presence of a major fluorescent compound (DA10), which underwent chromatographic purification and spectral analysis as follows:

Identification by thin-layer chromatography

The TLC analysis of the chloroform fraction revealed several distinct spots, indicating the presence of multiple phytochemical constituents. Notably, one spot exhibited a characteristic light-blue fluorescence under UV light at 365 nm, which is a well-documented feature of coumarin derivatives, particularly scopoletin (20, 21). The DA10 displayed a Rf value of 0.16, which is in close agreement with the Rf value of the scopoletin reference standard (Rf = 0.17) analysed under identical conditions. Such minor variation in Rf values is commonly attributed to slight differences in sample concentration, matrix effects, or solvent saturation conditions. These results are consistent with previously reported TLC characteristics of scopoletin in the literature and support its preliminary identification prior to further chromatographic purification and spectroscopic confirmation (22), as shown in Table 1 and Fig. 1A.

High-performance liquid chromatography of the chloroform fraction

Analytical HPLC profiling of the chloroform fraction revealed several peaks, suggesting a mixture of phytochemicals. Among these, one major peak exhibited a retention time comparable to that of the authenticated scopoletin standard analysed under identical chromatographic conditions (23, 24). In the present study, the DA10 showed a retention time of 7.92 min compared to 7.90 min for the reference standard, indicating a close agreement. A similar close agreement in retention time has been reported previously, where scopoletin identified in plant extracts exhibited an HPLC peak at 7.94 min, closely matching the retention time of the corresponding standard at 7.90 min (25). Such consistency in retention behaviour across different studies supports the identification of the major compound in the chloroform fraction as scopoletin prior to further spectroscopic confirmation, as shown in Table 2, Supplementary Fig. 1 and 2.

Isolation by preparative layer chromatography

Table 1. Retardation factor (Rf) values of DA10 in the chloroform fraction and scopoletin standard

No	Compound	Rf
1	DA10	0.16
2	Scop. St.	0.17

DA10 = Isolated Scopoletin, Scop. St. = Scopoletin standard.

Table 2. Retention time (R_t) values of DA10 in the chloroform fraction and scopoletin standard

No	Compound	R_t (min)
1	DA10	7.92
2	Scop. St.	7.90

Preparative layer chromatography of the chloroform fraction resulted in the successful isolation of a distinct fluorescent band corresponding to compound DA10. The appearance of a single, well-defined band under UV light at 365 nm indicates effective separation of the target compound from accompanying constituents. Such fluorescence behaviour is characteristic of coumarin derivatives and is consistent with previous reports on scopoletin isolation using planar chromatographic techniques. The clear resolution achieved in this step demonstrates the suitability of preparative layer chromatography for isolating scopoletin from complex plant matrices (26). The corresponding PLC chromatogram is presented in Fig. 2, while the yield and percentage recovery of the isolated compound DA10 are summarised in Table 3.

Characterisation of the isolated compound DA10

Thin-layer chromatography: The TLC analysis of the isolated compound DA10 obtained after preparative layer chromatography revealed a single light-blue fluorescent spot under UV light (365 nm), with no additional bands detected when compared with the scopoletin reference standard, as shown in Fig. 1 B. The presence of a single spot indicates a high degree of chromatographic purity of the isolated compound and reflects chromatographic behaviour consistent with that reported for scopoletin in previous studies. These findings further support the successful isolation and preliminary identification of DA10 as scopoletin (20, 21).

Table 3. Amount and percentage yield of isolated compound DA10 isolated from the chloroform fraction

Compound	Amount (g)	Yield %
DA10	0.12	0.013

UV-VIS spectroscopy: The UV–Vis spectrum of the isolated DA10 exhibited absorption bands characteristic of coumarin chromophores. The observed spectral pattern showed absorption maxima at 344, 297 and 228 nm, which were in close agreement with those of the authenticated scopoletin standard (345, 297 and 228 nm), indicating similar electronic transitions associated with the benzene ring fused to the α-pyrone lactone system. These absorption features are typical of π→π* transitions reported for naturally occurring coumarins (27). Similar UV–Vis absorption maxima for scopoletin have been previously reported, with characteristic bands observed around 339 nm, 295 nm and 226 nm confirming the diagnostic spectral behaviour of this compound (28). The close agreement between the absorption maxima of DA10, the reference standard and literature data further supports the identification of DA10 as scopoletin. The absorption maxima of DA10 and the reference standard are summarised in Table 4, while the corresponding spectra are presented in Supplementary Fig. 3.

High-performance liquid chromatography spiking analysis: Following isolation, the identity of compound DA10 was further examined by HPLC spiking analysis. The isolated compound (R_t = 7.99 min) was co-injected with the authenticated scopoletin standard (R_t = 7.90

Table 4. UV–Vis absorption maxima (λmax) of isolated compound DA10 and scopoletin standard.

No	Compound	λmax (nm)
1	DA10	344 297 228
2	Scop. St.	345 297 228

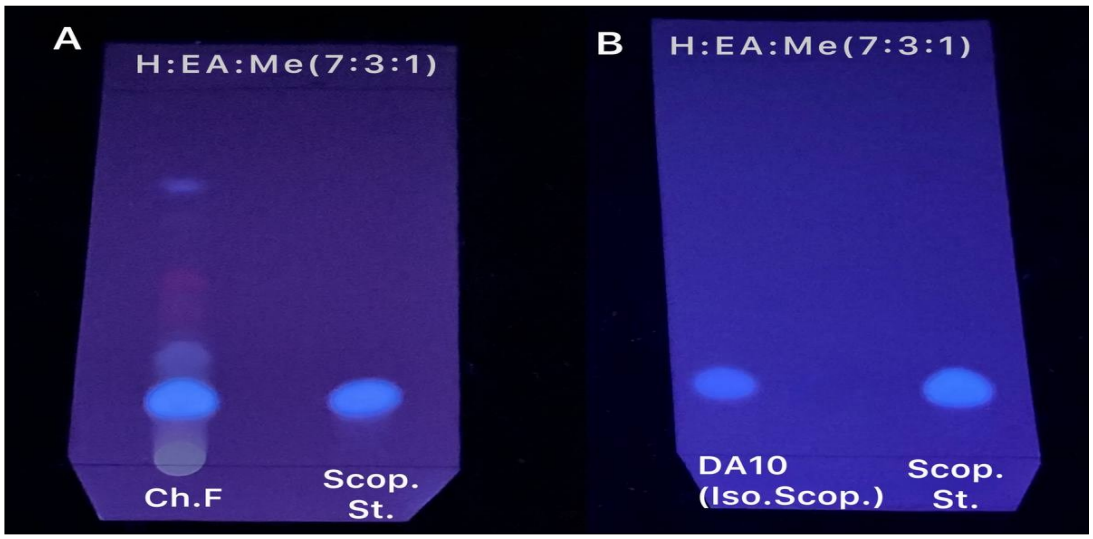


Fig. 1. Analytical Thin layer chromatography chromatograms developed in hexane: ethyl acetate: methanol (7:3:1 v/v/v) and visualised under UV light at 365 nm. (A) The chloroform fraction (Ch. F) of R. epapposum showing a fluorescent spot with the same R_f value as the scopoletin standard (Scop.St.), indicating the presence of scopoletin in the fraction; (B) Thin layer chromatography comparison of the isolated compound (DA10) obtained by preparative layer chromatography and the scopoletin standard (Scop.St.), demonstrating identical fluorescence and R_f values, confirming the successful isolation of scopoletin.

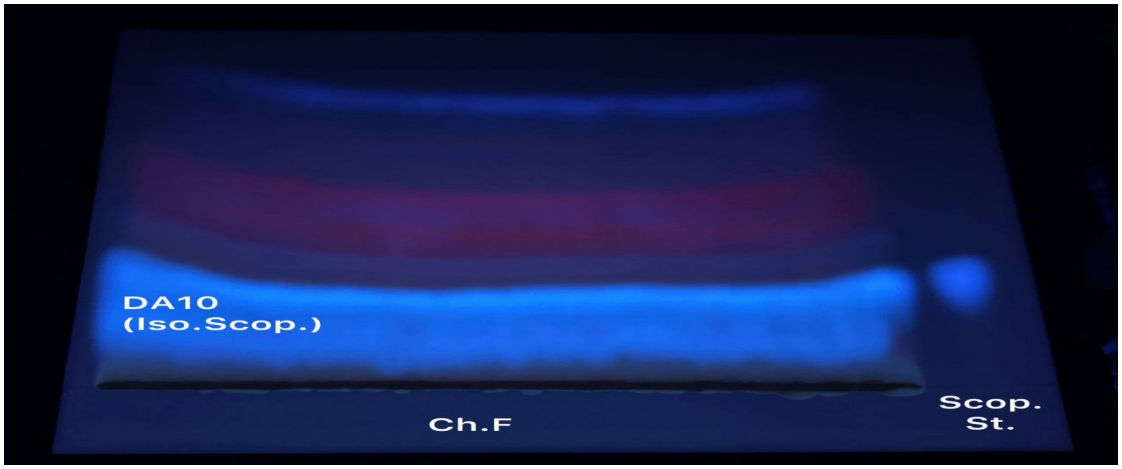


Fig. 2. Preparative layer chromatography of the isolated compound (DA10) obtained from the chloroform fraction of *R. epapposum*, developed in hexane: ethyl acetate: methanol (70:30:10 v/v/v) and visualised under UV light at 365 nm. The intense light-blue fluorescent band corresponding to DA10 shows the same migration behaviour as the scopoletin standard, indicating successful isolation of scopoletin.

min), resulting in a single sharp peak at a retention time of 7.99 min without peak splitting or additional signals, indicating complete co-elution. A slight shift in retention time upon spiking is commonly observed and can be attributed to increased sample load or minor matrix effects during co-injection. Similar HPLC spiking behaviour has been previously reported for scopoletin, where co-injection with a reference standard produced a single coincident peak, confirming compound identity (25). This chromatographic behaviour provides strong confirmatory evidence that the isolated compound DA10 corresponds to scopoletin, as shown in Supplementary Fig. 4.

Fourier transform infrared spectroscopy: The FTIR spectrum of the isolated DA10 displayed absorption bands characteristic of coumarin derivatives and showed a high degree of similarity to that of the authenticated scopoletin standard. As summarised in Table 5, DA10 displayed a broad absorption band around 3335 cm^{-1}, corresponding to O–H stretching vibrations, comparable to the band observed for the scopoletin standard at 3266 cm^{-1}. The presence of a strong absorption band at 1702 cm^{-1} in the region associated with the lactone carbonyl (C=O) stretching, along with characteristic aromatic C=C vibrations at 1605–1507 cm^{-1}, further confirms the coumarin skeleton of the isolated compound DA10. Minor shifts in band positions between DA10 and the reference standard can be attributed to differences in intermolecular hydrogen bonding or sample environment,

Table 5. Fourier transform infrared data of isolated compound DA10 and scopoletin standard

Functional group	ν (cm^{-1}) of DA10	ν (cm^{-1}) of Scop. St.	Assignment
O-H	3335	3266	Stretching vibration of phenolic OH (broad)
C-H	3050	3088	Stretching vibration of C-H aromatic
C-H	2925, 2857	2944	Stretching vibration of C-H aliphatic (asymmetric and symmetric)
C=O	1702	1694	Stretching vibration of C=O carbonyl of lactone group
C=C	1605–1507	1614–1503	Stretching vibration of C=C aromatic
C-H	1453, 1374	1445, 1417	Bending vibration of C-H
C-O / C-O-C	1274–1129	1285–1131	Stretching vibration of C-O /C-O-C phenolic and methoxy /ether groups
C-H	917–742	915–746	Bending vibration of C-H out of plane

*ν = wavenumber.

which are commonly reported in FTIR analyses of phenolic coumarins (29). Similar FTIR spectral features for scopoletin, including O–H stretching and lactone carbonyl bands, have been reported in previous studies (30), supporting the structural assignment of DA10 as scopoletin. The corresponding FTIR spectra are presented in Supplementary Fig. 5 and 6.

Proton nuclear magnetic resonance spectroscopy: The ^{1}H-NMR spectrum of the isolated DA10 displayed proton resonances characteristic of a substituted coumarin skeleton. As shown in Table 6, aromatic proton signals appeared in the range of δ 6.21–7.90 ppm, along with a singlet at δ 3.82 ppm corresponding to a methoxy group (–OCH_3) and a downfield singlet at δ 10.31 ppm attributable to a phenolic hydroxyl (–OH) proton. Comparison with the authenticated scopoletin standard revealed very close agreement in chemical shift values, multiplicity and integration patterns. The reference standard showed corresponding signals at δ 3.80 ppm for the methoxy group and δ 10.33 ppm for the phenolic hydroxyl proton, indicating similar proton environments. Minor variations in chemical shift values may arise from solvent effects or hydrogen-bonding interactions, which are commonly observed in phenolic coumarins. These spectral features are consistent with previously reported ^{1}H-NMR data for scopoletin isolated from plant sources, where aromatic proton signals, a methoxy singlet around δ 3.83 ppm and a phenolic hydroxyl proton near δ 10 ppm were observed, thereby supporting the identification of DA10 as scopoletin (31), as shown in Supplementary Fig. 7 and 8.

Gas chromatography–mass spectrometry analysis: The GC-MS analysis of the isolated compound DA10 showed a well-defined peak with a retention time (R_t= 15.971 min), which was in close agreement with that of the authentic scopoletin standard (R_t = 16.045 min). In addition, the mass spectrum of DA10 exhibited a high similarity index (95 %) when compared with the reference library spectrum of scopoletin, supporting its structural identity. Although the relative peak area of DA10 (74.81 %) was lower than that of the standard, this difference is attributable to sample concentration and matrix effects rather than structural variation, as shown in Table 7 and Supplementary Fig. 9 and 10.

Collectively, the chromatographic and spectroscopic analyses provide consistent and complementary evidence supporting the identity of compound DA10. Preliminary TLC and HPLC profiling suggested the presence of a coumarin-like constituent in the chloroform fraction, which was subsequently isolated as a single fluorescent compound by preparative layer chromatography. Post-isolation HPLC spiking demonstrated complete co-elution with the authenticated scopoletin standard, while UV–Vis, FTIR, ^{1}H-NMR and GC-MS analyses provided structural features consistent with a scopoletin framework. Taken together, these findings confirm that compound DA10 corresponds to scopoletin isolated from the chloroform fraction of Iraqi *R. epapposum.*

Scopoletin has previously been reported in *R. epapposum* based on GC-MS analysis of crude methanolic extracts, in which compound identification was primarily dependent on mass spectral library matching without prior chromatographic isolation or complementary spectroscopic validation (1). In the present study, scopoletin (DA10) was successfully isolated and purified from the chloroform fraction of the Iraqi chemotype of *R. epapposum*. The identity of the isolated compound was supported by consistent chromatographic behaviour, agreement in HPLC retention times, characteristic UV–visible absorption features, post-isolation spiking analysis, FTIR functional group analysis, diagnostic ^{1}H-NMR chemical shift patterns and GC-MS analysis. Notably, GC-MS comparison revealed a close agreement in retention time between DA10 and the authentic scopoletin standard, accompanied by a high mass spectral similarity index, providing confirmatory evidence for compound identity. Differences in retention time reported in earlier extract-based studies can be attributed to variations in extraction procedures, fractionation steps and GC-MS operational conditions, which are known to influence analyte stationary phase interactions. Collectively, these findings extend previous reports by providing the first fully integrated chromatographic and spectroscopic confirmation of scopoletin isolated from Iraqi *R. epapposum*, rather than its tentative identification at the crude extract level based solely on GC-MS detection.

Scopoletin is a naturally occurring phenolic coumarin, chemically identified as 7-hydroxy-6-methoxycoumarin (7-hydroxy-6-methoxy-2H-1-benzopyran-2-one), with the molecular formula $C_{10}H_8O_4$ as shown in Fig. 3 and a molecular weight of 192.17 g/mol. It appears as an amorphous light-yellow powder and exhibits limited solubility in water and cold ethanol, while being soluble in hot ethanol, hot acetic acid and chloroform and almost insoluble in benzene (32). Scopoletin is widely distributed among plant species, where it functions as a phytoalexin involved in plant defense mechanisms against biotic and abiotic stresses (33). Beyond its ecological role, scopoletin has attracted considerable scientific interest due to its broad spectrum of biological activities, including anticancer and anti-angiogenic effects (34), antimicrobial activities (35–39) and neuroprotective properties (40). The successful isolation and comprehensive characterisation of scopoletin from the Iraqi chemotype of *R. epapposum* in the present study provide a plausible chemical explanation for some of the medicinal properties previously attributed to this species. The detection of this bioactive coumarin underscores the phytochemical

Table 7. Gas chromatography–mass spectrometry data of isolated compound DA10 and scopoletin standard

No	Compound	R_t (min)	A %	S.I %
1	DA10	15.971	74.81	95
2	Scop.St.	16.045	100.00	99

*A % = Area. R_t = Retention time, S.I % = Similarity Index.

Table 6. Proton nuclear magnetic resonance data of isolated compound DA10 and scopoletin standard (500 MHz, DMSO-d_6)

Carbon atom	δ ppm of DA10	δ ppm of Scop. St.	Integration	Multiplicity	Assignment
C_6-OH	10.31	10.33	1H	s	Phenolic hydroxyl proton (-OH)
C_9-H	7.90	7.90	1H	d	Aromatic H
C_{10}-H	7.20	7.20	1H	d	Aromatic H
C_7-H	6.76	6.76	1H	s	Aromatic H
C_4-H	6.21	6.21	1H	d	Aromatic H
C_5-OCH_3	3.82	3.80	3H	s	Protons of methoxy group (-OCH_3)

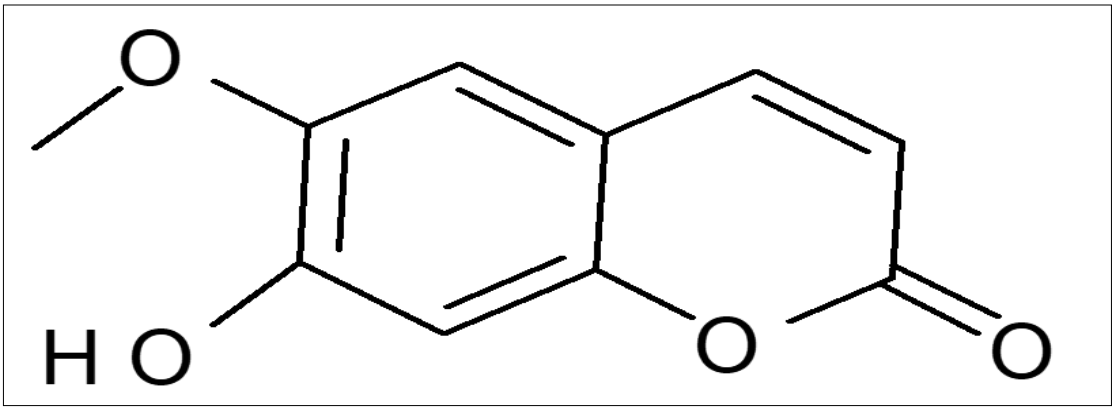


Fig. 3. Chemical structure of scopoletin.

importance of *R. epapposum* and supports its potential as a promising source for further pharmacological and biological investigations.

Conclusion

In this study, scopoletin was successfully isolated from the chloroform fraction of the whole plant of Iraqi *Rhanterium epapposum* using solvent fractionation followed by preparative layer chromatography, with a yield of 0.013 %. The structure of the isolated compound (DA10) was conclusively confirmed through a combination of chromatographic and spectroscopic techniques, including TLC, HPLC, UV–visible spectroscopy, HPLC spiking, FTIR, ^{1}H-NMR and GC-MS analyses, showing excellent agreement with an authenticated scopoletin standard. This work represents the first confirmed isolation and comprehensive spectroscopic characterisation of scopoletin from *R. epapposum* collected in Iraq, thereby contributing to the phytochemical profiling of the Iraqi chemotype of this species. Given that scopoletin is a biologically active coumarin widely reported for its anticancer, antimicrobial, anti-inflammatory and neuroprotective properties, the confirmed presence of this compound provides a strong chemical basis for the traditional and potential medicinal relevance of *R. epapposum*.

Future studies should focus on evaluating the biological activities of the isolated scopoletin, particularly its cytotoxic, antioxidant, anti-inflammatory and antimicrobial potential, as well as investigating other solvent fractions of the plant for additional bioactive constituents. Such studies will help to further clarify the pharmacological significance of Iraqi *R. epapposum* and assess its potential as a natural source of therapeutically valuable coumarins.

Acknowledgements

The authors gratefully acknowledge the Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad, for providing laboratory facilities and technical support.

Authors' contributions

DAE carried out the isolation and characterisation and drafted the manuscript. EJK supervised the research and provided critical revision. Both authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Quill bot, Grammarly and Chat GPT (Open AI) to improve English language, grammar, clarity of expression and to reduce unintended textual similarity through careful language editing. After using these tools/services, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

1. El-Ashmawy IM, Aljohani ASM, Soliman AS. Studying the bioactive components and phytochemicals of the methanol extract of *Rhanterium epapposum* Oliv. Appl Biochem Biotechnol. 2024;196 (6):2414–24. https://doi.org/10.1007/s12010-023-04574-y
2. Omar SAS, Bhat NR. Alteration of the *Rhanterium epapposum* plant community in Kuwait and restoration measures. Int J Environ Stud. 2008;65(1):139–55. https://doi.org/10.1080/00207230701823332
3. Phondani PC, Bhatt A, Elsarrag E, Horr YA. Ethnobotanical magnitude towards sustainable utilization of wild foliage in Arabian desert. J Tradit Complement Med. 2016;6(3):209–18. https://doi.org/10.1016/j.jtcme.2015.03.003
4. Demirci B, Yusufoglu HS, Tabanca N, Temel HE, Bernier UR, Agramonte NM, et al. *Rhanterium epapposum* Oliv. essential oil: chemical composition and antimicrobial, insect-repellent and anticholinesterase activities. Saudi Pharm J. 2017;25(5):703–8. https://doi.org/10.1016/j.jsps.2016.10.009.
5. Rajendrasozhan S, El Moll H, Snoussi M, Romeilah RM, Shalaby EA, Younes KM, et al. Phytochemical screening and antimicrobial activity of various extracts of aerial parts of *Rhanterium epapposum*. Processes. 2021;9(8):1351. https://doi.org/10.3390/pr9081351.
6. Shahat AA, Mahmoud EA, Al-Mishari AA, Alsaid MS. Antimicrobial activities of some Saudi Arabian herbal plants. Afr J Tradit Complement Altern Med. 2017;14(2):161–5. https://doi.org/10.21010/ajtcam.v14i2.17
7. Awad M, Abdelwahab A. Chemical diversity of essential oils from flowers, leaves and stems of *Rhanterium epapposum* Oliv. growing in the northern border region of Saudi Arabia. Asian Pac J Trop Biomed. 2016;6(9):767–70. https://doi.org/10.1016/j.apjtb.2016.07.010
8. Al Qahtani MSJ, Al-Debaiky SAA, Sayed M. Antifungal and cytotoxic activities of biosynthesized silver, zinc and gold nanoparticles by flower

extract of *Rhanterium epapposum*. Open J Appl Sci. 2020;10:663–74. https://doi.org/10.4236/ojapps.2020.1011046

9. Mahmood MA, Abd AH, Kadhim EJ. Assessing the cytotoxicity of phenolic and terpene fractions extracted from Iraqi *Prunus arabica* against AMJ13 and SK-GT-4 human cancer cell lines. F1000Res. 2024;12:433. https://doi.org/10.12688/f1000research.131336.3
10. Suksaeree J, Chankana N, Luprasong C, Monton C. Optimization of dynamic maceration of *Clausena anisata* (Willd.) Hook. f. ex Benth. leaves to maximize trans-anethole content. Sci Nat. 2021;108(3):1–9. https://doi.org/10.1007/s42452-021-04509-4
11. Abdul-Jalil TZ. Ultrasound-assisted extraction and cytotoxic activity of fennel leaves: process optimization and thin layer chromatography. Iraqi J Pharm Sci. 2024;33(3):94–103. https://doi.org/10.31351/vol33iss3/pp94-103
12. Zych M, Pyka-Pająk A. TLC in the analysis of plant material. Processes. 2025;13:3497. https://doi.org/10.3390/pr13113497
13. Premnath SM, Zubair M. Chromatography. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024.
14. Patel P, Raval M, Patel N, Patel S, Vyas N, Patel A. Quantification of scopoletin from the roots of *Argyreia speciosa* (Linn. f.) Sweet using HPLC through the concept of design of experiment. J AOAC Int. 2021;104(4):1167–80. https://doi.org/10.1093/jaoacint/qsab013
15. Abbas A, Naqvi SAR, Rasool MH, Noureen A, Mubarik MS, Tareen RB. Phytochemical analysis, antioxidant and antimicrobial screening of *Seriphidium oliverianum* plant extracts. Dose Response. 2021;19(1):15593258211004739. https://doi.org/10.1177/15593258211004739.
16. Tan DC, Quek A, Kassim NK, Ismail IS, Lee JJ. Rapid quantification and validation of biomarker scopoletin in *Paederia foetida* by qNMR and UV–Vis for herbal preparation. Molecules. 2020;25(21):5162. https://doi.org/10.3390/molecules25215162
17. Hamad MN. Detection and isolation of flavonoids from *Calendula officinalis* (Asteraceae) cultivated in Iraq. Iraqi J Pharm Sci. 2017;25(2):1–6. https://doi.org/10.31351/vol25iss2pp1-6
18. Firmansyah A, Winingsih W, Manobi JDY. Review of scopoletin: isolation, analysis process and pharmacological activity. Biointerface Res Appl Chem. 2021;11(4):12006–19. https://doi.org/10.33263/BRIAC114.1200612019
19. Yan J, Xing Z, Lei P, Sikandar A, Yang R, Wang Y, et al. Evaluation of scopoletin from *Penicillium janthinellum* Snef1650 for the control of *Heterodera glycines* in soybean. Life. 2021;11:1143. https://doi.org/10.3390/life11111143
20. Thomaz DV, Couto RO, Oliveira RA, Oliveira LAR, Leite KCS, Bara MTF, et al. Assessment of noni (*Morinda citrifolia* L.) product authenticity by solid state voltammetry. Int J Electrochem Sci. 2018;13:8983–94. https://doi.org/10.20964/2018.09.390
21. Rohman A, Gandjar IG. Kimia farmasi analisis. Yogyakarta: Pustaka Pelajar; 2007. 298–304.
22. Dwivedi J, Gupta A, Paliwal S, Rawat AKS. Validated simultaneous HPTLC analysis of scopoletin and gallic acid in the methanolic fraction of *Jatropha glandulifera*. JPC J Planar Chromatogr Mod TLC. 2020;33:457–62. https://doi.org/10.1007/s00764-020-00063-3
23. Tasfiyati AN, Antika LD, Septama AW, Hikmat H, Kurniawan HH, Ariani N, et al. A validated HPLC-DAD method and comparison of different extraction techniques for analysis of scopoletin in noni-based products. Kuwait J Sci. 2023;50(3):276–81. https://doi.org/10.1016/j.kjs.2023.02.023
24. Wada M, Asoh M, Takada M, Noguchi K, Nishigaki T, Mutoh J, et al. Quantitation of nine scopoletin analogues using an HPLC-fluorescence method and its application to *Morinda citrifolia* L. products. Chromatography. 2025;46(2):89–93. https://doi.org/10.15583/jpchrom.2025.007
25. Elaf BD, Dhuha AAS. Isolation and characterization of scopoletin from Iraqi-cultivated *Leonotis leonurus* using maceration and chromatographic techniques. Plant Sci Today. 2025;12(2):1–8. https://doi.org/10.14719/pst.8534
26. Suryati, Efdia M, Astuti SH, Aziz H. Isolation of scopoletin from *Spilanthes paniculata* Wall. ex DC. Der Pharma Chemica. 2016;8(9):99–104.
27. Fatima S, Mansha A, Asim S, Shahzad A. Absorption spectra of coumarin and its derivatives. Chem Pap. 2022;76(2):1–9. https://doi.org/10.1007/s11696-021-01902-6
28. Bhatt MK, Dholwani KK, Saluja AK. Isolation and structure elucidation of scopoletin from *Ipomoea reniformis*. J Appl Pharm Sci. 2011;1(5):138–44.
29. Topala CM, Ducu CM, Tataru LD. ATR-FTIR spectra fingerprinting of medicinal herb extracts prepared using microwave extraction. Open Access J Med Aromat Plants. 2017;8(1):1–6.
30. Aldi Y, Handayani D, Bakhtiar A, Wardi A, Yanwirasti, Nasrul E, et al. Effects of scopoletin from noni fruit (*Morinda citrifolia* L.) on IL-10 levels in male white mice with type I hypersensitivity. Res J Pharm Biol Chem Sci. 2016;7(4).
31. Chen G, Zhang Y, Chen X, Yuan J, Zhang Y. Scopoletin as a key component of a novel acaricidal agent and its production methodology. Nat Prod Commun. 2024;19(12):1–9. https://doi.org/10.1177/1934578X241306808
32. Gao XY, Li XY, Zhang CY, Bai CY. Scopoletin: a review of its pharmacology, pharmacokinetics and toxicity. Front Pharmacol. 2024;15:1268464. https://doi.org/10.3389/fphar.2024.1268464
33. Ihnatowicz A, Siwinska J, Perkowska I, Grosjean J, Hehn A, Bourgaud F, et al. Genes to specialized metabolites: accumulation of scopoletin, umbelliferone and their glycosides in natural populations of *Arabidopsis thaliana*. BMC Plant Biol. 2024;24:806. https://doi.org/10.1186/s12870-024-05491-w
34. Hasan NK, Faisal N, Al-Sarrag NF. A new study on the effect of scopoletin and silver nanoparticles of fenugreek extract as pancreatic cancer inhibition agents. Acad Sci J. 2024;2(2):62–77. https://doi.org/10.24237/ASJ.02.02.809B
35. Napiroon T, Bacher M, Balslev H, Tawaitakham K, Santimaleeworagun W, Vajrodaya S. Scopoletin from *Lasianthus lucidulus* Blume (Rubiaceae): a potential antimicrobial against multidrug-resistant *Pseudomonas aeruginosa*. J Appl Pharm Sci. 2018;8:1–6. https://doi.org/10.7324/JAPS.2018.8901
36. Buathong R, Chamchumroon V, Schinnerl J, Bacher M, Santimaleeworagun W, Kraichak E, et al. Chemovariation and antibacterial activity of extracts and isolated compounds from species of *Ixora* and *Greenea* (Ixoroideae, Rubiaceae). PeerJ. 2019;7:e6893. https://doi.org/10.7717/peerj.6893
37. Mamatova AS, Korona-Glowniak I, Skalicka-Woźniak K, Józefczyk A, Wojtanowski KK, Baj T, et al. Phytochemical composition of wormwood (*Artemisia gmelinii*) extracts in respect of their antimicrobial activity. BMC Complement Altern Med. 2019;19(1):288. https://doi.org/10.1186/s12906-019-2719-x
38. Mogana R, Adhikari A, Tzar MN, Ramliza R, Wiart C. Antibacterial activities of the extracts, fractions and isolated compounds from *Canarium patentinervium* Miq. against bacterial clinical isolates. BMC Complement Med Ther. 2020;20(1):55. https://doi.org/10.1186/s12906-020-2837-5
39. Yang L, Ding W, Xu Y, Wu D, Li S, Chen J, et al. New insights into the antibacterial activity of hydroxycoumarins against *Ralstonia solanacearum*. Molecules. 2016;21(4):468. https://doi.org/10.3390/molecules21040468
40. Kashyap P, Ram H, Shukla SD, Kumar S. Scopoletin: antiamyloidogenic, anticholinesterase and neuroprotective potential of a natural compound present in *Argyreia speciosa* roots by in vitro and in silico study. Neurosci Insights. 2020;15:2633105520937693. https://doi.org/10.1177/2633105520937693

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

Copyright: © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited (https://creativecommons.org/licenses/by/4.0/)

Publisher information: Plant Science Today is published by HORIZON e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.