



RESEARCH ARTICLE

Isolation and characterization of scopoletin from Iraqi-cultivated *Leonotis leonurus* (lion's ear) using maceration and chromatographic techniques

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Received: 18 January 2025; Accepted: 05 May 2025; Available online: Version 1.0: 21 May 2025

Cite this article: Elaf BD, Dhuha AAS. Isolation and characterization of scopoletin from Iraqi-Cultivated *Leonotis leonurus* (lion's ear) using maceration and chromatographic techniques. Plant Science Today (Early Access). <https://doi.org/10.14719/pst.8534>

Abstract

The perennial herb *Leonotis leonurus* (*L. leonurus*), is commonly recognized by the names lion's ear and wild dagga family (Lamiaceae) is indigenous to Southern Africa and is commonly regarded for its ethnobotanical value. The plant is well-known for its medicinal as well as psychotropic properties. The leaves or blossoms are used in poultices or drinks. It's smoked for its mild euphoric effects, said to mimic those of cannabis, but weaker. The research focuses on the first-time isolation of coumarin (scopoletin) from *L. leonurus* specifically cultivated in Iraq and its several biological effects, including anti-inflammatory, antioxidant, antibacterial and antidiabetic ones, from the leaf methanolic extract of the spontaneously growing plant species in Iraq *Leonutus leonurus*. The extraction method employed is the simple maceration extraction technique, where the plant material is soaked in a solvent without applying any heat. The method involves first soaking the plant with the non-polar solvent hexane and then continuing with 85 % methanol. The yield of the maceration method extraction is 15.19 g. By using analytical thin-layer chromatography, high-performance liquid chromatography (HPLC), ultraviolet (UV) spectrophotometry and Fourier Transform Infrared Spectroscopy (FTIR), it was demonstrated that the isolated scopoletin was present and possessed a structure identical to the same respective standard. Isolation of scopoletin alone from the methanolic leaf extract of *Leonotis leonurus* proved its place in the leaf part, which shows that the leaf is signifying as one of the sources of bioactive compounds This finding is significant as it establishes the presence of scopoletin in Iraqi-grown *L. leonurus*, which may suggest potential variations in the phytochemical profile of this species based on geographical location and cultivation conditions. Further pharmacological investigations are warranted to explore the specific bioactivity of scopoletin derived from this Iraqi source. This requires further pharmacological investigations for their confirmation of bioactivity.

Keywords: coumarin (scopoletin); FTIR; HPLC; *Leonotis leonurus*; TLC; UV

Introduction

The evergreen bush *Leonotis leonurus* (*L. leonurus*), was commonly recognized as lion's ear and the wild dagga family (Lamiaceae), is indigenous to Southern Africa and is widely valued for its ethnobotanical significance. The plant is known for both therapeutic and mild psychoactive effects. The leaves or blossoms are used in various ways, from decoctions or infusions. Having mild euphoric activity somewhat comparable to cannabis (although less potent) (1), it is smoked for its euphoric effects, while its leaves and flowers have, historically, been used in African Indigenous medicine for the treatment of several health issues, including hypertension, diabetes, inflammatory and microbial diseases (2).

L. leonurus has also been employed to treat respiratory disorders (3). The volatile oil in the secretory structures of this plant is probably responsible for its therapeutic activity (4). In South Africa, particularly in the Eastern Cape Province, leaves are used to treat asthma, coughs and other respiratory diseases. Its leaves are commonly used in the form of pak, in

cases of pruritus as well as eczema. They are also marketed for their antioxidant properties (5). Although extensively used in traditional medicine, the phytochemical profile of *L. leonurus* is poorly defined since previous works have been limited to diterpenoids (1), alkaloids (6) and flavonoids (5). So far, there has been no systematic approach to extracting specific secondary metabolites, especially coumarins, from this species.

Scopoletin (7-hydroxy-6-methoxycoumarin), a naturally occurring coumarin derivative, has attracted considerable attention for its various pharmacological actions, such as anti-inflammatory, antioxidant, antibacterial and antidiabetic properties (7, 8).

Scopoletin proves to be a likely candidate with the potential for colon cancer treatment due to its modulation of multiple signaling pathways carried out by the disease. The pathways are related to oxidative stress and inflammation, among many others - apoptosis and autophagy, cell proliferation and insulin sensitization. The compound may

exert its protective effects against colon cancer. By regulating the nuclear factor erythroid-related factor-2 (Nrf2) signaling pathway and other inflammation-associated pathways, Scopoletin can potentially exert its anti-tumor activities (9). In addition, scopoletin has been shown to enhance the efficacy of conventional chemotherapeutic agents, such as cisplatin, in cholangiocarcinoma cells, leading to additive cytotoxic effects (10)

Scopoletin is a natural compound that is detected in nature and is commonly associated with the plant's defense response to infection by pathogens and microbes (7). *Morinda citrifolia* is one of the medicinal plants from which this compound has been isolated. Coumarins are relatively simple to detect, as they display fluorescent blue properties under UV light 366 (11), such as the compound scopoletin.

Despite both the Rutaceae and the Umbelliferae (12) families containing coumarin, no such molecule has been previously observed in *L. leonurus*. Scopoletin detection among the plant sources is of high interest because it would be able to elucidate chemotaxonomic relationships and indicate bioactive leads for pharmacological development. Maceration methods were the extraction process, strategic to isolate the scopoletin compounds existing in the plant (13).

An integral methodology that is frequently acknowledged is maceration, which entails the immersion of botanical substrates in a low-temperature solvent, permitting the solvent to permeate at ambient temperatures, accompanied by the application of constant agitation. This protocol is designed to facilitate the softening of the plant cell walls, thereby aiding in the liberation of solubilized phytochemicals. (14). To date, this is the first report on the isolation and identification of scopoletin from the methanolic leaf extract of *L. leonurus*. Preliminary identification and separation of the molecule were carried out using spectroscopic methods - UV and FTIR - and chromatographic methods - TLC and HPLC. Therefore, the main objectives of this study were to isolate and identify scopoletin from the methanolic leaf extract of *Leonotis leonurus* grown in Iraq. Furthermore, characterize the purified scopoletin using spectroscopic (UV and FTIR) and chromatographic (TLC and HPLC) techniques.]

Materials and Methods

Plant Materials

The *Leonotis leonurus* plant was obtained in April 2024 from Babil City. Approximately 100 g of dried leaves were used for this study. Professor Assistant Dr. Sukaina Abbas (certified botanist) from the Department of Biology, College of Science, University of Baghdad, identified and confirmed the authenticity of the fresh *Leonotis leonurus* plant seen in Fig. 1.

Extraction

1 kg of fresh leaves were collected and dried in a well-ventilated room. Then, the dried leaves were crushed with a mechanical blender to make fine powder. 100 gm of powdered dried leaves macerated first with 1000 mL hexane for 3 days with continuous agitation to remove fatty and waxy unwanted material. The Defatted powder is then macerated with 85 % methanol (15) for 9 days with continuous agitation; every 3 days, the solvent is replaced with fresh solvent. After filtration, the methanolic extract was concentrated with a rotary evaporator. The resulting marc is shown on Fig. 2. 100 g of dried leaf provided us with a yield of 15.19 g of methanolic extract, which represents a percentage yield of 15.19 %. This entire maceration and extraction process was performed on a single batch of plant material for the isolation of scopoletin.

Identification of scopoletin by thin-layer chromatography

For the first identification of bioactive chemicals in phytochemical research, thin-layer chromatography (TLC) is a fast, reasonably priced method extensively utilized. This work used TLC to identify and validate scopoletin presence in *Leonotis leonurus* methanolic leaf extract. Silica gel 60 F254 pre-coated aluminum plates (Merck, Deutschland) were used as the stationary phase.

The crude methanolic extract and scopoletin standard (MACKLIN, China) were dissolved in methanol (1 mg/mL). Two solvent systems were used for the effective separation of coumarins: S1: Toluene: Acetone: Chloroform (45: 55: 5 v/v) was selected based on a previous study that demonstrated its effectiveness in the isolation of coumarins (16). S2: Toluene: Dioxan: Glacial acetic acid (90: 25: 4 v/v) was chosen based on its reported ability to effectively separate a broader range of phytochemical compounds (17). After development, we visualized the TLC under UV light (366 nm) to see the blue fluorescence of coumarin see Fig. 3.



Fig. 1. Fresh and dried *Leonotis leonurus* plants.

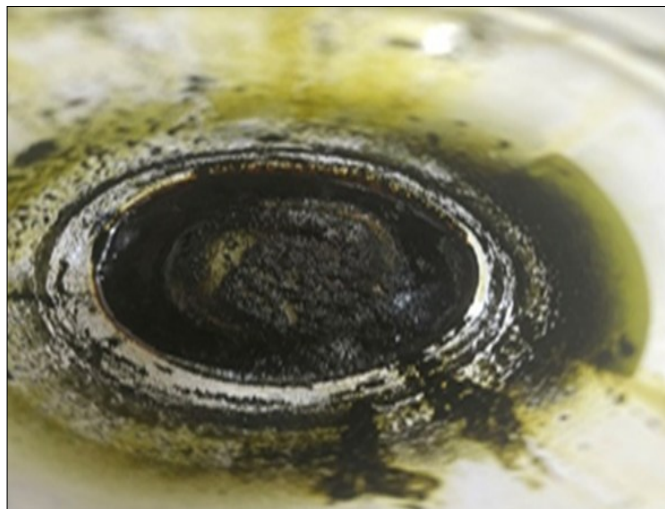


Fig. 2. Concentrated methanolic leaf extract.

Identification and isolation of scopoletin by reverse-phase HPLC

In this study, the isolation and the qualitative analysis of methanolic leaf extract were performed by reverse HPLC model with fraction collector (SYKAM /Federal Republic of Germany) using a movable phase = methanol: D.W = (80: 20), Support = C18 – ODS (25cm * 4.6 mm), a sensor of UV–366 nm, and a flow rate = 1.0 milliliter/minute (18, 19). We identify the compound by comparing its retention period to the scopoletin standard with purity of 98 % cas no.: 56445-51-8 and the batch number BP1275 (Phytopurify.com, China) exhibited a retention time of 7.90 min with a peak area of 466.21 mAU. In the methanolic leaf extract, scopoletin was identified at a retention time of 7.94 min, with a corresponding peak area of 2587.00 mAU as shown in Fig. 4, 5 and 6. To confirm co-elution, the spiking method of the sample with the standard was used as shown in Fig. 7. The calibration curve for scopalamine was linear ($R^2 = 0.9998385$) over a range of 5 to 20 ppm. The equation for the calibration curve was ($Y = 93.93333 * X$) as shown in Fig. 8.

The identification and characterization of isolated scopoletin

The identification of scopoletin isolated from the leaves of *Leonotis leonurus* was confirmed through a multi-technique approach: Thin layer chromatography (TLC), HPLC spiking method (SYKAM Germany in Department of Environment) utilized To determine the optimal uniqueness, a measurable and equivalent quantity of the standard was merged before the finalization of volume with the solvent; an instrumental signal consistent to the standard solutions and samples was recognized separately and subsequently, when combined, FTIR (SHIMADZU through ATR system in Department of Environment and water research) Through the attenuated total reflection (ATR) method, structural assignments were linked to distinctive bands.

Ultimately, UV spectroscopy (utilizing SHIMADZU equipment within the BPC analytical center) was employed to investigate the isolated scopoletin, subsequently compared with the established standards, which served as a reference point and both were examined under analogous conditions.

Results and Discussion

Thin Layer Chromatography (TLC)

The TLC results of the methanolic extract showed a prominent fluorescence spot with an R_f value of 0.62 in S1 and 0.4 in S2 Fig. 3A and B While Fig. 3. C shows the isolated scopoletin with that of the standard spot.

High-Performance Liquid Chromatography (HPLC)

The interpretation of HPLC shows a well-defined peak at a retention time of 7.94 min, corresponding to the scopoletin standard, which has a retention time of 7.90 min as shown in Fig. 4,5,6.

Spiking method by HPLC

The spiking method of the standard with the isolated resulted in a single, sharp peak with no shoulder formation, verifying the purity and successful elution of scopoletin under the chosen chromatographic conditions, as shown in Fig. 7.

FTIR spectroscopy

Table 1 shows the data obtained from FTIR Spectroscopy and the possible functional groups present. The broad band at 3274.0 and 3282.6 cm^{-1} in the infrared spectrum analysis is most likely caused by the phenol OH group's O-H stretching vibrations. Because of -CH₃, the peaks at 2940.1 and 2940.1 cm^{-1} displayed C-H stretching. The existence of the -C=O, carbonyl group is indicated by the peaks at 1710.3 and 1703.14. The existence of the -CH=CH group was shown by the peaks at 1560.3 & 1561.9. The existence of the benzene ring is indicated by the peaks at 1612.5 and 1612.6. The existence of an aromatic co-group was indicated by the peaks at 1295.7 and 1295.9. The existence of benzene ring Di substitution was demonstrated by the peaks at 852.5 and 852.6 in the isolated and scopoletin standards, respectively Fig. 9 and 10 (20, 21).

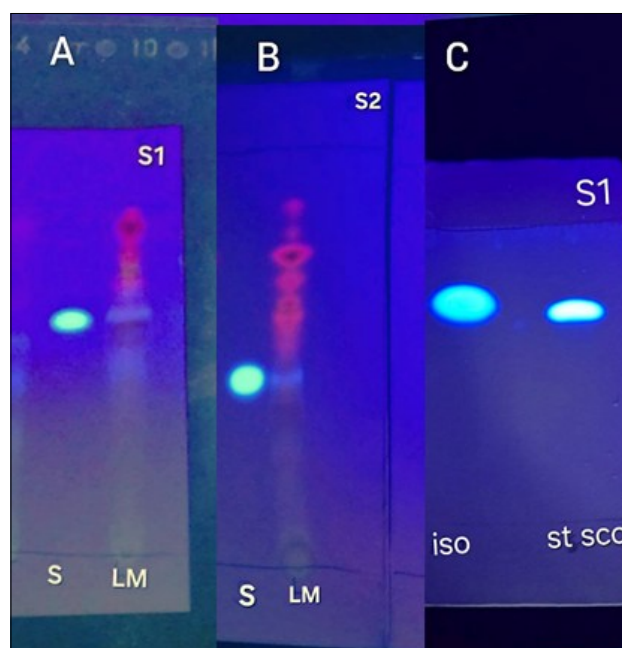


Fig. 3. (A) Analytical TLC of S1 mobile phase below UV light 366 nm; (B) Analytical TLC OF S2 mobile phase below UV light 366 nm; (C) Analytical TLC chromatogram of isolated scopoletin by HPLC (iso) and scopoletin standard (st sco) in S1 mobile phase.

LM= leaves part, **s**= scopoletin standard.

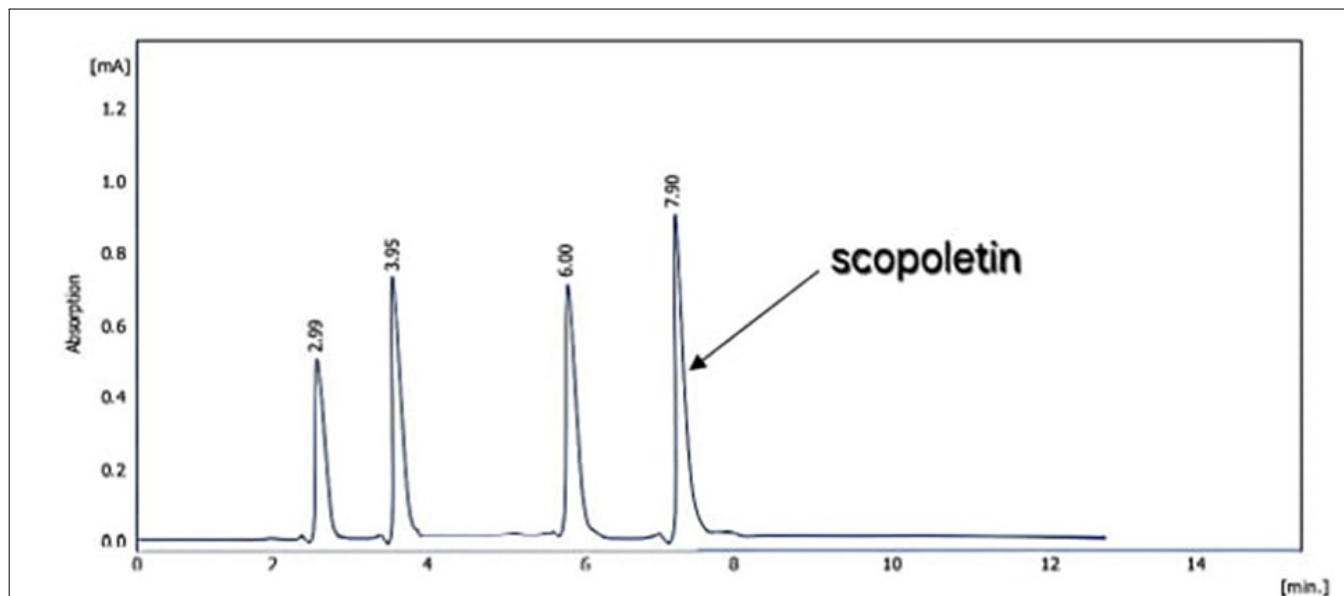


Fig. 4. Chromatographic record of *L. leonurus* methanolic leaves extract.

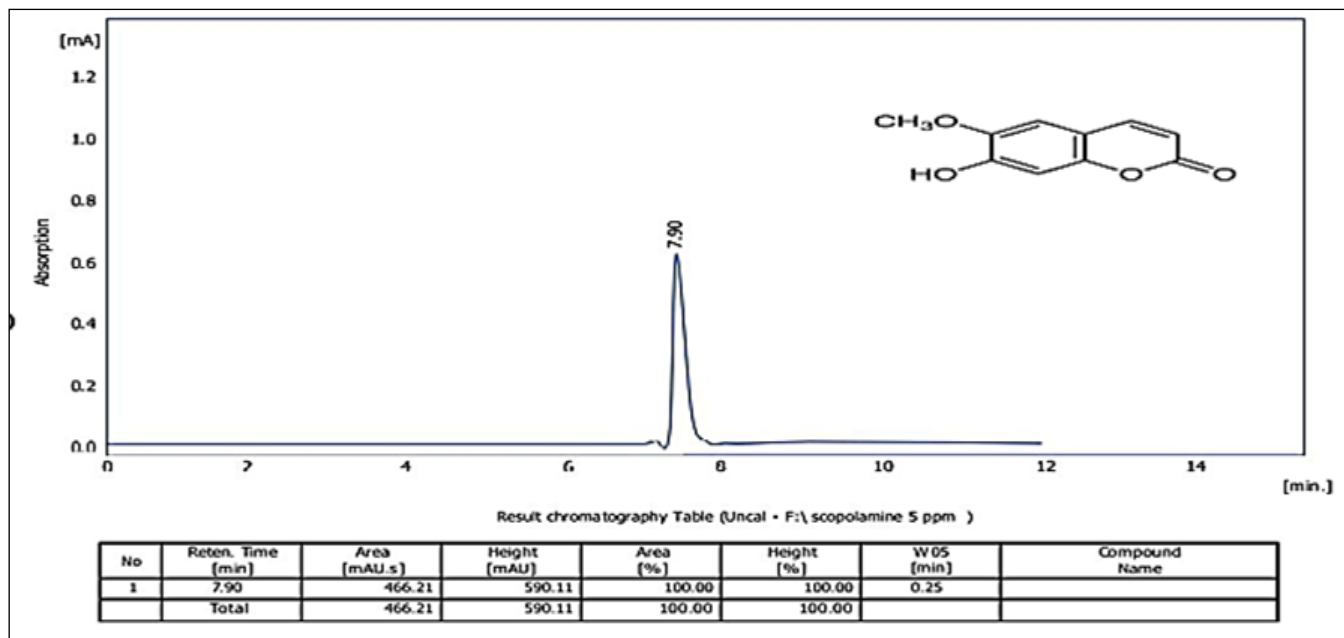


Fig. 5. Chromatographic record of standard scopoletin.

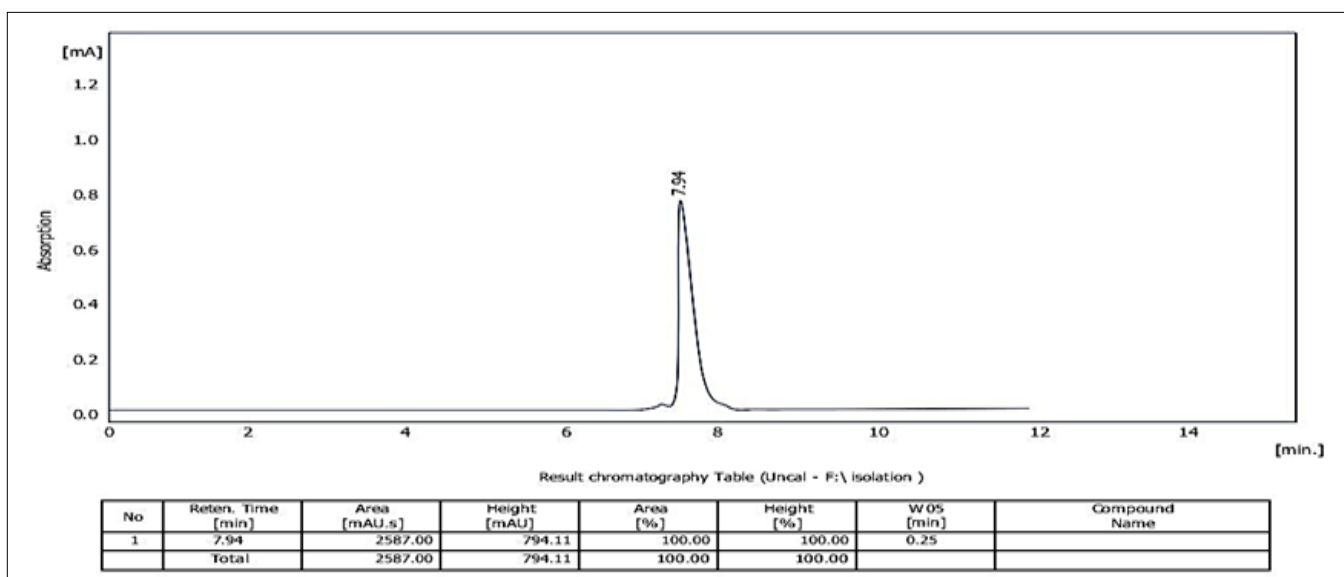


Fig. 6. Chromatographic record of isolated scopoletin.

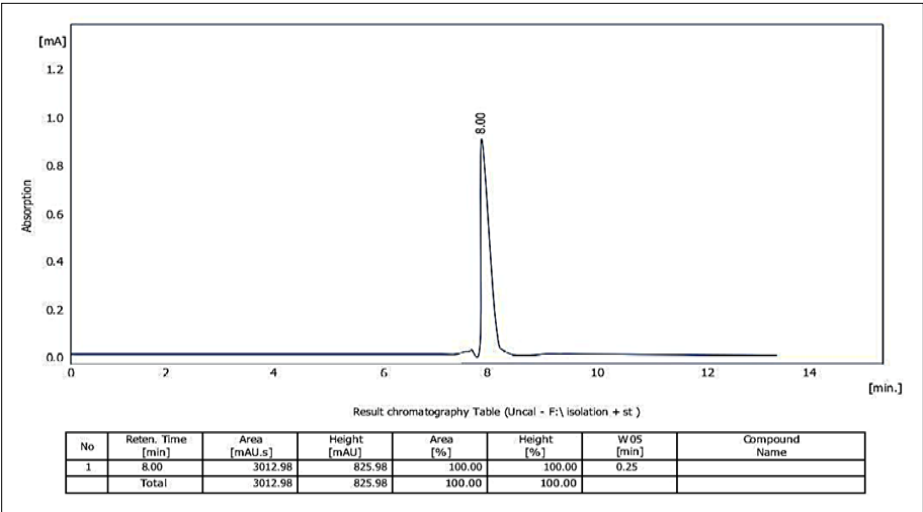


Fig. 7. Chromatographic record spiking method of isolated scopoletin.

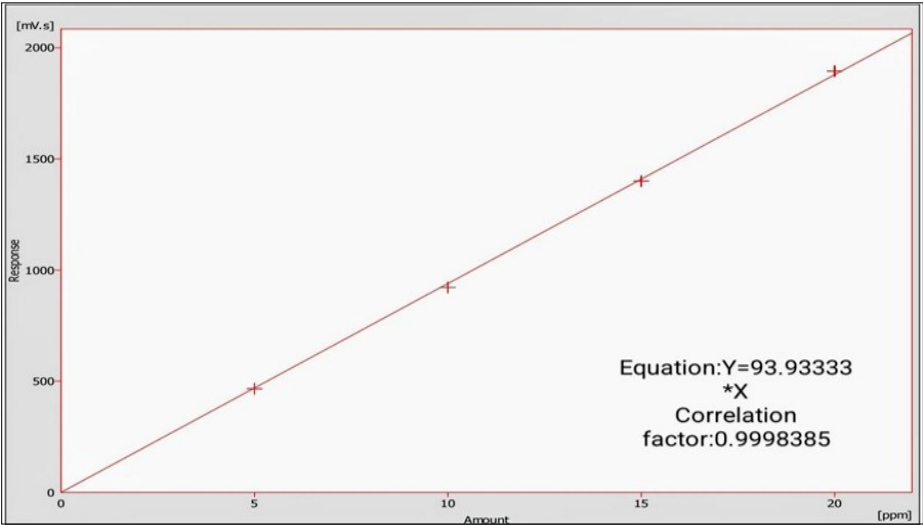


Fig. 8. HPLC chromatography calibration plot of scopoletin.

Table 1. FTIR data of isolated and standard scopoletin

Wavenumber (cm ⁻¹) of the isolated	Wavenumber (cm ⁻¹) of the	Functional group of the isolated	Functional group of the standard	Type of vibration of the isolated	Type of vibration of the standard
3274.0	3282.6	O-H (Hydroxyl)	O-H (Hydroxyl)	Stretching	Stretching
2940.1	2940.1	C-H (Alkyl)	C-H (Alkyl)	Stretching	Stretching
1710.3	1703.14	C=O (Carbonyl)	C=O (Carbonyl)	Stretching	Stretching
1560.3	1561.9	C=C (Alkene)	C=C (Alkene)	Stretching	Stretching
1612.5	1612.6	C=C (Aromatic)	C=C (Aromatic)	Stretching	Stretching
1295.7	1295.9	C-O (Ether)	C-O (Ether)	Stretching	Stretching
852.5	852.6	C-H (Aromatic)	C-H (Aromatic)	Bending	Bending

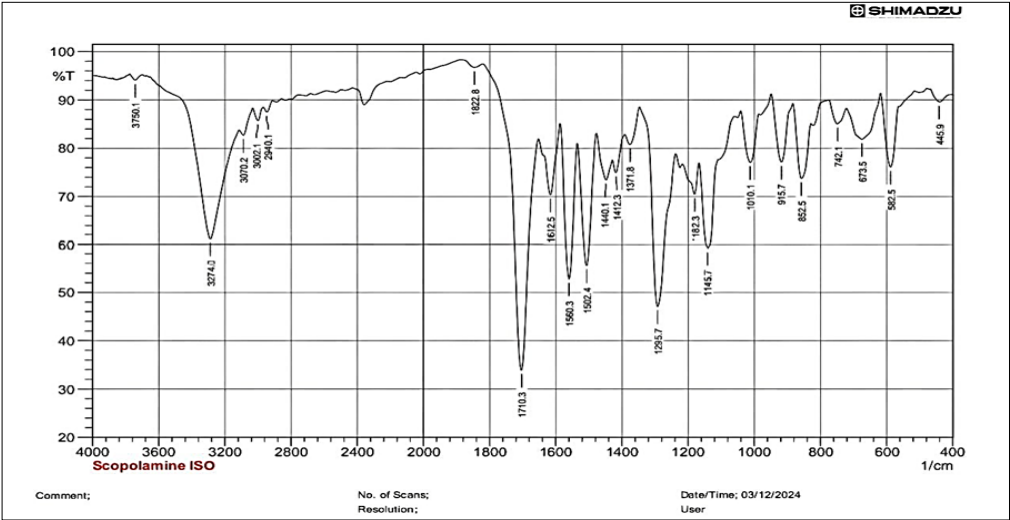


Fig. 9. FTIR of isolated scopoletin.

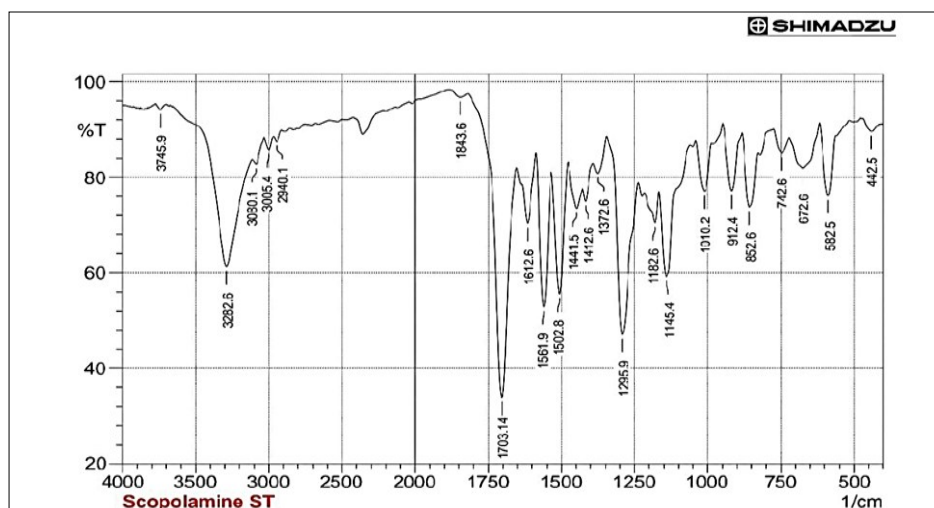


Fig. 10. FTIR of scopoletin standard.

UV-Visible spectroscopy

The UV-Visible spectra of the isolated compound and the scopoletin standard in methanolic solution were recorded and presented in Table 2. The UV spectra of standard scopoletin and isolated scopoletin have the following characteristic bands:

SCO - Scopoletin isolated

Notable peaks are observed at 346.0 nm (Absorbance: 1.110) and 229.0 nanometers (Absorbance: 1.291). These peaks are consistent with the expected UV absorption profile of scopoletin, a coumarin derivative known for its characteristic UV absorption properties (22) and (23). The strong absorbance values at these wavelengths suggest a high degree of conjugation within the molecule, typical of coumarins as shown in Fig. 11.

SCOS - Scopoletin standard

The standard shows absorbance values at 345.0 nm (Absorbance: 0.196) and 229.0 nm (Absorbance: 0.223). These are characteristics of scopoletin, confirming the standard's purity and identical to the data in the literature (22, 23) as shown in Fig. 12.

The mix of SCO and SCOS

The mixed data combines the characteristics of both isolated scopoletin and the standard. The peaks at 346.0 nm and 229.0 nm in the isolated sample are consistent with the standard's peaks at 345.0 nm and 229.0 nm. The UV-Vis spectrum of the mixed sample (SCO and SCOS), as shown in Fig. 13, confirms this interpretation. The mixed sample exhibits peaks at 229.0 nm and 346.0 nm, consistent with the peaks observed in the individual samples. This confirms that both the isolated compound and the standard contribute to the spectral profile of the mixture and further supports the conclusion that the

Table 2. Characteristic UV-Visible absorption bands of isolated compound and scopoletin standard

Compound	λ_{max} (nm)	Absorbance
Isolated scopoletin	346.0	1.110
	229.0	1.291
Scopoletin standard	345.0	0.196
	229.0	0.223

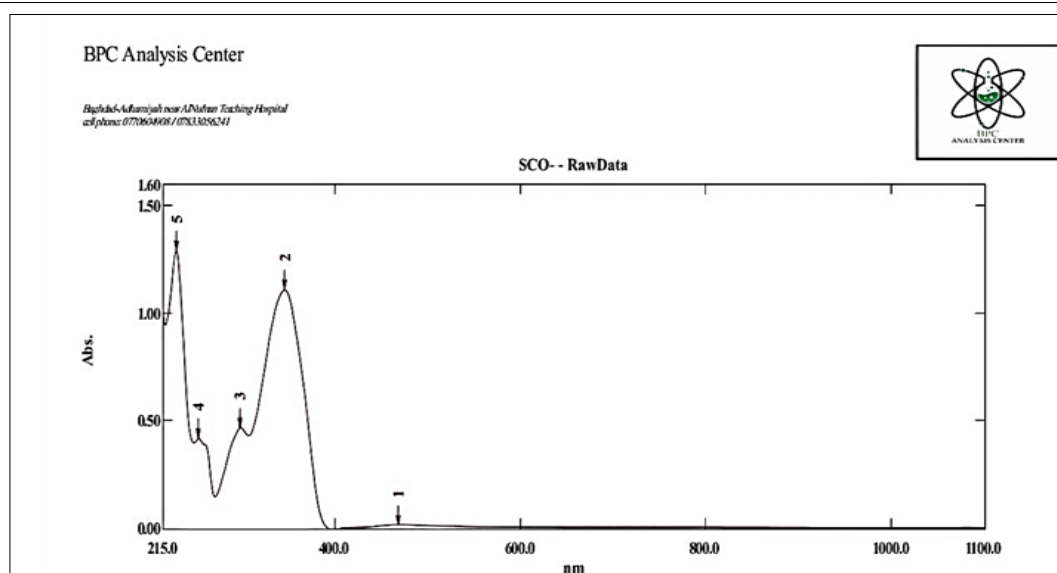


Fig. 11. UV spectra of isolated scopoletin (sco).

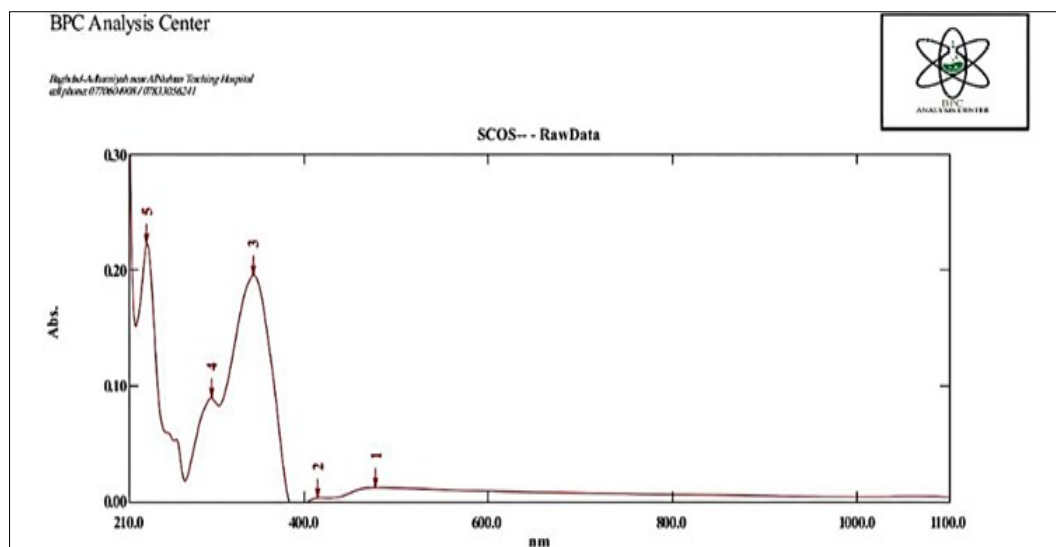


Fig. 12. UV spectra of scopoletin standard (SCOS).

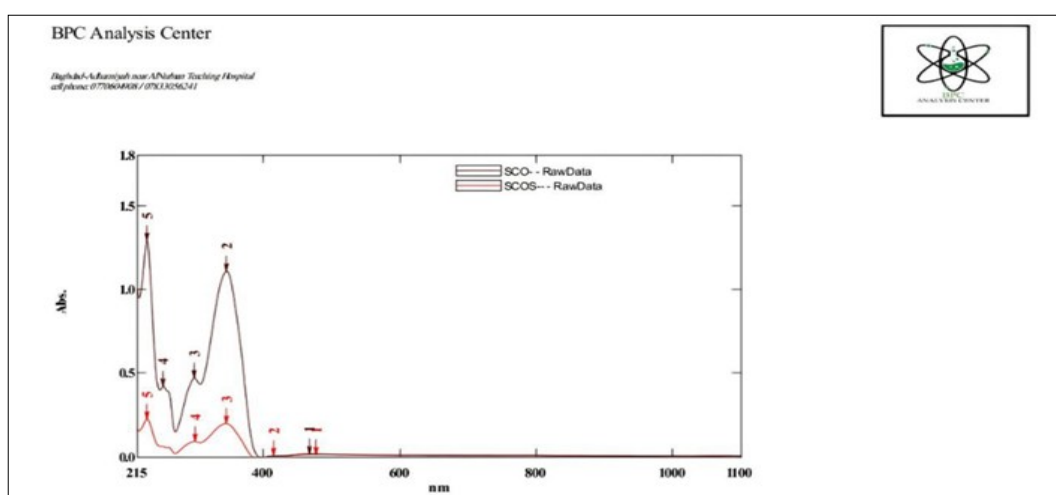


Fig. 13. Mix UV spectroscopy of isolated scopoletin (SCO) and scopoletin standard (SCOS).

isolated compound is indeed scopoletin. The minor shift in the second λ_{max} (346.0 nm in SCO vs. 345.0 nm in SCOS) could be attributed to several factors: matrix effects. The isolated sample contains a mixture of compounds extracted from the plant material. The presence of these other compounds could interact with scopoletin molecules, influencing their electronic transitions and causing a slight shift in the absorption maximum (24). Solvent effects: although both samples were dissolved in methanol, subtle variations in solvent composition, pH, or the presence of trace impurities can affect the UV absorption properties of the compounds (25). The higher absorbance values observed for the isolated sample (SCO) compared to the standard (SCOS) likely reflect differences in sample concentration according to the Beer-Lambert Law that state the absorbance is directly proportional to the concentration of the absorbing species in a solution, expressed as $A = \epsilon \cdot b \cdot c$.

Conclusion

This work presents the first effective isolation and thorough characterization of a bioactive coumarin derivative, scopoletin, from the methanolic leaf extract of *L. leonurus* grown in Iraq. Using hexane and 85 % methanol in successive maceration enhanced chromatographic and spectroscopic analysis. This finding emphasizes the possibilities of Iraqi-cultivated

L. leonurus as a useful source of scopoletin for the next pharmacological studies. Future studies should prioritize *in vivo* models of inflammation that allow for the specific assessment of COX-1 and COX-2 inhibition by *L. leonurus* extracts and isolated scopoletin and investigate the potential synergistic or additive effects of *L. leonurus* extracts or scopoletin when administered in combination with conventional non-steroidal anti-inflammatory drugs (NSAIDs), including selective COX-2 inhibitors, in *in vivo* models.

Acknowledgements

We would like to express our deepest gratitude to the College of Pharmacy at the University of Baghdad for providing the essential resources and support that made this research possible.

Authors' contributions

DAA was responsible for designing and refining the extraction protocol and optimizing the chromatography conditions for TLC and HPLC to ensure effective separation and identification of scopoletin. EBD was responsible for the extraction process, data collection, analysis, and manuscript writing.

Compliance with ethical standards

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

Ethical issues: None

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