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RESEARCH ARTICLE

Scopoletin: A new coumarin isolated from four parts of Iraqi *Eichhornia crassipes*: its extraction, isolation and structure elucidation

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Abstract

The primary focus of the current study was to confirm the presence of scopoletin as a novel metabolite in Iraqi *Eichhornia crassipes*. Since there are currently no studies on Iraqi *E. crassipes* coumarins, particularly scopoletin, in the literature survey, the aim of the current work is to compare the effectiveness of ultrasound-assisted extraction using both a probe and a bath with the traditional extraction method (using Soxhlet) for extracting coumarins, particularly scopoletin, from Iraqi *E. crassipes* flowers, leaves, stems and roots. The objective of study was accomplished using a quick and efficient method to identify and quantify scopoletin, a bioactive compound, in 4 parts of the *E. crassipes* plant. High-performance thin-layer chromatography (HPTLC) was employed to verify the presence of coumarins, including scopoletin, in these plant parts. The findings revealed that the leaves contained the highest concentration of scopoletin (0.1876 mg/mL), followed by the flowers (0.145 mg/mL), stems (0.1396 mg/mL) and the roots, which had the lowest concentration (0.089 mg/mL). The leaves were extracted using ultrasound-assisted extraction (UAE), resulting in the most significant yield of scopoletin. Silica gel column chromatography was used to isolate and purify coumarins (scopoletin) from *E. crassipes* leaves. Finally, the melting point of the extracted compound (scopoletin) was measured and it was confirmed and characterized by several techniques, which include liquid chromatography/ mass spectroscopy (LC/MS), ^{1}H nuclear magnetic resonance spectroscopy (NMR), Fourier transforms infrared spectroscopy (FT-IR) and measuring the melting point.

Keywords: *Eichhornia crassipes*; high-performance thin layer chromatography (HPTLC); scopoletin; ultrasound-assisted extraction (UAE)

Introduction

Numerous plants, fungi and microbes contain the secondary metabolite family called coumarins. Coumarins are classified into 3 primary classes based on their biosynthesis pathways: the phenylalanine metabolic pathway, the cinnamic acid pathway and the shikimate acid pathway (1). One common member of the coumarin family is 7-hydroxy-6-methoxy coumarin, known as scopoletin (2). *In vitro* pharmacological activity of scopoletin (SPT) has been documented, encompassing antibacterial, antifungal, antitubercular and antihypertensive characteristics (3–6).

Eichhornia crassipes, commonly known as water hyacinth, belongs to the Pontederiaceae family and is a free-floating aquatic monocotyledonous plant. Native to Brazil and the Amazon basin, it has spread widely across tropical and subtropical regions (7). The plant is known for its rapid growth, widespread dispersal and resilience in pH, nutrients and temperature variations. Therefore, the International Union for Conservation of Nature has listed it among the 100 most aggressive invasive species and is considered one of the ten worst weed plants worldwide (8).

Plant extracts contain flavonoids, tannins, alkaloids and phenols, which have biological qualities like antiviral, antifungal, anticancer and antibacterial effects. Water hyacinth has a large concentration of cytotoxic qualities, tissue healing, non-enzymatic antioxidant processes and oxidative enzymes (9). Because of its capacity to grow in contaminated water and absorb heavy metals, it has been employed as a phytoremediation agent for wastewater treatment (10). Additionally, it has been contemplated as a possible source of biofertilizers and bioenergy (11, 12). Internal worms, diarrhea, digestive issues and flatulence are among the gastrointestinal conditions the herb has historically used to cure. Furthermore, the beans were utilized to ensure a healthy splenic function (13).

The current study's goals are to evaluate the qualitative and quantitative determination of scopoletin in the 4 parts (flowers, leaves, stems and roots) of the widely grown Iraqi *E. crassipes* and offer scientific insight into which part has the higher concentration of scopoletin, which will benefit herbal consumers and the nutraceutical industry.

Materials and Methods

Collection and authentication of plant

Four fresh parts (flowers, leaves, stems and roots) of Iraqi *E. crassipes* were collected in April 2024 from the Euphrates River in Karbala Governorate. These were identified, confirmed and authenticated by the taxonomist of Department of Biology, college of Science/University of Baghdad, at BUH No. 50777.

Extraction procedure

The extraneous materials were removed initially from flowers, leaves, stems and roots of the Iraqi *E. crassipes*, followed by drying, grinding and weighing processes before starting the extraction procedure.

The extraction procedure, Fig. 1, involved 2 methods: method A, which is the conventional and classical method using Soxhlet and method B, which is a non-conventional and fast method using ultrasound-assisted extraction (UAE).

Method A: This method was conducted by subjecting approximately 250 g of each grounded part of water hyacinth (flowers, leaves, stems and roots) to extraction using a Soxhlet apparatus and 85 % methanol as a solvent. Each part's crude methanol extract has been filtered, concentrated under reduced pressure and suspended in distilled water. After partitioning each fraction with petroleum ether (BP, 60 - 80 °C), chloroform, ethyl acetate and finally n-butanol, 500 mL was used per fraction. The procedure was executed thrice. Subsequently, the initial 3 fractions from each plant part were dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure utilizing a rotary evaporator, weighed and designated for further analysis. Fig. 2 illustrates the fractionation procedure of crude extracts from methanolic flowers, leaves, stems and roots (14, 15).

Method B: The UAE method was achieved using 85 % methanol as a solvent and a probe and bath ultrasonicator UAE was carried out. The intricacy of the herbals' chemical makeup and the components' affinities for many variable parameters necessitated the selection of an appropriate ideal condition through several experiments to compare the UAE (probe and bath) and Soxhlet extraction methods equally (16).

The UAE was conducted under the subsequent experimental conditions:

The probe ultrasonicator operates at a temperature of 25 °C for 20 min, utilizing a solvent-to-solid ratio of 8:1 mL/gm, a sonication frequency of 20 KHz and a power output of 60 watts.

The ultrasonic waves in a bath ultrasonicator pass through the glass before contact with plant material. The sonication frequency is 60 kHz and the power is 60 watts. The temperature is 50 °C, 60 min in time, the solvent-to-solid ratio is 8:1 mL/gm and the sonication frequency is 60 kHz.

The present study employed both instruments to investigate the impact of direct and indirect ultrasonic wave contact on extracting 4 components of the Iraqi *E. crassipes* plant. Using the same schematic procedure as the usual method (Soxhlet), the crude methanolic extract was filtered, concentrated, suspended in distal water and partitioned. Afterward, each plant part's fraction was dried, weighed and designated for additional examination (17–19).

Phytochemical examination of different extract fractions: The plant may be considered as biosynthetic laboratory stock for constituents like coumarins (scopoletin); for checking the presence or absence of this bioactive constituent, the extract fractions of different plant parts were subjected to conflict of analysis by thin layer chromatography (TLC) technique. TLC aims to validate the identity of a compound within a mixture by comparing its R_f value to that of a reference compound to obtain clearly delineated and distinctly differentiated spot locations. TLC was conducted by using readymade aluminum foil plates of silica gel GF254, UV light detector at wavelength 366 nm, reference sample of scopoletin (Biopurify, China) in comparison with 3 different developing solvent systems S1-3, S1 = di-chloromethane: ethyl acetate (2:1), S2 = toluene: ether: saturated with acetic acid 0.1 % (5:5) and S3 = ether: benzene (1:1) (20).

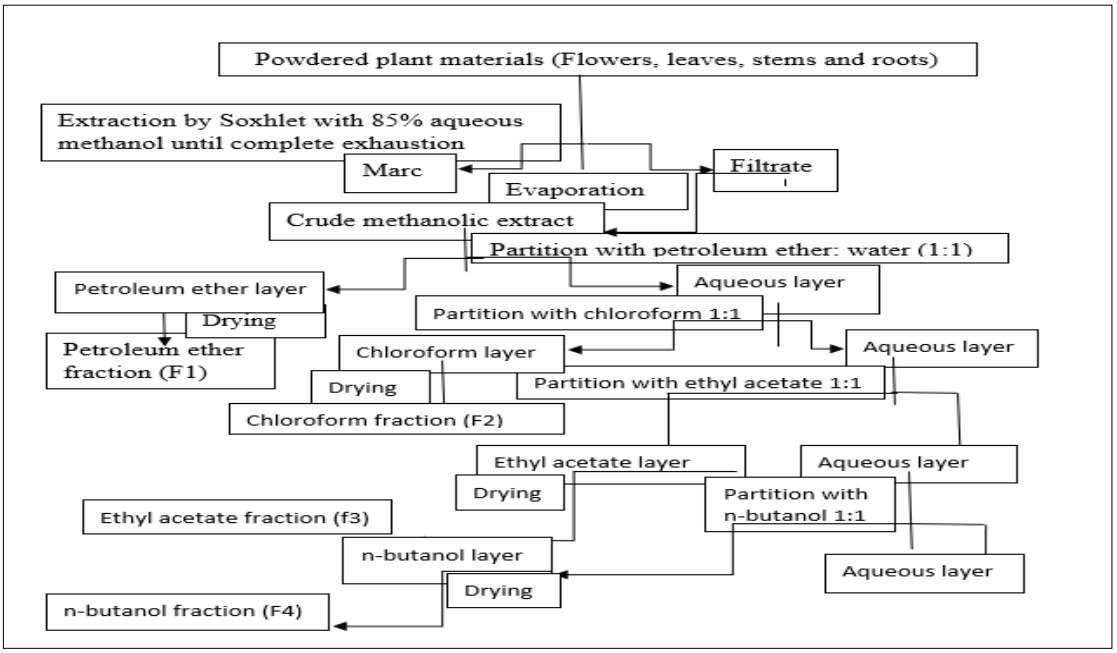


Fig. 1. The fractional method of crude methanolic flowers, leaves, stems and root extracts of *E. crassipes.*

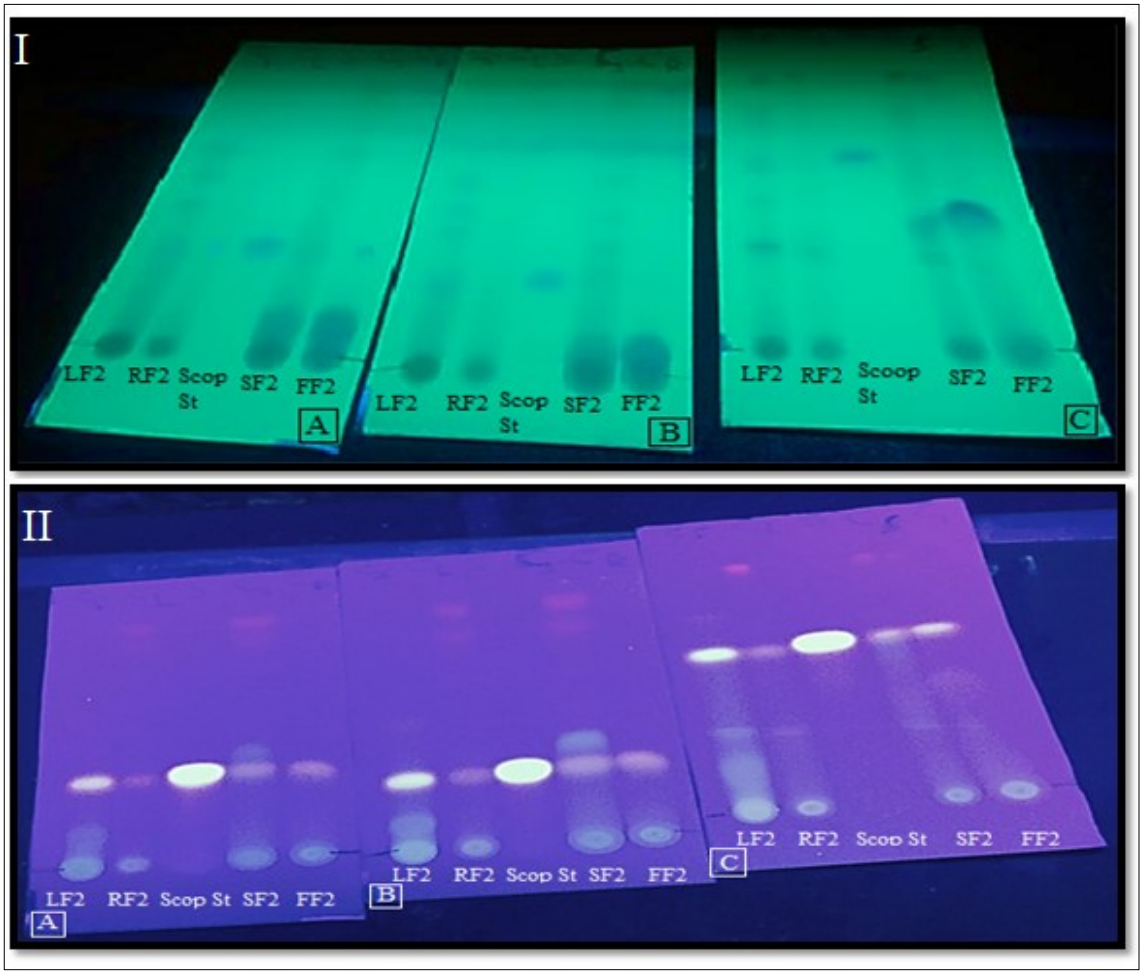


Fig. 2.TLC chromatogram of F2 for 4 parts of *E. crassipes* using silica gel GF254 nm as adsorbent.

* S3 = ether: benzene (1:1), S2 = toluene: ether: saturated with acetic acid 0.1 % (5:5) and S1 = di-chloromethane: ethyl acetate (2:1) as a mobile phase for A, B and C respectively. Detection by UV light at 254nm for I and 366 for II. LF2= leaves chloroform fraction, RF2 = roots chloroform fraction, Scop St = scopoletin standard SF2 = stems chloroform fraction, FF2= flowers chloroform fraction.

Qualitative and quantitative estimation by high-performance thin layer chromatography (PHTLC): With a Camag Linomat 5 Microliter Syringe (Switzerland) 8.0 mm in width, the solution was spotted into bands of 8.0 mm width on a silica gel glass plate 60F254 (size 20 x 10 cm, thickness 250 m; E.Merck KJaA). The constant application volume was 4 L and 3.3 mm was the space between the 2 bands. The slit size was maintained at 4.0 mm by 0.3 mm, with a scanning speed of 20 mm/s. Each sample was subjected to 3 scanning iterations and baseline correction was applied. The mobile phase utilized was a mixture of dichloromethane and ethyl acetate in a volume ratio of 2:1 (v/v), with 10 mL of this mobile phase employed for each chromatography run.

Linear ascending development was conducted in a 20 cm x 10 cm automatic developing chamber (Camag, ADC 2) saturated with Whatmann No. 1 filter paper in the mobile phase. The optimal chamber saturation duration for the mobile phase was 5 min at ambient temperature (25 °C ± 2) and relative humidity of 50 % ± 5. The chromatogram run measured 7.5 cm in length. After scanning, the TLC plates were dried for 5 min using an air dryer. Densitometry scanning was conducted using a Camag TLC scanner in reflectance absorbance mode at 366 nm, driven by Win CATS software with a tungsten light, all accomplished at Baghdad College of Pharmacy (21).

The quantitative determination of scopoletin was conducted utilizing a calibration curve, wherein a series of diluted solutions (0.5, 0.25, 0.125, 0.0625 mg/mL) were generated from a scopoletin standard stock solution (0.5 mg/mL) (22).

Isolation and purification of proposed scopoletin by silica gel column chromatography

Column chromatography was performed on the chloroform fraction (F2) and ethyl acetate fraction (F3) of the leaves produced from probe ultrasonic-assisted extraction, utilizing a glass column (50 cm x 2 cm) packed with silica gel slurry. A perforated filter paper disc was positioned at the top of the column, followed by an approximately 0.5 cm layer of sea sand and then another perforated filter paper disc. Two grams of the samples (F2 + F3) were dissolved in 10 mL of ethanol and placed at the top of the column.

The column was eluted by gradient elution technique using methanol: chloroform 4 %, 6 % and 8 % as a mobile phase to obtain 3 fractions, FA, FB and FC. FA was further chromatographed using a column of silica gel and eluted successively with 4 % methanol: chloroform solvent for further purification.

After re-dissolving the isolated chemical in hot methanol and allowing it to crystallize for 4 - 5 hr in the refrigerator, a pale-yellow crystal was produced after filtration (23).

Spectral identification of the isolated compound : The spectral identification was done by using the following instruments:

Liquid chromatography/ mass spectroscopy (LC/MS) [Shimadzu-Japan], Fourier transforms infrared (FT-IR) spectroscopy [Jusco-Japan] and ^{1}H nuclear magnetic resonance spectroscopy (300MHz for ^{1}H-NMR) analysis [Euro-vector EA 3000A-Italy].

Results and discussion

Comparison of yield extracted fractions

Conventional techniques of phytochemical extraction have been associated with a high consumption of organic solvents, which limits the application of bioactive extracts due to solvent toxicity. Long-term extraction is required, which involves high energy consumption, causing an incremental cost. All these reasons lead to the implementation of nonconventional extraction technologies, such as ultrasound -assisted extraction methods (24, 25).

The extraction yields attributed to the UAE depend on a series of factors that modulate the effectiveness of this extraction technique. Still, they also affect their efficiency as a sustainable procedure, aiming at the achievement of the lowest consumption of energy and non-renewable resources. These parameters can be classified into 3 groups, according to their nature, physical parameters (sonication frequency and power output) and those related to medium and matrix effects (temperature, time and the solvent-to-solid ratio) (26, 27). Therefore, the validated optimized conditions were conducted in this study (28).

Tables 1, 2 and 3 compare the percentage yields of the 3 extraction techniques under the optimized conditions. The results indicate that probe UAE yielded the highest extraction fraction, followed by Soxhlet extraction, whereas bath UAE generated the lowest percentage yield. The advantage of probing UAE is enhanced due to the decreased extraction time and reduced solvent use (28).

In this study, scopoletin was considered due to its interesting bioactive characteristics such as antibacterial, antifungal, antiparasitic, anticancer, anti-inflammation, hepatoprotective, antihyperlipidemic, antidiabetic, neuroprotective, antioxidant, anti-angiogenesis, anti-hypertensive, analgesic, anxiolytic, immunomodulatory, anti-osteoporosis, anti-allergic, anti-aging and anti-gout activities. Additionally, it has importance in the industrial field, for example, it can be used as an electro monomer in the synthesis of polymers (29).

Phytochemical screening by TLC: Based on TLC analysis of extract fractions obtained from 4 parts of Iraqi *E. crassipes*, a single hydroxycoumarin, C1, has been detected in the chloroform fraction (F2) as well as the ethyl acetate fraction (F3) in all studied plant parts (flowers, leaves, stems and roots).

In all of these developing solvent systems, C1 appeared as a single compact spot with the same color and R_f value as scopoletin standard on TLC plates, detected with UV-light at 254 nm and 366 nm, as shown in Fig. 2 and 3.

Table 1. Percentage yield difference of extract's content of different parts for Iraqi *E. crassipes* by Soxhlet extraction method

Fractions	Flowers extracts	Leaves extracts	Stems extracts	Roots extracts
Crude extracts	16.17 %	18.66 %	10.22 %	12.94 %
Petroleum ether fraction F1	3.4 %	4.2 %	1.28 %	2.12 %
Chloroform fraction F2	2.59 %	2.7 %	1.57 %	2.33 %
Ethyl acetate fraction F3	3.94 %	4.25 %	2.15 %	2.75 %
n-butanol fraction F4	3.18 %	4.5 %	2.4 %	2.87 %

Table 2. The difference in yield percentages of extract's content of different parts for Iraqi *E. crassipes* by probe UAE method

Fractions	Flowers extracts	Leaves extracts	Stems extract	Roots extracts
Crude extracts	18.63 %	20.76 %	11.97 %	15.58 %
Petroleum ether fraction F1	3.68 %	4.52 %	1.52 %	2.76 %
Chloroform fraction F2	2.85 %	3.08 %	1.92 %	2.88 %
Ethyl acetate fraction F3	4.31 %	4.72 %	2.56 %	3.37 %
n-butanol fraction F4	3.72 %	4.91 %	2.74 %	3.55 %

Table 3. Difference in yield percentages of extract's content of different parts for Iraqi *E. crassipes* by bath UAE method

Fractions	Flowers extracts	Leaves extracts	Stems extract	Roots extracts
Crude extracts	13.95 %	16.59 %	8.59 %	10.87 %
Petroleum ether fraction F1	2.98 %	3.76 %	1.14 %	1.92 %
Chloroform fraction F2	2.10 %	2.29 %	1.23 %	2.06 %
Ethyl acetate fraction F3	3.37 %	3.93 %	1.98 %	2.27 %
n-butanol fraction F4	2.86 %	4.07 %	2.05 %	2.30 %

*S1 = di-chloromethane: ethyl acetate (2:1), S2 = toluene: ether: saturated with acetic acid 0.1 % (5:5) and S3 = ether: benzene (1:1).

For the chloroform fraction, R_f values of C1 and its corresponding standard (scopoletin) were calculated and presented in Table 4, while for the ethyl acetate fraction, Rf values are presented in Table 5.

Results illustrated in Fig. 3 and Table 5 indicate that TLC analysis has shortcomings: C1 was not detected in the ethyl acetate fractions (F3) of either the stems or the roots due to low resolution, low sensitivity and the challenge of detecting trace components (30).

Qualitative and quantitative estimation by HPTLC: To investigate extraction efficiency, efficacy and reproducibility, 2 optimally conditioned UAE techniques and the traditional Soxhlet method were used. Extraction yield percentage was used as a key metric to compare the performance of the different extraction techniques. Following extraction, the fractions obtained from the flowers, leaves, stems and roots of Iraqi *E. crassipes* were subjected to HPTLC analysis to identify and quantify a putative active constituent, designated as C1.

HPTLC, an advanced form of thin layer chromatography, is effective for both qualitative and quantitative analysis. When the sample is administered automatically, the droplet size variation that could happen when the sample is applied manually is avoided and the resolution is increased by obtaining more accurate qualitative and quantitative measurements through automation in various processes (31).

Qualitative and quantitative HPTLC analysis for the analyzed fractions of 4 plant parts that were obtained by UAE (probe and bath) and Soxhlet revealed the presence of C1 in 4 plant parts (fruits, leaves, stems and roots) was performed using a mixture of equal portions of the chloroform (F2) and

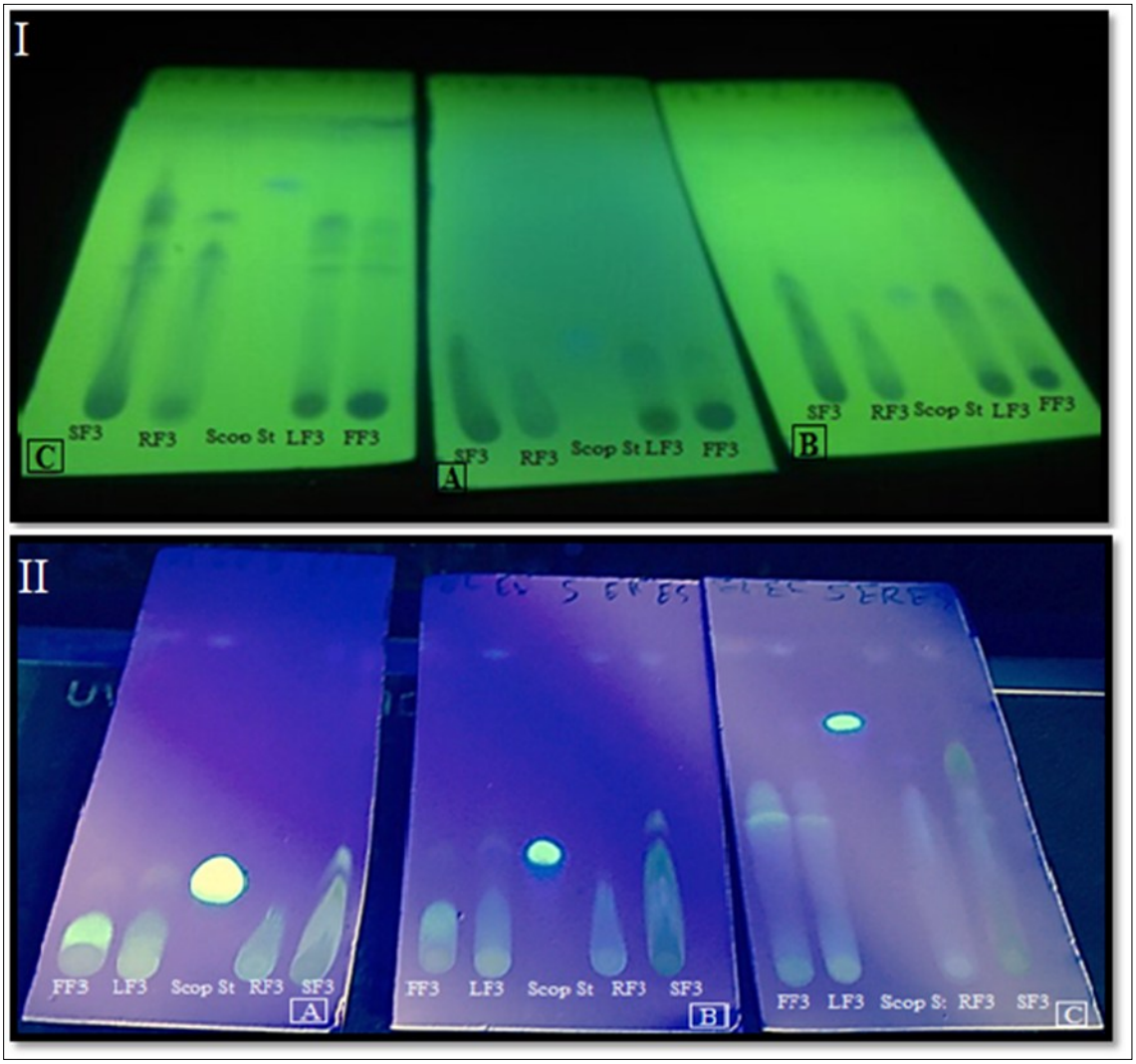


Fig. 3. TLC chromatogram of F3 for four parts of *E. crassipes* using silica gel GF254nm as adsorbent * S2 = toluene: ether: saturated with acetic acid 0.1 % (5:5), S3 = ether: benzene (1:1) and S1 = dichloromethane: ethyl acetate (2:1) as a mobile phase for A, B and C respectively. Detection by UV light at 254nm for I and 366 for II. LF3= leaves ethyl acetate fraction, RF3 = roots ethyl acetate fraction, Scop St = scopoletin standard SF3 = stems ethyl acetate fraction, FF3= flowers ethyl acetate fraction.

Table 5. Rf value of C1 obtained from ethyl acetate fraction of 4 parts of Iraqi E. crassipes compared to scopoletin standard in different developing solvent systems

Solvent systems	Scopoletin standard	C1 in flowers ethyl acetate fraction	C1 in leaves ethyl acetate fraction	C1 in stems ethyl acetate fraction	C1 in roots ethyl acetate fraction
S1	**0.746**	**0.745**	**0.746**	**Undetected**	**Undetected**
S2	**0.306**	**0.305**	**0.306**	**Undetected**	**Undetected**
S3	**0.367**	**0.366**	**0.367**	**Undetected**	**Undetected**

*****S1 = di-chloromethane: ethyl acetate (2:1), S2 = toluene: ether: saturated with acetic acid 0.1 % (5:5) and S3 = ether: benzene (1:1).

ethyl acetate (F3) fractions to get the highest possible concentration of C1 (scopoletin) which were obtained by 3 different extraction methods (probe UAE, bath UAE and Soxhlet).

As shown in Fig. 4 - 7, qualitative identification was performed by comparing the maximum (R_f) value and UV spectrum of C1 of each part of the plant with its corresponding scopoletin standard.

In order to construct the calibration curve for quantification measures, the area under the curve (AUC) was plotted against 4 concentration levels of the scopoletin standard. The analyte concentration was determined using the linear regression equation derived from the calibration curve in each section of the *E. crassipes* plant, as shown in Fig. 8.

The quantitative concentration of C1 (scopoletin) in mixed F2 + F3 fractions showed that the leaves had the highest concentration, followed by the flowers, stems and roots had the lowest. The elevated levels of scopoletin in the leaves of Iraq *Eichhornia crassipes* may be ascribed to the presence of biochemical components deemed crucial for synthesizing this secondary metabolite's building blocks.

In Table 6 and Fig. 9, the probe UAE shows the highest concentration, followed by Soxhlet and, finally, the bath UAE.

Isolation and purification of scopoletin (C1) by silica gel column chromatography

One hundred fractions obtained from silica gel column chromatography of leaves mixed (F2 + F3) fraction probe UAE (highest quantity) were monitored by HPTLC. After successive elution with gradient solvent methanol: chloroform (4 %, 6 % and 8 %), the consecutive fractions that have the same number of spots and the same R_f values were collected together to get 3 sub-fractions (FA, FB and FC). The results showed that FA (fractions numbered 1 - 40) gave a spot similar to that of the scopoletin standard when checked by TLC, as shown in Fig. 10(I).

FA was chromatographed again for further purification using 4 % methanol: chloroform as a solvent to give C1 based on TLC results, as shown in Fig. 10(II).

Identification, characterization and structural elucidation of C1

The identification and characterization of white needles isolated C1 constituent was done by using the following techniques:

A- Melting point: One physical characteristic that provides valuable information and aids in identifying the sample and its purity is the melting point (32). The isolated C1 constituent had a melting point of 204 °C - 205 °C (with decomposition) compared with standard scopoletin, which has a melting point of 200 °C - 207 °C (with decomposition).

B- FTIR: A useful technique for characterizing and elucidating the structure of components or functional groups found in unknown compounds of plant extracts is Fourier transform infrared spectroscopy (FTIR). Pure constituents' FTIR spectra are typically sufficiently distinct that they resemble molecular fingerprints (33). The FTIR spectroscopy was carried out for the identification and characterization of isolated C1 compared with the scopoletin standard, as shown in Fig. 11.

The IR spectrum of isolated C1 showed the characteristic band frequencies reported for scopoletin standard as listed in Table 7.

C- Nuclear magnetic resonance (NMR) spectroscopy: It is a highly developed and potent analytical method for determining the structures of organic compounds and identifying molecular interactions. It is generally accepted to be the most effective approach. For isolated C1, the results demonstrated that the NMR spectral data were quite similar to those found in scopoletin literature as shown in Fig. 12 and Table 8 (34–37).

D- LC/MS: The isolated C1 ingredient, obtained using silica gel column chromatography, was further characterized and its structure was clarified using this technique. The [M + H] + ion with m/z 193 was chosen as a precursor ion from the full scan mass spectra of C1. The results of the product ion scan mode reveal that the most abundant fragment was recorded at m/z 132, which consistently showed as [M+H-CH3-CO-OH] +, as shown in Fig. 13. These LC/MS results were all quite similar to those for scopoletin that were published in the literature (38, 39).

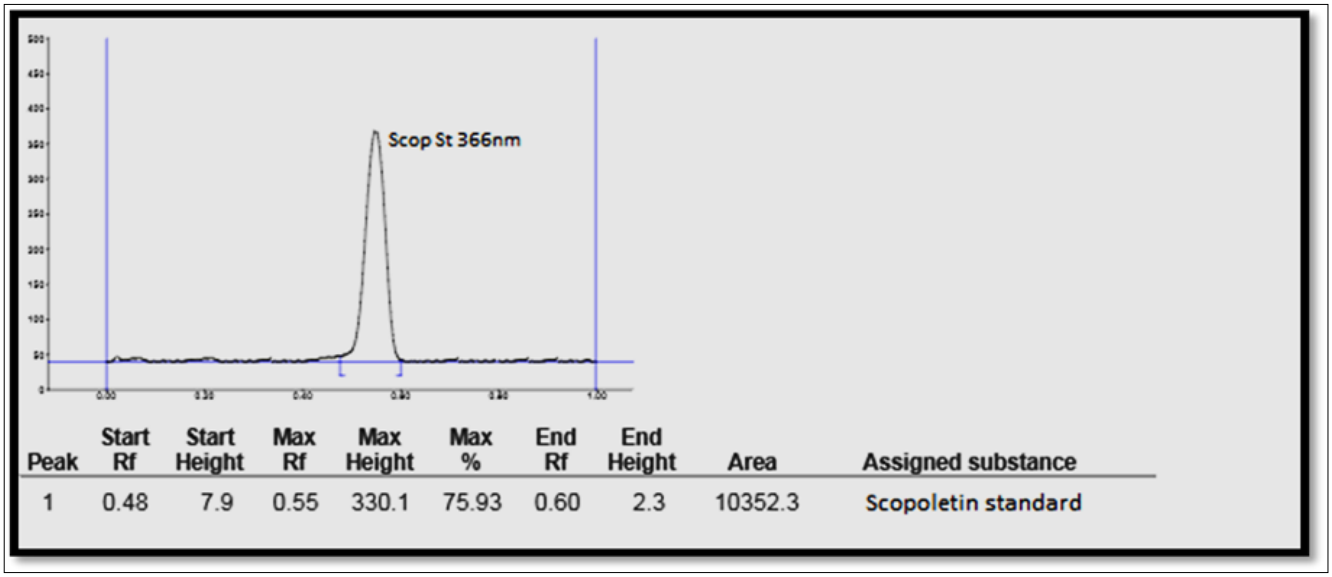
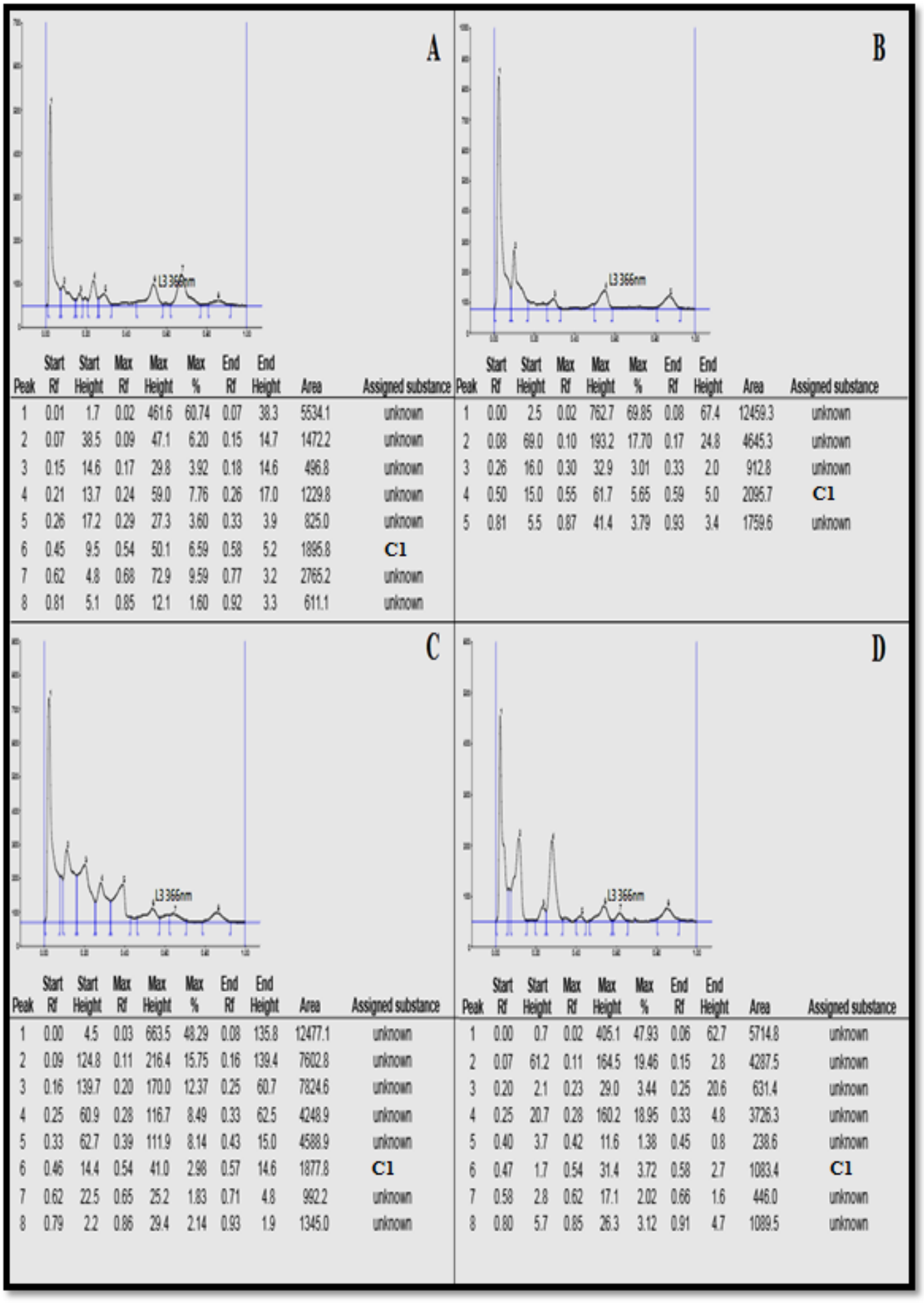


Fig. 4. Standard HPTLC chromatogram for scopoletin.



A

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance
1	0.01	1.7	0.02	461.6	60.74	0.07	38.3	5534.1	unknown
2	0.07	38.5	0.09	47.1	6.20	0.15	14.7	1472.2	unknown
3	0.15	14.6	0.17	29.8	3.92	0.18	14.6	496.8	unknown
4	0.21	13.7	0.24	59.0	7.76	0.26	17.0	1229.8	unknown
5	0.26	17.2	0.29	27.3	3.60	0.33	3.9	825.0	unknown
6	0.45	9.5	0.54	50.1	6.59	0.58	5.2	1895.8	C1
7	0.62	4.8	0.68	72.9	9.59	0.77	3.2	2765.2	unknown
8	0.81	5.1	0.85	12.1	1.60	0.92	3.3	611.1	unknown

B

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance
1	0.00	2.5	0.02	762.7	69.85	0.08	67.4	12459.3	unknown
2	0.08	69.0	0.10	193.2	17.70	0.17	24.8	4645.3	unknown
3	0.26	16.0	0.30	32.9	3.01	0.33	2.0	912.8	unknown
4	0.50	15.0	0.55	61.7	5.65	0.59	5.0	2095.7	C1
5	0.81	5.5	0.87	41.4	3.79	0.93	3.4	1759.6	unknown

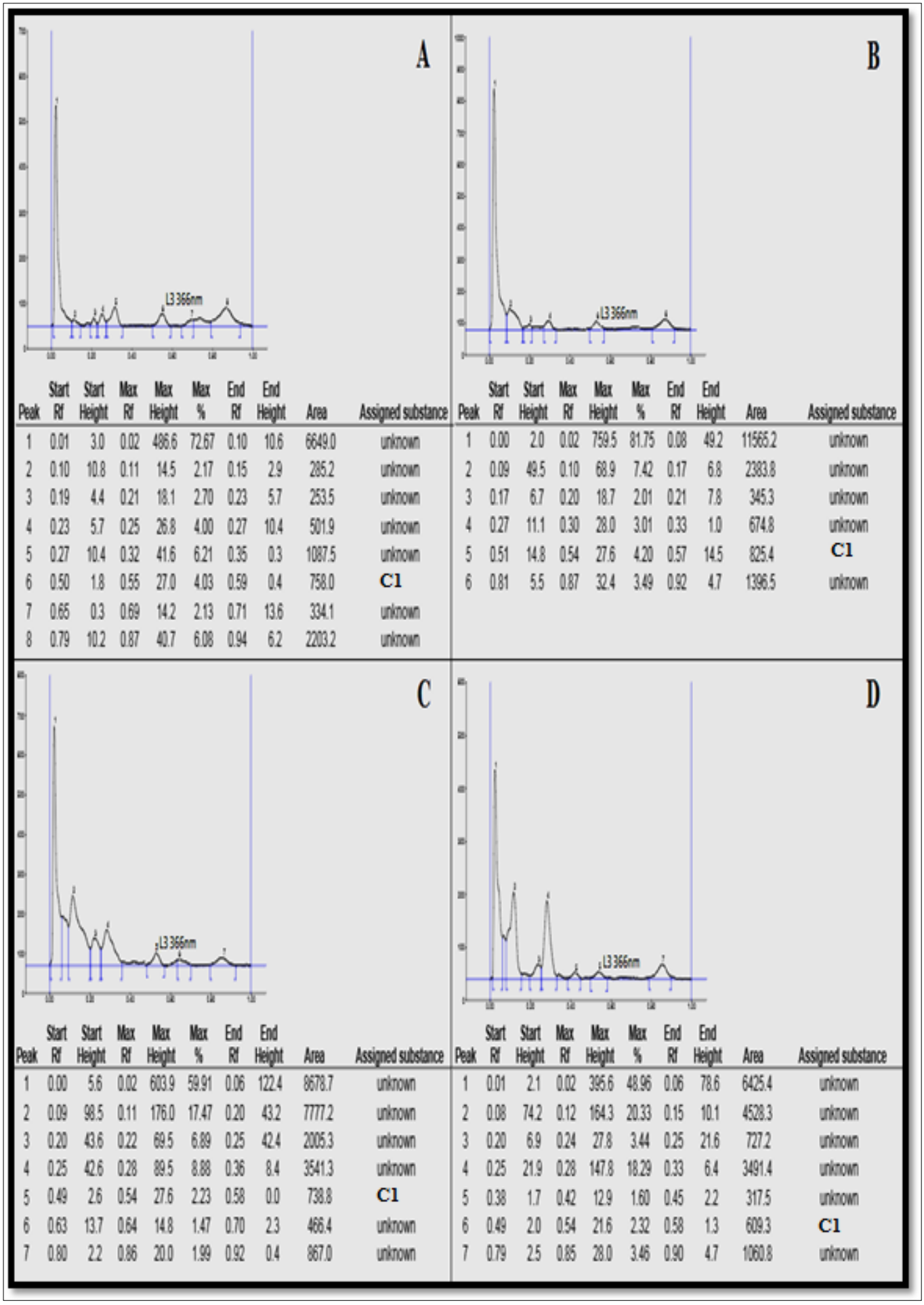
C

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance
1	0.00	4.5	0.03	663.5	48.29	0.08	135.8	12477.1	unknown
2	0.09	124.8	0.11	216.4	15.75	0.16	139.4	7602.8	unknown
3	0.16	139.7	0.20	170.0	12.37	0.25	60.7	7824.6	unknown
4	0.25	60.9	0.28	116.7	8.49	0.33	62.5	4248.9	unknown
5	0.33	62.7	0.39	111.9	8.14	0.43	15.0	4588.9	unknown
6	0.46	14.4	0.54	41.0	2.98	0.57	14.6	1877.8	C1
7	0.62	22.5	0.65	25.2	1.83	0.71	4.8	992.2	unknown
8	0.79	2.2	0.86	29.4	2.14	0.93	1.9	1345.0	unknown

D

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance
1	0.00	0.7	0.02	405.1	47.93	0.06	62.7	5714.8	unknown
2	0.07	61.2	0.11	164.5	19.46	0.15	2.8	4287.5	unknown
3	0.20	2.1	0.23	29.0	3.44	0.25	20.6	631.4	unknown
4	0.25	20.7	0.28	160.2	18.95	0.33	4.8	3726.3	unknown
5	0.40	3.7	0.42	11.6	1.38	0.45	0.8	238.6	unknown
6	0.47	1.7	0.54	31.4	3.72	0.58	2.7	1083.4	C1
7	0.58	2.8	0.62	17.1	2.02	0.66	1.6	446.0	unknown
8	0.80	5.7	0.85	26.3	3.12	0.91	4.7	1089.5	unknown

Fig. 5. HPTLC chromatogram of C1 in mixed F2+F3 fraction of 4 parts of *E. crassipes* plant by UAE probe method. A = flowers, B = leaves, C = stems, D = roots.



A

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance
1	0.01	3.0	0.02	486.6	72.67	0.10	10.6	6649.0	unknown
2	0.10	10.8	0.11	14.5	2.17	0.15	2.9	285.2	unknown
3	0.19	4.4	0.21	18.1	2.70	0.23	5.7	253.5	unknown
4	0.23	5.7	0.25	26.8	4.00	0.27	10.4	501.9	unknown
5	0.27	10.4	0.32	41.6	6.21	0.35	0.3	1087.5	unknown
6	0.50	1.8	0.55	27.0	4.03	0.59	0.4	758.0	C1
7	0.65	0.3	0.69	14.2	2.13	0.71	13.6	334.1	unknown
8	0.79	10.2	0.87	40.7	6.08	0.94	6.2	2203.2	unknown

B

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance
1	0.00	2.0	0.02	759.5	81.75	0.08	49.2	11565.2	unknown
2	0.09	49.5	0.10	68.9	7.42	0.17	6.8	2383.8	unknown
3	0.17	6.7	0.20	18.7	2.01	0.21	7.8	345.3	unknown
4	0.27	11.1	0.30	28.0	3.01	0.33	1.0	674.8	unknown
5	0.51	14.8	0.54	27.6	4.20	0.57	14.5	825.4	C1
6	0.81	5.5	0.87	32.4	3.49	0.92	4.7	1396.5	unknown

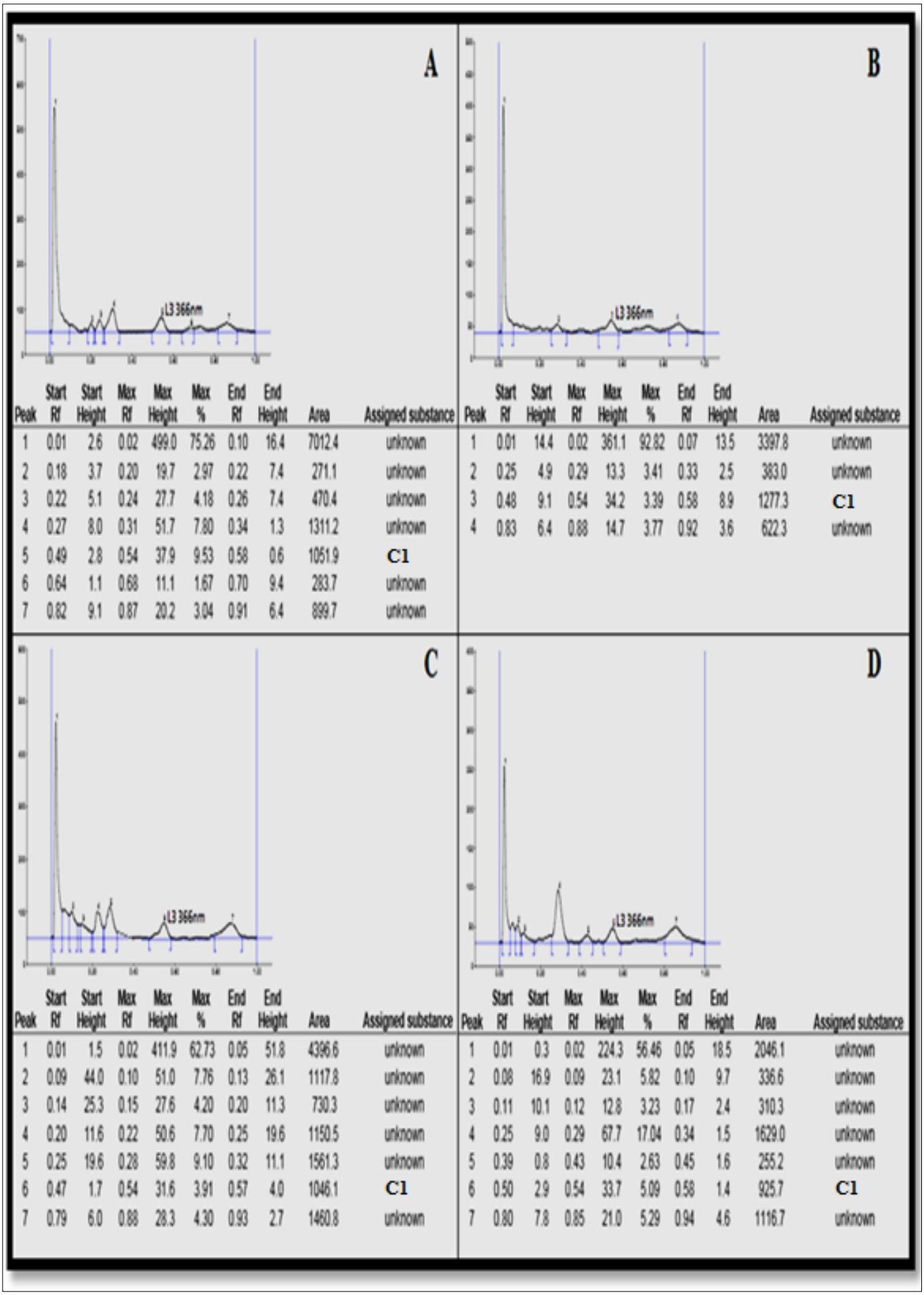
C

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance
1	0.00	5.6	0.02	603.9	59.91	0.06	122.4	8678.7	unknown
2	0.09	98.5	0.11	176.0	17.47	0.20	43.2	7777.2	unknown
3	0.20	43.6	0.22	69.5	6.89	0.25	42.4	2005.3	unknown
4	0.25	42.6	0.28	89.5	8.88	0.36	8.4	3541.3	unknown
5	0.49	2.6	0.54	27.6	2.23	0.58	0.0	738.8	C1
6	0.63	13.7	0.64	14.8	1.47	0.70	2.3	466.4	unknown
7	0.80	2.2	0.86	20.0	1.99	0.92	0.4	867.0	unknown

D

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance
1	0.01	2.1	0.02	395.6	48.96	0.06	78.6	6425.4	unknown
2	0.08	74.2	0.12	164.3	20.33	0.15	10.1	4528.3	unknown
3	0.20	6.9	0.24	27.8	3.44	0.25	21.6	727.2	unknown
4	0.25	21.9	0.28	147.8	18.29	0.33	6.4	3491.4	unknown
5	0.38	1.7	0.42	12.9	1.60	0.45	2.2	317.5	unknown
6	0.49	2.0	0.54	21.6	2.32	0.58	1.3	609.3	C1
7	0.79	2.5	0.85	28.0	3.46	0.90	4.7	1060.8	unknown

Fig. 6. HPTLC chromatogram of C1 in mixed F2+F3 fraction of 4 parts of *E. crassipes* plant by UAE bath method. A = flowers, B = leaves, C = stems, D = roots.



A

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance
1	0.01	2.6	0.02	499.0	75.26	0.10	16.4	7012.4	unknown
2	0.18	3.7	0.20	19.7	2.97	0.22	7.4	271.1	unknown
3	0.22	5.1	0.24	27.7	4.18	0.26	7.4	470.4	unknown
4	0.27	8.0	0.31	51.7	7.80	0.34	1.3	1311.2	unknown
5	0.49	2.8	0.54	37.9	9.53	0.58	0.6	1051.9	**C1**
6	0.64	1.1	0.68	11.1	1.67	0.70	9.4	283.7	unknown
7	0.82	9.1	0.87	20.2	3.04	0.91	6.4	899.7	unknown

B

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance
1	0.01	14.4	0.02	361.1	92.82	0.07	13.5	3397.8	unknown
2	0.25	4.9	0.29	13.3	3.41	0.33	2.5	383.0	unknown
3	0.48	9.1	0.54	34.2	3.39	0.58	8.9	1277.3	**C1**
4	0.83	6.4	0.88	14.7	3.77	0.92	3.6	622.3	unknown

C

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance
1	0.01	1.5	0.02	411.9	62.73	0.05	51.8	4396.6	unknown
2	0.09	44.0	0.10	51.0	7.76	0.13	26.1	1117.8	unknown
3	0.14	25.3	0.15	27.6	4.20	0.20	11.3	730.3	unknown
4	0.20	11.6	0.22	50.6	7.70	0.25	19.6	1150.5	unknown
5	0.25	19.6	0.28	59.8	9.10	0.32	11.1	1561.3	unknown
6	0.47	1.7	0.54	31.6	3.91	0.57	4.0	1046.1	**C1**
7	0.79	6.0	0.88	28.3	4.30	0.93	2.7	1460.8	unknown

D

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Assigned substance
1	0.01	0.3	0.02	224.3	56.46	0.05	18.5	2046.1	unknown
2	0.08	16.9	0.09	23.1	5.82	0.10	9.7	336.6	unknown
3	0.11	10.1	0.12	12.8	3.23	0.17	2.4	310.3	unknown
4	0.25	9.0	0.29	67.7	17.04	0.34	1.5	1629.0	unknown
5	0.39	0.8	0.43	10.4	2.63	0.45	1.6	255.2	unknown
6	0.50	2.9	0.54	33.7	5.09	0.58	1.4	925.7	**C1**
7	0.80	7.8	0.85	21.0	5.29	0.94	4.6	1116.7	unknown

Fig. 7. HPTLC chromatogram of C1 in mixed F2+F3 fraction of 4 parts of *E. crassipes* plant by Soxhlet extraction method. A = flowers, B = leaves, C = stems, D = roots.

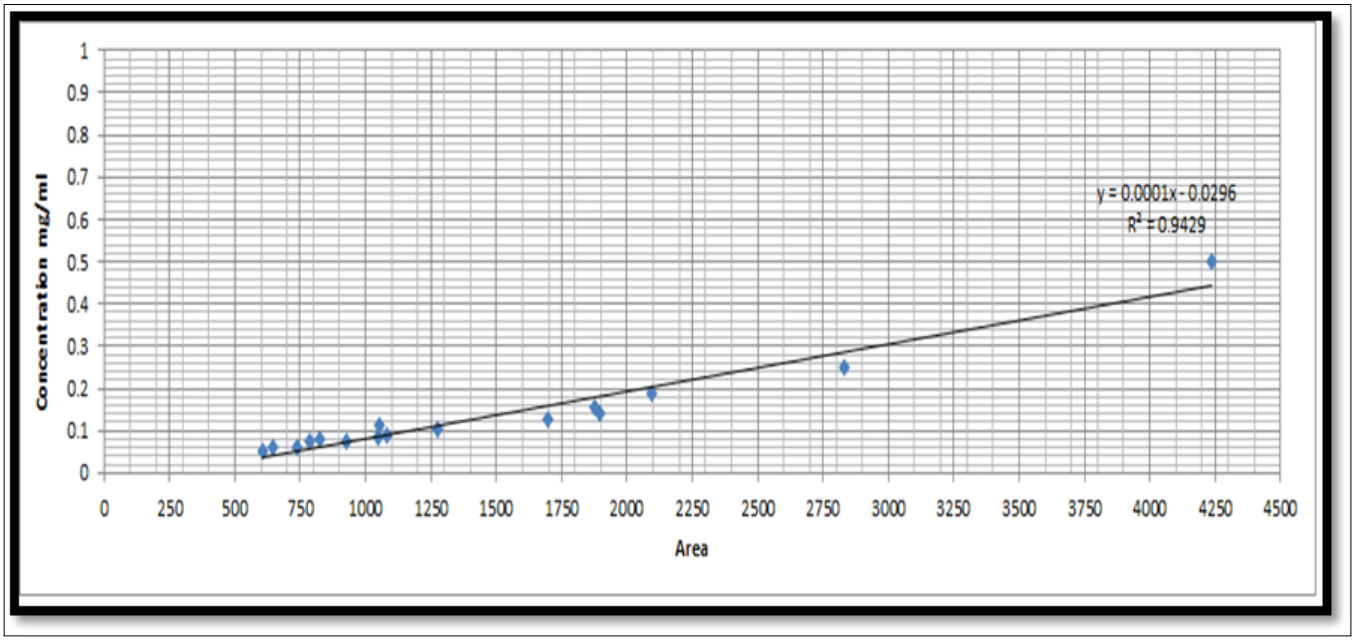


Fig. 8. Calibration curve of scopoletin on HPTLC.

Table 6. Quantitative analysis of scopoletin (C1) in each part of Iraqi *E. crassipes* by HPTLC

Part of plant	Concentration mg/mL by probe UAE	Concentration mg/mL by bath UAE	Concentration mg/mL by Soxhlet
Flowers	0.145	0.0762	0.105
Leaves	0.1876	0.08	0.114
Stems	0.1396	0.0605	0.086
Roots	0.089	0.05	0.076

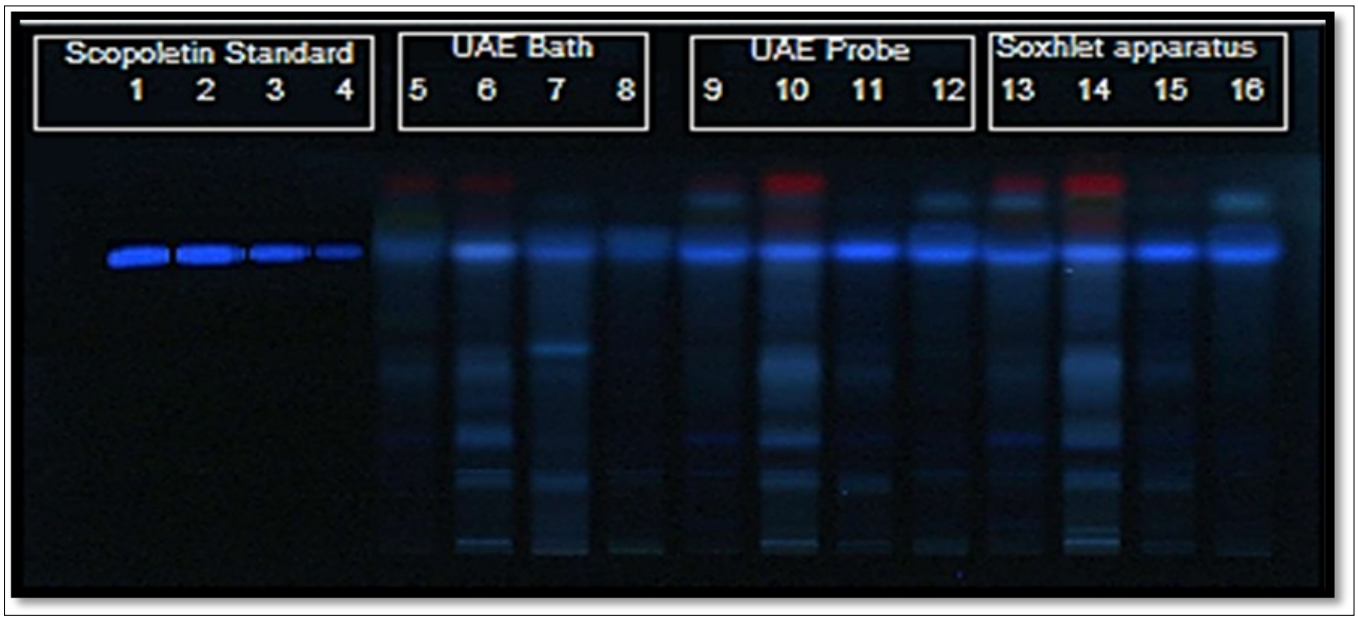


Fig. 9. HPTLC, track 1-4 = scopoletin standard and tracks 5-16 = mixed F2 + F3 fraction obtained by bath UAE, probe UAE and Soxhlet methods tracks for stems, leaves, flowers and roots respectively in each track detected at 366nm UV light.

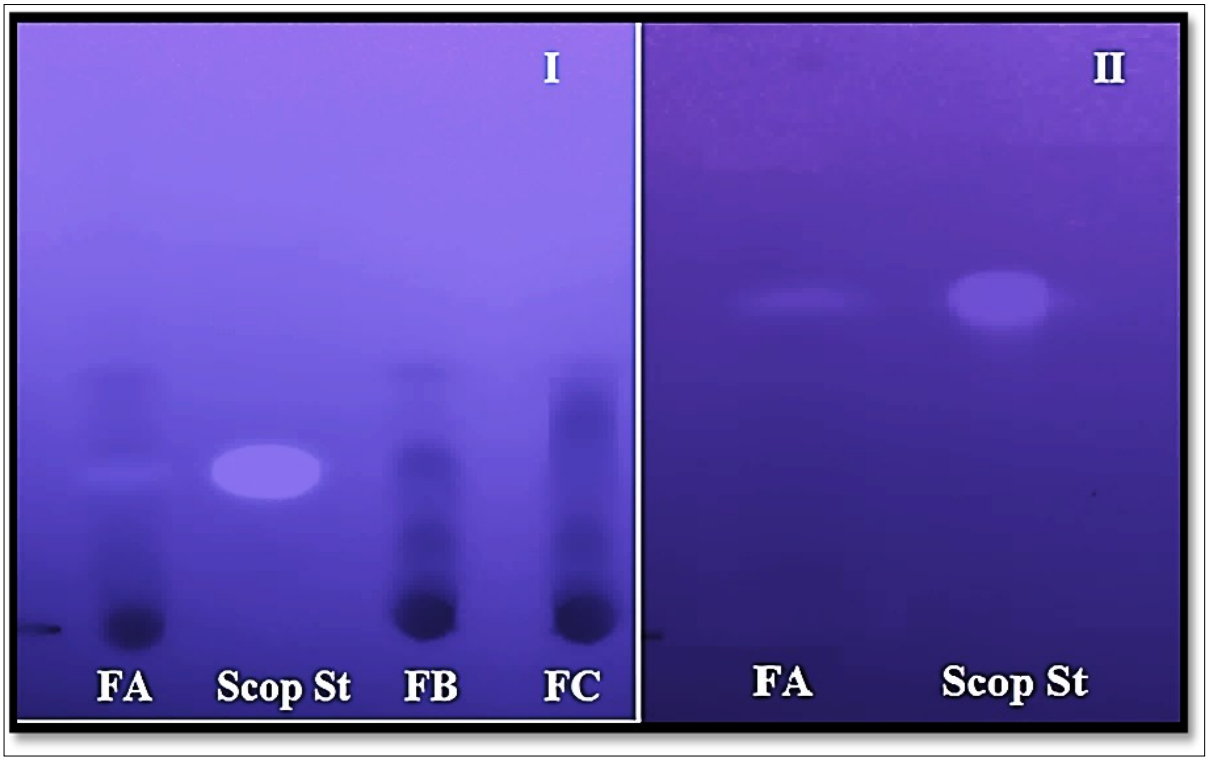


Fig. 10. TLC chromatogram of: I = fractions (FA, FB, FC) and II = purified fraction A, compared to scopoletin standard (Scop St) obtained from silica gel column chromatography using S3 [ether: benzene (1:1)] as a mobile phase and detected by 366nm UV light.

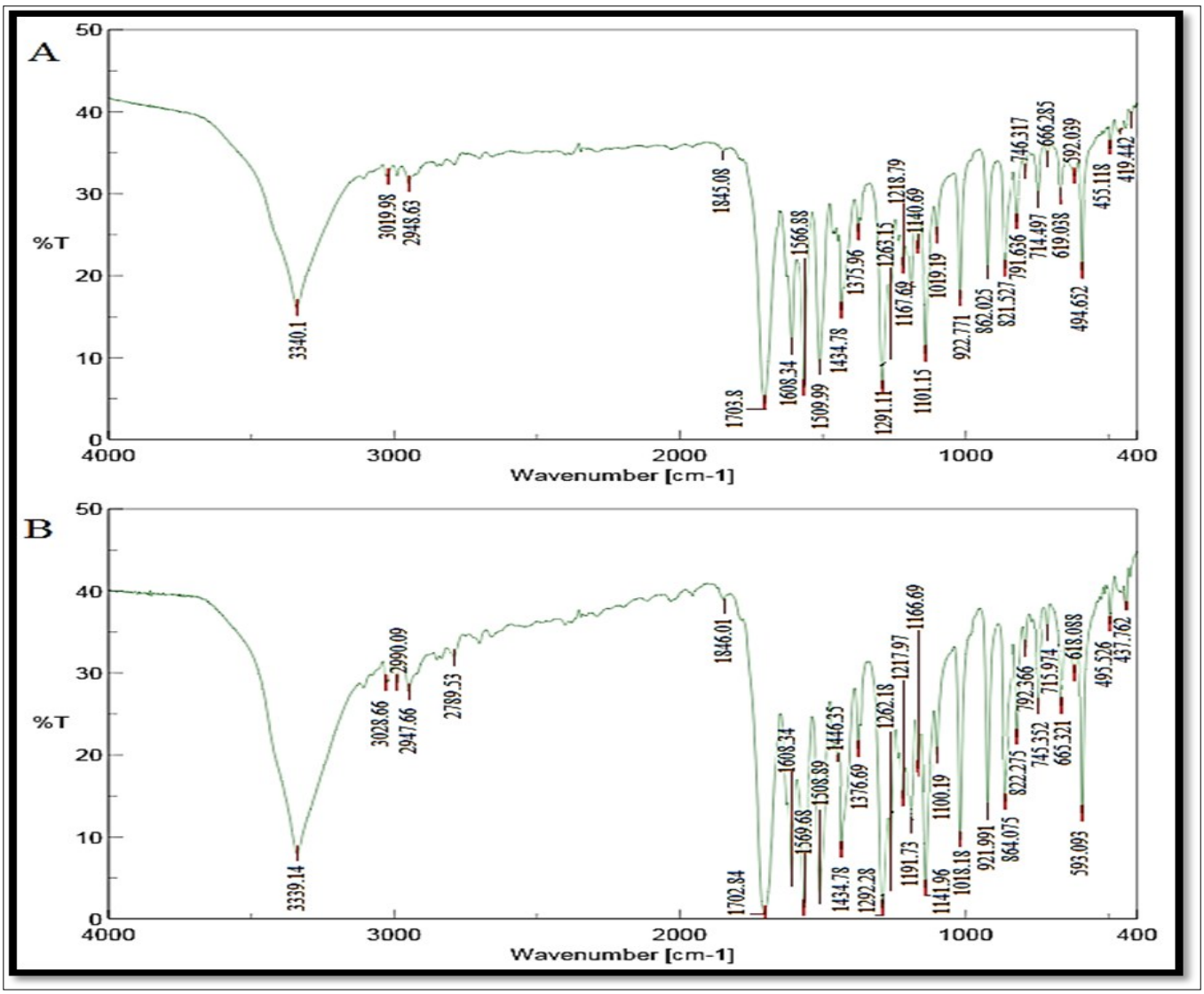
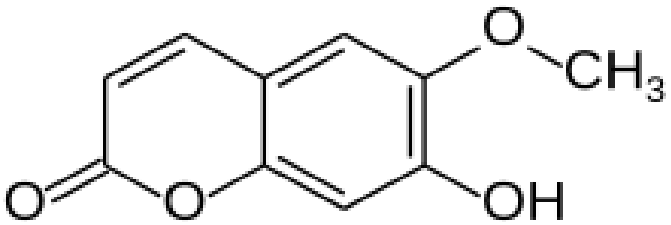
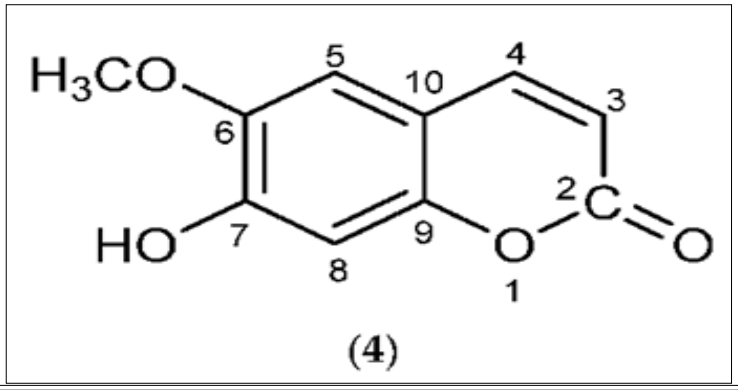


Fig. 11. FTIR spectra of A = isolated C1 and B = scopoletin standard.

Table 7. Characteristic FTIR absorption bands (cm^{-1}) of the isolated C1 constituent (23)

Functional group	Group frequency wave number (in cm^{-1})		Main attributed
	Isolated C1	Scopoletin standard	
Phenol O-H	3340.1	3339.14	O-H stretching vibration
C=C-H	3019.98	3028.66	C=C-H stretching of aromatic ring
C-H	2948.63	2947.66	Asymmetric and symmetric stretching of CH2
C=O	1703.8	1702.84	C=O stretching vibration
C=C	1608.34, 1566.88	1608.34, 1569.68	C=C stretching of aromatic ring
O-H	1434.78	1434.78	O-H bending of carboxyl group
Ar-C-O	1291.11	1292.28	Ar-C-O stretching of phenol
O-H	1263.15	1262.18	O-H bending of phenol group
Ar-C-O-C	1218.79	1217.97	Ar-C-O-C stretching of ether
C-H	1019.19	1018.18	C-H in plane bending of aromatic ring
C=C	922.71	921.99	C=C out plane bending vibration
C-H	862,791,666	864,792,665	Benzene ring C-H out plane bending vibration





(4)

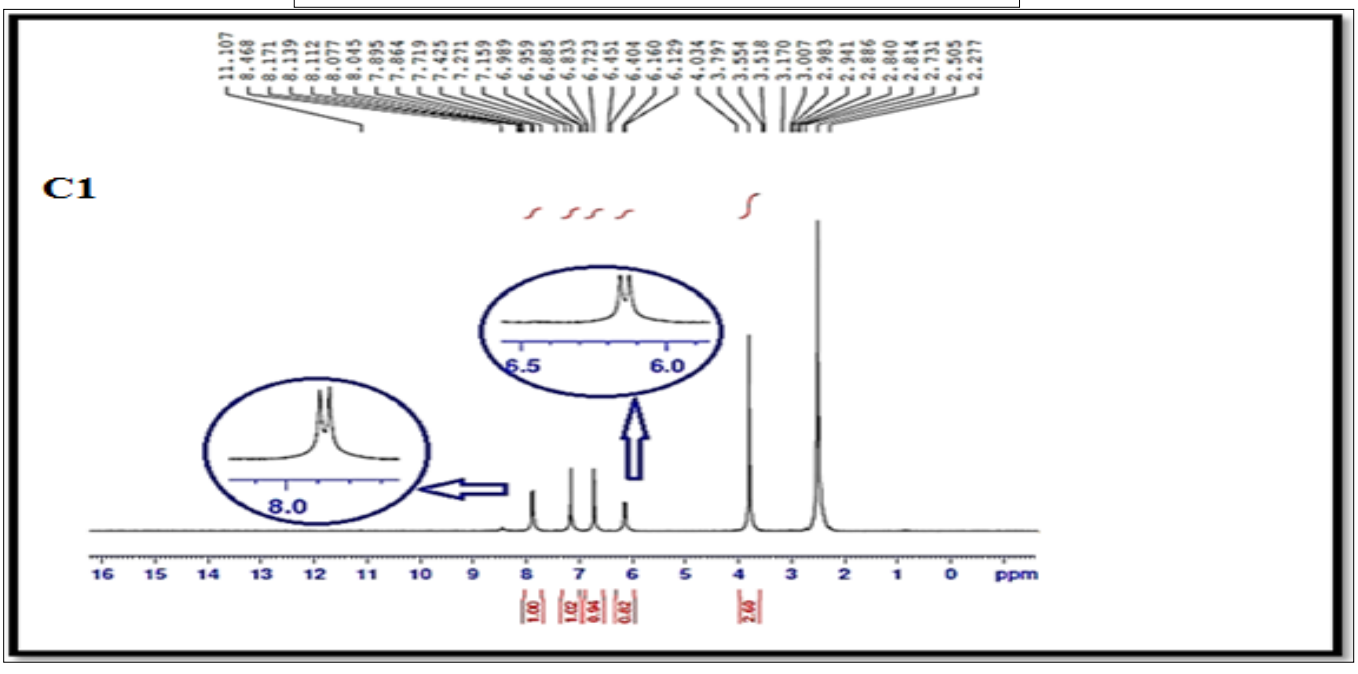
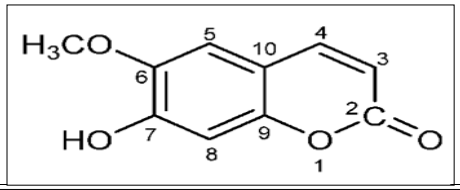


Fig. 12. ^{1}H-NMR spectrum of C1.

Table 8. Assignment of ^{1}H-NMR spectral data of C1

Ppm	Integration	Multiplicity	Assignment
8.46	1H	Singlet broad	O-H
7.86	1H	Doublet	C4-H
7.15	1H	Singlet	C5-H
6.72	1H	Singlet	C8-H
6.16	1H	Doublet	C3-H
3.79	3H	Singlet	O-CH_3



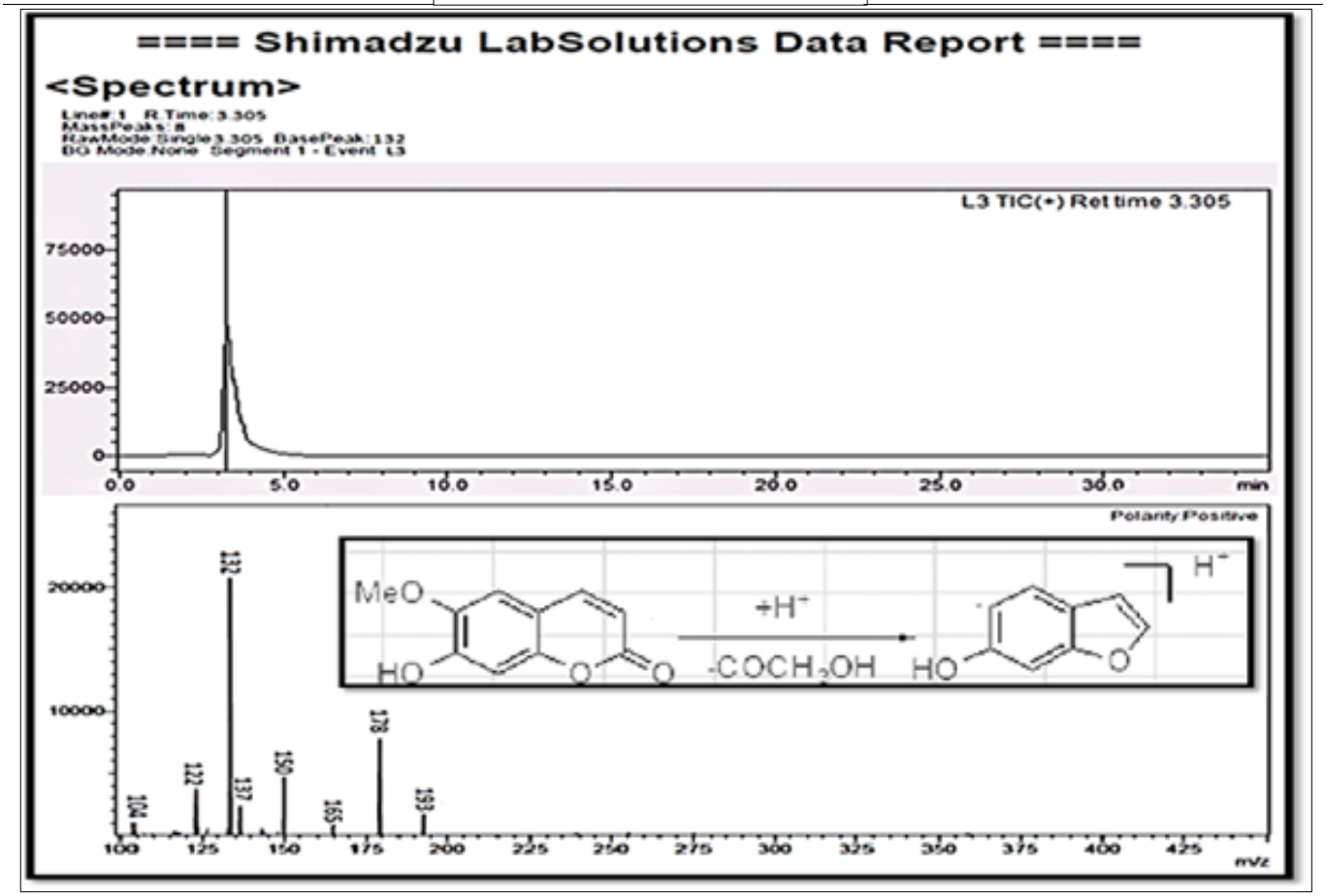


Fig. 13. Representative full scan product ion LC chromatogram and mass fragmentation spectra of isolated C1.

Conclusion

The study findings indicate that probe UAE use is significantly simpler and more effective than traditional Soxhlet and bath UAE extraction procedures for extracting scopoletin from the flowers, leaves, stems and roots of the Iraqi *E. crassipes* plant. HPTLC enabled identification and quantification of scopoletin by comparing sample retention factor (R_f) values with those of authenticated standards. Scopoletin was detected in the chloroform and ethyl acetate fractions derived from all 4 plant parts. Spectral analyses including FTIR, ^{1}H-NMR and LC-MS confirmed that the isolated compound (C1) is chemically identical to standard scopoletin.

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Authors' contributions

SHA participated in the design, conducted the study and performed the statistical analysis. TZAJ designed the study, contributed to its sequences and aligned the drafted manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

References

1. Annunziata F, Pinna C, Dallavalle S, Tamborini L, Pinto A. An overview of coumarin as a versatile and readily accessible scaffold with broad-ranging biological activities. Int J Mol Sci. 2020;21 (13):4618. https://doi.org/10.3390/ijms21134618
2. Gay NH, Suwanjang W, Ruankham W, Songtawee N, Wongchitrat P, Prachayasittikul V, et al. Butein, isoliquiritigenin and scopoletin attenuate neurodegeneration via antioxidant enzymes and SIRT1/ ADAM10 signaling pathway. RSC Advances. 2020;10(28):16593–606. https://doi.org/10.1039/C9RA06056A
3. Napiroon T, Bacher M, Balslev H, Tawaitakham K, Santimaleeworagun W, Vajrodaya S. Scopoletin from *Lasianthus lucidus* Blume (Rubiaceae): A potential antimicrobial against multidrug-resistant *Pseudomonas aeruginosa*. J Appl Pharma Sci. 2018;8(9):1–6. https://doi.org/10.7324/JAPS.2018.8901
4. Novie E, Hendro W, Hadi S, Tri NK. Anti-tubercular activity of extract and coumpounds of noni (*Morinda citrifolia* Linn.). Int J Pharm Pharma Sci. 2017;9(1):1–4. http://doi.org/10.22159/ijpps.2017v9i12.19841
5. Ndam NY, Nyegue MA, Mounjouenpou P, Kansci G, Kenfack MJ, Eugene EE. LC-MS quantification of scopoletin in cassava (*Manihot esculenta* Crantz) varieties, local derived foods and activity on some food spoilage fungi. J Food Process Preserv. 2020;44 (4):e14387. https://doi.org/10.1111/jfpp.14387
6. Anwar K. Hypotensive activity of ethanolic extracts of *Morinda citrifolia* L. leaves and fruit in dexamethasone-induced hypertensive rat. J Evid Based Complementary Altern Med. https://doi.org/10.1177/2156587216653660
7. Dersseh MG, Melesse AM, Tilahun SA, Abate M, Dagnew DC. Water hyacinth: review of its impacts on hydrology and ecosystem services—lessons for management of Lake Tana. In: Melesse AM, Abtew W, Setegn SG, editors. Extreme Hydrology and Climate Variability. 2019. p. 237–51. https://doi.org/10.1016/B978-0-12-815998-9.00019-1
8. Patel S. Threats, management and envisaged utilizations of aquatic weed *Eichhornia crassipes*: An overview. Rev Environ Sci Biotechnol. 2012;11:249–59. https://doi.org/10.1007/s11157-012-9289-4
9. Rufchaei R, Abbas-Mohammadi M, Mirzajani A, Nedaei S. Evaluation of the chemical compounds and antioxidant and antimicrobial activities of the leaves of *Eichhornia crassipes* (water hyacinth). Jundishapur J Nat Pharma Prod. 2022;17(1):e116103. https://doi.org/10.5812/jjnpp.101436
10. Mustafa HM, Hayder G. Recent studies on applications of aquatic weed plants in phytoremediation of wastewater: A review article. Ain Shams Eng J. 2021;12(1):355–65. https://doi.org/10.1016/j.asej.2020.05.009
11. Sayago UF, Rodriguez C. Design and construction of a biohydrogen and bioethanol production system from the biomass of the *Eichhornia crassipes* [preprint]; 2018. Available from: https://www.preprints.org/manuscript/201805.0393
12. Manyuchi MM, Mbohwa C, Muzenda E, Mutusva TN, Mpeta M. Degradation of water hyacinth (*Eichhornia crassipes*) to vermicompost through application of the vermicomposting technology. In: Proceedings of the International Conference on Industrial Engineering and Operations Management. IEOM; 2019. p. 79–88. https://doi.org/10.33965/ste2019_201901L010
13. Sharma AK, Sharma V, Sharma V, Sharma JK, Singh R. Multifaceted potential of *Eichhornia crassipes* (water hyacinth) ladened with numerous value aided and therapeutic properties. Plant Arch. 2020;20(2):2059–65.
14. Abdul-Jalil TZ. Screening of rutin from seeds and leaves extracts of dill, coriander and fennel cultivated in Iraq. Pharmacie Globale. 2013;4(3):1.
15. Hamad MN. Detection and isolation of flavonoids from *Calendula officinalis* (F. Asteraceae) cultivated in Iraq. Iraqi J Pharma Sci. 2016;25(2):1–6. https://doi.org/10.31351/vol25iss2 pp1-6
16. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: extraction, isolation and identification of bioactive compounds from plant extracts. Plants. 2017;6(4):42. https://doi.org/10.3390/plants6040042
17. Medina-Torres N, Ayora-Talavera T, Espinosa-Andrews H, Sanchez -Contreras A, Pacheco N. Ultrasound assisted extraction for the recovery of phenolic compounds from vegetable sources. Agron. 2017;7(3):47. https://doi.org/10.3390/agronomy7030047
18. Samaram S, Mirhosseini H, Tan CP, Ghazali HM. Ultrasound-assisted extraction (UAE) and solvent extraction of papaya seed oil: yield, fatty acid composition and triacylglycerol profile. Mol. 2013;18(10):12474–87. https://doi.org/10.3390/molecules181012474
19. Tatke P, Rajan M. Comparison of conventional and novel extraction techniques for the extraction of scopoletin from *Convolvulus pluricaulis*. Ind J Pharma Educ Res. 2014;48(1):27–31. https://doi.org/10.5530/ijper.48.1.5
20. Mohammad A, Bhawani SA, Sharma S. Analysis of herbal products by thin-layer chromatography: A review. Int J Pharm Biosci. 2010;1(2):1–4.
21. Tasfiyati AN, Antika LD, Septama AW, Hikmat H, Kurniawan HH, Ariani N. 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
22. Abdul-Jalil TZ. Phytochemicals screening by GC/MS and determination of some flavonol in cultivated Iraqi *Eruca sativa* dried leaves extract and its biological activity as antioxidant. Int J Pharma Phytochem Res. 2016;8(10):1722–30.
23. Suryati EM, Astuti SH, Aziz H. Isolation of scopoletin from Subang-Subang plants (*Spilanthes paniculata* Wall. ex DC.). Der Pharma Chemica. 2016;8(9):94–104.
24. Carreira-Casais A, Otero P, Garcia-Perez P, Garcia-Oliveira P, Pereira AG, Carpena M, et al. Benefits and drawbacks of ultrasound-assisted extraction for the recovery of bioactive compounds from marine algae. Int J Environ Res Public Health. 2021;18(17):9153. https://doi.org/10.3390/ijerph18179153
25. Picot-Allain C, Mahomoodally MF, AK G, Zengin G. Conventional versus green extraction techniques-A comparative perspective. Curr Opin Food Sci. 2021;40:144–56. https://doi.org/10.1016/j.cofs.2021.02.009
26. Chemat F, Rombaut N, Sicaire A, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M. Ultrasound assisted extraction of food and natural products: mechanisms, techniques, combinations, protocols and applications– A review. Ultrason Sonochem. 2017;34:540–60. https://doi.org/10.1016/j.ultsonch.2016.06.035
27. Xu Y, Pan S. Effects of various factors of ultrasonic treatment on the extraction yield of all-trans-lycopene from red grapefruit (Citrus paradise Macf.). Ultrason Sonochem. 2013;20(4):1026–32. https://doi.org/10.1016/j.ultsonch.2013.01.006
28. Abdul-lalil ZT. Ultrasound-assisted extraction of fennel leaves: process optimization, thin layer chromatography and cytotoxic activity of ethanolic extract. Iraqi J Pharma Sci. 2024;33(1):94–103. https://doi.org/10.31351/vol33iss1pp94-103
29. Gao XY, Li X, Zhang C, Bai C. Scopoletin: A review of its pharmacology, pharmacokinetics and toxicity. Front Pharmacol.

2024;15:1268464. https://doi.org/10.3389/fphar.2024.1268464

30. Kawambe A. Determination of insecticidal and seed protective properties of pawpaw extracts against bean weevils (*Acanthoscelides obtectus*) in storage. PhD [dissertation]. Uganda Martyrs University; 2018
31. Khamees AH. Isolation, characterization and quantification of a pentacyclic triterpinoid compound ursolic acid in *Scabiosa palaestina* L. distributed in the north of Iraq. Plant Sci Today. 2022;9(1):178–82. https://doi.org/10.14719/pst.1398
32. Vinod TK, Craine LE. Lab manual for organic chemistry: A short course. Cengage Learning; 2011
33. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 2010;8 (1):1–10. https://doi.org/10.4314/ajtcam.v8i1.60483
34. Valizadeh H, Kordi MF, Koohkan R, Bahadori MB, Farimani M. Isolation and structure elucidation of coumarin and cinamate derivatives from *Lycium ruthenicum*. Iranian Chem Commun. 2014;2(4):236–325.
35. Mehul BK, Kishor DK, Ajay SK. Isolation and structure elucidation of scopoletin from *Ipomoea reniformis* (Convolvulaceae). J Appl Pharma Sci. 2011;1(05):138–44.
36. Nahata A, Patil UK, Dixit VK. Effect of *Evolvulus alsinoides* Linn. on learning behavior and memory enhancement activity in rodents. Phytother Res. 2010;24(4):486–93. https://doi.org/10.1002/ptr.2932
37. Wong KC. Review of NMR spectroscopy: basic principles, concepts and applications in chemistry. J Chem Edu. 2014;91(8):1103–04. https://doi.org/10.1021/ed500324w
38. Zeng Y, Li S, Wang X, Gong T, Sun X, Zhang Z. Validated LC-MS/MS method for the determination of scopoletin in rat plasma and its application to pharmacokinetic studies. Mol. 2015;20(10):18988–9001. https://doi.org/10.3390/molecules201018988
39. Tine Y, Renucci F, Costa J, Wele A, Paolini J. A method for LC-MS/MS profiling of coumarins in *Zanthoxylum zanthoxyloides* (Lam.) B. Zepernich and Timler extracts and essential oils. Mol. 2017;22 (1):174. https://doi.org/10.3390/molecules22010174

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