International Journal of Plant Pathology and Microbiology

E-ISSN: 2789-3073
P-ISSN: 2789-3065
Impact Factor (RJIF): 5.78
www.plantpathologyjournal.com
IJPPM 2025; 5(2): 53-59
Received: 21-05-2025
Accepted: 24-06-2025

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Comparative study on the extraction of apigenin from parsley leaves (*Petroselinum crispum* L.) by liquid-liquid extraction and ultrasonic-assisted extraction methods

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DOI: https://www.doi.org/10.22271/27893065.2025.v5.i2a.144

Abstract
Apigenin is a flavone abundantly present in various plants such as parsley, and Parsley's bioactive component (*Petroselinum crispum* L.) has a variety of medicinal qualities. In this work, apigenin was isolated from parsley leaves using liquid-liquid extraction and ultrasound-assisted extraction (UAE).
Several parameters, including solvent type and concentration, extraction duration, ultrasonic power, ultrasonic frequency, and temperature, were evaluated for their influence on apigenin extraction yield. Under optimal conditions, Soxhlet extraction yielded 9.87 (mg apigenin/g parsley), whereas UAE yielded 6.53 (mg apigenin/g parsley).
The duration of the extraction in the Soxhlet device was 6 hours, using (300 ml) of ethanol, and a temperature of 70 degrees Celsius, while the duration of the Ultrasonic-assisted extraction was (20-70) minutes, using (10 ml) of solvent (ethanol, water), and the temperature Ranging from (20-60) degrees Celsius.
The findings indicated that the best mobile phase for isolating Apigenin from the column was (hexane - ethyl acetate - ethanol) (2:2:1), followed by thin layers with a displacement combination (hexane - dichloromethane) (1:2), with a valuable RF of 0.88.
The FTIR and NMR tests indicated that apigenin's structure remained unchanged following extraction.

Keywords: *Petroselinum crispum* L, flavones, apigenin, liquid-liquid extraction, ultrasonic assisted extraction, bioactive component. IR, NMR

1. Introduction
Medicinal plants receive great attention at present in many of the countries that produce them, especially after plant treatment became based on scientific foundations, as it became the primary source of medicinal drugs or active substances that are used in the formulation of medicines [1], Parsley leaves have a wide range of therapeutic applications, including diabetes [2], eczema, kidney stones, nosebleed, halitosis, hypertension, anemia, constipation, baldness, odontalgia, skin disease, hepatotoxicity, cardiac disease [3], renal disease, and urinary tract illnesses [4].

Parsley is a glabrous biennial plant raised as an annual to produce edible leaves. The leaves have a triangular shape and are divided into three cuneate lobes. The umbels are loose, and the blooms have yellow petals that are inflexed at the tip [5]. The blooming stem may grow up to 75 cm tall and has sparser leaves. Figure 1 shows the *Petroselinum crispum* L. plant. Flat-topped umbels 3-10 cm in diameter, with many 2 mm yellow to yellowish-green blooms [6]. Figure (1), Table (1).

Parsley is native to Europe, but it might not grow well in other parts of the world, like North America. There are three kinds: curly-leaf parsley, Italian or flat-leaf parsley, and root parsley, which is grown for its tasty tuberous root. For each type, there are several variations [8]. A lot of vitamin A and C, as well as chlorophyll, can be found in parsley. It is one of the most important herbs used in European cooking [9].

Phytochemical testing of parsley (*Petroselinum crispum*) has shown that it contains a number of different types of flavonoids [10]. The main flavonoids found in parsley (*Petroselinum crispum*) and other apiaceous plants are flavones (apigenin and luteolin) and flavanols (kaempferol and quercetin), both of which exist in nature in the form of glycosidic bonds [11].

Apigenin (4',5,7,-trihydroxyflavone), a flavonoid with the chemical formula C15H10O5 and a molecular weight of 270.24 g/mol, Figure (2) [12], is entirely insoluble in water, which restricts its therapeutic applications. Apigenin has garnered interest as a beneficial health agent due to its low intrinsic toxicity [13]. Parsley serves as a significant source of apigenin, contributing to a range of therapeutic properties associated with this plant [14].

Fig 1: shows the *Petroselinum crispum* L. plant. Flat-topped umbels 3-10 cm in diameter, with many 2 mm yellow to yellowish-green blooms

Table 1: Represents the scientific classification of *Petroselinum crispum* L. [7]

Taxonomic Rank	Classification
Class	Magnoliopsida - Dicotyledons
Family	Apiaceae
Genus	*Petroselinum*
Species	*P. crispum*

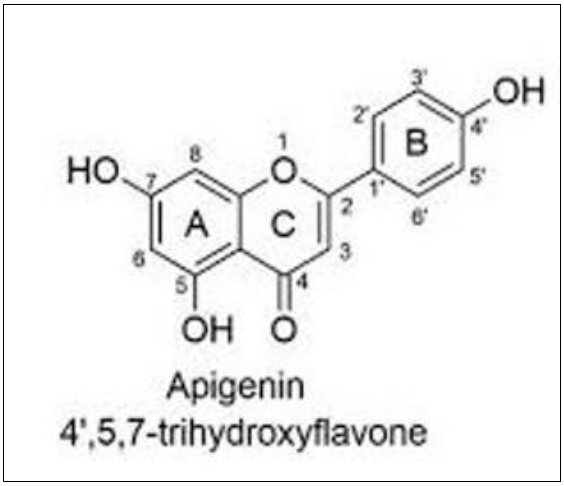


Fig 2: Chemical formula of Apigenin ($C_{15}H_{10}O_5$)

A study conducted in Korea aimed to explore the active effects of 70% ethanol extracts of parsley (*Petroselinum crispum*) and assess their potential as a functional cosmetic ingredient. Parsley was extracted using 70% ethanol to evaluate its anti-inflammatory, anti-aging, and skin-brightening properties, along with its total polyphenol and flavonoid content, DPPH radical scavenging activity, and capacity to synthesize nitric oxide (NO) [15]. Soxhlet extraction is a continuous solvent extraction technique that employs solvents at ambient pressure and boiling temperature to selectively isolate target compounds from solid materials [16]. This method is widely recognized for its effectiveness in extracting bioactive compounds, including lipids, sterols, and flavonoids [17].

The research on medicinal plants commences with the extraction of bioactive chemicals, a crucial process in obtaining bioactive components from plant material. Conventional extraction methods encompass maceration, decoction, Soxhlet extraction, and heat reflux techniques. There are several drawbacks, including the need for significant energy and extended extraction durations, which are typically observed in small manufacturing enterprises and research environments [18].

Contemporary extraction techniques, including supercritical extraction and ultrasonic assisted extraction (UAE), are being evaluated for their potential to enhance extraction yield while minimizing costs. These methods offer notable benefits, such as decreased solvent and energy usage, as well as reduced extraction duration [19].

2 Materials and working methods

2. 1. Plant samples used

Samples of leaves of the parsley plant were collected from Iraq, where the samples were dried in the shade for (15) days at a temperature of 25°C. Then grind the leaves, and place them in a dark, tightly closed glass container in the refrigerator at a temperature of 4°C until extraction. Figure (3) shows the dried leaves.

Fig 3: The dried leaves of the parsley plant

2. 2. Preparation of Ethanolic extract for the plant:

Fixed oils were extracted using a Soxhlet extractor device, where (30 grams) of the studied plant sample of the ground parsley plant was placed in the paper of the organic solvent (300 ml ethanol) for (6) hours at the boiling point solvent (70 degrees Celsius), where the extraction process continued until the solvent used became colorless so that the temperature did not increase. The organic solvent used in the device exceeded (80) C°. The organic solvent was evaporated with a rotary evaporator at a temperature of (40) Co to concentrate the sample, then it was put in a Petri dish to dry completely by $CaCl_2$, as we noticed that the Weight and yield for the organic plant extract were (2.6g, 8.66%). Figure (4) shows the Soxhlet device.

Fig 4: The Soxhlet device

2.3 Ultrasonic-Assisted extraction (UAE)

Ultrasonic-Assisted Extraction the extraction of parsley leaf extract, rich in apigenin, was conducted utilizing an ultrasonic extraction method. Ultrasonic extraction was conducted using 30 g of powder combined with an 80:20 (v/v) solution of ethanol and water. The extraction was accomplished using an Elm sonic ultrasonic bath for 30 minutes at 40 °C., the frequency of the ultrasound waves is (37-80) kHz, and the power of the waves is (60-100) watts, as we noticed that the Weight and yield for the organic plant extract were (1.2g, 4%). Figure (5) shows the Ultrasonic-Assisted device.

Fig 5: The ultrasonic-assisted device

2.4 The ultrasonic-assisted device

Chromatographic isolation of apigenin from the ethanolic extract of the plant:

Thin layer chromatography (TLC) was initially used to determine the best displacement mixture, which was (hexane ethyl acetate ethanol) (2:2:1), then (1) g of ethanolic extract was injected into a chromatography column, the chemical compounds were isolated from the organic extracts using a chromatography column (70 x 10 cm), Which was filled by the wet method (dissolved 50 g of Silkia Gel in 150 ml of hexane). The column flow rate was (15) drops per minute, and the isolation process in the column took (7) hours.

The isolation resulted in several compounds. The purity of apigenin was confirmed by calculating the value of the retention factor (RF) with the displacement mixture (hexane dichloromethane) (1:2), for the single spot formed.

The solvent was evaporated from the sample in a rotary evaporator, and then a stream of nitrogen gas was passed through to dry it completely.

2.5 Analysis of apigenin using infrared spectroscopy

Infrared spectroscopy device, model 460 PLUS, JASCO, single beam, using a KBr disc.

2.6 Analysis of apigenin using nuclear magnetic resonance spectroscopy

Nuclear Magnetic Resonance (NMR) spectroscopy model Bruker 400MHz AVANCE SPETROMETER Spectra were recorded using the solvent DMSO and using TMS as an internal standard.

3 Results and discussion

3. 1. Results of chromatographic isolation of ethanolic extract of plant leaves

Chromatographic isolation resulted in a pure compound, and its purity was confirmed by measuring the value of the retention factor of the single spot formed and the results of the isolated compound are shown in the table (2).

Table 2: Physical properties and yield of the compound (apigenin)

Extraction method	Color	Weight	Rf (hexane:CH_2Cl_2) (1:2)
Liquid-Liquid Extraction	Green	9.87 mg	0.88
Ultrasonic-assisted extraction	Green	6.53 mg	0.88

3. 2. Study of the chemical structure of the isolated compound (apigenin)

3.2.1. Infrared spectrum (IR)

Vibrational spectroscopy (IR) [Figure (6)] of the isolated compound showed the appearance of a broad absorption band at 3411 cm^{-1} to the hydroxyl group (OH), an absorption band at 3041 cm^{-1} of $(CH)_{aromatic}$, An absorption band at 1675 cm^{-1} corresponding to the carbonyl group (C=O), an absorption band at 1610 cm^{-1} associated with the double bond (C=C), an absorption band at 1265 cm^{-1} for the ether (C-O-C), and an absorption band at 1160 cm^{-1} related to the (C-C-O) extension of the ketone.

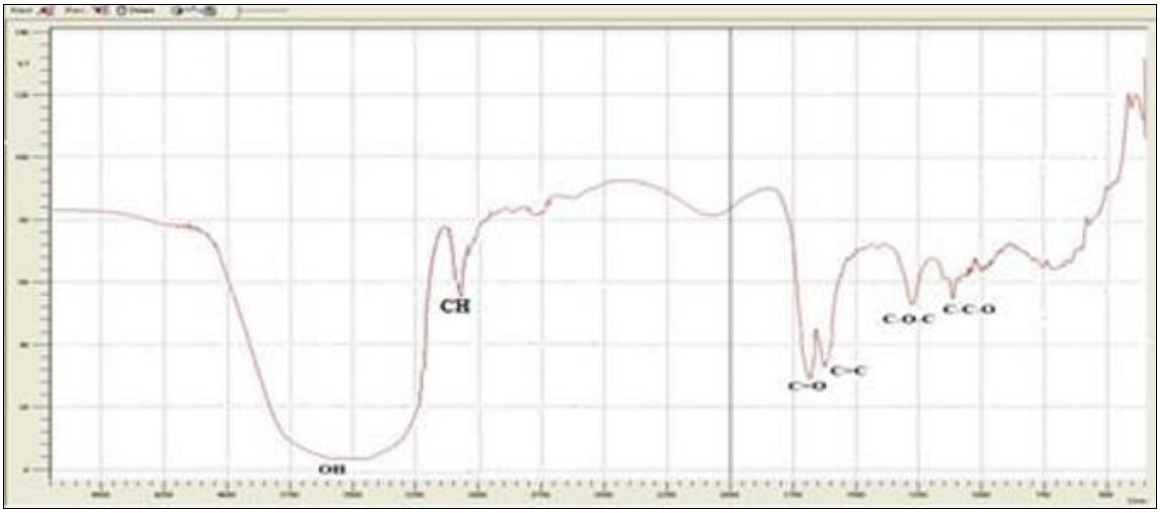


Fig 6: Infrared spectrum of apigenin (KBr)

3.2.2. (^{1}HNMR) Spectrum (DMSO, 400 MHZ, δ PPM)

The proton nuclear magnetic resonance (^{1}HNMR) spectrum of the isolated compound [Figure 7, Table 3] includes a group of peaks characteristic of the protons of the compound. The spectrum includes single peak (s) At 6.2 ppm, a single peak (s) the signal at 6.4 ppm indicates a single peak (s) for the proton associated with carbon C_8. At 6.5 ppm, another single peak (s) is observed for the proton linked to carbon C_3. The double peak (d) at 6.6 ppm corresponds to the protons connected to carbons C_3' and C_5'. A double peak is also noted at 6.9 ppm for the protons attached to carbons C_2' and C_6'. Finally, at 9.2 ppm, a single peak is present for the protons of the hydroxyl groups.

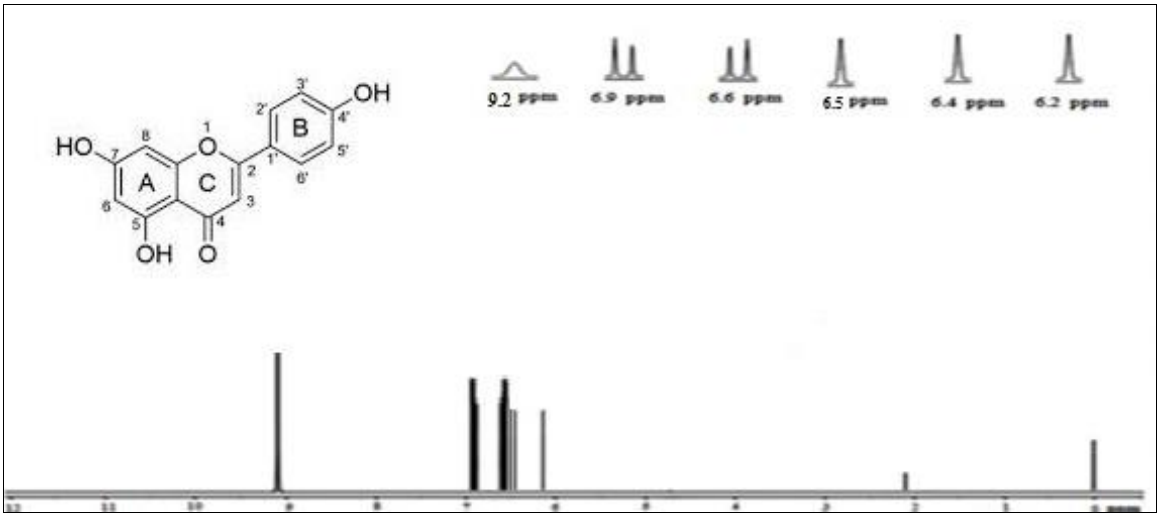


Fig 7: ^{1}HNMR of apigenin (DMSO)

Table 3: Results of ^{1}HNMR spectrum analysis of the isolated compound (400MHz, DMSO, ppm)

Number of peak	Shape of peak	Number of protons		Carbon atom number	Chemical shift Δ(ppm)
1	S	1H	CH	6	6.2
2	S	1H	CH	8	6.4
3	S	1H	CH	3	6.5
4	D	2H	2[CH]	3', 5'	6.6
5	D	2H	2[CH]	2', 6'	6.9
6	S	3H	3[OH]	-	9.1
		The total number of protons: 10			

3.2.3. (^{13}CNMR) Spectrum (DMSO, 100 MHZ, δ PPM)

The carbon nuclear magnetic resonance (^{13}CNMR) spectrum of the isolated compound [Figure 8, Table 4] showed (12) signals. These signals are characteristic of the carbon atoms of the aromatic rings within the range, (93.2-164.4) ppm, and of the carbonyl group (C=O) was characterized at 177.1 ppm, and a signal for the solvent (DMSO) appeared at 39.3 ppm.

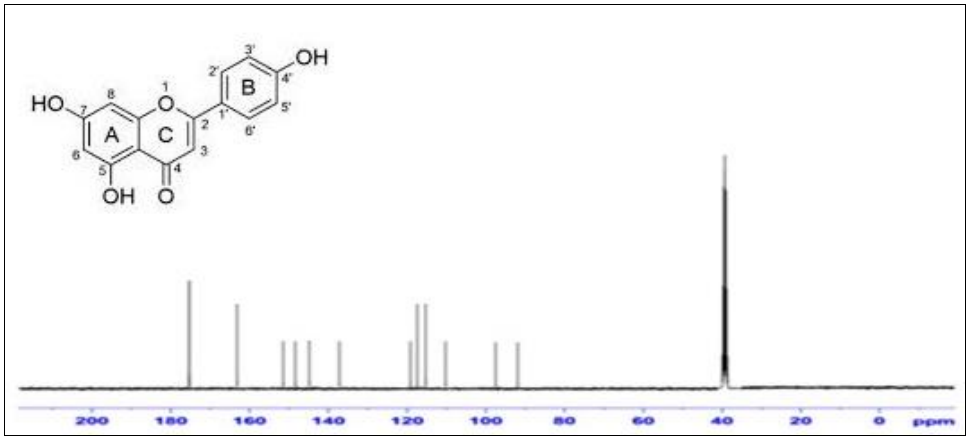


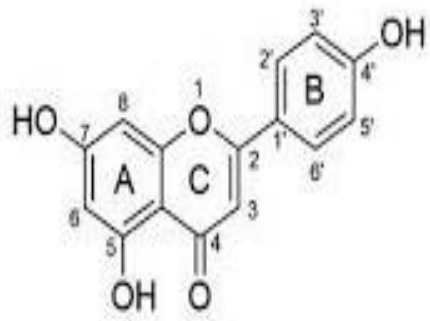
Fig 8: ^{13}CNMR of apigenin (DMSO)

Table 4: Results of ^{13}CNMR spectrum analysis of the isolated compound (100MHz, DMSO, ppm)

Number of signs	Carbon atom number	Chemical shift Δ(ppm)
1	C_8	93.2 ppm
2	C_6	97.5 ppm
3	C10	110.1 ppm
4	C_3', C_5'	115.1 ppm
5	C_2', C_6'	118.3 ppm
6	C_1'	119.2 ppm
7	C_3	138.3 ppm
8	C_4'	145.5 ppm
9	C_9	149.3 ppm
10	C_2	150.5 ppm
11	C_5, C_7	164.4 ppm
12	C_4	177.1 ppm
Total number of carbon atoms: 15		

The results obtained confirmed that the structure of the compound isolated from the ethanolic extract of Apigenin is derived from the ethanolic extract of the plant's leaves. The compound isolated from the plant's leaves is apigenin, and Table 5 presents the physical properties and chemical formula of this compound:

Table 5: Physical properties and chemical formula of isolated compound (apigenin)

M.W$_t$	M.F	Rf (hexane:CH_2Cl_2) (1:2)	Compound color
270	C15H10O5	0.88	Green
	 **Apigenin**		

The results of the current study showed the effectiveness of both extraction methods, liquid-liquid and ultrasonic extraction, as the ultrasonic extraction method was characterized by requiring less energy and time, and a smaller amount of solvent than the liquid-liquid extraction method, while this method is characterized by a lower yield of ethanolic extract. For more parsley leaves than the first method.

The cutting resulting from isolation using a chromatographic column from the ethanolic extract of the leaves resulting from extraction using the two previously mentioned methods is pure, and its purity was confirmed by thin layers and the appearance of a single spot, and the value of the retention coefficient was: RF= 0.88,

However, the ethanolic extract obtained by the first method was superior in that it contained a higher amount of the isolated compound (apigenin), which has a green colour. The isolation gave a compound with a higher yield using the liquid-liquid extraction method.

A comparison was conducted regarding the quantity of apigenin extracted from the ethanolic extract of parsley leaves in our present investigation, alongside many reference studies, as shown in Table 6.

Table 6: Comparison of the apigenin concentration of the parsley leaves extract produced by liquid-liquid and UAE extraction in this investigation with several reference studies on plant extracts containing apigenin.

Plant sample	Extraction method	AC (mg/g)	Ref
P. crispum L.	Liquid-liquid extraction	9.87 mg	This study
	UAE extraction	6.53 mg	
Chamomilla matricaria L.	SWE	3.33 mg	[20]
Scutellaria barbata D. Don	USC-CO2-E	2.43 mg	[21]

AC=apigenin content; UAE= ultrasonic extraction; SWE= subcritical water extraction; USC-CO2-E= ultrasound-assisted supercritical CO2 extraction.

We noticed from the previous table that the amount of apigenin isolated in our current study from the ethanolic extract of parsley leaves is higher than the extraction methods used in reference studies of other plants.

Chromatographic isolation of the ethanolic extract of plant leaves by a method (UAE) in our current study gave an amount of apigenin (6.53 mg/g) which is less than the amount isolated from the same plant in the reference study [22], which used the same extraction method, where the isolation gave an amount of apigenin equal to (9.48 mg/g).

When comparing the isolated amount of apigenin in our current study with reference studies [23-24], we noticed the superiority of the extraction methods used in our current study over other methods, such as pressurized liquid extraction, Vortex mixing respectively which demonstrated the inability to isolate apigenin from the plant extract.

Conclusions

The extraction using the liquid-liquid extraction method of parsley leaves using ethanol as a solvent was superior to the extraction using the ultrasound method in terms of the amount of extract.

- Ultrasonic extraction of parsley leaves is superior to liquid-liquid extraction in terms of saving time, energy, and the amount of solvent used.
- Column chromatographic and thin-layer isolation of the ethanolic extract of *Petroselinum crispum* L. leaves resulted in a highly pure compound. The identity of the isolated pure compound was determined using spectrophotometry (IR, NMR), and it is apigenin that belongs to flavonoids (flavones).
- The yield of apigenin isolated from the ethanolic extract of plant leaves using the liquid-liquid extraction method is higher than the yield using the Ultrasonic extraction method.

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