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COMPARATIVE EXTRACTION AND QUANTITATIVE ESTIMATION OF KHELLIN AND FUMARIC ACID FROM *AMMI MAJUS* L. AND *FUMARIA PARVIFLORA* L. BY HPLC AND HPTLC METHODS

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Abstract:
The present study was undertaken to evaluate different extraction techniques for the isolation and quantitative estimation of Khellin from Ammi majus *L. and Fumaric acid from* Fumaria parviflora *L. using UV spectrophotometric, HPLC, and HPTLC methods. Various extraction methods including maceration, reflux extraction, Soxhlet extraction, and ultrasound-assisted extraction (UAE) were employed using solvents of different polarities such as methanol, ethanol, chloroform, acetone, petroleum ether, and n-hexane. Among all extraction techniques, ultrasound-assisted extraction demonstrated the highest extraction efficiency for both phytoconstituents. The methanolic UAE extract of* Ammi majus *L. showed the maximum Khellin content (6.22% w/w), whereas methanolic UAE extract of* Fumaria parviflora *L. exhibited the highest Fumaric acid content (6.6% w/w). Quantitative estimation by HPLC revealed that the methanolic extract of* Ammi majus *L. contained 6.01% w/w of Khellin at a retention time of 12.394 minutes, while the methanolic extract of* Fumaria parviflora *L. contained 6.35% w/w of Fumaric acid at a retention time of 4.45 minutes. Calibration curves of standard Khellin and Fumaric acid showed good linearity between concentration and peak area, confirming the suitability of the developed HPLC method. HPTLC fingerprinting profiles of both plant extracts revealed the presence of several phytoconstituents at different Rf values. Khellin was identified at an Rf value of 0.73 in* Ammi majus *L. extract, whereas Fumaric acid was identified at an Rf value of 0.06 in* Fumaria parviflora *L. extract. Quantitative HPTLC analysis showed 6.38% w/w Khellin and 6.69% w/w Fumaric acid in methanolic extracts, respectively. The HPTLC findings were found to be comparable with HPLC results, confirming the reliability and reproducibility of both analytical methods. The results of the present investigation indicate that ultrasound-assisted extraction using methanol is an effective extraction method for isolation of Khellin and Fumaric acid. Furthermore, HPLC and HPTLC methods were found to be accurate, sensitive, rapid, and reproducible techniques for qualitative and quantitative analysis of these phytoconstituents. The study may be useful for standardization, quality control, and further pharmacological evaluation of these medicinal plants and their bioactive compounds.*
Keywords: Ammi majus *L.,* Fumaria parviflora *L., Khellin, Fumaric acid, Ultrasound-assisted extraction, HPLC, HPTLC, Phytochemical analysis, Quantitative estimation, Medicinal plants.*

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INTRODUCTION:

Medicinal plants have been used since ancient times as an important source of therapeutic agents for the treatment and prevention of various diseases. The growing interest in herbal medicines and plant-derived bioactive compounds has increased the need for scientific validation, standardization, and quality control of medicinal plants [1]. Phytoconstituents isolated from medicinal plants possess diverse pharmacological activities including antioxidant, antimicrobial, anti-inflammatory, anticancer, and immunomodulatory effects. Among these bioactive compounds, Khellin and Fumaric acid are considered important naturally occurring phytochemicals with significant therapeutic potential [2].

Ammi majus L., belonging to the family Apiaceae, is a medicinal plant widely distributed in Mediterranean and Asian regions. The plant is well known for the presence of furanochromones, particularly Khellin and Visnagin, which possess several pharmacological activities [3]. Khellin has gained considerable attention due to its bronchodilator, vasodilator, antispasmodic, anti-inflammatory, and photochemotherapeutic properties. Traditionally, *Ammi majus* L. has been used in the treatment of vitiligo, psoriasis, renal colic, and respiratory disorders. The therapeutic significance of Khellin has encouraged researchers to develop efficient extraction and analytical methods for its isolation and quantification from plant sources [4].

Similarly, *Fumaria parviflora* L., a member of the family Fumariaceae, is an important medicinal herb extensively used in traditional systems of medicine. The plant contains several bioactive constituents including alkaloids, flavonoids, phenolic compounds, and organic acids. Among these constituents, Fumaric acid is considered one of the major active compounds responsible for various biological activities such as antioxidant, hepatoprotective, anti-inflammatory, antimicrobial, and immunomodulatory effects. Fumaric acid derivatives are also clinically important in the management of psoriasis and multiple sclerosis. Therefore, quantitative estimation of Fumaric acid is essential for quality assessment and standardization of *Fumaria parviflora* L. extracts [5].

The extraction process plays a crucial role in obtaining maximum yield of phytoconstituents from plant materials. The efficiency of extraction depends on several factors including solvent polarity, extraction time, temperature, and extraction technique employed [6]. Conventional extraction methods such as maceration, reflux extraction, and Soxhlet extraction are widely used for phytochemical isolation; however, these methods often require longer extraction time, higher solvent consumption, and may lead to degradation of heat-sensitive compounds. In recent years, ultrasound-assisted extraction (UAE) has emerged as a promising alternative technique due to its advantages such as shorter extraction time, improved extraction efficiency, reduced solvent consumption, and enhanced recovery of phytoconstituents through ultrasonic cavitation [7].

Accurate qualitative and quantitative analysis of phytoconstituents is essential for herbal drug standardization and quality control. Chromatographic techniques such as High-Performance Liquid Chromatography (HPLC) and High-Performance Thin-Layer Chromatography (HPTLC) are extensively employed for identification, separation, and quantification of plant-derived compounds. HPLC is considered a highly sensitive, precise, and reproducible analytical technique for quantitative estimation of phytoconstituents, whereas HPTLC offers advantages such as simplicity, rapid analysis, low solvent consumption, and fingerprint profiling of herbal extracts. The combined use of HPLC and HPTLC provides reliable analytical information regarding the quality, purity, and phytochemical composition of herbal formulations and plant extracts [8].

Although several studies have reported the pharmacological importance of *Ammi majus* L. and *Fumaria parviflora* L., limited information is available regarding comparative evaluation of different extraction techniques for isolation of Khellin and Fumaric acid along with their simultaneous chromatographic characterization using HPLC and HPTLC methods. Therefore, the present study was designed to compare different extraction methods including maceration, reflux extraction, Soxhlet extraction, and ultrasound-assisted extraction for efficient isolation of Khellin and Fumaric acid from *Ammi majus* L. and *Fumaria parviflora* L., respectively. Furthermore, the study aimed to quantitatively estimate these phytoconstituents using validated HPLC and HPTLC methods and to establish chromatographic fingerprint profiles for quality evaluation and standardization of the plant extracts.

MATERIAL AND METHODS:

Material

The materials used in the present study included dried plant materials of *Ammi majus* L. and *Fumaria parviflora* L. for the extraction of Khellin and Fumaric acid, respectively. Standard Khellin and Fumaric acid were procured from Sigma, India.

Various analytical grade solvents such as methanol, ethanol, chloroform, acetone, petroleum ether, n-hexane, ethyl acetate, toluene, formic acid, acetonitrile, ammonium acetate, and diethylamine were used for extraction and chromatographic analysis. HPLC-grade methanol and other solvents were utilized for HPLC and HPTLC studies. Chromatographic analysis was carried out using a Shimadzu HPLC system equipped with a UV-visible detector and a Lichrospher C18 column, while HPTLC analysis was performed using aluminium-backed silica gel plates and a CAMAG Linomat applicator with WinCATS software.

Methods

Extraction of Khellin from *Ammi majus* L. and Fumaric Acid from *Fumaria parviflora* L. by Different Methods

Different extraction techniques including maceration, Soxhlet extraction, reflux extraction, and ultrasound-assisted extraction (UAE) were employed for the isolation of Khellin from *Ammi majus* L. and Fumaric acid from *Fumaria parviflora* L. Various solvents were used for extraction in order to determine the most suitable extraction method and solvent system for obtaining maximum yield of phytoconstituents [9].

Table 1: Solvents Used for Extraction of Khellin and Fumaric Acid

Phytocompound	Solvents Used
Khellin	Ethanol, Methanol, Chloroform, n-Hexane
Fumaric acid	Acetone, Chloroform, Methanol, Petroleum ether

Extraction by Maceration Technique

In the maceration method, 5–10 g of dried powdered plant material was extracted with different solvents in a drug-to-solvent ratio of 1:10 for four to five days at room temperature. The obtained extracts were filtered and concentrated under reduced pressure using a rotary vacuum evaporator (HAHN SHIN, HS-2005 V-N, Korea) in an inert atmosphere. The concentrated extracts were dried completely, reconstituted with suitable solvent, weighed, and preserved for further analysis.

Extraction by Reflux Technique

The dried plant materials of *Ammi majus* L. and *Fumaria parviflora* L. were coarsely powdered and subjected to hot solvent extraction using a reflux condenser assembly for six hours with different solvents. The obtained extracts were concentrated under reduced pressure to obtain a semisolid mass. The extracts were then reconstituted with solvent, evaporated to dryness, weighed, and stored for further studies.

Extraction by Soxhlet Apparatus

The coarse powdered plant materials of *Ammi majus* L. and *Fumaria parviflora* L. were extracted separately using a Soxhlet apparatus for six hours with different solvents in a drug-to-solvent ratio of 1:10. The extracts obtained were concentrated at 40°C under reduced pressure using a rotary vacuum evaporator (HAHN SHIN, HS-2005 V-N, Korea) to obtain a concentrated mass. The extracts were subsequently reconstituted with solvent, dried completely, weighed, and stored for further investigation.

Ultrasound-Assisted Extraction (UAE) Technique

Ultrasound-assisted extraction was carried out using coarse powdered plant materials of *Ammi majus* L. and *Fumaria parviflora* L. The extraction process was performed for 20 minutes at 50°C using different solvents. The obtained extracts were concentrated under reduced pressure to obtain a crude mass. The extracts were further reconstituted with suitable solvent, evaporated to dryness, weighed accurately, and preserved for further analysis.

Analytical Methodology

Quantitative Analysis of Khellin and Fumaric Acid by UV Spectrophotometric Method

A simple, rapid, economical, and reproducible UV-spectrophotometric method was employed for the quantitative estimation of Khellin in *Ammi majus* L. extracts and Fumaric acid in *Fumaria parviflora* L. extracts. The method provided reliable and accurate results for determining the concentration of the phytoconstituents extracted using various extraction techniques [10].

Preparation of Calibration Curve of Khellin and Fumaric Acid

A stock standard solution was prepared by dissolving 1 mg of standard Khellin or Fumaric acid (Sigma, India) in 10 ml of methanol. From the stock solution, working standard solutions of concentrations 2, 4, 8, 16, and 20 µg/ml were prepared using methanol as solvent. The UV spectrum of the 8 µg/ml standard solution was scanned to determine the λmax value of the compounds.

Preparation of Sample Solutions

Accurately weighed 10 mg of each extract was transferred into a 10 ml volumetric flask and dissolved in methanol to obtain a final concentration of 1000 µg/ml. From this solution, 500 µl was diluted with methanol to prepare a final concentration of 50 µg/ml. The absorbance of the sample solutions was measured between 200–400 nm. The selected λmax values for Khellin and Fumaric acid were found to be 282 nm and 230 nm

respectively, based on the peak reports of standard compounds.

Quantitative Analysis of Khellin and Fumaric Acid by HPLC Technique

Quantitative estimation of Khellin and Fumaric acid was performed using a Shimadzu HPLC Quaternary System equipped with LC-10AT VP pumps and a UV-visible detector. A reverse-phase Lichrospher C18 column (25 × 4.6 mm, 5 µm particle size; Merck, Germany) was used for chromatographic separation. Samples were injected through a 20 µl fixed loop Rheodyne injector. Standard and sample solutions were filtered through a 0.2 µm syringe filter (Axiva) prior to injection [11].

Table 1: Chromatographic Conditions

S. No.	Phytocompound	Mobile Phase	Flow Rate	Retention Time	Wavelength
a.	Khellin from *Ammi majus* L.	Methanol : Water (75:25 v/v) (Isocratic mode)	1.5 ml/min	15 min	250 nm
b.	Fumaric acid from *Fumaria parviflora* L.	Acetonitrile : Ammonium acetate (70:30 v/v) (Isocratic mode)	1.5 ml/min	15 min	220 nm

Preparation of Standard Calibration Curve

A stock solution of standard compounds (1 mg/ml) was prepared in HPLC-grade methanol. Various working standard solutions were prepared by suitable dilution and stored at –20°C. All solutions were filtered through a 0.2 µm membrane filter (Axiva). Calibration curves were constructed by plotting concentration versus peak area for both Khellin and Fumaric acid.

Preparation of Sample Solution for HPLC Analysis

Ten milligrams of each extract of *Ammi majus* L. and *Fumaria parviflora* L. was dissolved in HPLC-grade methanol to obtain a concentration of 1 mg/ml. The solution was filtered through a 0.2 µm membrane filter, and 20 µl of the filtrate was injected into the HPLC system. The content of Khellin and Fumaric acid in the extracts was calculated using the calibration curve equations.

High-Performance Thin-Layer Chromatography (HPTLC)

Sample Preparation and Extraction

Ten grams of powdered plant material were extracted using different solvents and extraction techniques for three hours. The obtained extracts were concentrated under vacuum and re-dissolved in appropriate solvents prior to HPTLC fingerprinting analysis.

Chromatographic Analysis

Thin-layer chromatography was performed on aluminium-backed HPTLC plates (E. Merck, Germany). Sample solutions (1 µl) were applied as 4 mm wide bands using a CAMAG Linomat applicator. Plates were developed in a CAMAG twin-trough chamber using suitable mobile phases as shown in Table. The developed plates were air-dried to remove residual mobile phase and scanned at the selected wavelengths.

Table 2: Mobile Phases Used for HPTLC Fingerprinting

S. No.	Phytocompound	Mobile Phase	Wavelength
a.	*Ammi majus* L. extract	Ethyl acetate : Toluene : Formic acid (5.4:4:0.5 v/v/v)	254 nm
b.	*Fumaria parviflora* L. extract	Toluene : Ethyl acetate : Diethylamine (7.5:2:0.5 v/v/v)	260 nm

Quantitative Estimation of Khellin and Fumaric Acid by HPTLC Technique

Selection of Mobile Phase and Wavelength

Suitable mobile phases and detection wavelengths were selected to achieve optimum chromatographic separation of Khellin and Fumaric acid. Standard and extract samples were analyzed after post-chromatographic derivatization for quantitative estimation.

Preparation of Calibration Curve and Standard Solutions

Standard stock solutions were prepared by dissolving 1 mg of Khellin and Fumaric acid separately in 1 ml of solvent. Different spot volumes ranging from 0.1–1.0 µl were applied on HPTLC plates to obtain calibration curves.

Preparation of Sample Solutions

The extracts of *Ammi majus* L. and *Fumaria parviflora* L. were dissolved in HPLC-grade solvent to obtain a final concentration of 1 mg/ml. The solutions were filtered through a 0.2 µm membrane filter and 2 µl of the filtrate was applied to the HPTLC plates for analysis. The concentration of Khellin and Fumaric acid in the extracts was calculated using calibration curve equations.

Chromatographic Analysis

HPTLC analysis was carried out on aluminium-backed HPTLC plates using a CAMAG Linomat 5 applicator. Samples were applied as 4 mm wide bands at a distance of 10 mm from the base of the plate. Nitrogen gas was used during spotting to

facilitate drying. The plates were developed in a CAMAG twin-trough chamber using the selected mobile phases. After development, plates were dried completely and scanned using WinCATS software in absorption-reflection mode with deuterium and tungsten lamps [12].

RESULTS AND DISCUSSION:

The present study was carried out to evaluate different extraction techniques for the isolation and quantitative estimation of Khellin from *Ammi majus* L. and Fumaric acid from *Fumaria parviflora* L. using UV spectrophotometry, HPLC, and HPTLC techniques. The investigation also aimed to compare the efficiency of various solvents and extraction methods for obtaining maximum phytoconstituent yield.

Different extraction methods including maceration, reflux extraction, Soxhlet extraction, and ultrasound-assisted extraction (UAE) were employed using solvents of varying polarity. The obtained results clearly demonstrated that both extraction method and solvent type significantly influenced the extraction efficiency of Khellin and Fumaric acid. Among the solvents used, methanol showed the highest extraction efficiency for both phytoconstituents in all extraction techniques. This may be attributed to the polar nature of methanol, which enhances penetration into plant tissues and improves solubilization of phytoconstituents.

In the maceration method, methanolic extract of *Ammi majus* L. showed the highest Khellin content (4.02% w/w), while methanolic extract of *Fumaria parviflora* L. demonstrated the highest Fumaric acid content (5.3% w/w). Ethanol and acetone extracts also exhibited appreciable phytoconstituent content, whereas non-polar solvents such as n-hexane and petroleum ether showed comparatively poor extraction efficiency. The results suggest that polar solvents are more effective for extraction of Khellin and Fumaric acid than non-polar solvents.

The reflux extraction method produced comparatively lower phytoconstituent yield than other extraction techniques. Methanolic extracts obtained by reflux extraction contained 2.64% w/w of Khellin and 3.2% w/w of Fumaric acid. The lower extraction efficiency observed in reflux extraction may be due to prolonged heating, which could lead to partial degradation of thermolabile compounds. Similar findings have been reported in previous studies where heat-sensitive phytoconstituents showed reduced recovery after prolonged thermal treatment.

The Soxhlet extraction method demonstrated moderate extraction efficiency with methanolic extracts showing 3.09% w/w of Khellin and 4.3% w/w of Fumaric acid. Continuous solvent recycling and prolonged contact between solvent and plant matrix may have contributed to improved extraction efficiency compared to reflux extraction. However, the extraction yield was still lower than that obtained by UAE technique.

Among all extraction methods employed, ultrasound-assisted extraction (UAE) showed the highest extraction efficiency for both phytoconstituents. Methanolic UAE extracts exhibited 6.22% w/w of Khellin and 6.6% w/w of Fumaric acid. The superior performance of UAE may be attributed to ultrasonic cavitation, which enhances solvent penetration, disrupts plant cell walls, and facilitates rapid release of intracellular compounds into the extraction medium. In addition, UAE reduces extraction time and minimizes thermal degradation of bioactive constituents. Therefore, UAE can be considered a highly efficient and economical extraction method for phytochemical isolation.

The HPLC method developed for quantitative estimation of Khellin and Fumaric acid demonstrated good resolution, reproducibility, and sensitivity. Standard Khellin exhibited a retention time of 12.56 minutes, while the methanolic UAE extract of *Ammi majus* L. showed a corresponding peak at 12.394 minutes. Quantitative analysis revealed that the extract contained 6.01% w/w of Khellin. Similarly, standard Fumaric acid showed a retention time of 4.61 minutes, whereas the methanolic UAE extract of *Fumaria parviflora* L. displayed a peak at 4.45 minutes with a Fumaric acid content of 6.35% w/w.

The calibration curves prepared for both Khellin and Fumaric acid demonstrated excellent linearity between concentration and peak area, confirming the suitability of the HPLC method for quantitative analysis. The slight variation in retention time between standard and sample chromatograms may be due to the presence of additional phytoconstituents in the extracts.

HPTLC fingerprinting analysis of methanolic extracts of *Ammi majus* L. and *Fumaria parviflora* L. revealed the presence of multiple phytoconstituents at different Rf values. In *Ammi majus* L. extract, Khellin was identified at an Rf value of 0.73, whereas Fumaric acid was detected at an Rf value of 0.06 in *Fumaria parviflora* L. extract. The presence of several additional peaks in both extracts indicates the existence of various secondary metabolites that may contribute to the therapeutic potential of these medicinal plants.

The HPTLC quantitative analysis also demonstrated good linearity between concentration and peak area for both Khellin and Fumaric acid.

Quantification of Khellin in methanolic extract by HPTLC revealed 6.38% w/w content, while Fumaric acid content was found to be 6.69% w/w. These values were in close agreement with HPLC analysis, confirming the reliability, precision, and reproducibility of both analytical techniques.

The regression coefficients obtained from HPTLC calibration plots for Khellin (0.9908) and Fumaric acid (0.9927) further support the accuracy and linearity of the developed method. The close agreement between HPLC and HPTLC findings validates the analytical procedures used in the present study.

The study demonstrated that ultrasound-assisted extraction combined with methanol as solvent was the most effective approach for extraction of Khellin and Fumaric acid from *Ammi majus* L. and *Fumaria parviflora* L., respectively. Furthermore, HPLC and HPTLC methods proved to be accurate, reliable, sensitive, and reproducible techniques for qualitative and quantitative estimation of these phytoconstituents. The obtained results may be useful for standardization, quality control, and further pharmacological investigation of these medicinal plants and their bioactive compounds.

Table 3: Percentage Content (% w/w) of Khellin and Fumaric Acid in Different Solvent Extracts by Maceration Method

S. No.	Khellin Sample	Content of Khellin (% w/w)	Fumaric Acid Sample	Content of Fumaric Acid (% w/w)
1	Ethanol	3.28	Acetone	4.2
2	Methanol	4.02	Chloroform	3.3
3	Chloroform	2.42	Methanol	5.3
4	n-Hexane	1.5	Petroleum Ether	2.5

Table 4: Percentage Content (% w/w) of Khellin and Fumaric Acid in Different Solvent Extracts by Reflux Technique

S. No.	Khellin Sample	Content of Khellin (% w/w)	Fumaric Acid Sample	Content of Fumaric Acid (% w/w)
1	Ethanol	1.85	Acetone	2.8
2	Methanol	2.64	Chloroform	1.4
3	Chloroform	2.24	Methanol	3.2
4	n-Hexane	0.9	Petroleum Ether	1.1

Table 5: Percentage Content (% w/w) of Khellin and Fumaric Acid in Different Solvent Extracts by Soxhlet Method

S. No.	Khellin Sample	Content of Khellin (% w/w)	Fumaric Acid Sample	Content of Fumaric Acid (% w/w)
1	Ethanol	2.25	Acetone	3.8
2	Methanol	3.09	Chloroform	2.8
3	Chloroform	1.62	Methanol	4.3
4	n-Hexane	1.1	Petroleum Ether	1.9

Table 6: Percentage Content (% w/w) of Khellin and Fumaric Acid in Different Solvent Extracts by UAE Technique

S. No.	Khellin Sample	Content of Khellin (% w/w)	Fumaric Acid Sample	Content of Fumaric Acid (% w/w)
1	Ethanol	4.24	Acetone	5.1
2	Methanol	6.22	Chloroform	3.9
3	Chloroform	3.12	Methanol	6.6
4	n-Hexane	2.1	Petroleum Ether	2.8

Table 7: Percentage Content (% w/w) of Khellin and Fumaric Acid in Different Solvent Extracts by UAE Technique

S. No.	Khellin Sample	Content of Khellin (% w/w)	Fumaric Acid Sample	Content of Fumaric Acid (% w/w)
1	Ethanol	4.24	Acetone	5.1
2	Methanol	6.22	Chloroform	3.9
3	Chloroform	3.12	Methanol	6.6
4	n-Hexane	2.1	Petroleum Ether	2.8

Table 8: Concentration of Standard Khellin and Corresponding Area Under Curve (AUC)

S. No.	Concentration of Standard Khellin (µg/ml)	Area Under Curve (AUC)
1	20	276048
2	40	336668
3	60	465851
4	80	548621
5	100	652102

Table 9: Khellin Content in Methanolic Extract of *Ammi majus* L. by HPLC

Compound	Extract	Retention Time	Content (% w/w)
Ammi majus L.	10 mg	12.394 min	6.01%

Table 10: Concentration of Standard Fumaric Acid and Corresponding Area Under Curve (AUC)

S. No.	Concentration of Standard Fumaric Acid (µg/ml)	Area Under Curve (AUC)
1	20	1624729
2	40	2725835
3	60	3393674
4	80	4465875
5	100	5350637

Table 11: Fumaric Acid Content in *Fumaria parviflora* L. Extract by HPLC

Compound	Extract	Retention Time	Content (% w/w)
Fumaria parviflora L.	10 mg	4.45 min	6.35%

Table 12: HPTLC Fingerprinting Profile of Methanolic Extract of *Ammi majus* L.

Peak	Rf Value	Area (AU)	Compound
1	0.02	124.7	Unknown
2	0.09	216.5	Unknown
3	0.12	362.7	Unknown
4	0.19	138.5	Unknown
5	0.24	129.0	Unknown
6	0.34	59.7	Unknown
7	0.49	29.5	Unknown
8	0.61	26.2	Unknown
9	0.73	126.8	Khellin
10	0.93	78.9	Unknown

Table 13: HPTLC Fingerprinting Profile of Methanolic Extract of Fumaria parviflora L.

Peak	Rf Value	Area (AU)	Compound
1	0.03	10.3	Unknown
2	0.06	672.0	Fumaric acid
3	0.09	136.6	Unknown
4	0.16	88.8	Unknown
5	0.27	20.2	Unknown
6	0.36	19.6	Unknown
7	0.86	28.1	Unknown

Table 14: Concentration of Standard Khellin versus Area

S. No.	Concentration (µl)	Track No.	Rf Value	Area (AU)
1	0.1	1	0.74	214.4
2	0.2	2	0.73	494.0
3	0.4	3	0.72	601.9
4	0.8	4	0.72	613.0
5	1.0	5	0.72	477.7

Table 15: Khellin Content in Methanolic Extract of *Ammi majus* L. by HPTLC

S. No.	*Ammi majus* L. Extract	Rf Value	Content of Khellin (% w/w)
1	10 mg	0.73	6.38%

Table 16: Concentration of Standard Fumaric Acid versus Area

S. No.	Concentration (µl)	Track No.	Rf Value	Area (AU)
1	0.2	1	0.10	145.8
2	0.4	2	0.06	195.6
3	0.6	3	0.09	227.3
4	0.8	4	0.06	262.2
5	1.0	5	0.09	312.8

Table 17: Fumaric Acid Content in Methanolic Extract of *Fumaria parviflora* L. by HPTLC

S. No.	*Fumaria parviflora* L. Extract	Rf Value	Content of Fumaric Acid (% w/w)
1	10 mg	0.06	6.69%

CONCLUSION:

The present study demonstrated that ultrasound-assisted extraction (UAE) using methanol was the most effective method for extracting Khellin from *Ammi majus* L. and Fumaric acid from *Fumaria parviflora* L. Among all extraction techniques, UAE produced the highest phytoconstituent yield. HPLC and HPTLC methods successfully quantified and characterized both compounds with good accuracy, sensitivity, and reproducibility. The chromatographic fingerprint profiles confirmed the presence of Khellin and Fumaric acid in the methanolic extracts. Overall, the study established efficient extraction procedures and reliable analytical methods for the quality control and standardization of these medicinal plants and their bioactive constituents.

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