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HPLC Estimation of Khellin in *Ammi majus* L. Extracts and Fumaric acid in *Fumaria parviflora* L. Extracts

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Abstract

This study presents the quantitative analysis of two important phytocompounds: Khellin from *Ammi majus* L. and Fumaric acid from *Fumaria parviflora* L. using High-Performance Liquid Chromatography (HPLC). The analysis was conducted by optimizing chromatographic conditions, developing calibration curves, and applying these to determine the concentration of these bioactive compounds in the plant extracts. The results show that *Ammi majus* contains 6.01% Khellin, and *Fumaria parviflora* contains significant levels of Fumaric acid. This analytical approach highlights the precision and applicability of HPLC in herbal phytocompound analysis.

Keywords: Khellin, Fumaric Acid, *Ammi majus* L., *Fumaria parviflora* L., HPLC, Quantitative Analysis

Introduction

Natural products are increasingly being studied for their medicinal and therapeutic potential, and plants like *Ammi majus* L. and *Fumaria parviflora* L. are key sources of bioactive compounds such as Khellin and Fumaric acid, respectively. Khellin, a furanochromone, has been recognized for its vasodilatory, anti-inflammatory, and antispasmodic properties, while Fumaric acid, a dicarboxylic acid, is widely known for its role in the treatment of skin conditions such as psoriasis and its antioxidant properties [1]. Given the therapeutic relevance of these compounds, precise quantification in herbal extracts is critical. High-Performance Liquid Chromatography (HPLC) is an ideal tool for this purpose due to its high precision, sensitivity, and reproducibility in analyzing complex mixtures of phytochemicals [2-4].

In this study, we aim to quantitatively analyze Khellin and Fumaric acid in extracts of *Ammi majus* and *Fumaria parviflora*, respectively, using HPLC. The study also seeks to optimize chromatographic conditions to achieve reliable separation and quantification of these compounds.

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Material and Method

Plant Material Procurement and Authentication

Procurement of plant material: The plant materials, *Ammi majus* L. and *Fumaria parviflora* L., were collected from the Herbal Garden and authenticated by a taxonomist from the Department of Botany at SCLS.

Preparation of plant material: The plant materials were cleaned, air-dried, and ground into powder using a grinder (Sujata Powermatic plus, 810 watts). The powdered samples were stored in airtight containers until further use.

Quantitative Analysis by HPLC Technique

The quantitative analysis of Khellin and Fumaric acid was performed using the HPLC Quaternary System (Shimadzu, Japan) with a UV-visible detector. A Lichrospher C18 reverse-phase column (5 µm, 25 x 4.6 mm) was used for the separation of both compounds [5]. The mobile phases and chromatographic conditions are described in Table 1.

Table-1: Chromatographic Conditions

Phytocompound	Mobile Phase	Flow Rate	Retention Time	Wavelength
Khellin	Methanol (75:25 v/v) [6]	1.5 ml/min	12.56 mins	250 nm
Fumaric Acid	Acetonitrile acetate (70:30 v/v) [7]	1.5 ml/min	4.61 mins	220 nm

Standard Calibration Curve Preparation

Stock solutions of 1 mg/ml of Khellin and Fumaric acid were prepared in HPLC-grade methanol. Dilutions were made to produce a range of concentrations (20 µg/ml to 100 µg/ml), filtered through a 0.2 µm membrane filter, and injected into the HPLC system [8]. Calibration curves were plotted for concentration versus peak area to develop the standard equation for quantifying the compounds in plant extracts.

Sample Solution Preparation

Extracts of *Ammi majus* L. and *Fumaria parviflora* L. were prepared by dissolving 10 mg of each extract in HPLC-grade methanol to achieve a concentration of 1 mg/ml. The solutions were filtered using a 0.2 µm membrane filter before being injected into the HPLC system for analysis [9].

Results and Discussion

Quantitative Analysis of Khellin in *Ammi majus* L. Extracts

The calibration curve for Khellin was developed using five concentrations of the standard solution (20 µg/ml to 100 µg/ml). The retention time for Khellin was found to be 12.56 minutes, and the calibration plot showed a linear relationship between concentration and peak area (R^2 = 0.9986) [10]. The equation derived from the plot was used to quantify Khellin in the extract.

The content of Khellin in the methanolic extract of *Ammi majus* (obtained via ultrasonic-assisted extraction) was calculated as 6.01% w/w, with a retention time of 12.39 minutes (Figure 2) [12].

Quantitative Analysis of Fumaric Acid in *Fumaria parviflora* L. Extracts

The calibration curve for Fumaric acid was prepared using the same concentration range (20 µg/ml to 100 µg/ml). The retention time for Fumaric acid was 4.61 minutes. The equation obtained from the calibration curve allowed for the quantification of Fumaric acid in the extract [13].

Table -2: Linearity data for Khellin

Concentration of Khellin (µg/ml)	Area under the curve (AUC)
20	276048
40	336668
60	465851
80	548621
100	652102

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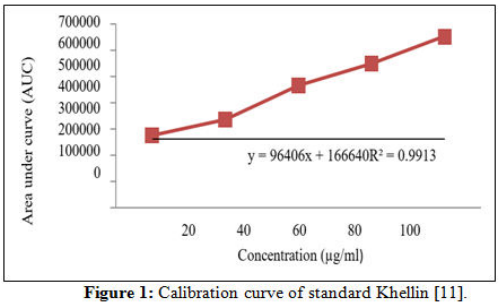

Figure 1: Calibration curve of standard Khellin [11].

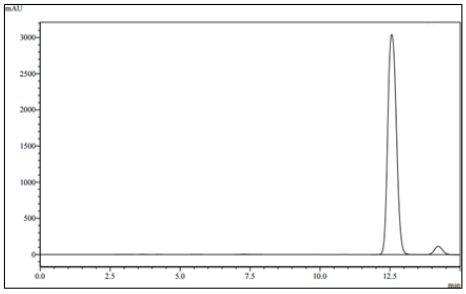

Figure 2: HPLC chromatogram of standard Khellin (100µg/ml)

Table -3: Linearity data for Fumaric acid

Concentration of Fumaric Acid (µg/ml)	Area under the curve (AUC)
20	1624729
40	2725835
60	3393674
80	4465875
100	5350637

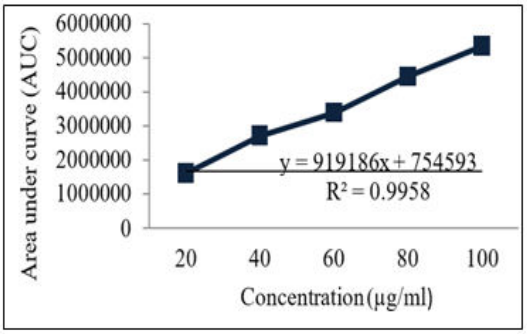

Figure 3: Calibration curve of standard Fumaric acid [14].

The HPLC analysis of the methanolic extract of *Fumaria parviflora* showed the presence of Fumaric acid with significant retention time and content in the extract [15].

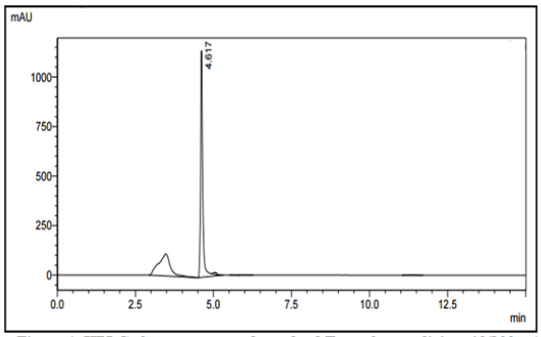

Figure 4: HPLC chromatogram of standard Trans-butenedioic acid(100µg/ml); Retentiontime-4.61minutes

The peak area with the retention time was utilised to determine the amount of Trans-butenedioic acid in the extract. To determine the amount of Trans-butenedioic acid inthe extract, an equation from the calibration curve was employed. The plot equationwasasfollows:Y=919186x+754593

Table 4: Trans-butenedioic acid content in Pitpapra extract by HPLC

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

The content of trans butenedioic acid was found to be 6.35% in UAE methanoli cextract (Retentiontime-4.45mins)

Conclusion

This study demonstrated the successful application of HPLC in the quantitative analysis of Khellin and Fumaric acid in extracts of *Ammi majus* and *Fumaria parviflora*, respectively. The content of Khellin in the methanolic extract of *Ammi majus* was found to be 6.01%, and Fumaric acid was quantified in the *Fumaria parviflora* extract using an optimized HPLC method. These results contribute to the understanding of the phytochemical profiles of these plants, facilitating their use in pharmaceutical and therapeutic applications.

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