

## Research Article

# Identification and Quantification of Coumarins in Four *Ferulago* Species (*Apiaceae*) Growing in Turkey by HPLC-DAD

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Received Date: 08 August, 2017; Accepted Date: 12 September, 2017; Published Date: 29 September, 2017

## Abstract

*Ferulago* species have been used since ancient times for the treatment of intestinal worms, hemorrhoids and as tonic, digestive, aphrodisiac and sedative. They have also been used as salad or spice due to their special odors. Samples were analyzed on a ACE 5 CN S/N-A21532 column (250 x 4.6 mm, 10 µm) with hexane and ethyl acetate as mobile phases at a flow rate of 1.0 mL x min<sup>-1</sup> and the method was successfully validated according to ICH guideline acceptance criteria for selectivity, linearity, precision, and accuracy. The structures of isolated compounds were elucidated by detailed analyses of 1D and 2D NMR and HR-ESI-MS data. Osthol (1), prantschimgin (2), felamidin (3) and umbelliferone (4) contents of roots and aerial parts from *F. blancheana*, *F. pachyloba*, *F. trachycarpa* *F. bracteata* were found to be 0.39, 2.76, 1.26, 0.20, 0.10, 1.07, 0.26, 0.05; 0.14, 2.83, 0.56, 0.44, 0.23, 0.59, 0.26, 0.11; 0.11, 2.45, 1.29, 0.24, 0.27, 0.72, 0.43, 0.26 and 0.30, 2.28, 0.48, 0.21, 0.40, 0.83, 0.78, 0.05 %, respectively. A new Coumarin, peucedanol-2'-benzoate (5) was only found to be from roots of *F. bracteata* 1.12 %.

## Keywords

*Apiaceae*; Coumarin; *Ferulago*; Peucedanol-2'-benzoate; Validation; HPLC

## Introduction

*Ferulago* W Koch, belonging to *Apiaceae* family, is represented by approximately 50 taxa throughout the world and 34 taxa in

Turkey 19 of which are endemic. Therefore, Anatolia is considered to be the gene center of this genus [1]. *F. blancheana*, *F. pachyloba* and *F. bracteata* are endemic perennial species, growing only in Kayseri, Nigde and Gaziantep, Turkey, respectively [2,3].

*Ferulago* species have been used in folk medicine for their aphrodisiac, digestive, tonic, sedative, antiseptic, carminative and vermifuge properties as well as for the treatment of hemorrhoids, ulcers, snake bites, spleen diseases and headache [4,5]. Except medicinal usage, they have been consumed as salad or spice due to their special odor, also as food for goats and deer's [6].

Previous phytochemical studies indicated that Coumarins are the most common metabolites on *Ferulago* species [7]. Coumarins have various biological activities such as anticancer [8,9], antiinflammatory [10,11], anticoagulant [12,13], antiadipogenic [14], antitubercular [15], antihyperglycemic [16,17], antiviral [18], antifungal [19,20], antibacterial [21], antihypertensive [22,23], anticonvulsant [24], antioxidant [25,26], neuroprotective [27] and antidiabetic [28,29].

This study aims to give first report to identify and establish the content of several Coumarins like osthol, prantschimgin, felamidin, umbelliferone in *F. blancheana*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata* by using HPLC-DAD. Also, a new Coumarin, peucedanol-2'-benzoate was isolated from roots of *F. bracteata* and established the content of in all species. Furthermore, the method was validated on the basis of selectivity, linearity, precision, and accuracy. To the best of our knowledge, this is the first report on the HPLC analysis and isolation of Coumarins from *F. blancheana*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata*.

## Material and Methods

### Plant material

The aerial parts and roots of *Ferulago blanchiana*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata* were collected during flowering time in 2014 from Kayseri, Niğde, Antalya and Gaziantep (Turkey),

respectively and identified by Prof. Dr. Hayri Duman, a plant taxonomist in the Department of Biological Sciences, Faculty of Art and Sciences, Gazi University. The voucher specimens are kept in the Herbarium of Ankara University, Faculty of Pharmacy (Herbarium numbers are AEF 26673, AEF 26674, AEF 26677 and AEF 26676, respectively). Collected locations of these species are given in table 1.

Species	Collected Location	Voucher Numbers
<i>F. blanchiana</i>	B6 Kayseri: Between Pınarbası-Sarız, 4 Km to Sarız, rocky slopes, 1633 m, 13.07.2014.	AEF 26673
<i>F. pachyloba</i>	C5 Niğde: Camardı Demirkazık village, Cimbar channel, rocky slopes, 1696 m, 13.07.2014.	AEF 26674
<i>F. trachycarpa</i>	C3 Antalya: Akseki-Seydisehir road 35. Km, calcareous slopes, 1785 m, 12.07.2014.	AEF 26677
<i>F. bracteata</i>	C6 Gaziantep: Nurdagi Antep road 22. Km, calcareous cliff slopes, 1642 m, 14.07.2014.	AEF 26676

**Table 1:** Collected locations of *Ferulago* species.

### Reagents and chemicals

Hexane and ethyl acetate were HPLC-grade (Sigma, Germany). Analytical grade solvents of dichloromethane, ethyl acetate (Merck, Darmstadt, Germany), *n*-butanol (Analar NORMAPUR, Fontenay, France), methanol (Sigma, France), sulphuric acid (Merck, Germany), and also silica gel (Merck-737, Germany) were used. Osthol, prantschimgin, felamidin and umbelliferone were isolated from the dichloromethane extracts of *F. blanchiana*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata* roots using silica gel columns. NMR spectra were recorded on a Varian Mercury Plus at 400 MHz for <sup>1</sup>H NMR and 100 MHz for <sup>13</sup>C NMR by using TMS as the internal standard. The chemical shifts were measured in CDCl<sub>3</sub>. Mass spectra were recorded on a Waters Micromass ZQ Mass Spectrophotometer ESI and Agilent 6530 Accurate- Mass Q-TOF LC/MS. UV spectra were measured using Thermo Scientific Multiskan GO microplate and cuvette spectrophotometer. IR spectra were run on a Bruker VERTEX 70v FT-IR Spectrophotometer.

TLC analyses were carried out on silica gel F<sub>254</sub> TLC plates aluminum sheets (Merck). Compounds were detected by UV fluorescence and spraying 1% H<sub>2</sub>SO<sub>4</sub> reagent, followed by heating at 105°C for 1-2 min.

### Extraction, fractionation and isolation

Extracts were obtained from the aerial parts and roots of *Ferulago blanchiana*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata* with Methanol (MeOH). Aerial parts and roots were grounded and macerated (Heidolph MR3001, Germany) for 8 hours/3 days with methanol in a water bath not exceeding 45°C using a Heidolph mechanical mixer (300 rpm). The extracts, filtered and concentrated till dryness using a Heidolph rotary evaporator (Heidolph VV2000, Germany), were dispersed in methanol-water (1:9) and fractionated three times with 400 mL of dichloromethane, ethyl acetate, *n*-butanol and aqueous residue in a separatory funnel, respectively. Each fraction was then concentrated to dryness. Amounts of the powdered plants and obtained extracts are given in table 2.

Species	Used parts	Powdered (g)	MeOH (g)	CHCl <sub>3</sub> (g)	EtOAc (g)	<i>n</i> -BuOH (g)	Aqueous residue (g)
<i>F. blanchiana</i>	Root	750	86.62	28.52	2.32	12.24	23.35
	Aerial part	50	3.22	1.89	0.46	0.57	0.39
<i>F. pachyloba</i>	Root	600	83.25	23.63	1.53	13.13	21.29
	Aerial part	50	3.32	1.78	0.45	0.59	0.45
<i>F. trachycarpa</i>	Root	450	86.77	26.29	2.41	13.55	22.08
	Aerial part	50	3.41	1.67	0.5	0.61	0.55
<i>F. bracteata</i>	Root	450	60.94	17.96	2.44	14.98	13.98
	Aerial part	50	3.65	1.55	0.61	0.59	0.61

**Table 2:** Amounts of the powdered plants and obtained extracts.

As a result of the previous bioguided fractionation study [30], the effective dichloromethane extract was first submitted to a silica gel column (Merck 737, 70-230 Mesh) and eluted with a gradient of *n*-hexane:ethyl acetate (100:0 → 0:100, v/v) and ethyl acetate:methanol (100:0 → 0:100, v/v), and five fractions

(Fr. A-E) were obtained. Repetitive silica gel column chromatography with *n*-hexane-ethyl acetate (90:10 and 95:5) solvent system on Fr. A gave compound 1 (220 mg). Eluting with *n*-hexane-ethyl acetate (80:20) over silica gel column of Fr. B gave compound 2 (420 mg). Fr. C was fractionated by column chromatography over

silica gel using *n*-hexane:ethyl acetate mixtures (70:30 and 90:10) consecutively and compound 3 was obtained (330 mg). Fr. D was submitted on a silica gel column using *n*-hexane:ethyl acetate (65:35) and the resulting fraction was chromatographed on silica gel column using *n*-hexane:ethyl acetate (90:10) to give compound 4. Fr. E gave compound 5 (240 mg), eluting with *n*-hexane-ethyl acetate (80:20) over silica gel column. Compounds 1, 2, 3 and 4 were isolated by the same chromatographic methods in all species. Compound 5 was only isolated from dichloromethane fraction from the roots of *F. bracteata*.

### Peucedanol-2'-benzoate (5)

White powder; IR  $\nu_{\max}$  (KBr)  $\text{cm}^{-1}$ : 1702, 1623, 1565; UV  $\lambda_{\max}$  ( $\text{CH}_2\text{Cl}_2$ ) nm (log  $\epsilon$ ): 350 (4.20);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) spectroscopic data, (Table 3); HR-ESI-MS at  $m/z$  367.1999 [ $\text{M}-\text{H}$ ] $^-$  (Calcd for  $\text{C}_{21}\text{H}_{19}\text{O}_6$  367.1181).

No	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$
2		161.38
3	6.26 (d, 9.4)	112.37
4	7.64 (d, 9.4)	143.62
5	7.28 (s)	123.22
6		124.55
7		163.49
8	6.80 (s)	98.01
9		155.84
10		112.71
1'	3.38 (m)	29.67
2'	5.16 (dd, 9.2, 7.3)	89.12
3'		82.93
4'	1.72 (s)	22.16
5'	1.71 (s)	21.38
1''		165.4
2''		131.04
3''	7.73 (m)	129.39
4''	7.32 (m)	128.27
5''	7.51 (m)	132.86
6''	7.32 (m)	128.27
7''	7.73 (m)	129.39

**Table 3:**  $^1\text{H}$  NMR (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data of 5 in  $\text{CDCl}_3$ .

### Preparation of sample solutions and standard solutions

Quantitative determinations of osthol, prantschimgin, umbelliferone, felamidin and peucedanol-2'-benzoate obtained from the dichloromethane fractions of the roots of these species were performed as follows: 5 mg of the isolated compounds were weighed precisely, then dissolved in ethyl acetate and completed to 10 mL with in a volumetric flask. The retention times of reference compounds were determined individually. Then 5  $\mu\text{L}$  of different dilutions were injected to the HPLC device 3 times

and the calibration curve was established by plotting peak areas against the curve equation. The extract was transferred into a 10 mL volumetric flask which was made up to its volume with ethyl acetate and filtered through a 0.45  $\mu\text{m}$  Econofilter (Agilent Technologies, USA) prior to injection into the HPLC system. Chromatograms obtained from dichloromethane extracts of plants were compared with standard chromatograms.

### HPLC analysis

All analyses were performed on an Agilent Series 1100 (Agilent Technologies, USA) consisting of a LC/MSD Trap system, a quaternary pump, an autosampler, equipped with a vacuum degasser, a column compartment, a diode-array detector and an ion-trap mass spectrometer with electrospray ionization interface, controlled by Agilent LC/MSD Trap Software. ACE 5 CN S/N-A21532 column (250x4.6 mm, 10  $\mu\text{m}$ ) was used. The reference compounds and samples were separated using an isocratic mobile phase consisting of *n*-hexane (76%) and ethyl acetate (24%). The flow rate was 1 mL/min and the injection volume was 10  $\mu\text{L}$ . The column temperature was maintained at 28°C. DAD data was collected from 190 to 400 nm. Identification of the main Coumarins was facilitated by HPLC-DAD. All standard working solutions were analyzed in foregoing HPLC conditions. The analyte peak area values were graphed against the corresponding concentrations of the analytes (expressed in mg/mL). In order to quantify the intermediate precision (interday precision) and repeatability (intraday precision) the same stock solutions with four different osthol, prantschimgin, felamidin, umbelliferone and peucedanol-2'-benzoate concentrations were injected into the HPLC system 3 times on the same day and 3 times over different days. The percentage Relative Standard Deviations (RSD, %) of the data in this way obtained were calculated. Accuracy was evaluated by means of recovery assays performed by adding known amounts of the reference compounds to the sample solutions.

### Quantification of the main coumarins in roots and aerial parts of *F. blancheana*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata*

For the quantification of main Coumarins found in the roots and aerial parts of *Ferulago blancheana*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata* HPLC-DAD method has been validated using the external standard osthol, prantschimgin, felamidin, umbelliferone and peucedanol-2'-benzoate.

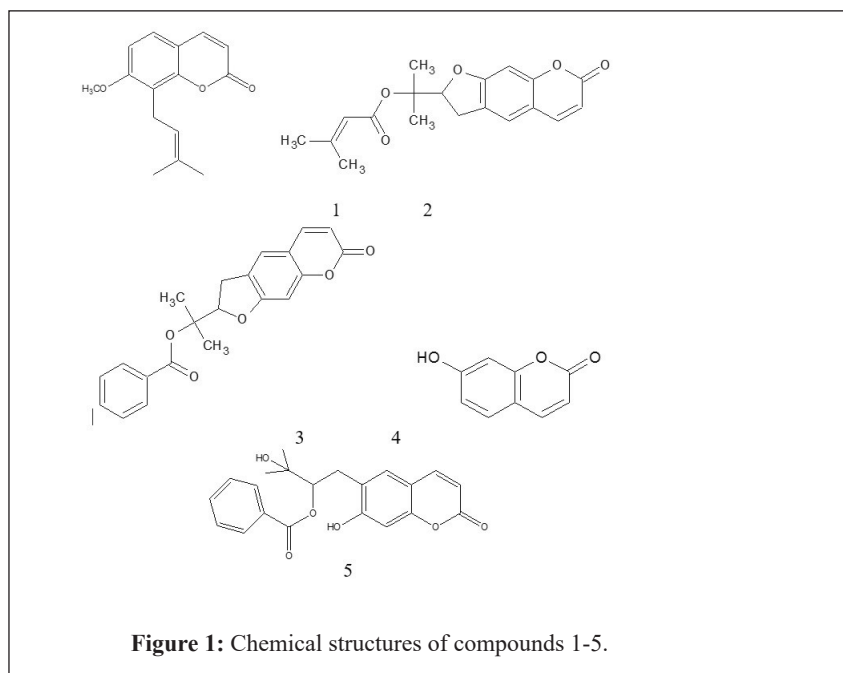
### Statistical analysis

All results are expressed as mean  $\pm$  SE and differences between means were statistically analyzed using One-way analysis of ANOVA followed by Bonferroni's complementary analysis, with  $P < 0.05$  considered to indicate statistical significance.

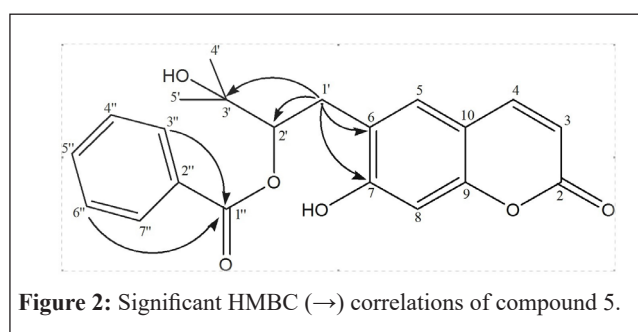
## Results and Discussion

Methanol extract of the roots of these species were fractionated using solvents with different polarities (*n*-hexane, dichloromethane, ethyl acetate and *n*-butanol). The active dichloromethane extract was subjected to column chromatography over silica gel. As the

result, a new Coumarin, peucedanol-2'-benzoate (5), together with four known ones, osthol (1) [31], prantschimgin (2) [32], felamidin (3) [33] and umbelliferone (4) [34] (Figure 1) were isolated and identified.



Peucedanol-2'-benzoate (5) was isolated as a white powder with the molecular formula of  $C_{21}H_{20}O_6$  as determined by the HR-ESI-MS at  $m/z$  367.1999  $[M-H]^+$  (Calcd for  $C_{21}H_{19}O_6$  367.1181). The IR spectrum of 5 showed absorption bands for C=O groups ( $1702\text{ cm}^{-1}$ ) and -CH=CH- bonds ( $1623, 1565\text{ cm}^{-1}$ ). The  $^1\text{H}$  NMR spectrum of compound 5 displayed two AB type system protons at  $\delta_H$  6.26 and 7.64 (each 1H, d,  $J = 9.4\text{ Hz}$ ) which was attributed to the H-3 and H-4 protons of the Coumarin nucleus. The two single aromatic proton signals at  $\delta_H$  7.28 and 6.80 were assigned to H-5 and H-8 protons. The  $^{13}\text{C}$  NMR spectrum revealed the presence of 9 carbons resonances including four methine [ $\delta_C$  112.37 (C-3), 143.62 (C-4), 123.22 (C-5), 98.01 (C-8)], three oxygenated quaternary [ $\delta_C$  161.38 (C-2), 163.49 (C-7), 155.84 (C-9)] and two non-oxygenated quaternary carbons [ $\delta_C$  124.55 (C-6), 112.71 (C-10)] for Coumarin skeleton. Two tertiary methyls at  $\delta_H$  1.72 (3H, s, H-4'), 1.71 (3H, s, H-5') and at  $\delta_C$  22.16 (C-4'), 21.38 (C-5') with the hydroxyl group; an oxygenated methine at  $\delta_H$  5.16 (1H, dd,  $J = 9.2/7.3\text{ Hz}$ , H-2') and at  $\delta_C$  89.12 (C-2'); and a methylene at  $\delta_H$  3.38 (2H, m, H-1') and at  $\delta_C$  29.67 (C-1') confirmed the 2',3'-dihydroxy-3'-methyl butyl moiety. HMBC correlation (Figure 2) between H-1' ( $\delta_H$  3.38) and C-6 ( $\delta_C$  124.55) suggested that it was attached to C-6 position. Characteristic signals of a benzoyl moiety were also exhibited, including a pair of 2H at  $\delta_H$  7.73 (H-3'', H-7'') and 7.32 (H-4'', H-6'') and 1H at 7.51 (H-5'') in the  $^1\text{H}$  NMR spectrum and aromatic carbons at  $\delta_C$  131.04 (C-2''), 129.39 (C-3'', C-7''), 128.27 (C-4'', C-6''), 132.86 (C-5'') with a carbonyl carbon at  $\delta_C$  165.40 (C-1'') in the  $^{13}\text{C}$  NMR spectrum. The linkage of the benzoyl group to the 2',3'-dihydroxy-3'-methyl butyl moiety was deduced from the downfield shifted signal of H-2' ( $\delta_H$  5.16) and C-2' ( $\delta_C$  89.12). Thus, the structure of the compound 5 was characterized as peucedanol-2'-benzoate.



## Validation of the method

Validation of the method was carried out for 3 days by testing the following criteria: linearity, response function, trueness, accuracy, precision (repeatability and intermediate precision), Limits of Detection (LOD) and Limits of Quantification (LOQ), and quantification range. Analyte standard solutions at four different concentrations were injected into the HPLC system. The procedure was evaluated in triplicate for each concentration. The analyte concentrations were as follows: 100, 200, 300, and 400 mg/mL for osthol, prantschimgin, felamidin, umbelliferone and peucedanol-2'-benzoate. The calibrations were curves constructed via the least-squares method. The methods were validated in accordance with the ICH guidelines on the validation of analytical methods [35,36].

## HPLC method development

Obtained chromatograms with baseline separation of the five marker compounds in plants, selection of the column, mobile phase composition, conditions for isocratic flow and temperature



was performed by HPLC. Initially a number of solvent systems were tried to develop a separation method for the isolates from the plants extract solutions starting from pure *n*-hexane and ethyl acetate. Finally, a simple isocratic method comprising of A (*n*-hexane-76%) and B (ethyl acetate-24%) at a flow rate of 1 mL/min provided a best base line separation with elution of all marker compounds in less than 20 minutes. Maximally efficient detection was observed at a fixed wavelength of 320 nm. Compounds 1, 2, 3, 4 and 5 were found to elute at 4.86, 5.89, 6.58, 7.86 and 4.98 min respectively with highly symmetric and well resolved peaks.

### Identification of the constituents

Identification of all constituents was performed by HPLC-DAD analysis by comparing the retention time and the UV spectra of the peaks in the samples with those of authentic reference

samples or isolated compounds and in some cases data reported in the literature. The developed method indicated a fair amount of specificity. Comparison between the peaks of the spectra and the retention times confirmed the specificity of the developed analytical methods.

### Linearity

Linearity of the developed method was validated by analysing four concentrations of each analyte ranging between 1 and 10.2 mg/mL. A 10.0 mL volume of standard solution was injected and analysed using the following HPLC method. The analysis were carried out in triplicate for all samples. Calibration curves for all compounds were obtained using linear regression analysis. The concentration range is usually selected as per ICH guidelines (International Conference on Harmonization i.e., 70% and 130% of the nominal concentration). All the results are indicated in table 4.

Compounds	1	2	3	4	5
Retention Time	4.86	5.89	6.58	7.86	4.98
Linearity range (µg.mL-1)	1-10.2	1-10.2	1-10.21	1-10.2	1-10.2
Slope	251.2	227.5	207.3	508.9	265.4
Intercept	534.4	430	389.9	502.3	567.3
Correlation coefficient	0.997	0.997	0.997	0.998	0.997
Standard error of the slope	0.0641	0.0312	0.0152	0.0451	0.0491
Standard error of the intercept	0.2516	0.3055	0.1527	0.4509	0.5671
Limit of detection	0.1311	0.2637	0.3431	0.1469	0.1873
Limit of quantification	0.437	0.879	1.1449	0.4897	0.5642
Repeatability (RSD %)	0.287	0.167	0.461	0.111	0.278
Reproducibility (RSD %)	0.441	0.219	0.345	0.256	0.345

**Table 4:** Calibration values for compounds 1-5.

### Specificity

In order to validate the specificity of the developed method, standard solutions of the analytes were prepared. An isocratic system of *n*-hexane and ethyl acetate was used as a blank control and fixed injection volumes of each sample, standard and blank solutions were applied and analysed using the developed HPLC method.

### Accuracy

The accuracy of the developed HPLC method was carried out by spiking the known amounts of analytes into extract solution of *Ferulago blanchena*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata*. After addition of known amounts of each analyte to the previous analysis extracts solutions recovery studies were carried out and the results were shown in table 5.

Marker compounds	Amount present in the extract (mg)	Amount found (mg)	Recovery (%) ±SS	RSD (%)
1	0.158	0.099	99.02±0.987	0.00996
		0.21	105.04±0.903	0.0086
		0.398	99.51±0.789	0.0079
2	0.5	0.231	92.43±0.580	0.0062
		0.494	98.96±0.720	0.0073
		0.923	92.34±0.091	0.00098
3	1.11	0.486	97.25±0.098	0.001
		0.998	99.88±0.810	0.0081
		1.945	97.39±0.840	0.0086
4	0.079	0.039	99.08±0.023	0.00023
		0.079	99.85±0.650	0.0065
		0.154	97.58±0.510	0.012
5	0.43	0.297	96.78±0.670	0.0345
		0.591	97.92±0.910	0.0654
		1.19	95.45±0.072	0.03478

**Table 5:** Recoveries for the assay of marker compounds in the species.

The recovery rates of the analytes ranged between 92.3 and 99.8% with relative standard deviations in the range of 0.091-0.987% i.e., less than 5% indicating high accuracy of the developed method.

### Precision

Based on the triplicate analysis performed each day and also per day over a three-day period, the intra as well as inter day precision levels for the developed method was analysed. The results are

shown in table 6. The intraday variations were carried out by analyzing triplicate at four different concentrations (1, 4.2, 6.8 and 10.2) in a day. The interday variations were identified by analyzing triplicate at four different concentrations at three days and the results are expressed as  $RSD (\%) = (SD/mean) \times 100\%$ . The RSD was well below 5 % showing good precision of the developed method. To analyze the repeatability of the method, the Relative Standard Deviation (RSD) values of retention times was determined and found to be <0.007801 %.

Marker compound	Intraday				Interday		
	Concentration (mg/mL)	Found (mg/mL) (Mean±SD)	Accuracy (%)	Precision (RSD) (%)	Found (mg/mL) (Mean±SD)	Accuracy (%)	Precision (RSD) (%)
1	1	1.0311±0.0069	103.11	0.0087	1.13±0.06	103	0.0264
	4.2	4.4258±0.1660	105.37	0.0007	4.53±0.21	102.9	0.0042
	6.8	6.9503±0.0080	102.21	0.0002	7.24±0.34	97.5	0.0022
	10.1	10.309±0.0180	102.07	0.0009	11.03±0.1	101.2	0.0015
2	1	1.0855±0.0270	101.19	0.0015	1.15±0.3	98.2	0.0007
	4.2	4.1746±0.0070	99.396	0.0001	4.34±0.67	103.3	0.0039
	6.8	6.9534±0.2620	102.25	0.0018	6.96±0.45	102.3	0.0014
	10.2	10.564±0.1530	102.59	0.0013	10.52±0.8	103.1	0.0005
3	1	1.0787±0.0060	101.45	0.0041	1.18±0.4	101.3	0.0034
	4.2	4.1331±0.0140	98.206	0.0072	4.23±0.08	100.7	0.0028
	6.8	6.7930±0.0220	99.705	0.0021	7.00±0.05	102.9	0.0029
	10.1	10.309±0.0090	102.15	0.0011	10.60±0.8	99	0.0032
4	1.1	1.135±0.0060	103.17	0.0078	1.16±0.3	101.61	0.0093
	4	4.0332±0.0160	100.83	0.0044	3.77±0.34	94.41	0.1509
	6.8	6.7539±0.0950	99.322	0.0012	6.77±0.67	99.5	0.001
	10.2	10.415±0.0110	101.21	0.0027	10.60±0.31	103.9	0.0007
	1	1.037±0.0170	101.34	0.0091	1.56±0.65	101.61	0.0093
	4	4.029±0.0220	99.93	0.0051	4.72±0.51	98.23	0.2398
	6.8	6.7599±0.0390	100.22	0.0072	6.56±0.45	98.41	0.0176
	10.2	10.551±0.0290	101.41	0.0092	10.76±0.17	102.01	0.0573

**Table 6:** Intra and inter-day accuracies and precisions area of HPLC method of marker compounds 1-5.

Percentage of osthol, prantschimgin, felamidin, umbelliferone and peucedanol-2'-benzoate in the dichloromethane extracts of the species roots and aerial parts. The amounts of isolated constituents present in dichloromethane extracts *F. blancheana*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata* roots and aerial parts are shown in table 7. Prantschimgin is the major Coumarin component of the roots and aerial parts of all species.

Species		1	2	3	4	5
<i>F. blancheana</i>	Root	0.39	2.76	1.26	0.2	-
	Aerial part	0.1	1.07	0.26	0.05	-
<i>F. pachyloba</i>	Root	0.14	2.83	0.56	0.44	-
	Aerial part	0.23	0.59	0.26	0.11	-
<i>F. trachycarpa</i>	Root	0.11	2.45	1.29	0.24	-
	Aerial part	0.27	0.72	0.43	0.26	-
<i>F. bracteata</i>	Root	0.3	2.28	0.48	0.21	1.12
	Aerial part	0.4	0.83	0.78	0.05	-

**Table 7.** Content of compounds 1-5 (w/w %) in dichloromethane extracts of roots and aerial part of the species determined by HPLC.

Coumarins represent interesting compounds in the *Apiaceae* family. Recent studies have shown their broad spectrum of biological activities and their potential as new drug leads [37]. Although several HPLC methods for the analysis of Coumarins were developed and published, as far as we are concerned, this is the first study on the Coumarin content of *F. blancheana*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata*. The potential of Coumarins has not been fully exploited despite their biological importance; thus, discovery of new drug substances and molecules can be a model for synthetic drugs. So, it was studied on the development of standard HPLC methods (resolution and quantification) of the natural products isolated from *F. blancheana*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata*.

Overall, the Coumarin content of the dichloromethane extracts is potentially high enough to exert biological effects. The developed method was suitable for the identification and quantification of the compounds in these plants. A highest percentage was found 2.76%, of prantschimgin in roots of *F. blancheana* and the lowest was found in aerial parts of *F. pachyloba* with 0.59%.

In conclusion, the above-described method is well suited for fast profiling and quantification of the main Coumarins like osthol, prantschimgin, felamidin, umbelliferone and peucedanol-2'-benzoate for quality control of the drugs and various preparations for this reason. As far as we know, this is the first report on the HPLC analysis and isolation of Coumarins from *F. blancheana*, *F. pachyloba*, *F. trachycarpa* and *F. bracteata*.

## Acknowledgments

The authors report no conflict of interest in this study. Analytical experiments and processes were carried out at the Laboratory of the Faculty of Pharmacy of Ankara University so the authors would like to thank Ankara University for their support during the completion of this study. This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK 115S009).

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