Indian Journal of Traditional Knowledge
Vol 19(4), October 2020, pp 728-735

The biological activities and phytochemical content of *Ferulago humulis* Boiss.

S Süzgeç-Selçuk*[a,+], S Anıl[a], E Rayaman[b], Ü S Gürer[b], N Özsoy[c] & E A Uruşak[d]

[a]Deparment of Pharmacognosy, Faculty of Pharmacy, Istanbul University, 34416, Istanbul, Turkey

[b]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Marmara University, 34668, Istanbul, Turkey

[c]Department of Biochemistry, Faculty of Pharmacy, Istanbul University, 34416, Istanbul, Turkey

[d]Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University, 34416, Istanbul, Turkey

*E-mail: [+]sevdasuzgec@hotmail.com

Received 22 April 2019; revised 19 October 2020

Ferulago humulis Boiss. is an endemic species growing in Turkey. The aim of the study was to compare *in vitro* antioxidant and antimicrobial activities of the aerial parts (HFH) and rhizomes (RFH) of *F. humulis*. According to the results of antimicrobial and antioxidant activities of the extracts obtained from different parts of the plant, their phytochemical contents were evaluated. Petroleum ether (HFH-PE; RFH-PE), dichloromethane (HFH-DM; RFH-DM) and methanol (HFH-ME; RFH-ME) extracts from aerial parts (HFH) and rhizomes (RFH) of *F. humulis* were obtained for antimicrobial activity and examined by the agar hole diffusion and microdilution methods. Chromatographic and spectroscopic (^{1}H NMR, LS-MS and UV) techniques were used for the isolation of coumarin compounds from petroleum ether (RFH-PE) and dichloromethane (RFH-DM) extracts. Furthermore, antioxidant activity were assayed by the 4 different methods in methanol extracts (HFH-ME; RFH-ME). HFH-PE (MIC=6.25 mg/mL), RFH-PE (MIC=12.5 mg/mL) and RFH-DM (MIC=11 mg/mL) extracts against *Staphylococcus aureus*, and RFH-PE (MIC= 3.125 mg/mL), HFH-PE (MIC=6.25 mg/mL) extracts showed antimicrobial activity against *S. epidermidis*. HFH-PE (MIC=1.56 mg/mL), RFH-PE (MIC= 6.25 mg/mL) extracts exhibited antifungal activity against *Candida tropicalis*. From the rhizomes of *F. humulis* isoimperatorin, bergapten, oxypeucedanin, marmesin senecioate and oxypeucedanin hydrate known as furanocoumarins derivatives were isolated.

Keywords: Antimicrobial activity, Antioxidant activity, *Ferulago humulis*, Isolation coumarin

IPC Code: Int. Cl.[20]: A01P 1/00, A61P 17/18, H02B 11/12

The genus *Ferulago* W. Koch belonging to the Umbelliferae family is known as "çakşırotu" "kişniş", "asaotu", "kuzubaşı" and "kuzukemirdi" in Turkey since Dioscorides times. It is naturally grown mainly in Europe, Northwest and Central Asia, Caucasus, North, and Northwest Africa and Turkey. It is represented by 34 species of which 18 are endemic in Turkey[1]. The species are commonly known in traditional medicine as carminative, digestive, sedative, tonic, aphrodisiac as well as in the treatment of intestinal worms and hemorrhoids[2,3].

The researchers have focused screening studies on active extracts of medicinal plants which is used in traditional treatment[4]. Plant extract and metabolites from *Ferulago* genus have many biological activities such as antioxidant, antibacterial, acetylcholinesterase inhibitory and antiproliferative activity[5-8]. Several phytochemical studies conducted on essential oils and extracts obtained from the different parts of *Ferulago* species so far. A number of coumarin and furanocoumarin derivatives being the most frequent metabolites with flavonoids, sesquiterpenes, fatty acids and phytosterols have also been reported from the roots and aerial parts as the chemical constituents of the *Ferulago* plants[9-15]. There are also some reports about antioxidant and antimicrobial activities of plant extracts and isolated coumarins from *F.carduchorum*[5], *F.macedonica*[6], *F. nodosa*[16], *F. bernardii*[17], *F. campestris*[18]. In previous study, it was reported that marmesin senecioate (prantchimgin) as coumarin compound, apigenin, rutin, isorhamnetin 3-galactoside, quercetin 3-0-glucoside, luteolin, rhamnetin as flavonoid compounds; 1-acetylhydroquinone 4-galactoside and quinol monoacetate as aromatic compound have been isolated from aerial parts of *F. humulis*[19,20]. In previous study it was studied the composition of essential oil and antimicrobial activities of *F. humulis*. *(z)-β*-Ocimene (32.4%), limonene (12.1%) and *trans*-chrysanthenylacetate (12.1%) were detected as the main components and its essential oil

*Corresponding author

was also tested for its antifungal and antibacterial activities[11,21]. In this study the antioxidant and antimicrobial activities of the aerial parts (HFH) and rhizomes (RFH**)** of *F. humulis* were compared and also the results of antimicrobial and antioxidant activities of the extracts obtained from different parts of the plant were evaluated with their phytochemical contents. There are no reports on antimicrobial and antioxidant activity from aerial parts and rhizomes of *F. humulis*. Also, we firstly reported the isolation, structure elucidation of coumarin compounds obtained from antimicrobial active extracts from rhizomes of *F. humulis* which is an endemic species to Turkey.

Material and Methods

Plant material

F. humulis Boiss. (Apiaceae), aerial parts and rhizomes collected from Zeytinli-Mehmetalan village, Edremit-Turkey and identified by Prof. Dr. Emine Akalın Uruşak. A voucher specimen is deposited in the Herbarium of Faculty of Pharmacy, Istanbul University with Nr. ISTE: 74477.

Test microorganisms

The microbial strains were from the Standart ATCC strains collection of Department of Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy at Marmara University.

Instruments

Bruker Avance III, 500 MHz, Shimadzu UV-1800 spectrophotometer, and a quadrupole time-of-flight mass spectrometer (Q-TOF LC/MS 6530, Agilent Technologies) instruments were used for ^{1}H NMR, UV spectra, and LC-MS analysis.

Extraction

Preparation of methanol extracts for antioxidant activity

The methanol extract was prepared with 30 g of dried and powdered aerial parts (HFH) and rhizomes (RFH) of *F. humulis* and it was percolated with 600 mL methanol. Methanol extracts were obtained from aerial parts (HFH-M) and rhizomes (RFH-M) of *F. humulis* by using percolation method. The obtained percolates were concentrated in rotavapor at 45 °C and raw extracts were obtained. The extracts were lyophilized. The obtained methanol extracts (HFH-M, RFH-M) were used for antioxidant activity determinations.

Preparation of the extracts for antimicrobial activity

The amount of 30 g plant material (HFH; RFH) were extracted with 600 mL of petroleum ether (PE), dichloromethane (DM) and methanol (M) solvents as fractionally by percolation method. Petroleum ether (HFH-PE; RFH-PE), dichloromethane (HFH-DM; RFH-DM), and methanol (HFH-ME; RFH-ME) extracts from aerial parts (HFH) and rhizomes *F. humulis* (RFH) were obtained by using percolation method. The obtained percolates were concentrated in rotavapor at 45 °C and raw extracts were obtained. The crude extracts were dried on a lyophilizer.

Preparation of extract for isolation

The amount of 175 g plant material (HFH; RFH) were extracted with 6 L of petroleum ether (PE), dichloromethane (DM) and methanol (M) solvents as fractionally. Petroleum ether (HFH-PE; RFH-PE), PE), dichloromethane (HFH-DM; RFH-DM), and methanol (HFH-ME; RFH-ME) extracts from aerial parts (HFH) and rhizomes (RFH) *F. humulis* were obtained by using percolation method. The obtained percolates were concentrated in rotavapor at 45 °C and raw extracts were obtained. During the condensation of the extracts to the rotavapor, the collapse of the petroleum ether extracts (RFH-PE) occurred. These extracts were left in the refrigerator overnight and then the sediment was removed by filtration. The crude extracts were dried on a lyophilizer.

For the isolation of the compounds from the petroleum ether rhizome extract (RFH), the sediment (RFH-PE-S) and the filtrate (RFH-PE-F) were separately studied.

Determination of total phenolic and flavonoid content

The extracts of total phenolic compounds were evaluated using Folin-Ciocalteu reagent according to Slinkard and Singleton[22] method and expressed as means mg gallic acid equivalents (GAE)/g of extract. Total flavonoids were evaluated by $AlCl_3$ colorimetric method described by Sakanaka et al.[23] and expressed as mg catechin equivalents (CE)/g of DW. Inhibition or radical scavenging activities (%) of the extract were calculated according to the equation: Inhibition or radical scavenging activity (%) = [1 – (Absorbance of sample at 517 nm/Absorbance of control at 517 nm)] x 100.

Antioxidant activity

The extract was evaluated for its antioxidant activity using thiobarbarbituric acid (TBA)[24] test based on the $FeCl_3$/ascorbic acid stimulated lipid peroxidation (LPO) in a liposome model[25], 2,2-

diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging[26], Trolox equivalent antioxidant capacity (TEAC)[27] and ferric reducing ability of plasma (FRAP)[28] assays.

Statistical analysis

All measurements were made in triplicate. The results were statistically analyzed using GraphPad Prism version 7.00. Results were considered significant at $p < 0.05$.

Antimicrobial activity

Agar well diffusion method

The antimicrobial activities of petroleum ether, dichloromethane and methanol extracts of *F. humulis* were investigated using agar well diffusion test. Bacterium cultures were prepared to 1-2 x 10^8 cfu/mL of microorganism equivalent to Mc Farland 0.5 standards of turbidity. The yeast cultures were subcultured twice prior to use at SDB at 35 ^{0}C for 48 hours and were prepared in Saboraud Dextrose Broth up to 10^7 cfu/mL, 0.1 mL of which were spread on the agar by sterile swab. Then 6 mm wells were opened and 50 µL of the extracts were put in them. The solvents of the extracts, meropenem (10 µg) and fluconazole (100 µg) were used as a control. The culture plates were incubated at 35 °C 18-24 hours for bacteria and 48 hours for yeasts. After it the inhibition zones were measured with calliper and noted as mm. All the experiments were done in three replicates. Data were given as mean±standard deviation[29,30].

Antibacterial activity test

Minimal inhibitory concentration (MIC) against of bacteria were determined using broth microdilution method in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines[31]. Meropenem, sterile medium and extract's solvents were used as controls in the study. After covering the plates were incubated at 35–37 °C for 24 hours. The MIC was defined as the lowest concentration of extract which gives complete inhibition of visible growth[31].

Antifungal activity test

Minimal inhibitory concentration (MIC) against of were determined using broth microdilution method according to Clinical Laboratory Standards Institute (CLSI) guidelines. Fluconazole, sterile medium and extract's solvents were used as controls in the study. After covering the plates were incubated at 35 °C and MIC endpoints were read after 48 hours of incubation. The MICs of all the extracts were defined as the lowest concentration which resulted in a obvious decrease in turbidity compared with that of growth-control wells[32].

Isolation and identification of coumarin compounds

Isolation was performed on RFH-PE and RFH-DM extracts of rhizomes (RFH) of *F. humulis* plant showing antimicrobial activities.

The petroleum ether filtrate extract (RFH-PE-F, 1.6 g) was subjected to silica gel (0.063-0.200 mm Merck) column chromatography (2.5 cm x 100 cm) and eluted with a solvent system increasing polarity from with n-hexane-ethyl acetate and ethyl acetate-methanol. Each 80 mL, 124 fractions were collected. After checking the thin-layer chromatography (TLC), the similar fractions were combined. Compound I (4 mg) was isolated by preparative thin layer chromatography (pTLC) from combined fraction 15-16 using toluene-chloroform-methanol (6:3:1) solvent system. Fraction 21-23 was chromatographed on pTLC with cyclohexane-ethyl acetate (1:1) solvent system and compound II (6 mg) was isolated. Compound III (9 mg) was isolated by pTLC from combined fraction 26-27 using cyclohexane-ethyl acetate (2:1) solvent system.

The petroleum ether sediment extract (RFH-PE-S, 1.7 g) was subjected to Sephadex LH 20 column chromatography (2.5 cm x 100 cm) and eluted with solvent system, n-hexane-dichloromethane-ethanol (7:4.5:0.5, 7:4:0.5, 7:4:1, 7:3.5:1.5, 7:3:2, 0:2:1, 0:0:1) increasing polarity. Each 40 mL, 95 fractions were collected. After checking TLC, the similar fractions were combined. Fraction 4-5 and 7-9 was chromatographed by pTLC with toluene-ethyl acetate-methanol (7: 3.5: 1.5) solvent system and compound IV (11 mg) was isolated. Compound I (5 mg) which is same compound as in the petroleum ether filtrate extract was isolated from fraction 8-9 by pTLC using with cyclohexane-ethyl acetate (1: 1) solvent system.

The dichloromethane extract (RFH-DM, 1.8 g) was subjected to silica gel (0.2-0.5 mm Merck) column chromatography (3 cm x 100 cm) and eluted with a solvent system, petroleum ether-chloroform, chloroform-ethyl acetate and ethyl acetate-ethanol. Each 200 mL, 54 fractions were collected. After checking the TLC, the similar fractions were combined. From combined fraction 23-25, compound V (8 mg) was isolated by pTLC with cyclohexane-ethyl acetate (1: 1) solvent system.

The structures of the compounds were identified by spectral methods (UV, ^{1}H NMR, and LS/MS).

Results and Discussion

Antioxidant activity

The results given in Table 1 showed that the amount of extractable phenolic compounds and flavonoids in HFH-M extract is higher than detected in RFH-M extract ($p<0.05$). So, the aerial parts of *F. humulis* is a rich source of phenolics and flavonoids. In past years, the capacity of flavonoids to act as antioxidants *in vitro* has been the subject of several studies and the importance of structure-activity relationships for the antioxidant activity have been established[33,34]. A similar content of flavonoids was reported by Süzgeç et al. for *F. trojana*[7].

The antioxidant activity was tested using four *in vitro* assays including lipid peroxidation inhibition, scavenging effect on DPPH and ABTS radicals, and FRAP assays. For comparison, Table 2 presents the results of the antioxidant activities, expressed as $EC_{50,}$ TEAC and FRAP values. As can be seen from the EC_{50} values, the methanol extract of aerial parts showed a higher scavenging effect on DPPH and ABTS radicals and reducing power when compared to its capability to inhibit lipid peroxidation. TEAC value was similar to the FRAP value, which indicates that the extract is effective in donating electrons. The aerial parts, containing the highest amount of total phenolics and flavonoids (HFH-M) showed the better antioxidant activity than rhizomes (RFH-M) in DPPH and FRAP assays. However, the results showed a weak antioxidant activity for both extracts when compared to the reference compound, quercetin. Although the extract (HFH-M) was less active than the quercetin ($p < 0.05$), it was seen that it has hydrogen and a single electron donor activities, thus could serve as the antioxidant. It was concluded that the structural differences in the phenolic and other compounds presenting aerial parts and rhizomes of *F. humulis* significantly affected the antioxidant activity results.

Table 1 — Total phenolic compounds (PC) (as gallic acid equivalents) and total flavonoids (as catechin equivalents) in methanol extracts from *F. humulis*

Extract (mg/g extract)	PC	Flavonoids (mg/g extract)
HFH-M	82.07 ± 4.04[a]	73.99 ± 3.50[a]
RFH-M	25.13 ± 2.29[b]	21.61 ± 0.54[b]

Values were the means of three replicates ± standard deviation.
Values with different letters in the same column were significantly ($p < 0.05$) different

Antimicrobial activity

The antimicrobial activity aerial parts and rhizomes of *F. humulis* has been studied for the first time. The antimicrobial activity results of extracts (HFT, RFT) prepared from *F. humulis* plants are shown in Table 3 and Table 4. RFH-PE, RFH-DM and HFH-PE, HFH-DM extracts showed antibacterial activity, while RFH-PE and HFH-PE extracts have shown antifungal activity. HFH-PE, RFH-PE, RFH-DM, and HFH-DM extracts against *Staphylococcus aureus*; HFH-PE and RFH-PE extracts showed antibacterial activity against *S. epidermidis* while the activity was not observed against *E. coli, K. pneumoniae, P. vulgaris* and *P. aeruginosa* for any of the extracts. The HFH-PE extract was found most effective extract because of showing antimicrobial activity against *S. aureus* and *S. epidermidis* strains with MIC values of 6,25 mg/L and also antifungal activity against used all the yeast

Table 2 — EC_{50}, TEAC and FRAP values of methanol extracts from *F. humulis*

Extract	LPO [a] EC_{50} (mg/mL)	DPPH [a] EC_{50} (mg/mL)	ABTS [a] EC_{50} (mg/mL)	TRAP [b*] (mM/L TEAC)	FRAP value [c*] (mM/L Fe^{2+})
HFH-M	3.07 ± 0.14[a]	0.96 ± 0.01[a]	1.96 ± 0.065[a]	2.13 ± 0.03[a]	2.19 ± 0.11[a]
RFH-M	N.d	3.39 ± 0.24[b]	5.04 ± 0.17[b]	1.33 ± 0.071[b]	1.35 ± 0.11[b]
Quercetin	0.034 ± 0.006[b]	0.069 ± 0.001[c]	0.113 ± 0.002[c]	2.15 ± 2.42[a] (at 0.16 mg/ml)	2.15 ± 0.011[a] (at 0.16 mg/ml)

Values were the means of three replicates ± standard deviation
[a] EC_{50} value: The effective concentration at which the antioxidant activity was 50 %; DPPH and ABTS radicals were scavenged by 50 % . EC_{50} value was obtained by interpolation from linear regression analysis
[b] Expressed as mmol Trolox equivalents per gram of dry weight
[c] Expressed as mmol ferrous ions eqivalents per gram of extract
∗ - Determined at 5 mg/mL
N.d. Not determined

Table 3 — Antimicrobial activities of petroleum ether, dichloromethane and methanol extracts of *F. humulis* plants against various bacterial strains

Extracts	*Staphylococcus aureus* ATCC 25923		*Staphylococcus epidermidis* ATCC 12228		*Escherichia coli* ATCC 25922		*Pseudomonas aeruginosa* ATCC 27853		*Proteus vulgaris* ATCC 13315		*Klebsiella pneumoniae* ATCC 4352	
	Zone*	MIC**	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC
RFH-PE	11,08	12,5	9,82	3,12	0	-	0	-	0	-	0	-
RFH-DM	9,83	11	0	-	0	-	0	-	0	-	0	-
RFH-ME	0	-	0	-	0	-	0	-	0	-	0	-
HFH-PE	14,57	6,25	10,84	6,25	0	-	0	-	0	-	0	-
HFH-DM	10,6	45	0	-	0	-	0	-	0	-	0	-
HFH-ME	0	-	0	-	0	-	0	-	0	-	0	-
Meropenem (10 µg)	42,98	0,12	52,88	0,12	44,38	<0,03	28,42	1	46,63	<0,03	36,75	<0,03

* Zone values have been measured in mm

** MIC concentrations have been given in mg/mL for extracts and µg/mL for meropenem

-: Untested

Table 4 — Antimicrobial activities of petroleum ether, dichloromethane and methanol extracts of *F. humulis* plants against *Candida* strains

Extracts	*Candida albicans* ATCC 90028		*Candida glabrata* ATCC 90030		*Candida guilliermondii* KUEN 998		*Candida tropicalis* KUEN 1021		*Candida parapsilosis* ATCC 90018		*Candida krusei* ATCC 6258	
	Zone*	MIC**	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC	Zone	MIC
RFH-PE	0	-	0	-	0	-	9,37	6,25	0	-	0	-
RFH-DM	0	-	0	-	0	-	0	-	0	-	0	-
RFH-ME	0	-	0	-	0	-	0	-	0	-	0	-
HFH-PE	13,73	3,125	15,05	3,12	15,50	<0,049	14,76	1,56	16,54	0,19	29,37	<0,049
HFH-DM	0	-	0	-	0	-	0	-	0	-	0	-
HFH-ME	0	-	0	-	0	-	0	-	0	-	0	-
Fluconazole (100 µg)	0	64	22	≥256	19	2	0	≥256	0	≥256	18	16

* Zone values have been measured in mm

** MIC concentrations have been given in mg/mL for extracts and µg/mL for fluconazole

-: Untested

strains. As a result of this study, the HFH-PE extract can be used as antimicrobial therapy in the presence of microorganisms after the toxicity studies of its are examined.

In a previous study, essential oil of fruits from *F. humulis* showed antibacterial activity against *E. coli* and *S. aureus* strains with MIC values of 250 and 125 µg/mL, respectively and showed antifungal activity against *C. albicans* 125 µg/mL[21]. Therefore, these results demostrated that the essential oil obtained from fruits of *F. humulis* against *S. aureus* strain had higher antimicrobial activity than the extracts obtained from the aerial parts and rhizomes. Also, it is thought that flavonoids and aromatic compounds which have been found in the aerial parts may be related to high activity when compared with the rhizomes.

On the other hand, the antimicrobial activity of extracts from other *Ferulago* species has been reported[35]. In our previous study, the extracts n-hexane of rhizomes from *F. trachycarpa* showed antibacterial activity with a MIC value of 3.9 mg/mL and 19.5 mg/mL against *S. aureus* and *E. feacalis*, *P. aeruginosa* and antifungal activity with a MIC value of 4,8 mg/mL, 4.8 mg/mL and 625 mg/mL against *C. albicans, C. tropicalis* and *C. parapsilosis* respectively by the microdilution method[36]. HFH-PE extract demonstrated antifungal activity with a MIC value of 3.125, <0,049 mg/mL and 0,19 mg/mL against *C. albicans, C. tropicalis* and *C. parapsilosis* in our study. However, we did not find antifungal activity of rhizomes from *F. humulis* against *C. albicans* and *C. parapsilosis.*

In our study, it can be said that these coumarin compounds are responsible for the antimicrobial activity in RFH-PE and RFH-DM.

Identification of isolated compounds from rhizomes of F. humulis

Examination of the petroleum ether (RFH-PE) and dichloromethane (RFH-DM) extracts of *F. humulis* rhizomes led to the isolation of five known furanocoumarins: isoimperatorin **(I)**, bergapten **(II)**, oxypeucedanin **(III)**, marmesin senecioate ((-)-prantschimgin) **(IV)**, oxypeucedanin hydrate (prangol) **(V)**. To the best of our knowledge, all determined coumarins are reported for the first time from rhizomes of the plant.

The molecular formula of all compounds were obtained with the help of UV, ^{1}H NMR, as and LS-MS. The structures of isolated compounds are shown in Figure 1.

Compound **I** was isolated from RFH-PE-F and RFH-PE-S extracts. Its molecular formula $C_{16}H_{14}O_4$ was determined by HR-ESI-MS at m/z 271.0958 $[M+H]^+$ (calcd. for $[C_{16}H_{15}O_4]^+$ 271.0964) according to extensive spectroscopic analyses including HR-ESI-MS and ^{1}H-NMR and UV spectroscopy, the structure of **I** identified as isoimperatorin[37,38].

Compound **II** was isolated from RFH-PE-F extract. Its molecular formula $C_{12}H_8O_4$ was determined by HR-ESI-MS at m/z 217.0486 $[M+H]^+$ (calcd. for $[C_{12}H_9O_4]^+$ 217.0492) according to extensive spectroscopic analyses including HR-ESI-MS and ^{1}H-NMR and UV spectroscopy, the structure of **II** identified as bergapten[38,39].

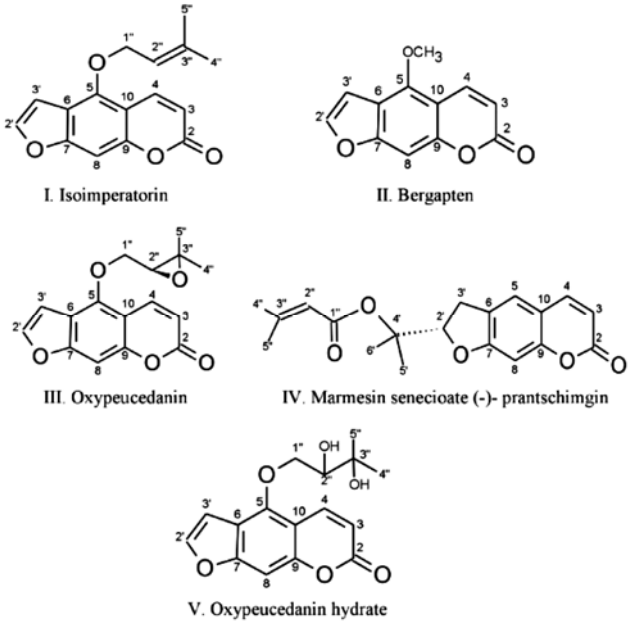

Fig. 1 — Structures of compunds I-V

Compound **III** was isolated from RFH-PE-F extract. Its molecular formula $C_{16}H_{14}O_5$ was determined by HR-ESI-MS at m/z 287.0979 $[M+H]^+$ (calcd. for $[C_{16}H_{15}O_5]^+$ 287.0984) according to extensive spectroscopic analyses including HR-ESI-MS and ^{1}H-NMR and UV spectroscopy, the structure of **III** identified as oxypeucedanin[37,38].

Compound **IV** was isolated from RFH-PE-S extract. Its molecular formula $C_{19}H_{20}O_5$ was determined by HR-ESI-MS at m/z 329.1392 $[M+H]^+$ (calcd. for $[C_{19}H_{21}O_5]^+$ 329.1395) according to extensive spectroscopic analyses including HR-ESI-MS and ^{1}H-NMR and UV spectroscopy, the structure of **IV** identified as marmesin senecioate ((-)-prantschimgin)[8,36].

Compound **V** was isolated from RFH-DM extract. Its molecular formula $C_{16}H_{16}O_6$ was determined by HR-ESI-MS at m/z 305.1021 $[M+H]^+$ (calcd. for $[C_{16}H_{17}O_6]^+$ 305.1015) according to extensive spectroscopic analyses including HR-ESI-MS and ^{1}H-NMR and UV spectroscopy, the structure of **V** identified as oxypeucedanin hydrate (prangol)[37,38].

Conclusion

In this study, the phytochemical properties, antioxidant activity and antimicrobial activities in various solvent extracts according to their polarity obtained from different parts of *F. humulis* were investigated. So, the study revealed that *F. humulis* contains coumarin derivatives as the main components of the rhizomes and presented diverse antimicrobial activity. Coumarins are among the most characteristic chemical compounds of the genus, as well as of the Apiaceae family. The flavonoid and aromatic compounds were detected in the aerial parts of *F. humulis* while these compounds were not found in the rhizomes.

To the best of our knowledge, all determined coumarins were reported for the first time from rhizomes of the plant. (-)-Prantschimgin has been isolated from both aerial parts and rhizomes of *F. humulis*. The previous study showed that *F. humulis* plant contains only one coumarin compound and is richer in terms of flavonoid and aromatic compounds in the aerial parts. So, flavonoids and aromatic compounds present in the aerial parts of the *F. humulis* may be responsible for significant antioxidant and antimicrobial activity than

the rhizomes and it can be considered for the *Ferulago* genus that the flavonoid and aromatic compounds found in the aerial parts as chemical components are more active than the coumarin compounds.

In spite of the results demonstrated the use of rhizomes of *Ferulago* genus in traditional medicine in Turkey the health-promoting potential of *F. humulis* comes from aerial parts. Therefore, aerial parts of *F. humulis* can be considered as a potential source of natural radical scavenger and the antimicrobial agent to be used in food and pharmaceutical industries. Our further investigations will be focused on identification of active constituents in aerial parts of *F. humulis* especially HFH-PE extract and the molecular mechanisms of their action and also we will aim to explain the mechanism of antimicrobial activity according to the phytochemical content.

Acknowledgments

This study was supported by Marmara University Scientific Research Project Coordination Unit (BAPKO) (Project number: SAG-B-120514-0132).

Conflict of interests

The authors declare no conflicts of interest.

Author Contributions

SSS elucidated the chemical structure, prepared the article, and conceived the project. SSS and SA carried out the extraction, isolation and purification process. ÜSG and ER performed the antimicrobial activity assay. NÖ performed antioxidant activity assay. EAU collected and identified the plant material.

References

1 Akalın E & Kızılarslan Ç, Fruit anatomy of some *Ferulago* (Apiaceae) species in Turkey, *Turk J Bot,* 37 (2013), 434-445.

2 Akalın E, *Taxonomical studies on the genus Ferulago in Western Anatolia*. PhD Thesis, (Istanbul University, Istanbul), 1999.

3 Baytop T, *Therapy with Medicinal Plants in Turkey, Past and Present*, second ed., (Nobel Tıp Basımevi, Istanbul) 1999, pp 348-349.

4 Turker A U, Yıldırım A B, Karakaş F P & Turker H, *In vitro* antibacterial and antitumor efficiency of traditional some medicinal plants from Turkey, *Indian J Tradit Know,* 17 (1) (2018) 50-58.

5 Golfakhrabadi F, Ardakani M R Z, Saeidnia S, Akbarzadeh T, Yousefbeyk F *et al., In vitro* antimicrobial and acetylcholinesterase inhibitory activities of coumarins from *Ferulago carduchorum*, *Med Chem Res,* 25 (2016) 1623-1629.

6 Mileski K S, Džamić A M, Ćirić A D, Ristić M S, Grujić S M, *et al.,* Composition, antimicrobial and antioxidant properties of endemic species *Ferulago macedonica* Micevski & E. Mayer, *Rec Nat Prod,* 9 (2) (2015) 208-223.

7 Süzgeç-Selçuk S, Özsoy N, Özbek-Çelik B & Akalın-Uruşak E, Antioxidant and antimicrobial activitiy of *Ferulago trojana* E. Akalın & Pimenov., *Istanbul J Pharm,* 47 (3) (2017) 101–106.

8 Sajjadi S-E, Jamali M, Shokoohinia Y, Abdi G, Shahbazi B, *et al.,* Antiproliferative evaluation of terpenoids and terpenoid coumarins from *Ferulago macrocarpa* (Fenzl) Boiss. fruits, *Pharmacog Res*, 7 (4) (2015) 322-328.

9 Miski M, Moubasher H A & Mabry T J, Sesquiterpene aryl esters from *Ferulago antiochia, Phytochemistry,* 29 (1990) 881-886.

10 Jiménez B, Concepción Grande M, Anaya J, Torres P & Grande M, Coumarins from *Ferulago capillaris* and *F. brachyloba*, *Phytochemistry,* 53 (2000) 1025-1031.

11 Başer K H C, Demirci B, Özek T, Akalın E & Özhatay N, Micro-distilled volatile compounds from *Ferulago* species growing in Western Turkey, *Pharm Biol,* 40 (2002) 466-471.

12 Erdurak C S, Coşkun M, Demirci B & Başer K H C, Composition of the essential oil of fruits and roots of *Ferulago isaurica* Peşmen and *F. syriaca* Boiss. (Umbelliferae) from Turkey, *Flavour Fragr J,* 21 (2006) 118-121.

13 Başer K H C, Composition of the essential oil of fruits and roots of *Ferulago isaurica* Peşmen and *F. syriaca* Boiss. (Umbelliferae) from Turkey, *Flavour Fragr J,* 21 (2006) 118-121.

14 Erdemoğlu N, Akalın E, Akgöç M, Çıkrıkçı S & Bilsel G, Comparison of the seed oils of *Ferulago trachycarpa* Boiss. different localities with respect to fatty acids, *Rec Nat Prod*, 2 (1) (2008) 13-18.

15 Alkhatib R, Hennebelle T, Roumy V, Sahpaz S, Süzgeç S, *et al.,* Coumarins, caffeoyl derivatives and a monoterpenoid glycoside from *Ferulago asparigifolia, Biochem Syst Ecol,* 37 (2009) 230-233.

16 Ruberto G, Cannizzo S, Amico V, Bizzini M & Piattelli M, Chemical constituents of *Ferulago nodosa*, *J Nat Prod*, 57 (1994) 1731-1733.

17 Khalighi-Sigaroodi F, Hadjiakhoondi A, Shafiee A, Mozaffarian V A, Shahverdi A R, *et al*., Phytochemical analysis of *Ferulago bernardii,* Tomk and M. Pimen., *Daru,* 14 (2006) 214-221.

18 Basile A, Sorbo S, Spadaro V, Bruno M, Maggio A *et al.,* Antimicrobial and antioxidant activities of coumarins from the roots of *Ferulago campestris* (Apiaceaea), *Molecules,* 14 (2009) 939-952.

19 Doğanca S, Hırlak F, Tüzün O T & Gürkan E, Phytochemical investigation on *Ferulago confusa*, *J Fac Pharm Istanbul*, 31 (1995) 41-45.

20 Doğanca S, Tuzlacı E & Ulubelen A, Phytochemical investigation on *Ferulago humulis*, *J Fac Pharm Istanbul*, 26-28 (1990-92) 19-22.

21 Demirci F, İşcan G, Güven K, Kırımer N, Demirci B, *et al.,* Antimicrobial activities of *Ferulago* essential oils, *Z Naturforsch,* 55c (2000) 886-889.

22 Slinkard K & Singleton V L, Total phenol analysis: automation and comparison with manual methods, *Am J Enol Vitic*, 28 (1977) 49-55.

23 Sakanaka S, Tachibana Y & Okada Y, Preparation and antioxidant properties of extracts of *Japanese persimmon* leaf tea (kakinoha-cha), *Food Chem*, 89 (2005) 569-575.

24 Duh P D, Tu Y Y & Yen G C, Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium* Ramat), *Lebensm Wiss Technol*, 32 (1999) 269-277.

25 Buege J A & Aust S D, Lipid peroxidation, *Methods Enzymol,* 52 (1978) 302-310.

26 Brand-Williams W, Cuvelier M E & Berset C, Use of a free radical method to evaluate antioxidant activity, *Food Sci Technol*, 28 (1995) 25-30.

27 Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, *et al.,* Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radic Biol Med*, 26 (1999) 1231-1237.

28 Benzie I F F & Strain J J, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay, *Anal Biochem*, 239 (1996) 70-76.

29 Perez C, Pauli M & Bazerque P, An antibiotic assay by the agar-well diffusion method, *Acta Biol Med Experiment* (1990), 15, 113-115.

30 Magaldi S, Mata-Essayag S, Hartung de Capriles C, Perez C, Colella M T, *et al.,* Well diffusion for antifungal susceptibility testing, *Int J Infect Dis*, 8 (2004) 39-45.

31 *Clinical Laboratory Standards Institute (CLSI)*, Performance standards for antimicrobial susceptibility testing, Twenty-First Informational Supplement, M100-S21, Vol.31 No.1 (Wayne, Pennsylvania), (2011).

32 *Clinical Laboratory Standards Institute (CLSI),* Reference method for broth dilution antifungal susceptibility testing of yeast, Approved Standard-Third Edition, CLSI document M27-A3, (Wayne, Pennsylvania), (2008).

33 Pietta P G, Flavonoids as antioxidants, *J Nat Prod*, 63 (2000) 1035-1042.

34 Velderrain-Rodriguez G R, Palafox-Carlos H, Wall-Medrano A, Ayala-Zavala J F, Chen C-YO, *et al.,* Phenolic compounds: their journey after intake, *Food Funct,* 5 (2014) 189.

35 Azarbani F, Saki Z & Mohammadi A, Phenolic contents, antibacterial and antioxidant activities of flower, leaf and stem extracts of *Ferulago angulata* (Schlecht) Boiss. *Int J Pharm Pharm Sci,* 6 (2014) 123-125.

36 Dikpınar T, Süzgeç-Selçuk S, Çelik B Ö & Akalın Uruşak E, Antimicrobial activity of rhizomes of *Ferulago trachycarpa* Boiss. and bioguided isolation of active coumarin costituents, *Ind Crops Prod*, 123 (2018) 762-767.

37 Naseri M, Monsef-Esfehani H R, Saeidnia S, Dastan D & Gohari A R, Antioxidative coumarins from the roots of *Ferulago subvelutina*, *Asian J Chemi*, 25 (4) (2013) 1875-1878.

38 Malikov V M & Saidkhodzhaev A I, Coumarins. Plants, structure, properties, *Chem Nat Compd,* 34 (3) (1998) 345-368.

39 Sbai H, Zribi I, DellaGreca M & Haouala R, Bioguided fractionation and isolation of phytotozic compounds from *Apium graveolens* L. aerial parts (Apiaceae), *S Afr J Botan,* 108 (2017) 423-430.