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Antioxidant and Antimicrobial Activity of *Ferulago trojana* E. Akalın & Pimenov

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ABSTRACT

Ferulago W. Koch is a genus in the Apiaceae family comprising 34 species, of which 18 are endemic in Turkey. *Ferulago* species have been known since the time of Dioscorides and have been used in folk medicine for their sedative, tonic, digestive, carminative, and aphrodisiac effects, as well as for the treatment of intestinal worms and hemorrhoids. This study was conducted to evaluate the polyphenolic contents and antioxidant activities of methanol extracts of the aerial parts (HFT) and rhizomes (RFT) of *Ferulago* trojana E. Akalın & Pimenov by measuring their ability to inhibit lipid peroxidation induced by Fe^{3+}-ascorbate, their $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ scavenging activities, and their ferric reducing antioxidant power (FRAP value). The methanol extracts were also examined for their antimicrobial activity using the microbroth dilution technique. Results showed that the methanol extracts of the aerial parts of the plant, containing the highest amount of total phenolic content and flavonoids, exhibited antioxidative potential for the chain-breaking inhibition of lipid peroxidation and showed the strongest hydrogen and single electron donor activities, which could thus serve as a free radical scavenger, act as a reductant, and provide protection against oxidative stress. Although the methanol extract of rhizomes did not exhibit any inhibitory effect on lipid peroxidation, it is possible that it might also have protective effects against oxidative damage by scavenging free radicals and acting as a reductant. While both the methanol extracts of the aerial parts and rhizomes of *F. trojana* were effective against *Staphylococcus aureus, Methicillin-resistant S. aureus* (MRSA), and *S. epidermidis*, the extracts showed no activity against *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. In addition, the methanol extract of rhizomes of *F. trojana* exhibited antibacterial activity against *Proteus mirabilis* and antifungal activity against the yeast *Candida albicans*.

Keywords: *Ferulago, F. trojana*, antioxidant activity, antimicrobial activity

INTRODUCTION

Many medicinal plants and raw extracts, due to their traditional use in vitro antioxidants and antibacterial activities have been screened by many researchers. These activites have been observed on plants containing specially phenolic compounds (Wang et al. 1999; Kähkönen et al. 1999; Pietta 2000; Cushine & Lamb 2005; Rios & Recio 2005).

The genus *Ferulago* (Apiaceae/Umbelliferae) includes 49 species occuring throughout the Northern hemisphere. It is naturally grown mainly in Europe, Northwest and Central Asia, Caucasus, North and Northwest Africa and Turkey. 34 *Ferulago* species (18 endemic) are naturally grow in Turkey (Peşmen 1972; Davis et al. 1988; Pimenov and Leonov 1993; Özhatay and Akalın 2000; Solanas et al. 2000; Akalın and Pimenov 2004; Kandemir and Hedge 2007).

In different regions in Turkey, *Ferulago* species are known by different names, as the most common "çakşırotu", "kişniş", "asaotu", "kuzubaşı" ve "kuzukemirdi". Since Dioscorides, *Ferulago* species are used for the purpose tonic, digestive, carminative, aphrodi-

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siac as well as in the treatment of intestinal worms and hemorrhoids (Baytop 1999; Akalın 1999).

Ferulago trojana is endemic to Mount Ida which was first identified in 2004. On ISTE's samples, collected in Çanakkale and Balıkesir, previously determined as *F. sylvatica* (also this name was specified from same areas in the Flora of Turkey and East Aegean Islands). Later studies showed that they were different from the European sample. The results of the detailed examination of the Turkish '*F. sylvatica*' samples, they have been identified as a new species, *F. trojana*. The species of *F. sylvatica* is not considered to be in Turkey (Akalın and Pimenov 2004).

To date, there have been some studies on chemical composition of various *Ferulago* species. Essential oils, coumarins, flavonoids, sesquiterpenes, fatty acids and phytosterols were reported as the chemical constituents of the *Ferulago* plants. In these studies with respect to the essential oils of *Ferulago* genus, coumarins, monoterpenes and sesquiterpenes were characterized as the main components (Miski et al. 1990; Doğanca et al. 1991; Yoti&Assenov 1995; Rustaiyan et al. 1999; Jiménez et al. 2000; Başer et al. ; 2001; 2002; Erdurak et al. 2006; Kılıç et al. 2006; Erdemoğlu et al. 2008; Alkhatib et al. 2009).

In previous study, five compounds have been isolated from *F.trojana*. From these compounds bergapten, isoimperatorin, 3'-epidecursin and isomaltol are known compounds, isomaltol-3β-O-glucoside and 3,6-dimethoxy-7-isopropilcoumarin-4-tetradeca-13"-one have been isolated for the first time. Also antioxidant activities and anticholinesterase activities of dichloromethane and methanol extracts of *F. trojana* were determined (Çakar 2010). GS/MS analysis has resulted in the characterization of 19 compounds representing 99.3% of the oil with p-cymene (45.8%) as the main costituents. Monoterpenes and sesquiterpenes were reported from aerial parts of the essential oil of *F. sylvatica* (Chalchat et al. 1992).

The aim of this study was to evaluate and compare the antioxidant and antimicrobial activities of methanol extracts from the aerial parts (HFT) and rhizomes (RFT) of *F. trojana*. There are no reports on antimicrobial activity of *F.trojana*. The most active extract and parts of the plant will be detected and the compounds responsible for the antimicrobial activity in another study will be isolated.

MATERIALS AND METHODS

Plant material

Ferulago trojana E. Akalın & Pimenov a species growing in Turkey was collected from Kaz Dağları (Balıkesir) in June 2007, and identified by Dr. Emine Akalın. A voucher specimen (ISTE No: 74316) is deposited in the Herbarium of Faculty of Pharmacy, Istanbul University (ISTE).

Preparation of extracts

The dried and powdered aerial parts (30 g) (HFT) and rhizomes (30 g) (RFT) of the *F.trojana* were percolated with 600 mL methanol. The methanol extracts were evaporated to dryness under reduced pressure and controlled temperature (45 to 50°C) in a rotary evaporator. The extracts were lyophilized. The obtained methanol extracts (HFT, RFT) were used for antioxidant and antimicrobial activity determinations.

Chemicals

Soybean lecithin (L-a-phosphatidylcholine Type IV-S), 2,2-diphenyl-1-picrylhydrazyl (DPPH), quercetin and ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{+}$), 6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Fluka Chemical Co. (Bushs, Switzerland). Thiobarbituric acid (TBA), trichloroacetic acid (TCA) and ferric chloride were purchased from Merck (Darmstadt, Germany). All other chemicals used were of analytical grade.

Determination of total phenolic compounds

Total soluble phenolics in the methanol extract of *F.trojana* were determined with Folin-Ciocalteu reagent according to the method of (Slinkard and Singleton 1977) with some modifications. Aliquots (0.1 mL) of extracts were transferred into the test tubes and their volumes made up to 4.6 mL with distilled water. After addition of 0.1 mL Folin-Ciocalteu reagent (previously diluted 3-fold with distilled water) and 0.3 mL 2% Na_2CO_3 solution, tubes were vortexed and absorbance of mixture recorded after 2 h at 760 nm against a blank containing 0.1 mL of extraction solvent. Gallic acid (0.05 mg/mL–0.4 mg/mL) was used for calibration of a standard curve. The results were expressed as gallic acid equivalents (GAE)/g of extract. The data were presented as the average of triplicate analyses.

Determination of total flavonoid content

Total flavonoid content was determined by using a colorimetric method described by (Sakanaka et al. 2005). Briefly, 0.25 mL of the extract (0.625 mg/mL extract or (+)-catechin standard solution was mixed with 1.25 mL of distilled water or solvent in a test tube, followed by addition of 75 µL of a 5% (w/v) sodium nitrite solution. After 6 min, 150 µL of a 10% (w/v) $AlCl_3$ solution was added and the mixture was allowed to stand for a further 5 min before 0.5 mL of 1 M NaOH was added. The mixture was brought to 2.5 mL with distilled water and mixed well. The absorbance was measured immediately at 510 nm, using a spectrophotometer (Shimadzu UV-1208). (+)-Catechin standard solution (15–250 µL/mL) was used for the calibration of a standard curve. The results were expressed as means (± SD) mg of (+)-catechin equivalents per gram of extract.

Antioxidant activity

Antioxidant activity on liposome peroxidation

Lipid peroxidation assay was based on the method described by (Duh et al. 1999). Lecithin (300 mg) was suspended in 30 mL phosphate buffer (10 mmol/L, pH 7.4). This suspension was then sonicated with a rod using an ultrasonic homogenizer (Bandelin, Berlin, Germany) at 30 s intervals for 10 min until an opalescent suspension was obtained.

The sonicated solution (10 mg/mL), $FeCl_3$, ascorbic acid and the extracts (from 0.625 to 10 mg/mL) or quercetin (from 0.005 to 0.08 mg/mL) used as a reference antioxidant were mixed to produce a final concentration of 3.08 mg liposome/mL, 123.2 µmol $FeCl_3$ and 123.2 µmol ascorbic acid. After 1 h incubation at 37°C, the formation of lipid peroxidation products was assayed by the measurement of malondialdehyde (MDA) levels on the basis that MDA reacted with TBA at 532 nm according

to (Buege and Aust 1978). Briefly, 500 µL of this reaction mixture was mixed with 1000 µL TCA-TBA reagent (consisting of 15% w/v TCA and 0.375% TBA in 0.25 N HCl) and 14 µL BHT (2% in absolute ethanol). The mixture was vortexed and heated for 10 min in a boiling water bath. After cooling, an equal volume of n-butanol was added and the mixture was shaken vigorously. The n-butanol layer was separated by centrifugation at 3000 rpm for 5 min. The absorbance of the sample was read at 532 nm against a blank which contained all reagents except lecithin. The percentage inhibition of lipid peroxidation was calculated by comparing the results of the samples with those of controls not treated with the extract using the following equation: Inhibition effect (%) = [1-(Absorbance of sample at 532 nm/Absorbance of control at 532 nm)] x 100.

DPPH radical scavenging activity

The DPPH radical scavenging activity of the extract was measured according to the procedure described by (Brand-Williams et al. 1995). A 0.1 mL aliquot of extracts (from 0.16 to 15 mg/mL) or quercetin (from 0.01 to 0.16 mg/mL) in methanol was added to 3.9 mL of 6 x 10^{-5} M methanolic solution of DPPH. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. The decrease in absorbance of the resulting solution was then measured spectrophotometrically at 517 nm against methanol. All measurements were made in triplicate and averaged. Two controls were used for this test, a negative control (containing all reagents except the test sample) and positive controls (using the reference antioxidants). The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) = [1 - (Absorbance of sample at 517 nm/Absorbance of control at 517 nm)] x 100.

Total radical antioxidant potential (TRAP) assay

The total radical antioxidant potential of the extract was measured using the Trolox equivalent antioxidant coefficient (TEAC) assay as described by (Re et al. 1999) with minor modifications. ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation was produced by reacting $ABTS^{+}$ stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 hours before use. At the begining of the analysis day, an $ABTS^{\bullet+}$ working solution was obtained by the dilution in 96 % ethanol of the stock solution to an absorbance of 0.70 (± 0.02) at 734 nm. After addition of 990 µL of $ABTS^{\bullet+}$ solution to 10 µL of the extracts (from 0.625 to 15 mg/mL) or quercetin (from 0.01 to 0.16 mg/mL) or trolox standards (final concentration 0 - 20 µM/l) in absolute ethanol the decrease in absorbance at 734 nm was monitored exactly 6 min after the initial mixing. Appropriate methanol blanks were run in each assay. All determinations were carried out in triplicate.

The ability to scavenge $ABTS^{+}$ radical was calculated by the following equation: $ABTS^{+}$ radical scavenging activity (%) = [1 - (Absorbance of sample at 734 nm/Absorbance of control at 734 nm)] x 100.

The total antioxidant capacity value in a sample was assessed as TEAC. The TEAC value was calculated by using a regression equation between the Trolox concentration and the percentage of inhibition of absorbance at 734 nm at 6 minutes of incubation and was expressed as mmol TEAC.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the procedure of (Benzie and Strain 1996). The FRAP reagent contained 2.5 mL of 10 mM TPTZ solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3.6H_2O$ and 25 mL of 0.3 M acetate buffer, pH 3.6. FRAP reagent (900 µL), prepared freshly and incubated at 37°C, was mixed with 90 µL of distilled water and 30 µL of the extracts (from 1.25 to 10 mg/mL) or quercetin (from 0.02 to 0.31 mg/mL) or water for the reagent blank. The increase in absorbance at 593 nm was measured at 4 min. The standard curve was constructed using iron sulfate heptahydrate solution (125–2000 µM), and the results were expressed as mM Fe^{2+} equivalents. All the measurements were taken in triplicate and the mean values were calculated.

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 activitiy

Antimicrobial activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 14153, Methicillin-resistant (MRSA) ATCC 43300 and *Candida albicans* ATCC 10231 were determined by the microbroth dilutions technique the Clinical Laboratory Standards Institute (CLSI) recommendations (CLSI 2008; 2015). Mueller-Hinton broth for bacteria, RPMI-1640 medium buffered to PH 7.0 with MOPS for yeast strain were used as the test medium. Serial two-fold dilutions ranging from 5000 µg/mL to 4.9 µg/mL were prepared in medium. The inoculum was prepared using a 4-6h broth culture of each bacteria and 24h culture of yeast strains adjusted to a turbidity equivalent to a 0.5 Mc Farland standard, diluted in broth media to give a final concentration of $5x10^5$ cfu/mL for bacteria and $0.5x10^3$ to $2.5x10^3$ cfu/mL for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. The trays containing Mueller-Hinton broth were incubated at 35 °C for18-20h and the trays containing RPMI-1640 medium were incubated at 35 °C for 46-50h. The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of compound giving complete inhibition of visible growth. As control, antimicrobial effects of the solvents were investigated against test microorganisms. According to values of the controls, the results were evaluated.

RESULTS AND DISCUSSION

Antioxidant activity

It was reported that the oil, rhizomes, stems, leaves, and flowers of different *Ferulago* species contain phenolic compounds and show antioxidant activity (Azarbani et al. 2012; Mileski et al. 2015; Dikpınar 2017; Kiziltas et al. 2017).

The results given in Table 1 showed that the amount of extractable phenolic compounds and flavonoids in HFT extract is higher than that detected in RFT extract ($p<0.05$), so the aerial

parts of *Ferulago trojana* is a rich source of phenolics and flavonoids. A similar content of flavonoids was reported by (Kiziltas et al. 2017) for the flowers of *Ferulago angulata* (Schlecht.) Boiss. (Apiaceae).

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 of electrons. The aerial parts showed the better antioxidant activity than rhizomes in DPPH and FRAP assays. However, the results showed a weak antioxidant activity of both the extract compared to the referance antioxidant quercetin. Although the extract 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 antioxidant.

These results are in accordance with previous studies, which reported the efficiacy of *Ferulago* species to scavenge free radicals (Azarbani et al. 2012; Mileski et al. 2015; Dikpınar 2017; Kiziltas et al. 2017). Antioxidant activity against lipid peroxidation (LPO) has been reported for the dichloromethane extract of *F. trojana*, which is attributed to the richness of the coumarin in the extract (Çakar, 2010). Çakar (2010) also reported that the pure compounds isolated from the *Ferulago trojana* (bergapten, isoimperatorin, 3'-epidecursin,

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

Extract	PC (mg/g extract)	Flavonoids (mg/g extract)
HFT	64.49±4.47[a]	58.89±4.11[a]
RFT	6.51±0.82[b]	4.56±0.70[b]

HFT: Aerial parts of *F. trojana*
RFT: Rhizomes of *F. trojana*
Values were the means of three replicates ± standard deviation.
Values with different letters in the same column were significantly ($p<0.05$) different.

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

Extracts	Lipid peroxidation[a] EC_{50} (mg/mL)	DPPH[a] EC_{50} (mg/mL)	ABTS[a] EC_{50} (mg/mL)	Reducing power[a] EC_{50} (mg/mL)	Total Antioxidant potential[b]* (mM/L TEAC)	FRAP value[c]* (mM/L Fe^{2+})
HFT	3.01±0.045[a]	1.35±0.069[a]	1.79±0.22[a]	1.18±0.34[a]	2.15±0.005[a]	1.87±0.08[a]
RFT	N.d	16.69±0.34[b]	N.d.	10.73±0.54[b]	0.26±0.03[b]	0.27±0.03[b]
quercetin	0.034±0.006[b]	0.069±0.001[f]	0.113±0.002[d]	0.019±0.003[e]	2.15±2.42[a] (at 0.16 mg/mL)	2.15±0.011[a] (at 0.16 mg/mL)

HFT: Aerial parts of *F. trojana*; RFT: Rhizomes of *Etrojana*
[a] EC_{50} value: The effective concentration at which the antioxidant activity was 50%; DPPH and ABTS radicals were scavenged by 50% and the absorbance was 0.5 for reducing power. 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 dry weight
* - Determined at 5 mg/mL
N.d. Not determined
Values were the means of three replicates ± standard deviation

Table 3. Antimicrobial activities (MIC in mg/L) of methanol extracts from *F. trojana*

Extracts/ Pozitive control (mg/L)	*Staphylococcus aureus* ATCC 6538	Methicillin-resistant Staphylococcus aureus ATCC 33591	*Staphylococcus epidermidis* ATCC 12228	*Escherichia coli* ATCC 8739	*Klebsiella pneumoniae* ATCC 4352	*Pseudomonas aeruginosa* ATCC 1539	*Proteus mirabilis* ATCC 14153	*Candida albicans* ATCC 10231
HFT	4.8	48	78	-	-	-	-	-
RFT	78	19	625	-	-	-	156	312
Cefuroxime- Na	1.2	nt	9.8	4.9	4.9	nt	2.4	nt
Vancomycin	nt	2	nt	nt	nt	nt	nt	nt
Ceftazidime	nt	nt	nt	nt	nt	2.4	nt	nt
Klotrimazole	nt	nt	nt	nt	nt	nt	nt	4.9

MIC: miniumum inhibitory concentration; HFT: Aerial parts of *Etrojana*; RFT: Rhizomes of *Etrojana*
(–): Not active
(nt): Not tested

isomaltol, isomaltol-3β-O-glucoside and 3,6-dimethoxy-7-isopropilcoumarin-4- tetradeca-13"-one) showed antioxidant activity against lipid peroxidation investigated in a β-carotene-linoleic acid model system, but do not have free radical scavenging ability.

Antimicrobial activitiy

The antimicrobial activity of *F. trojana* has been studied for the first time. The antimicrobial activity results of methanol extracts prepared from aerial parts (HFT) and rhizomes (RFT) of *F. trojana* are shown in Table 3. In this study, both methanol extracts from aerial parts and rhizomes of *F. trojana* showed antibacterial activity against Gram positive bacteria such as *S. aureus,* MRSA, *S. epidermidis* while no activity was observed against *E. coli, K. pneumoniae* and *P. aeruginosa* for any of the extracts. Methanol extract from rhizomes of *F. trojana* showed antibacterial activity against *P. mirabilis* and antifungal activity the yeast *C. albicans*. When the results of the antimicrobial activity were evaluated, it was found that the RFT extract showed moderate antimicrobial activity against 4 bacteria and 1 fungal strain while the HFT extract showed activity against three bacterial strains. When compared with positive control results, the best activity was the HFT extract against the *S. aureus* strain with an MIC value of 4.8 mg/L; *S. epidermidis* with 78 mg/L. It is planned to identify the active compounds and to exhibit antimicrobial activity of the HFT extract.

CONCLUSION

It was concluded that methanol extract from the aerial parts of the plant, containing the highest amount of total phenolics and flavonoids, has the antioxidative potential for chain-breaking inhibition of lipid peroxidation and shows the strongest hydrogen and a single electron donor activities, thus could serves as free radical scavenger, acts as reductant and provide protection against oxidative stress. Although the methanol extract from rhizomes did not show any inhibitory effect on lipid peroxidation, it may also be expected to protect against oxidative damage by scavenging free radicals and acting as reductant. The results demonstrated the health promoting potential of aerial parts from *F. trojana*. On the other hand, because of the high antimicrobial activity of the plant, the aerial parts of the plant and its rhizomes could be source of antimicrobial effective new molecules.

REFERENCES

- Akalın E, Pimenov MG (2004). *Ferulago trojana* (Umbelliferae), a new species from western Turkey. *Bot J Linn Soc* **146:** 499-504. [CrossRef]
- Akalın E (1999). Taxonomical studies on the genus Ferulago in Western Anatolia. Unpublished PhD Thesis, Istanbul University, Istanbul.
- Alkhatib R, Hennebelle T, Roumy V, Sahpaz S, Süzgeç S, Akalın E, Mericli AH, Bailleul F (2009). Coumarins, caffeoyl derivatives and a monoterpenoid glycoside from *Ferulago asparigifolia. Biochem Syst Ecol* **37:** 230-233. [CrossRef]
- Azarbani F, Saki Z, Mohammadi A (2012). Phenolic contents, antibacterial and antioxidant activities of flower, leaf and stem extracts of *Ferulago angulata* (Schlecht) Boiss. *Int J Pharm Pharm Sci* **6:** 123-125.
- Başer KHC, Demirci B, Duman H (2001). Composition of the essential oil of *Ferulago asparigifolia* Boiss. from Turkey. *J Essent Oil Res* **13:** 134-135. [CrossRef]
- Başer KHC, Demirci B, Özek T, Akalın E, Özhatay N (2002). Microdistilled volatile compounds from *Ferulago* species growing in Western Turkey. *Pharm Biology* **40:** 466-471. [CrossRef]
- Baytop T. *Türkiye'de Bitkilerle Tedavi* (1999). İstanbul Üniversitesi Eczacılık Fakültesi Yayınları, Nobel Tıp Basımevi, 2.baskı, İstanbul, pp. 348-349.
- Benzie IFF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal Biochem* **239:**70-76. [CrossRef]
- Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *Food Sci Technol* **28:** 25-30. [CrossRef]
- Buege JA, Aust SD (1978). Lipid peroxidation. *Method Enzymol* **52:** 302-310. [CrossRef]
- Chalchat JC, Garry R, Gorunovic MS, Bogavac PM (1992). Composition of the essential oil of *Ferulago sylvatica* (Besser) Reichenb. *Pharmazie* **47:** 10-11
- Clinical and Laboratory Standards Institute (CLSI) (2015). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved Standard M7-A10. Wayne, Pennsylvania.
- Clinical and Laboratory Standards Institute (CLSI) (2008). Reference Method for Broth Dilution Antifungal Susceptbility Testing of Yeasts; Approved Standart Third Edition. Wayne M27-A3 Wayne, Pennsylvania.
- Cushnie TPT, Lamb AJ (2005). Antimicrobial activity of flavonoids. *Int J Antimicrob Agents* **26:** 343-356. [CrossRef]
- Çakar B (2010). Isolation of secondery metabolites of *Ferulago idaea* and *Ferulago trojana* plants, investigation of their antioxidant and anticholinesterase activities. Unpublished MSc Thesis, Istanbul Technical University, Istanbul.
- Davis PH, Mill RR, Tan K (1988). *Flora of Turkey and the East Aegean Islands*, Vol. 10, pp. 145–154 (Suppl. 1). Edinburgh University Press, Edinburgh.
- Dikpınar T (2017). The active constituents isolation from *Ferulago trachycarpa* Boiss. by antimicrobial activity-guided. Unpublished MSc Thesis, Marmara University, Istanbul.
- Doğanca S, Ulubelen A, Tuzlacı E (1991). 1-Acetylhydroquinone 4-galactoside from *Ferulago aucheri. Phytochem* **30:** 2803-2805. [CrossRef]
- Duh PD, Tu YY, Yen GC (1999). Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium Ramat*). Lebensm Wiss Technol **32:** 269-277. [CrossRef]
- Erdemoğlu N, Akalın E, Akgöç M, Çıkrıkçı S, Bilsel G (2008). Comparison of the seed oils of *Ferulago trachycarpa* Boiss. different localities with respect to fatty acids. *Rec Nat Prod* **2:** 13-18.
- Erdurak CS, Coşkun M, Demirci B, Başer KHC (2006). Composition of the essential oil of fruits and roots of *Ferulago isaurica* Peşmen and *F. syriaca* Boiss. (Umbelliferae) from Turkey. *Flavour Fragr J* **21:** 118-121. [CrossRef]
- Jiménez B, Concepción Grande M, Anaya J, Torres P, Grande M (2000). Coumarins from *Ferulago capillaris* and *F. brachyloba*. *Phytochem* **53:** 1025-1031. [CrossRef]
- Kähkönen MP, Hopia AN, Vuorela HJ, Rauha J-P, Pihlaja K, Kujala TS, Heinonen M (1999) Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem* **47:** 3954-3962. [CrossRef]
- Kandemir A & Hedge IC (2007). An anomalous *Ferulago* (Apiaceae) from eastern Turkey. *Willdenowia* **37:** 273-276. [CrossRef]
- Kılıç CS, Okada Y, Coşkun M, Okuyama T (2006). New furanocoumarins isolated from the roots of *Ferulago isaurica* Peşmen growing in Turkey. *Heterocycles* **69:** 481-486. [CrossRef]

- Kiziltas H, Eki, S, Bayramoglu M, Akbas E, Oto G, Yildirim S, Ozgokce F (2017). Antioxidant properties of *Ferulago angulata* and its hepatoprotective effect against N-nitrosodimethylamine-induced oxidative stress in rats. *Pharm Biol* **55:** 888-897. [CrossRef]
- Mileski KS, Džamić AM, Ćirić AD, Ristić MS, Grujić SM, Matevski VS, Marin, PD (2015). Composition, antimicrobial and antioxidant properties of endemic species *Ferulago macedonica* Micevski & E. Mayer. *Rec Nat Prod* **9:** 208-223.
- Miski M, Moubasher HA & Mabry TJ (1990). Sesquiterpene aryl esters from *Ferulago antiochia. Phytochem* **29:** 881-886. [CrossRef]
- Özhatay N, E Akalın (2000). A New species of *Ferulago* W.Koch (Umbelliferae) from North-west Turkey. *Bot J Linn Soc* **133:** 353-342. [CrossRef]
- Peşmen H (1972). *Ferulago* W.Koch In: Davis PH (ed), *Flora of Turkey and the East Aegean Islands*, Vol.4: 453-471, Edinburgh University Press, Edinburgh.
- Pietta P-G (2000). Flavonoids as antioxidants. *J Nat Prod* **63:** 1035-1042. [CrossRef]
- Pimenov MG, Leonov MV (1993). The Genera of the Umbelliferae, A Nomenclator. London: Royal Botanic Gardens Kew.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio Med* **26:** 1231-1237. [CrossRef]
- Rios JL & Recio MC (2005). Medicinal plants and antimicrobial activity. *J Ethnopharmacol* **100:** 80-84. [CrossRef]
- Rustaiyan A, Yari M, Masoudi S, Aghjani Z (1999). Chemical constituents of the essential oil of *Ferulago contracta* Boiss & et Hausskn., a species endemic to Iran. *J Essent Oil Res* **11:** 609-610. [CrossRef]
- Sakanaka S, Tachibana Y & Okada Y (2005). Preparation and antioxidant properties of extracts of *Japanese persimmon* leaf tea (kakinoha-cha). *Food Chem* **89:** 569-575. [CrossRef]
- Slinkard K & Singleton VL (1977). Total phenol analysis: automation and comparison with manual methods. *Am J Enol Viticult* **28:** 49-55.
- Solanas JL, Crespo MB & Martin FG (2000). Una nueva especie Iberica de *Ferulago* Koch (Apiaceae). *Anales del Jardín Botánico de Madrid* **58**: 101–107 (in Spanish with English abstract). [CrossRef]
- Yoti MJ & Assenov I (1995). Phyrochemical studies on *Ferulago sylvatica*. *Fitoterapia* **66:** 88-89.
- Wang M, Shao YLJ, Zhu N, Rangarajan M, LaVoie, EJ, Ho CT (1999). Antioxidative phenolic glycosides from Sage (*Salvia officinalis*). *J Nat Prod* **62:** 454-456. [CrossRef]