

# Phytochemical study and cytotoxic activity of *Ferulago angulata* (Schlecht) Boiss, from Kurdistan-region of Iraq

Baram AHMED HAMAH AMEEN\*

\*Department of Chemistry, School of Science, University of Sulaimani, Kurdistan-Iraq

**Abstract**—Four furanocoumarins and three sterol were isolated from the dichloromethane extract of the roots of *Ferulago angulata*, a folk Kurdish medicine and an herb as food additives. They were identified as xanthotoxin (1), isoimperatorin (2), oxypeucedanin (3), oxypeucedannin hydrate (4),  $\beta$ -sitosterol (5), stigmasterol (6) and  $\beta$ -sitosterol linoleate (7) based on the <sup>1</sup>H and <sup>13</sup>C-NMR data. The cytotoxic effects of compounds were evaluated on human cancer cell line, HeLa. Oxypeucedannin hydrate and isoimperatorin displayed the highest potency against HeLa cells with IC<sub>50</sub> of 16.7 and 24.5  $\mu$ M, respectively. The results of the present investigation indicated that furanocoumarins isolated from *Ferulago angulata* root extract can be considered as cancer chemopreventive agents.

**Keywords**— *Ferulago angulata*, Apiaceae, furanocoumarin, Cytotoxicity, HeLa Cells, food additive.

## I. INTRODUCTION

Since ancient times, herbs and spices have been added to different types of food to improve the flavor and to prevent from decay. Medicinal plants are a potential source of food additives in the food industry, today it has both nutritional and medicinal benefits. Many natural compounds isolated from plants have demonstrated a wide spectrum of biological activities [1].

The genus *Ferulago* (Apiaceae) is represented by 40 species in the world. In different regions of Turkey *F. terula* and *F. prangos* species have been used in folk medicine for their sedative, tonic, digestive, aphrodisiac properties and for treatment of intestinal worms and haemorrhoids [2],[3]. Moreover some of it have been used in folk medicine against snake bite, ulcers and for treatment of headache and spleen disorders[4].

Moreover *F. angulata* has been used in folk Kurdish medicine against ulcers and for treatment of hemorrhoids, *F. angulata* is one of the two species of the genus *Ferulago* occurring in Kurdistan Region of Iraq, Iran and Turkey[5][6][7]. Traditionally this plant was added to different productions such as oil ghee to prevent from decay and it keep the meat for longer time as well as give them a pleasant taste.

A literature review shows that *F. angulata* from Iran has previously been investigated for their essential oil compositions. The essential oil of the aerial parts of *F. angulata* have been reported to contain sixty-two volatile compounds. (Z)- $\beta$ -ocimene (35.5%), terpinolene (5.7%) and  $\alpha$ -phellandrene (5.4%) were major components[8]. The extracts of *F. angulata* showed antioxidant and cytotoxic activity [9][10], there is not any study on *F. angulata* from Iraq. In this study, we report the phytochemical investigation on a dichloromethane extract of the roots of *F. angulata* which led to the isolation and identification of four furanocoumarins.

The genus *Ferulago* is well documented as a rich source of biologically active compounds such as, coumarins, pyranocoumarins, furanocoumarins, sesquiterpenes, monoterpenes, aromatic compounds, flavonoids, phenylpropanoids and sesquiterpene lactones [11][12][13][14][15][16][17]. The furanocoumarins isolated from genus *Ferulago* have different biological activities such as antibacterial, anticoagulant, anti-inflammatory, antiviral, antioxidant and on their in vitro cytotoxic activity which have not been previously reported [18][19][20][21]

There are some studies about the cytotoxicity activity of furanocoumarins, such as Pangelin and oxypeucedanin hydrate acetone from *Angelica dahurica*, osthol and imperatorin from *Cnidium monnieri*, feroniellins A, B, and C from *Feroniella lucida*, as cytotoxic agents[22][23][24].

## II. MATERIALS AND METHODS

### II-1. General Experimental Procedures

NMR spectra were measured on a Bruker DRX-500 spectrometer. Semi-preparative HPLC was carried out with an Eurospher 100-7 RP C18 (250  $\times$  20 mm; Macherey Nagel) column with a MeOH-H<sub>2</sub>O gradient. Silica gel (70-230 and 230-400 mesh, Merck) and RP C18 (230-400 mesh, Fluka) were used for column chromatography. Silica TLC was performed on Merck F254 silica gel plates (10  $\times$  10 cm).

### II-2. Plant Material

Roots of *F. angulata* were collected from Sulaimanyia (Pinjwen), Kurdistan Region of Iraq, in July 2013 at an altitude of 800 meters, and identified. A voucher specimen (7104, 7105, 7106, 7107) is deposited in the Herbarium of the College of Science, Salahaddin University, Iraq.

### II.3. Extraction and Isolation

The roots of *F. angulata* (700 g) were crushed and extracted with dichloromethane ( $3 \times 3$  L, rt for 24 h) to obtain 17 g of extract. A portion (15 g) of extract was fractionated on a silica gel column eluted with mixtures of n-hexane-EtOAc of increasing polarity (100:0, 95:5, 90:10, 85:15, 80:20, 70:30, 50:50, 25:75, 0:100, respectively) to give seven fractions A1-A7.

Fraction A4 (3 g) was further purified on a silica gel column [n-hexane- EtOAc (8:2) to EtOAc], to afford five fractions (A4.1-A4.5). The fraction A4.2 was pure (2, 8 mg). Semi-preparative RP-HPLC (MeOH in H<sub>2</sub>O (75% to 100% MeOH) of fraction A4.4 gave 1 (12 mg) and 3 (10 mg). Oxypeucedannin hydrate (4, 10 mg) was obtained from fraction A7 on a silica gel column [CHCl<sub>3</sub>-MeOH (8:2 to 3:7)]. Fraction A2 (500 mg) was separated over a silica gel column [n - hexane – EtOAc (8:2 – 5:5)] to afford a mixture of  $\beta$ -sitosterol and stigmasterol (5 and 6, 20 mg). Silica gel chromatography [n-hexane– CHCl<sub>3</sub>– EtOAc (8:10:2– 2:10:8)] of fraction A3 gave  $\beta$ -sitosterol linoleate (7, 6 mg).

### II-4. Evaluation of cytotoxicity

Cytotoxicity of the furanocoumarins isolated from the roots of *F. angulata* was assessed by determination of their IC<sub>50</sub>s using HeLa-60 cell line. The ovarian cancer cell line (HeLa-60) was obtained from the Pasteur Institute of Iran and was cultured in RPMI 1640 medium, supplemented with 10% FBS, 100U/mL penicillin, and 100  $\mu$ g/mL streptomycin. Compounds 1–4 were dissolved in dimethyl sulfoxide (DMSO) to make a stock solution. The DMSO concentration was kept below 0.05% throughout the cell culture period and did not exert any detectable effect on cell growth or cell death. The cells were incubated at 37 °C and 5% CO<sub>2</sub> in 96-well plates. After incubation for 24 h, cells were treated with the four compounds at different concentrations. The cytotoxic effects of the compounds were investigated by colorimetric bioassay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) in a 24-well plate (triple holes). After incubation for 48 h, cell growth was measured by MTT assay. The percentage of cell growth inhibition was calculated as follows:

$$\text{Inhibition \% of cancer cells growth} = [A-B/A] \times 100$$

Where A is optical density of cancer cells and B is optical density of compound-treated HeLa cells [25].

## III. RESULTS AND DISCUSSION

The dichloromethane extract of *F. angulata* roots was separated by normal and reversed phase chromatography to give xanthotoxin (1), isoimperatorin (2), oxypeucedanin (3), oxypeucedannin hydrate (4),  $\beta$ -sitosterol (5), stigmasterol (6) and  $\beta$ -sitosterol linoleate (7). Compounds were identified by comparison of their spectral data (1H NMR and 13C NMR) with those reported in the literature [26], [27] and [28]. The NMR data of the compounds 1-4 have been summarized in Table I. The structures of the isolated compounds are shown in the Fig. I.

Isoimperatorin (2), oxypeucedanin (3) and oxypeucedannin hydrate (4) have been previously reported from various *F. angulata* species ([26], [27] and [28]). There are a report on xanthotoxin (1) isolated from *F. subvelutina*[17]. The presence of different furanocoumarins in *Ferulago* species could be used as chemotaxonomic marker in genus *Ferulago*.

Compounds 1-4 were tested for in vitro cytotoxicity in human cancer cell line, HeLa. (Table II). As shown, the least IC<sub>50</sub> (the highest cytotoxicity) was observed by oxypeucedannin hydrate (4) followed by isoimperatorin (2), xanthotoxin (1), and oxypeucedanin (3), respectively.

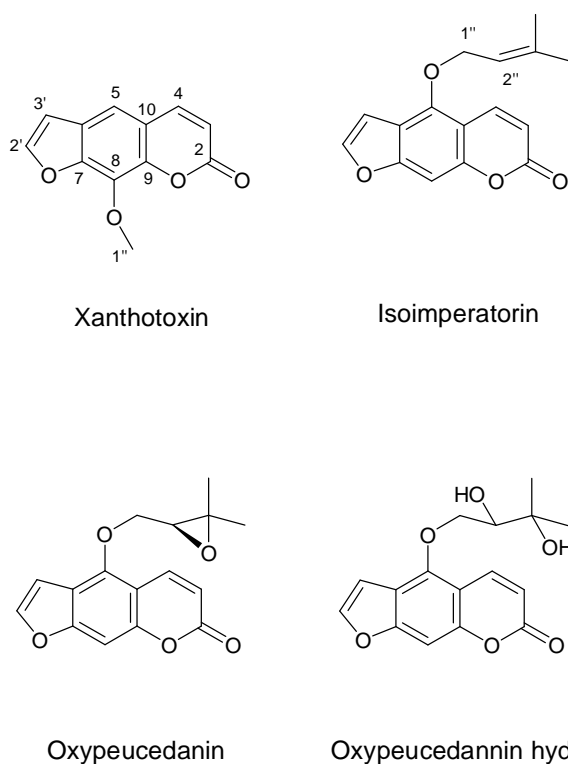
Recently, the cytotoxic activity of the isoimperatorin (2) and xanthotoxin (1) was evaluated against different tumor cell lines (leukemia, lung cancer, non-small lung carcinoma and liver cancer), and significant cytotoxicity was reported [22][29][30]and[31].

**Table I.** <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data for compounds **1-4** (CDCl<sub>3</sub>, 500 MHz for δ<sub>H</sub>, 125 MHz for δ<sub>C</sub>)<sup>a</sup>

Position	1 Xanthotoxin		2 Isoimperatorin		3 Oxypeucedanin		4 Oxypeucedannin hydrate	
	δ <sub>C</sub>	δ <sub>H</sub> (J in Hz, Integral)	δ <sub>C</sub>	δ <sub>H</sub> (J in Hz, Integral)	δ <sub>C</sub>	δ <sub>H</sub> (J in Hz, Integral)	δ <sub>C</sub>	δ <sub>H</sub> (J in Hz, Integral)
2	160.3		161.2		161.0		161.1	
3	114.4	6.37, d (9.5, 1H)	112.3	6.26, d (9.7, 1H)	113.1	6.30, d (9.7, 1H)	112.6	6.22, d (9.5, 1H)
4	144.1	7.76, d (9.5, 1H)	139.4	8.15, d (9.7, 1H)	139.0	8.20, d (9.7, 1H)	139.0	8.12, d (9.5, 1H)
5	112.7	7.34, s (1H)	148.7		148.2		148.3	
6	126.0		114.0		114.0		114.0	
7	147.4		158.0		158.1		158.0	
8	132.6		94.0	7.12, s (1H)	94.7	7.20, s (1H)	94.5	7.08, s (1H)
9	142.8		152.4		152.4		152.3	
10	116.2		107.1		107.3		107.0	
2'	146.4	7.69, d (2.2, 1H)	144.7	7.58, d (2.3, 1H)	145.1	7.61, s (1H)	145.0	7.56, d (2.5, 1H)
3'	106.5	6.80, d (2.2, 1H)	104.8	6.94, d (2.3, 1H)	104.2	6.95, s (1H)	104.6	6.96, d (2.5, 1H)
1''	61.1	4.26, s (3H)	69.5	4.91, d (6.8, 2H)	72.1	4.42, dd (10.3, 6.4, 1H) 4.59, dd (10.3, 6.6, 1H)	71.5	4.43, dd (9.5, 7.5, 1H) 4.53, dd (9.5, 3, 1H)
2''			119.0	5.53, t (6.8, 1H)	61.0	3.21, m (1H)	74.2	3.90, dd (7.5, 3, 1H)
3''			139.7		58.1		76.3	
4''			18.0	1.68, s (3H)	19.0	1.33, s (3H)	26.4	1.35, s (3H)
5''			25.7	1.79, s (3H)	24.5	1.41, s (3H)	25.0	1.30, s (3H)

<sup>a</sup> IC<sub>50</sub> in μM with standard deviation.

**Fig. I.** Chemical structures of the isolated furanocoumarins from *F. angulata* roots



**Table II.** Cytotoxic effects of compounds on HeLa human cancer cell line.

Compound	HeLa cells IC <sub>50</sub>
Xanthotoxin ( <b>1</b> )	32.4 ± 0.2 <sup>a</sup>
Isoimperatorin ( <b>2</b> )	24.5 ± 0.4
Oxypeucedanin ( <b>3</b> )	39.4 ± 0.3
Oxypeucedannin hydrate ( <b>4</b> )	16.7 ± 0.2

<sup>a</sup> IC<sub>50</sub> in μM with standard deviation.

#### IV. CONCLUSIONS

The results of this study suggest the possibility of using the furanocoumarins from *F. angulata* root extract as potential sources of cancer chemopreventive agent, and natural food preservatives for the pharmaceutical and food industry.

The presence of different furanocoumarins in *Ferulago* species could be used as chemotaxonomic marker in genus *Ferulago*.

#### ACKNOWLEDGMENT

Thanks are due to Michigan state university and Sulaimani University for using their facilities and to Dr.Chuanrai Zhang for his valuable comments on the manuscript and finally to my dear colleague Dr. Dara Dastan (Shahid Beheshti University) for interpretation of NMR spectra.

#### REFERENCES

- [1] C'avar, S., Maksimovic', M., Šolic', M. E., Jerkovic'-Mujkic', & A., Bešta, R. (2008). Chemical composition and antioxidant and antimicrobial activity of two Satureja essential oils. *Food Chemistry*, 111, 648-653.
- [2] Baser, K. H. C., Demirci, B., Demirci, F., Hashimoto, T., Asakawa, Y., & Noma, Y. (2002). Ferulagone: A new monoterpene ester from *Ferulago thirkeana* essential oil. *Planta Medica*, 68, 564-567.
- [3] Ozturk, B., Gur, S., Coskun, M., Kosan, M., Erdurak, C., Hafez, G., Ozgunes, O., & Cetinkaya, M. (2004). Relaxant effect of *Ferulago syriaca* root extract on human corpus cavernosum. *European Urology Supplements*, 3, 62-62.
- [4] Demetzos, C., Perdetzoglou, D., Gazouli, M., Tan, K., & Economakis, C. (2000). Chemical analysis and antimicrobial studies on three species of *Ferulago* from Greece. *Planta Medica*, 66, 560-563.
- [5] Fattah Saeed, J., Taher Raheem, M., & Dh Wadi, K. (2013). A systematic study about the genus *Ferulago* Boiss. (Umbelliferae) growing in Kurdistan Region of Iraq. *Advances in Bioresearch*, 4, 1-6.
- [6] Pimenov, M. G., & Leonov, M. V. (2004). The Asian Umbelliferae biodiversity database (ASIUM) with particular reference to South-West Asian taxa. *Turkish Journal of Botany*, 28, 139– 145.
- [7] Mozaffarian, V. (2007). *Flora of Iran: Umbelliferae*. Tehran: Research Institute of Forests and Rangelands Iran, (Vol 54).
- [8] Javidnia, K., Miri, R., Edraki, N., Khoshneviszadeh, M., & Javidnia, A. (2006). Constituents of the volatile oil of *Ferulago angulata* (schlecht.) Boiss. from Iran. *Journal of essential oil research*, 18, 548-550.
- [9] Khanahmadi, M., & Janfeshan, K. (2006). Study on antioxidation property of *Ferulago angulata* plant. *Asian Journal of Plant Science*, 5, 521-526.
- [10] Amirghofran, Z., Bahmani, M., Azadmehr, A., & Javidnia, K. (2006). Anticancer effects of various Iranian native medicinal plants on human tumor cell lines. *Neoplasma*, 53, 428-433.
- [11] De Pascual, T. J., Jimenez, B., Corrales, B., & Grande, M. (1979). Coumarins from *Ferulago granatensis* group: isovaleryl marmesin. *Anales De Quimica*, 75, 175-176.
- [12] Miski, M., Moubasher, H. A., & Mabry, T. J. (1990). Sesquiterpene aryl esters from *Ferulago antiochia*. *Phytochemistry*, 29, 881-886.
- [13] Doganca, S., Ulubelen, A., & Tuzlaci, E. (1991). 1-Acetylhydroquinone 4-galactoside from *Ferulago aucheri*. *Phytochemistry*, 30, 2803-2805.
- [14] Doganca, S., Tuzlaci, E., & Ulubelen, A. (1992). Constituents of *Ferulago asperigifolia*. *Fitoterapia*, 63, 552-555.
- [15] Jimenez, B., Grande, M. C., Anaya, J., Torres, P., & Grande, M. (2000). Coumarins from *Ferulago capillaris* and *F. brachyloba*. *Phytochemistry*, 53, 1025-1031.
- [16] Khalighi-Sigaroodi, F., Hadjiakhoondi, A., Shafiee, A., Mozaffarian, V. A., Shahverdi, A. R., & Alavi, S. H. R. (2006). Phytochemical analysis of *Ferulago Bernardii* Tomk and M. Pimen. *Daru*, 14, 214-221.
- [17] Naseri, M., Monsef-Esfahani, H. R., Saeidnia, S., Dastan, D., & Gohari, A. R. (2013). Antioxidant coumarins from roots of *Ferulago subvelutina*. *Asian Journal of chemistry*, 25, 1875-1878.
- [18] Widelski, J., Popova, M., Graikou, K., Glowinski, K., & Chinou, I. (2009). Coumarins from *Angelica lucida* L. - Antibacterial Activities. *Molecules*, 14, 2729-2734.
- [19] Piao, X. L., Park, I. H., Baek, S. H., Kim, H. Y., Park, M. K., & Park, J. H. (2004). Antioxidative activity of furanocoumarins isolated from *Angelicae dahuricae*. *Journal of Ethnopharmacology*, 93,243-246.
- [20] Razavi, S. M., Zahri, S., Nazemiyeh, H., Zarrini, G., Mohammadi, S., & Abolghassemi-Fakhri, M. A. (2009). A furanocoumarin from *Prangos uloptera* roots, biological effects. *Natural Product Research*, 23,1522-1527.
- [21] Abdelaal Selim, Y., & Hassan Ouf, N. (2012). Anti-inflammatory new coumarin from the *Ammi majus* L. *Organic and Medicinal Chemistry Letters*, 2, 1-4.
- [22] Thanh, P. N., Jin, W., Song, G., Bae, K., & Kang, S. S. (2004). Cytotoxic coumarins from the root of *Angelica dahurica*. *Archives of Pharmacal Research*, 27, 1211-1215.
- [23] Yang, L. L., Wang, M. C., Chen, L. G., & Wang, C. C. (2003). Cytotoxic activity of coumarins from the fruits of *Cnidium monnieri* on leukemia cell lines. *Planta Medica*, 69, 1091-1095.
- [24] Phuwapraisirisan, P., Surapinit, S., Sombund, S., Siripong, P., & Tip-pyang, S. (2006). Feroniellins A–C, novel cytotoxic furanocoumarins with highly oxygenated C10 moieties from *Feroniella lucida*. *Tetrahedron Letters*, 47,3685–3688.



- [25] Meng, H., Li, G., Huang, J., Zhang, K., Wei, X., Ma, Y., Zhang, C., & Wang, J. (2013). Sesquiterpenoid derivatives from *Ferula ferulaeoides* (Steud.) Korov. *Phytochemistry*, 86, 151-158.
- [26] Serkorov, S. V., Kagramanov, A. A., & Abbasov, R. M. (1976). Coumarins of *Prangos latiloba*. *Chemistry of Natural Compounds*, 12, 82-83.
- [27] Sklyar, Y. E., Andrianova, V. B., & Pimenov, M. G. (1982). Coumarins of the roots of *Ferulago sylvatica*. *Chemistry of Natural Compounds*, 18, 448-489.
- [28] Basile, A., Sorbo, S., Spadaro, V., Bruno, M., Maggio, A., Faronone, N., & Rosselli, S. (2009). Antimicrobial and Antioxidant Activities of Coumarins from the Roots of *Ferulago campestris* (Apiaceae). *Molecules*, 14, 939-952.
- [29] Jiang, H. Y., Wang, C. F., Fan, L., Yang, K., Feng, J. B., Geng, Z. F., Xu, J., Deng, Z. W., Du, S. S., & Yin, H. B. (2013). Cytotoxic Constituents from the Stems of *Clausena lansium* (Lour.) Skeels. *Molecules*, 18, 10768-10775.
- [30] Ruberto, G., Cannizzo, S., Amico, V., Bizzini, M., & Piattelli, M. (1994). Chemical constituents of *Ferulago nodosa*. *Journal of Natural Products*, 57, 1731-1733.
- [31] Satir, E., Kilic, S., & Coskun, M. (2009). Prantschimgin content of methanolic extract of roots of *Ferulago platycarpa*. *Chemistry of Natural Compounds*, 45, 872-873.