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

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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), β-sitosterol (5), stigmasterol (6) and β-sitosterol linoleate (7) based on the 1H and 13C-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 IC50 of 16.7 and 24.5 μM, 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)-β-ocimene (35.5%), terpinolene (5.7%) and α-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 acetonide 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 × 20 mm; Macherey Nagel) column with a MeOH-H2O 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 × 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 × 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 H2O (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 [CHCl3-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 β-sitosterol and stigmasterol (5 and 6, 20 mg). Silica gel chromatography [n-hexane – CHCl3 – EtOAc (8:10:2 – 2:10:8)] of fraction A3 gave β-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 IC50s 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 µg/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 ºC and 5% CO2 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:

Inhibition % of cancer cells growth = [A-B/A] ×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), β-sitosterol (5), stigmasterol (6) and β-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 TableI . 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 IC50 (the highest cytotoxicity) was observed by oxypeucedannin hydrate (4) followed by isoimperatorin (2), xanthotoxin (1), and oxypeucedanin (3), respectively.

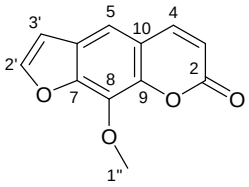
Recently, the cytotoxic activity of the isoimperatorin (2) and xanthotoxin (1) was evaluated against different tumor cell lines (leukemia , lung cancer, non-small lung carcinoma and liver cancer), and significant cytotoxicity was reported [22][29][30]and[31].

Table I. ^{1}H and ^{13}C NMR spectroscopic data for compounds **1-4** ($CDCl_3$, 500 MHz for δ_H, 125 MHz for δc)[a]

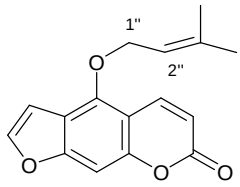
Position	**1** Xanthotoxin		**2** Isoimperatorin		**3** Oxypeucedanin		**4** Oxypeucedannin hydrate	
	δ_C	δH (J in Hz, Integral)	δ_C	δ_H (*J* in Hz, Integral)	δ_C	δ_H (*J* in Hz, Integral)	δ_C	δ_H (*J* in Hz, Integral)
2	160.3		161.2		161.0		161.1	
3	114.4	6.37, d (9.5, 1H)	112.3	6.26, d (9.7, 1H)	113.1	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, 1 H)
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)	71.5	4.43, dd (9.5, 7.5, 1H)
						4.59, dd (10.3, 6.6, 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)

[a] IC_{50} in µM with standard deviation.

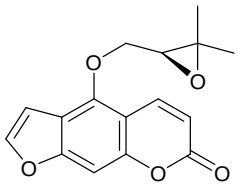
Fig. I. Chemical structures of the isolated furanocoumarins from *F. angulata* roots



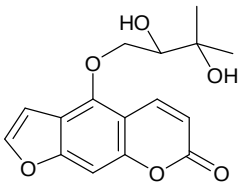
Xanthotoxin



Isoimperatorin



Oxypeucedanin



Oxypeucedannin hydrate

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

Compound	Hela cells IC_{50}
Xanthotoxin (**1**)	32.4 ± 0.2[a]
Isoimperatorin (**2**)	24.5 ± 0.4
Oxypeucedanin (**3**)	39.4 ± 0.3
Oxypeucedannin hydrate (**4**)	16.7 ± 0.2

[a] IC_{50} 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.

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