



FURANOCOUMARIN BIOACTIVES IN THE APIACEAE AND RUTACEAE FAMILIES OF PLANTS

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ABSTRACT

Citrus fruits, such as grapefruits and bitter oranges, contain large number of furanocoumarin bioactives which are potent inhibitors of hepatic P-450 cytochrome (CYP) enzymes. In comparison, relatively little is known of the inhibitory potency of furanocoumarin bioactives in plant-base traditional medicine, food supplements and nutraceuticals. In this study we identify and quantify the linear furanocoumarin bioactives in selected traditional herbal medicines and natural health products using gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography equipped with an ultra-violet detector (HPLC-UV). Ethanolic and aqueous extracts of 29 plant products belonging to the Apiaceae, Lamiaceae, Leguminosae, and Rutaceae families of plants were analyzed for the presence of linear furanocoumarins using GC-MS and HPLC-UV. Based on our experimental results, 8-Methoxypsoralen (8-MOP), 5-methoxypsoralen (5-MOP), and isopimpinellin (ISOP) were detected in the extracts of the following plant products: *Ammi majus* L. seeds, *Angelica archangelica* L. roots, *Angelica pubescens* Maxim. roots, *Apium graveolens* L. seeds, *Apium graveolens* L. flakes, *Cnidium monnieri* (L.) Cusson fruits, *Petroselinum crispum* (Mill.) Fuss leaves, *Pimpinella aniseum* L. seeds, and *Ruta graveolens* L. leaves. Different furanocoumarin bioactives, ranging from 0.016 to 11.468 mg/g dry weight, were found in these plant products. While, linear furanocoumarins such as 8-MOP, 5-MOP, and ISOP were commonly found in the Apiaceae and Rutaceae families of plants.

Keywords: GC-MS; HPLC-UV, furanocoumarins, natural health products, plant products.

Abbreviations (alphabetic order)

5-MOP, 5-methoxypsoralen; 8-MOP, 8-methoxypsoralen; FLD, Fluorescent Detectors; GC-MS, Gas Chromatography Mass Spectrometry; HPLC-UV, High Performance Liquid Chromatography Ultra-Violet Detection; ISOP, Isopimpinellin; LOD, Limit of Detection; LOQ, Limit of Quantitation; NIST, National Institute of Standards and Technologies

INTRODUCTION

Published literature have shown that furanocoumarin bioactives are often found in the following plant families: Amaranthaceae, Apiaceae (Umbelliferae), Compositae (Asteraceae), Cyperaceae, Dipsacaceae, Goodeniaceae, Guttiferae (Clusiaceae), Leguminosae (Fabaceae or Papilionaceae), Moraceae, Pittosporaceae, Rosaceae, Rutaceae, Samydaceae, Solanaceae, and Thymelaeaceae (Diawara and Trumble, 1997). The core structure of furanocoumarins consists of a furan ring fused with a coumarin molecule. The simplest linear and angular furanocoumarins are represented by psoralen and angelicin, respectively (Fig. 1).

In traditional Middle-Eastern medicine, a combination of sunlight exposure and *Ammi majus*, which contains furanocoumarins, consumption is used to treat vitiligo, a skin disease characterized by pigment loss (El-Mofty, 1964). However, consumption of furanocoumarin-containing plants may result in unintentional side-effects. For example, psoralen, angelicin, 8-MOP and 5-MOP can cause phototoxic reactions upon contact with skin (Quinn *et al.*, 2014). Also, furanocoumarin-rich fruits and vegetables such as grapefruits and bitter oranges have been shown to alter pharmaceutical drug metabolism by inhibiting cytochrome P450 (CYP) enzymes and result in potentially life-threatening food-drug and herb-drug interactions (Williamson, 2003).

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Previous studies have shown that both linear and angular

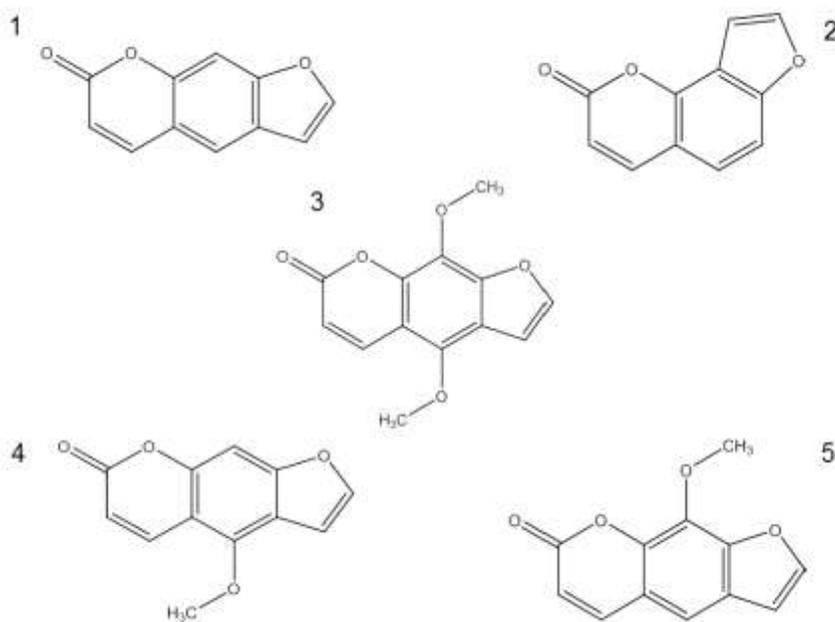


Fig. 1. Chemical structures of psoralen (1), angelicin (2), ISOP (3), 5-MOP (4), and 8-MOP (5). All these are linear furanocoumarins except angelicin which is an angular furanocoumarin.

furanocoumarins are present in plant products, vegetables, and fruits. Chromatographic techniques such as thin-layer chromatography (Ivie, 1978; Cieřla *et al.*, 2008), HPLC equipped with a UV detector (Beier *et al.*, 1994; Kamiński *et al.*, 2003; Frérot and Decorzant, 2004), fluorescent detector (FLD) (Frérot and Decorzant, 2004), or mass spectrometry (MS) detector (Dercks *et al.*, 1990; Frérot and Decorzant, 2004; Peroutka *et al.*, 2007), and Gas Chromatography (GC) equipped with a MS detector (Beier *et al.*, 1994; Peroutka *et al.*, 2007) or flame ionization detector (Cardoso *et al.*, 2000) are used to identify and quantify the furanocoumarins.

In the present study, the furanocoumarin bioactives in 29 food and herbal products were extracted, identified and quantified using GC-MS and HPLC-UV. Gas chromatography–mass spectrometry (GC-MS) was used initially to screen for the presence of linear and angular furanocoumarins in the ethanolic extracts of the plant products. The plant products that showed significant amounts of furanocoumarins also were extracted by the traditional method of decoction preparation in water (Duke, 2002) and were analyzed quantitatively using HPLC-UV to confirm results of GC-MS analysis.

MATERIALS AND METHODS

Sources of Herbs and Food

Plant products prepared from Apiaceae, Lamiaceae, Leguminosae, and Rutaceae families were obtained from commercial sources in Canada, USA, and Jordan (Table

1). The plant products were selected based on reports of finding furanocoumarins in these plants. All plant products used in our study were authenticated by the suppliers which certified that they were preservative-free, grown organically or wild-crafted.

Chemicals and Instrumentation

Acetonitrile ($\geq 99.9\%$), ethanol (99.9%), 8-MOP ($\geq 98.0\%$), and 5-MOP (99.0%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). ISOP (98.3%) was obtained from ChromaDex (Irvine, CA, USA). Ultrapure water was produced using a Millipore system (Billerica, MA, USA) with a minimum resistivity of 16.0 M Ω ·cm at 25°C. The detection of furanocoumarins in the ethanolic extracts was performed using a GC-MS system comprised of an Agilent 6890 GC, 5973 MS, 7683B auto sampler, and controlled by Chem Station software (version D.01.02.16) (Santa Clara, CA, USA). Furanocoumarins in aqueous extracts were quantified using an Agilent 1090 HPLC equipped with 79880A UV and 1046A FLD detectors. The High-performance liquid chromatography (HPLC) system was controlled by the Chem Station software (version A.10.02).

Experimental Methods

GC-MS analysis of linear and angular furanocoumarins in the ethanolic extracts of plant products

Ethanol was used to extract the plant products because the furanocoumarins were soluble in this organic solvent. Also, ethanol is a widely used solvent to prepare traditional decoctions and tinctures (Duke *et al.*, 2002).

Table 1. Selected plant products from the Apiaceae, Lamiaceae, Leguminosae, and Rutaceae plant families in this study.

Botanical Name	Common Name	Plant Family	Plant Part
<i>Ammi majus</i> L.	Khella Shaitani	Apiaceae	Seeds
<i>Ammi visnaga</i> L.	Khella	Apiaceae	Seeds
<i>Angelica archangelica</i> L.	Garden Angelica	Apiaceae	Roots
<i>Angelica dahurica</i> (Hoffm.) Maxim	Bai Zhi	Apiaceae	Roots
<i>Angelica pubescens</i> Maxim	Du Huo	Apiaceae	Roots
<i>Angelica sinensis</i> (Oliv.) Diels	Dong Gui	Apiaceae	Roots
<i>Anethum graveolens</i> L.	Dill	Apiaceae	Leaves
<i>Anethum graveolens</i> L.	Dill	Apiaceae	Seeds
<i>Anthriscus cerefolium</i> Hoffm.	Chervil	Apiaceae	Leaves
<i>Apium graveolens</i> L.	Celery	Apiaceae	Seeds
<i>Apium graveolens</i> L.	Celery	Apiaceae	Flakes
<i>Carum carvi</i> L.	Caraway	Apiaceae	Seeds
<i>Citri reticulatae</i> Blanco	Chen Pi	Rutaceae	Peels
<i>Cnidium monnieri</i> (L.) Cusson	Shi Chuang Zi	Apiaceae	Seeds
<i>Coriandrum sativum</i> L.	Coriander	Apiaceae	Seeds
<i>Cuminum cyminum</i> L.	Cumin	Apiaceae	Seeds
<i>Foeniculum vulgare</i> Mill.	Fennel	Apiaceae	Seeds
<i>Levisticum officinale</i> W.D.J. Koch	Lovage	Apiaceae	Root
<i>Levisticum officinale</i> W.D.J. Koch	Lovage	Apiaceae	Leaves
<i>Ligusticum chuanxiong</i> S.H.Qiu, Y.Q. Zeng, K.Y. Pan, Y.C.Tang & J.M. Xu	Chuan Xiong	Apiaceae	Roots
<i>Ligusticum porteri</i> J. M. Coult. & Rose	Osha	Apiaceae	Roots
<i>Ligusticum sinense</i> Oliv.	Gao Ben	Apiaceae	Roots
<i>Ocimum basilicum</i> L.	Basil	Lamiaceae	Leaves
<i>Pastinaca sativa</i> L.	Parsnip	Apiaceae	Roots
<i>Petroselinum crispum</i> (Mill.) Fuss	Parsley	Apiaceae	Leaves
<i>Petroselinum crispum</i> (Mill.) Fuss	Parsley	Apiaceae	Roots
<i>Pimpinella aniseum</i> L.	Anise	Apiaceae	Seeds
<i>Psoralea corylifolia</i> L.	Bu Gu Zhi	Leguminosae	Seeds
<i>Ruta graveolens</i> L.	Common Rue	Rutaceae	Leaves

Plant products were minced and reduced to fine powders using a Salton food processor (Dollard-des-Ormeaux, QC, Canada) model CG-1174. The plant powders were mixed with ethanol at 10 mg/mL and sonicated, using a Branson sonicator (Shelton, CT, USA), for 3 h under atmospheric pressure at 40-60°C. The extracts were cooled to room temperature for 5-10 min. Ethanol was added to replace the volume lost during extraction. The extract was filtered using a Millipore Millex-LG filter unit (0.2 µm) and analyzed using the GC-MS procedure described by Peroutka *et al.* (2007) with modifications. Our procedure used an Agilent HP-5 MS column (30 m × 0.25 mm, 0.25 µm). The following temperature program was used: 75°C

(0 - 2 min), 75-250°C (2 - 20 min), 250 - 280°C (20 - 25 min), and 280°C (25 - 30 min). Injection volume was 1.0 µL. High-purity helium was used at a constant flow rate of 0.5 mL/min. The MS was set at full scan with a 50 to 600 *m/z* range. The remaining settings and zone temperatures were similar to that of the published method (Peroutka *et al.*, 2007).

Quantitative analysis of 8-MOP, 5-MOP, and ISOP in the aqueous extracts of plant products using HPLC-UV.

Plant powders were mixed with 600.0 mL of filtered water and boiled under atmospheric pressure with occasional stirring on a model 11-493 Fisher Thermix hot

plate (Hampton, NH, USA) until half of the volume was evaporated. After cooling to room temperature for 5-10 min, the aqueous mixture was filtered by a Millipore Millex-LG filter unit (0.2 µm) and analyzed by HPLC-UV using the procedure described by Frerot and Decorzant (2004) with modification. Table 2 summarizes the amounts of herbal products used in the extraction.

The furanocoumarins were separated by gradient elution at room temperature. The mobile phase consisted of different proportions of acetonitrile (A) in ultrapure water at various run times: 0-35 min (A, 5-100%), 35-40 min (A, 100%), and 40-45 min (A, 100-5%). The flow rate was 1.0 mL/min. The injection volume was 5.0 µL. The

UV detector was set at 310 nm using 550 nm as the reference. The aqueous extracts were examined using a Phenomenex (Torrance, CA, USA) phenyl-based reverse-phase Kinetex pentafluorophenyl column (250 x 4.6 mm, 5 µm particle size).

Calibration curves and data analysis

The furanocoumarins in ethanolic extracts were measured semi-quantitatively using GC-MS and a one-point calibration curve (3140, 303, and 153 µg/mL for 8-MOP, 5-MOP, and ISOP, respectively). A full calibration curve was used to determine the concentration of individual furanocoumarins in the aqueous extracts using HPLC-UV. The calibration curves were prepared from different

Table 1. Weights of plant products used in present and previous studies.

Botanical Name	Reported Single	Amount Used in	Reference
	Dose Range (g)	Present Study (g)	
<i>A. majus</i> seeds	6.0 - 12.0 (dry)	6.0 (dry)	Lerner <i>et al.</i> (1953)
<i>A. archangelica</i> roots	0.5 - 4.5 (dry)	4.5 (dry)	Duke (2002)
<i>A. pubescens</i> roots	Up to 10.0 (dry)	12.0 (dry)	Jianhua (2009)
<i>A. graveolens</i> seeds	Up to 100.0 (fresh)	10.0 (dry)	Rastmanesh and Baer (2011)
<i>A. graveolens</i> flakes	1.0 – 13.0 (dry)	10.0 (dry)	Duke (2002)
<i>C. monnieri</i> fruits	3.0 – 10.0 (dry)	3.0 (dry)	Jianhua (2009)
<i>P. crispum</i> leaves	0.5 – 20.0 (dry)	10.0 (dry)	Duke (2002)
<i>P. aniseum</i> seeds	Up to 50.0 (fresh)	10.0 (dry)	Rastmanesh and Baer (2011)
<i>R. graveolens</i> leaves	0.5 – 10.0 (dry)	3.0 (dry)	Duke (2002)

Table 2. Semi-quantitative analyses of 8-MOP, 5-MOP, and ISOP in the ethanolic extracts of plant products using GC-MS.

Botanical Name	Plant part	Linear Furanocoumarins Content (µg/g) Dry Weight						
		8-MOP		5-MOP		ISOP		Total
<i>A. majus</i>	Seeds	2349.0	^a	380.9	^a	5553.7	^a	8283.6
<i>A. archangelica</i>	Roots	73.3	^a	180.5	^a	463.8	^a	717.6
<i>A. pubescens</i>	Roots	16.4	^a	11.5	^a	n.d.	^c	28.0
<i>A. graveolens</i>	Seeds	4.3	^b	7.3	^b	16.2	^b	27.8
<i>A. graveolens</i>	Flakes	7.4	^b	3.0	^b	8.2	^b	18.6
<i>C. monnieri</i>	Fruits	442.1	^a	252.5	^a	1480.4	^a	2175.1
<i>P. crispum</i>	Leaves	n.d.	^c	14.3	^b	n.d.	^c	14.3
<i>P. aniseum</i>	Seeds	5.8	^a	n.d.	^c	n.d.	^c	5.8
<i>R. graveolens</i>	Leaves	458.7	^a	171.2	^a	43.6	^a	673.4

^aIdentification confirmed by retention time and mass spectrum.

^bIdentification confirmed by retention time only.

^cn.d. = not detected.

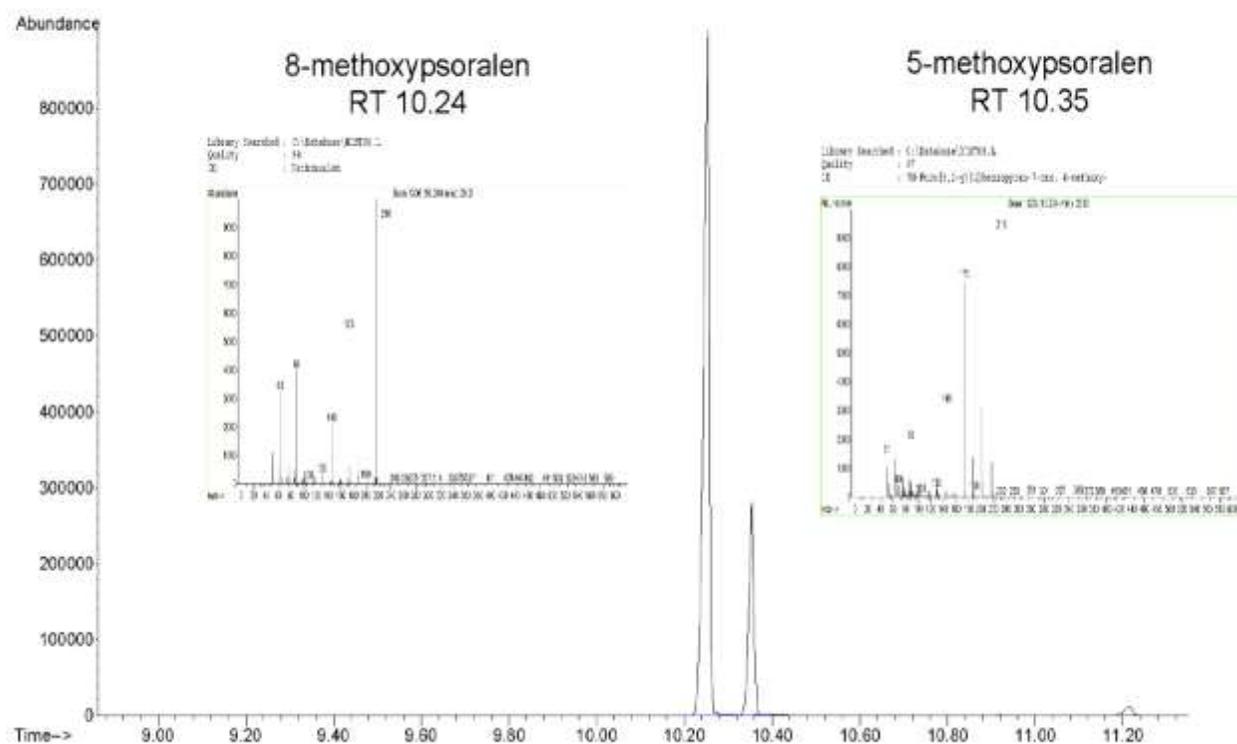


Fig. 1. GC-MS chromatograms and mass spectra of 8-MOP and 5-MOP.

concentration ranges of furanocoumarin standards: 8-MOP (6.13 – 196.25 $\mu\text{g/mL}$), 5-MOP (4.74 – 151.67 $\mu\text{g/mL}$), and ISOP (2.4 – 153.3 $\mu\text{g/mL}$). Concentrations of 8-MOP, 5-MOP, and ISOP in these aqueous extracts were determined by linear regression analysis using Microsoft Excel 2010 (Redmond, WA, USA). The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from the calibration curves based on DIN 32645 method (DIN, 1994) using B.E.N. software version 2 (Herbold and Schmitt, 2000).

RESULTS AND DISCUSSION

GC-MS Screening Results

The presence of furanocoumarins in 29 plant products from the Apiaceae, Lamiaceae, Leguminosae, and Rutaceae families of plants was screened initially with GC-MS. Furanocoumarins were found in the ethanolic extracts of *A. majus* roots, *A. archangelica* roots, *A. pubescens* roots, *A. graveolens* seeds and flakes, *C. monnieri* fruits, *L. officinale* roots, *P. crispum* leaves, *P. aniseum* seeds, *P. corylifolia* fruits, and *R. graveolens* leaves. The most common furanocoumarins found were 8-MOP, 5-MOP, and ISOP (Table 3). These findings

generally were consistent with results reported by EMEA (2007).

The furanocoumarins were identified based on their retention times and mass spectra in the GC-MS library. The retention times of 8-MOP, 5-MOP, and ISOP were 10.24, 10.35, and 11.20 min, respectively (Figures 2 and 3). The mass spectra of 8-MOP and 5-MOP showed a base peak of 216 m/z followed by ion 173 m/z . However, ISOP had a base peak at 231 m/z followed by ion 246 m/z . After the chromatographic peaks were identified using the National Institute of Standards and Technologies (NIST) library database; they were quantified using ions 216 m/z and 246 m/z because of interference by chemicals in the plant matrices.

Several less common furanocoumarins also were identified in the ethanolic extracts of different plant products but in very low concentrations. For example, pimpinellin was identified in *A. archangelica* roots with a retention time of 10.69 min and a NIST match score of 98%. Also, linear furanocoumarins such as sphondin, oxypeucedan in hydrate, neobyakangelicol, and byakangelicin were identified with retention times at 10.34,

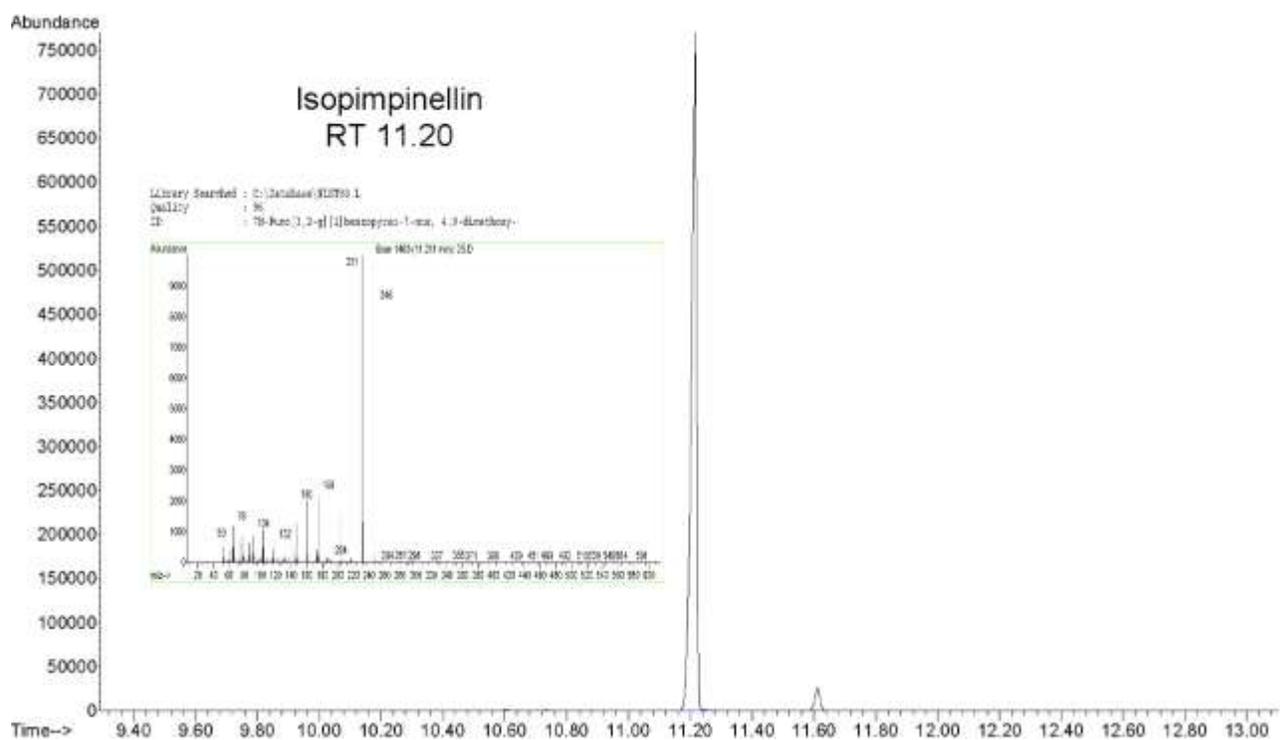


Fig. 2. GC-MS chromatogram and mass spectrum of ISOP.

14.34, 14.83, and 15.70 min and NIST match scores of 95, 93, 94, and 98%, respectively. An angular furanocoumarin, isobergapten was detected in *A. archangelica* and *L. officinale* roots with retention times of 10.2 and 10.19 min and NIST match scores of 91 and 93%, respectively. The angular furanocoumarin, angelicin was detected in *A. archangelica* roots and *P. corylifolia* fruits with 8.93 and 8.91 min retention times, respectively and a NIST match score of 97% for both herbs. The linear furanocoumarin psoralen was detected in *P. corylifolia* fruits and *R. graveolens* leaves with retention times of 9.19 and 9.18 min and NIST match scores of 98 and 96%, respectively.

Quantitative HPLC-UV Results

Three major linear furanocoumarins, 8-MOP, ISOP, and 5-MOP, were consistently found in the aqueous extracts of several plant products (Table 5); the HPLC retention times were 16.30, 17.23, 17.87 min, respectively (Fig. 4). As mentioned before, furanocoumarin concentrations in the aqueous extracts were determined using a HPLC-UV and full calibration curves.

Previous studies identified 8-MOP, 5-MOP, and ISOP in the aqueous extracts using different HPLC conditions: (a) Beier (1985) reported separating 8-MOP, 5-MOP, and ISOP with retention times from 5-10 min using a reverse-phase phenyl column and an isocratic system of methanol and water (44/56 v/v), (b) Liu *et al.* (2004) separated the

same furanocoumarins with retention times between 5 and 10 min using a gradient elution method of methanol-acetonitrile-water and a reverse-phase C-18 column, and (c) Li and Chen (2005) used a gradient elution method of methanol and water and a reverse-phase C-18 column to separate 8-MOP, 5-MOP, and ISOP with retention times ranging from 2 to 4 min. Although these HPLC methods successfully separated the furanocoumarins in the aqueous extracts of *C. monnieri* and *A. graveolens*, they were unable to separate the furanocoumarins in other plant extracts due to interference by the chemicals in the plant matrices. For example, *A. archangelica* root contained chemicals that interfered with the analysis of furanocoumarins on HPLC-UV. As a result, Wszelaki *et al.* (2011) used a gradient elution system of formic acid-acetonitrile-water (0.1/5/95 v/v/v) and formic acid-acetonitrile (0.1/100 v/v) with a reverse-phase C-18 (1.9 μm particle size) column to separate 8-MOP, 5-MOP, and ISOP in *A. archangelica* root; the retention times were between 5 and 7 min. Frerot and Decorzant (2004) also used a gradient elution system of water-acetonitrile-tetrahydrofuran (85/10/5 v/v/v) and acetonitrile-methanol-tetrahydrofuran (65/30/5) with a reverse-phase C-18 (3.1 μm particle size) column to separate 15 furanocoumarins in citrus oils. The retention times of 8-MOP, 5-MOP, and ISOP were between 20 and 25 min. Although these methods were able to separate the furanocoumarins, they could not be implemented in our HPLC system due to elevated backflow pressure.

Table 3. Analytical limits of linear furanocoumarin detection using HPLC-UV equipped with a pentafluorophenyl column.

Furanocoumarin	LOD ^a	LOQ ^b
	µg/mL	
8-MOP	7.8	29.1
5-MOP	2.1	7.8
ISOP	6.3	23.3

^aLimit of detection.^bLimit of quantitation.

Table 4. 8-MOP, 5-MOP, and ISOP concentrations in the aqueous extracts of furanocoumarin-containing plant products.

Botanical Name	Plant part	Linear Furanocoumarins Content (µg/g dry weight) ^a									
		8-MOP			5-MOP			ISOP			Total
<i>A. majus</i> ^b	Seeds	3213.5	±	219.7	717.2	±	6.5	7537.2	±	1492.9	11467.8
<i>A. archangelica</i> ^b	Roots	651.4	±	51.8	392.5	±	208	606.0	±	131.9	1650.0
<i>A. pubescens</i> ^c	Roots	25.6	±	20.0	32.5	±	20.0	n.d. ^d			58.1
<i>A. graveolens</i> ^b	Seeds	21.0	±	4.4	16.9	±	6.0	236.5	±	22.4	274.4
<i>A. graveolens</i> ^b	Flakes	12.1	±	1.6	243.2	±	39.7	9.5	±	0.9	264.9
<i>C. monnieri</i> ^b	Fruits	707.1	±	78.8	466.8	±	95.4	1788.1	±	152.3	2962.0
<i>P. crispum</i> ^b	Leaves	n.d. ^d			34.4	±	10.6	n.d. ^d			34.4
<i>P. aniseum</i> ^b	Seeds	15.8	±	5.8	n.d. ^d			n.d. ^d			15.8
<i>R. graveolens</i> ^b	Leaves	1342.4	±	135.7	534.0	±	120.6	294.9	±	49.5	2171.2

^aResults are expressed as mean ± SD.^bBased on three separate aqueous extracts.^cBased on five separate aqueous extracts.^dn.d. = not detected.

Using UV spectra to help identifying the furanocoumarins in plant extracts was not feasible as linear and angular furanocoumarins had similar UV spectra (Frerot and Decorzant, 2004). This is especially problematic if the furanocoumarins co-elute with each other (data not shown). Thus, in addition to retention times, 8-MOP, 5-MOP, and ISOP were identified by spiking extracts with standard chemicals. Although the furanocoumarins showed maximum absorbance at about 218 and 223 nm, the absorbance at 310 nm was used to quantify 8-MOP, 5-MOP, and ISOP in the aqueous extracts because it minimized the interference of plant matrices. Also, the LOD and LOQ at 310 nm were reasonable and acceptable for the present study (Table 4). The potential use of HPLC-FLD to detect furanocoumarins was explored but unsuccessful, a finding which was in agreement with Frerot and Decorzant (2004). The presence of

furanocoumarins in the aqueous extracts confirms previous findings that furanocoumarins are not degraded by conventional cooking or methods of decoction preparation (Ivie *et al.*, 1981).

The amounts of furanocoumarins in the plant products (Table 5) also are consistent with those reported in previous studies:

(a) *A. majus* seeds. Duke (1988) reported the content of 8-MOP in *A. majus* seeds to range from 2.3 to 5.8 mg/g, while we found 3.2 mg/g in our study. The content of 5-MOP *A. majus* seeds was reported to range from 0.4 to 3.1 mg/g, which agreed with our result of 0.72 mg/g. Krivot and Perel'son (1967) determined that the content of ISOP in *A. majus* seeds ranged from 0.47 to 0.64%, which was comparable to our result of 0.75%. The latter study also reported 8-MOP and 5-MOP contents to range from

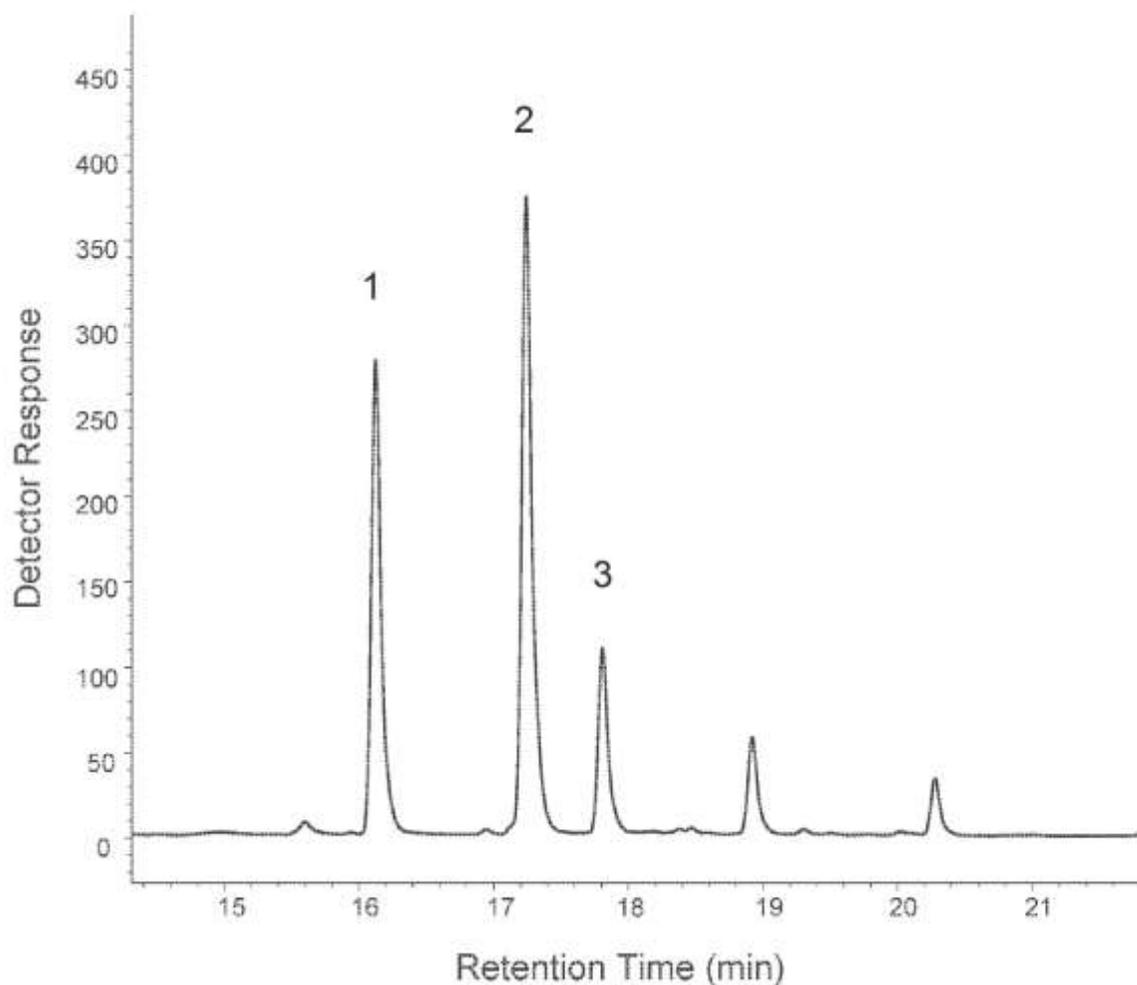


Fig. 3. HPLC-UV chromatogram of linear furanocoumarins using a pentafluorophenyl column. Peak 1 represents 8-MOP; peak 2 represents ISOP; peak 3 represents 5-MOP.

0.18 to 0.37% and 0.21 to 0.24%, respectively, which were in agreement with our results of 0.32 and 0.07%, respectively.

(b) *A. archangelica* roots. Several studies have identified the presence of 8-MOP, 5-MOP, and ISOP in *A. archangelica* roots (Härmälä *et al.*, 1992; Eeva *et al.*, 2004). Chalchat and Garry (1993) reported the presence of 5-MOP in the pentane extracts of *A. archangelica* roots to be 8.8% dry weight, which is significantly higher than our result of 0.04% dry weight. The discrepancy in results probably is due to the use of a different solvent to extract the *A. archangelica* roots. We are able to determine 8-MOP and ISOP in *A. archangelica* roots to be 0.07% and 0.06% dry weight, respectively although such information is not available in the literature.

(c) *P. anisum* seeds. Previous studies with ultrasensitive bioassays showed that about 5 ng/g dry weight each of 8-

MOP and 5-MOP were found in *P. anisum* seeds (Ceska *et al.*, 1986). In contrast, we found a much higher level of 8-MOP (15.8 $\mu\text{g/g}$ dry weight) but no 5-MOP in *P. anisum* seeds.

(d) *R. graveolens* leaves. Milesi *et al.* (2001) reported the contents of 8-MOP, 5-MOP, and ISOP in *R. graveolens* leaves to be 1.0 ± 0.6 mg/g, 1.6 ± 0.5 mg/g, and 0.1 ± 0.1 mg/g dry weight, respectively. We also found these furanocoumarins at comparable concentrations: 1.34 mg/g, 0.53 mg/g, and 0.30 mg/g dry weight, respectively.

(e) *A. graveolens*. Beier *et al.* (1983) detected 8-MOP, 5-MOP, and ISOP in the leaves of healthy *A. graveolens* from three different locations in the United States; total content of 8-MOP, 5-MOP, and ISOP was approximately 0.73 ng/g wet weight. Diawara *et al.* (1995) also reported finding 8-MOP and 5-MOP in various parts of healthy *A. graveolens*; total linear furanocoumarins found ranged

from 0.8 to 49.84 µg/g wet weight. We found significantly higher levels of linear furanocoumarins in *A. graveolens* (265 µg/g dry weight) since our results were based on the dry weights of leaves and stems.

(f) *A. pubescens* roots. Chen *et al.* (1995) detected 8-MOP and 5-MOP in the extracts of *A. pubescens* roots. Lin *et al.* (2009) reported the presence of 5-MOP in *A. pubescens* roots with an average content of 7.4 µg/g dry weight. However, they were unable to detect any 8-MOP in the extracts. We were able to detect both 8-MOP and 5-MOP in *A. pubescens* root extracts at 26 and 33 µg/g dry weight, respectively.

(g) *P. crispum* leaves. Beierer *et al.* (1994) reported that the levels of 8-MOP, 5-MOP, and ISOP in dried *P. crispum* leaves ranged between 5.3-53.0 µg/g, 56.7-146.7 µg/g, and 15.7-79.8 µg/g dry leaves weight, respectively. In contrast, we only found 5-MOP in *P. crispum* leaves at 34.0 µg/g dry weight.

(h) *C. monnieri* fruits. Yan *et al.* (2001) analyzed 53 samples of *C. monnieri* fruits from various parts of China and reported 8-MOP, 5-MOP, and ISO ranging from 0.03-0.30%, 0.01-0.26%, and 0.02-0.32% dry weight, respectively. Our results show an average of 0.07%, 0.05%, and 0.18% dry weight for 8-MOP, 5-MOP, and ISOP, respectively.

Many plant products including *A. visnaga* seeds (Sellami *et al.*, 2013), *A. dahurica* roots (Zhang *et al.*, 2009), *A. sinensis* roots (Noe, 1997, as cited in Al-Bareeq *et al.*, 2010), *Citrus* peels (Siskos *et al.*, 2008), *F. vulgare* seeds (Zaidi *et al.*, 2007), *Anethum graveolens* leaves (Szopa and Ekiert, 2015), *C. carvi* and *C. sativum* seeds (Ceska *et al.*, 1986), and *P. sativa* root (Lombaert *et al.*, 2001) are reported to contain some furanocoumarins. However, we are unable to find any linear furanocoumarins in these plant products (Table 1). The discrepancy in results may be related to various factors affecting the contents of furanocoumarins in plants during growth such as diseases and infection (Lord *et al.*, 1988), ultra-violet light, nutrient levels (Zangerl and Berenbaum, 1987), geographical locations (Sigurdsson *et al.*, 2012), seasonal changes (Zobel and Brown, 1990), pollution levels (Dercks *et al.*, 1990), fungicide use (Nigg *et al.*, 1997), and storage conditions (Chaudhary *et al.*, 1985).

CONCLUSION

In summary, 8-MOP, 5-MOP, and ISOP are among the most common furanocoumarin constituents found in plant products of this study. The HPLC method used to analyze the extracts is simple and cost-effective. Since the composition of furanocoumarins varies greatly among the plant products, it is important to identify and quantify the furanocoumarin bioactive(s) in these products before

embarking on a clinical pharmacokinetic study or safety assessment of furanocoumarin-containing plants.

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