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ORIGINAL ARTICLE

Screening of Fruits of Seven Plants Indicated for Medicinal Use in Iraq

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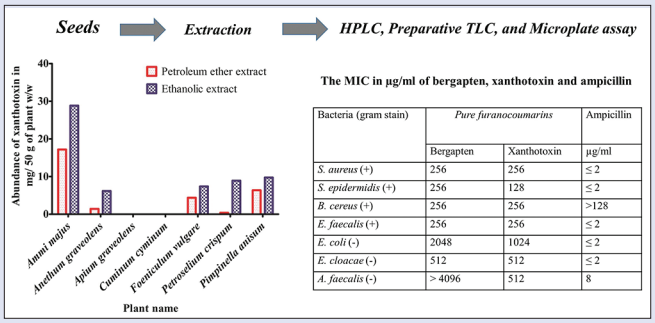
ABSTRACT

Introduction: Coumarins exert many biological effects in humans, animals, and plants, which make the evaluation of their biological activities and study of their role in ethnomedicine highly valued. **Objectives:** Here, we selected seven plants which have ethnopharmacological use as antimicrobial in Iraq and the aims were to quantify the two structural isomers bergapten and methoxsalen in their seeds, to evaluate the antibacterial activities against several clinical isolates, and to isolate bergapten and methoxsalen from *Ammi majus*. **Materials and Methods:** Seven plants were extracted by petroleum ether (PE) and ethanol (EtOH). Bergapten and methoxsalen were separated and purified by preparative thin-layer chromatography. Quantification of the furanocoumarins has been conducted by high-performance liquid chromatography, and all the plant extracts and pure compounds were checked for antibacterial activities utilizing alamar blue microplate assay. **Results:** *Cuminum cyminum* was deprived of bergapten and methoxsalen and methoxsalen was not detected from *Apium graveolens*. Bergapten was abundant in PE more than in EtOH; on the other hand, EtOH was rich in methoxsalen. The separation of the two structural isomers was performed using normal phase chromatography and ultraviolet light as an indicator. All extracts showed weak to moderate antibacterial activities against Gram-positive isolates which were more sensitive than the negative ones. *C. cyminum* extract was least active, uncover the antibacterial role of bergapten and methoxsalen. **Conclusion:** These findings support the medicinal use of seeds of seven plants from *Apiaceae* family and quantify the two pharmacologically important furanocoumarins (bergapten and methoxsalen).

Key words: Alamar blue, *Apiaceae*, bergapten, high-performance liquid chromatography, methoxsalen, thin-layer chromatography

SUMMARY

- This study was conducted to evaluate the antibacterial activities of seven plants seeds used in local medicine in Iraq. High-performance liquid chromatography was used to quantify bergapten and xanthotoxin in non-polar and polar extracts of these seeds. This study supports the medicinal use of these plants and clarifies the role of bergapten and xanthotoxin in antibacterial activities of these plants.



The MIC in µg/ml of bergapten, xanthotoxin and ampicillin

Bacteria (gram stain)	*Pure furanocoumarins*		Ampicillin
	Bergapten	Xanthotoxin	µg/ml
S. aureus (+)	256	256	≤ 2
S. epidermidis (+)	256	128	≤ 2
B. cereus (+)	256	256	>128
E. faecalis (+)	256	256	≤ 2
E. coli (-)	2048	1024	≤ 2
E. cloacae (-)	512	512	≤ 2
A. faecalis (-)	> 4096	512	8

Abbreviations used: EtOH: Ethanol; MIC: Minimum inhibitory concentration; PE: Petroleum ether; Rf: Retardation factor; Rt: Retention time.

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INTRODUCTION

Coumarins are consumed daily from diet and many herbal medicines, and these unsaturated lactones exert many biological effects in humans, animals, and plants, which make the evaluation of their biological activities and study of their role in ethnomedicine highly appreciated.[1] In plants, coumarins are working as phytoalexins defending the plants against microbes.[2,3] Chemically, there are several kinds of coumarins; furanocoumarins constitute one of the important bioactive coumarin groups which showed variable effects and antimicrobial is one of these effects.[4]

Bergapten and methoxsalen are furanocoumarins widely distributed in *Apiaceae* family which contains about 275 genera and 2850 species.[5] The *Apiaceae* plants have the abilities to synthesize and store bergapten and methoxsalen, the two methoxylated derivatives of psoralen; the enzyme O-methyl transferase is responsible for O-methylation of bergaptol and xanthotoxol to yield bergapten and methoxsalen, respectively [Figure 1].[6] Bergapten and methoxsalen have the abilities to retard cell division process by interacting with the DNA, and these two furanocoumarins have the abilities to fight fungus infecting plants in addition to their role in treatment of vitiligo and psoriasis.[3,7-9]

Several plants from this family are widely used in Iraq for the treatment of many ailments such as bronchitis and urinary tract infections. Here, we selected seven plants that have ethnopharmacological application as antimicrobial in Iraq and the aims were to quantify the two structural isomers bergapten and methoxsalen in their seeds, to evaluate the antibacterial activities against several clinical isolates, and to isolate bergapten and methoxsalen from *Ammi majus*. Table 1 shows the plants' profiles.

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Table 1: The plants' profiles of seven plants from *Apiaceae* family, have role in ethnomedicine

Scientific name	Iraqi name	Distribution in Iraq	Uses in local medicine	Literature review
Ammi *majus* L	Zand Al-Arus	Kut, Baghdad, and Hawija[10]	Treatment of asthma and stomach ulcer[11]	Bergapten and methoxsalen were previously detected in seeds.[12,13] In a recent study, several furanocoumarins were detected from petroleum ether extract of *Ammi majus* seeds such as methoxsalen, bergapten, isopimpinellin, imperatorin, and isoimperatorin[14]
Anethum graveolens L	Shbint	This plant is cultivated in most areas of Iraq as a winter crop[10]	The oil is used for assisting digestion[11]	Seeds of this plant contain volatile oils and furanocoumarins[11]
Apium graveolens L	Krafus	Most areas of Iraq[10]	The seeds decoction used for the treatment of urinary tract infections[11]	This crop contains volatile oils, flavonoids, bergapten, umbelliferone, and isopimpinellin.[15] Bergapten and methoxsalen were isolated from different varieties of *Apium graveolens*[16]
Cuminum cyminum L	Kammun	Rutba area[10]	Used for the treatment of urinary tract infections and respiratory infections[11]	Kammun has estrogenic, antimicrobial, and analgesic activities[10]
Foeniculum vulgare Mill	Habbat hulwa	It is wild in Sulaymaniyah and Mosul, while it is cultivated as a crop in the middle of Iraq[10]	The seeds used for treatment of cough and bronchitis[15]	Small quantities of furanocoumarins have been detected in this plant, previously[11]
Petroselinum crispum (Mill.) Fuss	Ma'danus	Most areas of Iraq[10]	Seeds are reported with antiparasitic activities[10]	This plant can cause allergic dermatitis which is believed to be caused by bergapten; the plant contains several furanocoumarins such as bergapten and methoxsalen in addition to flavonoids[10]
Pimpinella anisum L	Anisun	Most areas of Iraq[10]	Anisun is used for the treatment of cough, bronchitis, pharyngitis and as antibacterial[11,15]	Bergapten, umbelliferone, and quercetin were detected in this herb[15]

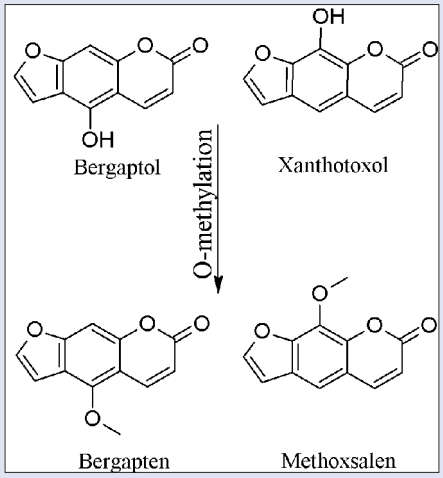

Figure 1: The structures of bergapten and methoxsalen synthesized by O-methylation

MATERIALS AND METHODS

Plants samples and extraction of active constituents

All plants were collected from the botany garden, College of Pharmacy, University of Baghdad, in 2005 and authenticated by the State Board for Seed Testing and Certification, Department of Plants, National Herbarium of Iraq. Voucher specimens (AM-050315-1, AG-050315-2, ApG-050315-3, CC-050315-4, FV-050315-5, PC-050315-6, and PA-050315-7) were kept at the Pharmacognosy Department, College of Pharmacy, University of Baghdad. The plant material was cleaned and pulverized by mechanical mills and weighed.

Each plant (50 g of seeds) was extracted by refluxing with 500 mL of petroleum ether (PE) at 70°C more than one time until exhaustion, the PE filtrates were combined and evaporated under vacuum at 45°C until complete dryness, and another 50 g of seeds of each plant was refluxed with 95% ethanol (EtOH) at 90°C until complete exhaustion. Ethanolic extracts (EEs) were dried under vacuum. PE and EtOH were supplied from Fisher Scientific, USA.

Equipment

Thin layer chromatography plates

SiO_2 thin-layer chromatography (TLC) plates from Merck, USA, silica gel 60 F_{254} precoated layer thickness of 0.25 mm (10 cm × 20 cm, 20 cm × 20 cm, 10 cm × 10 cm). Preparative TLC plates from Merck, USA, silica gel 60 F_{254} precoated layer thickness of 2 mm and 0.75 mm (20 cm × 20 cm).

High-performance liquid chromatography system

The quantification of bergapten and xanthotoxin in non-polar and polar extracts of the seven plants seeds, has been done using, Knauer high-performance liquid chromatography (HPLC) system and utilizing a ChromGate data system version 3.1 software for a personal computer linked to HPLC apparatus. The HPLC system composed of Knauer K501 pump connected to Knauer K2500 ultraviolet (UV)/visible detector.

Water distiller

Deionized water produced by water distiller, PURELAB Option-Q, UK.

Standards and development systems used for thin-layer chromatography

S_1: benzene/ethyl acetate 90:10, v/v; S_2: toluene/diethyl ether 50:50, v/v, toluene (50 mL), and diethyl ether (50 mL) were shaken for 5 min with 50 mL of 10% acetic acid in a separating funnel. The lower phase was discarded, and the toluene-diethyl ether mixture was used for TLC; S_3: toluene/ethyl acetate/formic acid 70:20:10, v/v; S_4: benzene/acetone 90:10, v/v; S_5: benzene/acetone 80:20, v/v; S_6: chloroform/methanol 99:1, v/v; S_7: chloroform 100%; S_8: ethyl acetate100%. The solvents were HPLC

grade from Fisher Scientific, USA. Bergapten and methoxsalen were purchased from Sigma-Aldrich, USA, with purity >99% by HPLC.

Preliminary thin-layer chromatography

PE extracts and EEs were dissolved in chloroform 1 gm/10 mL. 10 µL of each extract was applied aligned with bergapten and methoxsalen standards, SiO_2, 60 F_{254} precoated 20 cm × 20 cm plates, S_3: toluene/ethyl acetate/formic acid 70:20:10, v/v, UV light at wavelength 366 nm.

Purification of petroleum ether and ethanol extracts

PE extracts of each plant were washed several times with 80% EtOH to yield clear ethanolic filtrates, combined and evaporated to dryness under vacuum; the purified extract and marc residue were analyzed by TLC using S_1 and S_3 as development systems then kept aside for future studies.

The EE of each plant was dispersed in 200 mL of distilled water and washed with chloroform (200 mL eight times) and diethyl ether (200 mL eight times) successively. Aqueous, chloroform, and diethyl ether fractions were evaporated to dryness under vacuum. The purified fractions were subjected to TLC using two development systems S_1 and S_2, UV light at wavelength 366 nm. To define the best solvent system for separation of bergapten and methoxsalen, *A. majus* extracts were analyzed by TLC using development systems S_1–S_8. Retardation factor (Rf) of bergapten and methoxsalen was calculated in different development systems.

Separation of bergapten and methoxsalen by preparative thin-layer chromatography

PE extract 7.5 g of *Ammi majus* was dissolved in 10 mL of chloroform. Enough quantities were applied as bands on silica gel layer, SiO_2, 60 F_{254} precoated layer thickness 2 mm, 20 cm × 20 cm plates, S_1: benzene/ethyl acetate 90:10, v/v, UV light at wavelength 366 nm. The separated bands were scraped off and collected using a suitable spatula, and the compounds were extracted from SiO_2 using several fractions of chloroform and EtOH, which were combined later and evaporated under vacuum. These bands were analyzed by TLC using S_1 as the solvent system. The bands contained the desired materials were subjected to successive steps of preparative TLC using S_1 as the solvent system until purification of bergapten and methoxsalen.

High-performance liquid chromatography conditions

All plants extracts were prepared and ran through C-18 column 5 µm (4.6 mm × 150 mm), isocratic 37% acetonitrile/water solvent, flow rate 1 mL/min, λ_{max} for both coumarins standards was determined at $\lambda_{218\ nm}$ for bergapten and $\lambda_{249\ nm}$ for methoxsalen.

Calibration curves of bergapten and methoxsalen

The assay has been done in triplicate to ensure reproducibility. The lowest detection limit of the experiment was 0.004 mg/mL. Methoxsalen and bergapten with high purity were used as external standards to prepare the standard curves. Different combinations of acetonitrile and water were used separately to specify the best chromatographic mobile phase for the separation of the two structural isomers on the C-18 column using the reverse phase system.

Serial dilutions of bergapten and methoxsalen (1, 0.25, 0.125, 0.0625, 0.008, and 0.004 mg/mL) were prepared in methanol and 20 µL injected into the HPLC system; then, area under the curve (AUC) plotted versus the standard concentrations to construct the calibration curves (n = 3).

Y = 9E – 09X – 0.0071 ……(1)

Y = 1E – 08X – 0.0040 ……(2)

Y is the concentration in mg/mL, X is the AUC, 9E – 09 = 0.000000009, and 1E – 08 = 0.00000001.

The plant extracts prepared in methanol at a concentration of 100 mg/mL and 20 µL were analyzed using the HPLC method mentioned above (n = 3). The availability of bergapten and methoxsalen in the plant extracts was confirmed by matching of retention time (Rt) in minutes with those of the pure compounds and by the addition of internal standard using the same conditions of HPLC mentioned above. The abundance of bergapten and methoxsalen in the plant extract quantified utilizing equations (1) and (2), respectively.

Minimum inhibitory concentration

The antibacterial activities of bergapten, methoxsalen, PE, and EEs of all plants were determined by determination of minimal inhibitory concentration (MIC) against several clinical isolates provided by the Central Public Health Laboratory, Baghdad, using the microplate alamar blue assay.[17-19] Plant extracts and pure compounds were used in concentrations from 2 µg/mL to 4096 µg/mL with DMSO final concentration of 3%. The bacterial density used finally was 5 × 10^5 CFU/mL. Ampicillin used as a control for the assay in concentrations ranged from 2 µg/mL to 256 µg/mL; solvent control, sterility control, and growth control were used in the study which was conducted utilizing 96-well plates, and all plates were incubated at 37°C for 24 h. 20 µL of alamar blue was added to each well in the plate which was incubated for further 4 h at 37°C. MICs were verified visually as the lowest concentration of extracts/pure compounds that prevented a blue-to-pink alamar blue color change, (n=3). Alamar blue and 96-well plates were supplied from Fisher Scientific, USA.

RESULTS

The PE extract and EEs of all plants were examined first by TLC using S_3 as a development system; the results revealed unclear separation and chromatogram indicating the presence of some unwanted materials which affected the separation patterns. The purified PE extract and its marc residue were tested on TLC plates using S_1 and S_3 as development systems aligned with the standards of bergapten and methoxsalen. Results revealed that the marc residues were deprived of these furanocoumarins; Table 2 shows the weight of unpurified and purified PE extract.

The purification of EE yielded chloroform, diethyl ether, and aqueous fractions; these fractions were checked by TLC aligned with bergapten and methoxsalen utilizing S_1 and S_2 as development systems. The results suggested that the chloroform and ethyl ether fractions were similar in composition; hence, they were combined and marked as the EE for future work. Aqueous fraction did not contain any of the furanocoumarins under the study; Table 2 shows the weight of EEs.

To define the best development system for separation of the two structural isomers (bergapten and methoxsalen) on a SiO_2 thin layer, these compounds were analyzed individually and as a mixture on SiO_2

Table 2: The weight of petroleum ether extract and ethanolic extract (g) of several plants from *Apiaceae* family

Plant name	Weight of petroleum ether extract		Weight of ethanolic extract	
	Crude	Pure	Crude	Pure
Ammi majus	9.70	7.50	12.00	8.10
Anethum graveolens	10.70	8.25	13.37	9.05
Apium graveolens	8.60	6.50	11.70	8.02
Cuminum cyminum	7.00	3.59	8.80	5.07
Foeniculum vulgare	7.52	4.97	8.59	5.51
Petroselinum crispum	13.85	5.11	15.30	6.50
Pimpinella anisum	9.28	7.14	10.70	7.80

TLC plate utilizing eight different development systems (S_1–S_8). Table 3 shows the Rf of bergapten and methoxsalen in several development systems. The results showed that S_1 was the best development system which we used in preparative TLC. The Rt of the two standards was 17.133 min for bergapten and 13.133 min for methoxsalen determined in HPLC system [Figure 2].

Table 3: The retardation factor of bergapten and methoxsalen (cm) in eight solvent systems

Solvent system	Rf of bergapten	Rf of methoxsalen
S_1: Benzene:ethyl acetate (90:10)	0.500	0.433
S_2: Toluene:diethyl ether (50:50)	0.680	0.620
S_3: Toluene:ethyl acetate:formic acid (70:20:10)	0.620	0.570
S_4: Benzene:acetone (90:10)	0.690	0.650
S_5: Benzene:acetone (80:20)	0.740	0.710
S_6: Chloroform:methanol (99:1)	0.850	0.820
S_7: Chloroform (100)	0.792	0.752
S_8: Ethyl acetate (100)	0.860	0.850

Rf: Retardation factor

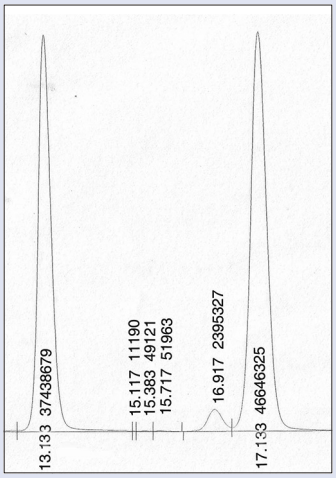

Figure 2: High-performance liquid chromatography chromatogram of methoxsalen and bergapten standards. The (retention time) of the two standards was 13.133 min for methoxsalen and 17.133 min for bergapten

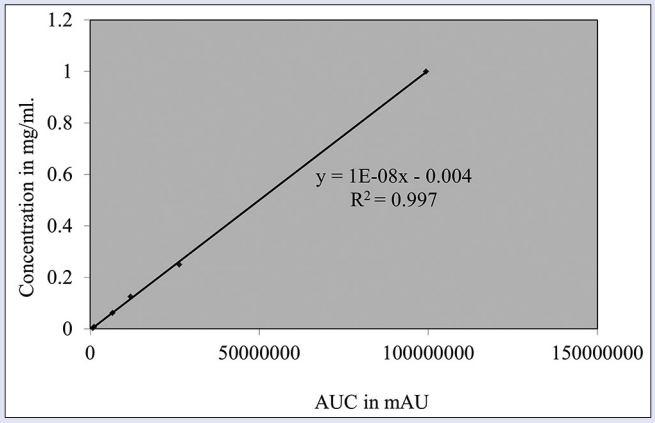

Figure 4: Calibration curve of methoxsalen

The quantities of bergapten and methoxsalen in the seeds of the plants under study were calculated utilizing calibration curves [Figures 3 and 4]. Results are shown in Table 4, and their quantities in 50 g of seeds of each plant were calculated and are shown in Table 5.

The separation of bergapten and methoxsalen from *A. majus* was accomplished using S_1 as the development system and 2 mm SiO_2 plates. Three major bands were detected by UV-366 nm, and they coded as A, B, and C [Figure 5]. Metabolites were washed from SiO_2 using chloroform and EtOH for several times. The contents of the bands were checked by TLC against the standards using two development systems (S_1 and S_2); the results revealed that bands A and B contain bergapten and methoxsalen. Bands A and B were mixed and evaporated to dryness yielding ≈ 42 mg crude mixture, and the band was coded as band AB.

Band AB was subjected to preparative TLC using S_1 as development system, yielding a characteristic band, coded as band bx, which contains bergapten and xanthotoxin [Figure 6]. Band bx was scraped off and extracted by chloroform and EtOH; as mentioned earlier, solvents evaporated to dryness to get ≈ 18.03 mg mixture of bergapten and methoxsalen, the mixture (band bx) was developed on SiO_2 layer 0.75 mm in thickness, development system was S_1, the mixture applied one time on each plate and the plate developed in the development system for three times to get better separation, two distinct bands were separated representing bergapten and xanthotoxin. Bergapten 2.8 mg was crystallized out of 95% EtOH as white needles and methoxsalen

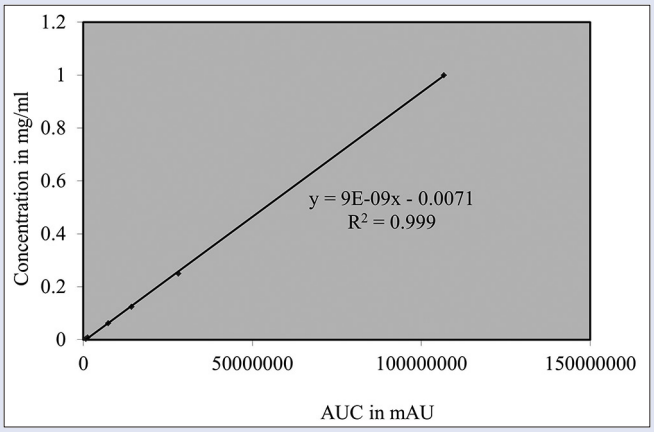

Figure 3: Calibration curve of bergapten

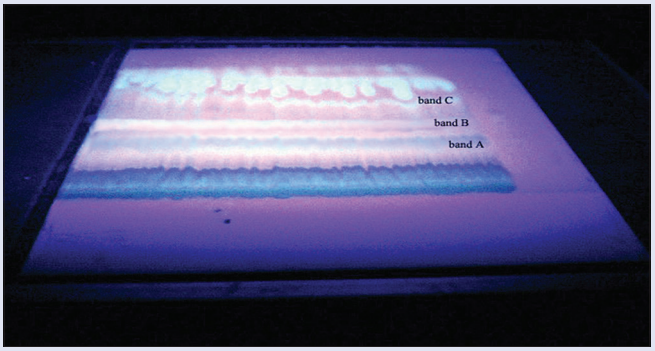

Figure 5: Preparative thin-layer chromatography of *Ammi majus* petroleum ether extract. Solvent system Benzene/Ethyl acetate 90:10, v/v. Stationary phase SiO_2, 60 F_{254}, layer thickness 2 mm. Detector ultraviolet light wavelength 366 nm

14.07 mg as silky needles from hot water. The melting points of these two compounds were measured as 185°C–188°C for bergapten and 147°C–149°C for methoxsalen, which were in agreement with melting points of bergapten and methoxsalen standards.

The isolated compounds were run on SiO_2 TLC plates using different development systems and in HPLC system against standards to confirm identity. The antibacterial activities of all extracts and pure compounds were ranged from weak to intermediate against several Gram-positive and Gram-negative bacteria [Tables 6 and 7].

Table 4: The abundance of bergapten and methoxsalen in mg/mL calculated by injection of 20 µL into the high-performance liquid chromatography system, concentration 100 mg/mL

Plant name	Concentration in mg/ml			
	AUC from petroleum ether extract		AUC from ethanolic extract	
	Bergapten	Methoxsalen	Bergapten	Methoxsalen
Ammi majus	6,088,731 0.0480	23,267,728 0.2290	1,047,454 0.002	36,028,652 0.356
Anethum graveolens	2,724,190 0.0174	2,121,465 0.0172	1,245,102 0.004	7,169,193 0.068
Apium graveolens	60,413,140 0.5370	- -	20,475,536 0.177	- -
Cuminum cyminum	- -	- -	- -	- -
Foeniculum vulgare	1,686,411 0.0080	9,181,694 0.0880	2,175,605 0.012	13,760,817 0.134
Petroselinum crispum	3,562,021 0.0250	1,167,039 0.0076	2,310,122 0.014	14,092,536 0.137
Pimpinella anisum	10,379,572 0.0860	9,287,827 0.0890	7,796,780 0.060	12,893,086 0.125

AUC: Area under curve

Table 5: The abundance of bergapten and methoxsalen in mg/50 g of seeds weight calculated as w/w

Plant name	Petroleum ether extract		Ethanolic extract	
	Bergapten	Methoxsalen	Bergapten	Methoxsalen
Ammi majus	3.600	17.175	0.162	28.836
Anethum graveolens	1.436	1.419	0.362	6.154
Apium graveolens	34.905	-	14.195	-
Cuminum cyminum	-	-	-	-
Foeniculum vulgare	0.397	4.374	0.066	7.383
Petroselinum crispum	1.277	0.388	0.910	8.905
Pimpinella anisum	6.140	6.355	4.680	9.750

DISCUSSION

EtOH extracted more materials than PE; the analysis of bergapten and methoxsalen by eight solvent systems revealed that methoxsalen was polar as compared to bergapten which showed less polarity. *Cuminum cyminum* was deprived of bergapten and methoxsalen, and methoxsalen was not detected from *Apium graveolens*. Bergapten was abundant in PE more than in EtOH; on the other hand, EtOH was rich in methoxsalen, which is attributed to lower polarity of bergapten as compared to methoxsalen. Bergapten and methoxsalen were separated efficiently by preparative TLC in a recovery percentage of 81%.

Xanthotoxin and isopimpinellin were isolated from the fruits of *A. majus* using column and partition chromatography.[13] All plants showed weak to moderate antibacterial activities, *C. cyminum* was the least active extract compared to other plants, and all the extracts were more efficient

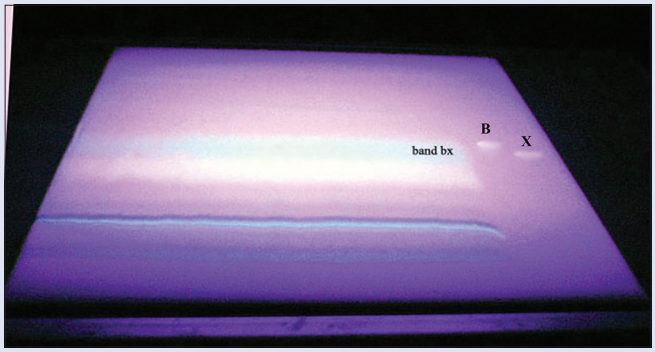

Figure 6: Preparative thin layer chromatography of *Ammi majus* (band AB). Band (bx) is the crude mixture of bergapten and methoxsalen. Standards bergapten (B) and methoxsalen (X). Solvent system Benzene/Ethyl acetate 90:10, v/v. Stationary phase SiO_2, 60 F_{254}, layer thickness 2 mm. Detector ultraviolet light wavelength 366 nm

Table 6: The minimal inhibitory concentration in µg/mL of petroleum ether extract and ethanolic extract of several plants seeds (family: *Apiaceae*), *n*=3

Bacteria (Gram-stain)	*Ammi majus*		*Anethum graveolens*		*Apium graveolens*		*Cuminum cyminum*		*Foeniculum vulgare*		*Petroselinum crispum*		*Pimpinella anisum*	
	PEE	EE	PEE	EE	PEE	EE	PEE	EE	PEE	EE	PEE	EE	PEE	EE
Staphylococcus aureus (+)	512	256	2048	1024	1024	256	4096	2048	1024	256	2048	256	2048	256
Staphylococcus epidermidis (+)	128	128	512	128	1024	256	2048	1024	2048	256	256	256	2048	128
Bacillus cereus (+)	512	256	512	128	1024	1024	4096	4096	2048	2048	2048	2048	2048	2048
Enterococcus faecalis (+)	1024	256	>2048	2048	256	128	2048	2048	2048	2048	2048	2048	2048	1024
Escherichia coli (−)	2048	2048	>4096	>4096	>4096	2048	>4096	4096	4096	2048	>4096	2048	2048	512
Enterobacter cloacae (−)	2048	2048	2048	2048	>4096	2048	>4096	>4096	>4096	>4096	2048	2048	2048	512
Alcaligenes faecalis (−)	>4096	2048	2048	2048	>4096	2048	>4096	>4096	>4096	>4096	>4096	>4096	>4096	>4096

PEE: Petroleum ether extract; EE: Ethanolic extract

Table 7: The minimal inhibitory concentration in μg/mL of bergapten, methoxsalen, and ampicillin (*n*=3)

Bacteria (Gram-stain)	*Pure furanocoumarins*		Ampicillin (μg/mL)
	Bergapten	Methoxsalen	
Staphylococcus aureus (+)	256	256	≤2
Staphylococcus epidermidis (+)	256	128	≤2
Bacillus cereus (+)	256	256	>128
Enterococcus faecalis (+)	256	256	≤2
Escherichia coli (−)	2048	1024	≤2
Enterobacter cloacae (−)	512	512	≤2
Alcaligenes faecalis (−)	>4096	512	8

against Gram-positive isolates than negative bacterial cells. In general, methoxsalen showed more potent antibacterial effects than bergapten against all bacterial isolates used in this study.

Anethum graveolens ethanolic fraction affecting *Helicobacter pylori* one of stomach ulcer-causative agents,[20] the essential oil of Shbint was active *in vitro* against several *Candida* with MIC of 0.3–0.6 μL/mL.[21] The methanolic extract of the leaves showed weak to moderate antibacterial activities with MIC of 5000 μg/mL against *Escherichia coli* and 2500 μg/mL against *Pseudomonas aeruginosa* and *Staphylococcus aureus*,[22] which come in accordance with our results on the other hand, the extraction of *A. graveolens* by MeOH leading to the production of moderate antibacterial fraction with MIC of 1250 μg/mL against *E. coli* and *S. aureus* and 5000 μg/mL against *P. aeruginosa*.[22] *A. graveolens* oil showed some antibacterial activities against *Alcaligenes faecalis* MIC equal to 5 μL/mL.[23] The leaves extract of *A. graveolens* acted against several *Candida* species with MIC ranged from 78 to 312 μg/mL.[22]

C. cyminum seeds oil fraction affecting *Salmonella typhimurium* and *E. coli* potently with MIC of 0.25 μL/mL and 0.5 μL/mL, respectively,[24] and showed weak antibacterial activities against several *S. aureus* resistant strains.[25] In another study, the antibacterial activities of the oil against *S. aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, and *E. coli* were reported to be 78 μg/mL.[26] Furthermore, the oil showed some antifungal activities against *Candida albicans* and *Candida dubliniensis* with MIC of 280 μg/mL,[27] the effect of methanolic extract of Kammun against *E. coli*, *S. aureus*, and *P. aeruginosa* was remarkably weak with MIC of 5000 μg/mL.[28]

Foeniculum vulgare var. *dulce* aerial parts contain bergaptol.[29] In another study, several coumarins such as psoralen, bergapten, and imperatorin were separated from the stems of this plant and showed weak activities against *E. coli*;[30] however, our results confirm that the seeds of *F. vulgare* contain methoxsalen in addition to bergapten.

The oil of *F. vulgare* showed very high MIC of 10000 μg/mL against *S. aureus* and 250 μg/mL against *E. coli*,[31] while in another study, the oil showed MIC of 62.5 μg/mL against *E. coli*.[32] The seeds EE showed moderate antibacterial activities from 62.5–500 μg/mL against *E. coli*, *Bacillus subtilis*, and *S. aureus*.[33] *Mycobacterium tuberculosis* growth was inhibited by the methanolic extract of *F. vulgare* stems at MIC of >200 μg/mL and at 200 μg/mL of its hexane extracts.[34]

Petroselinum crispum aerial parts essential oil showed good antibacterial activities with MIC of 11–44 μg/mL and minimum bactericidal concentration of 2810–11,250 μg/mL against different *Vibrio* strains.[35] The levels of psoralen, methoxsalen, oxypeucedanin, and bergapten from the methanolic extract of *P. crispum* aerial parts were measured before and they were 1.77–46.04 mg/kg of plant weight.[36] *Pimpinella anisum* oil was active against Gram-positive bacteria (*B. cereus* and *S. aureus*) with MIC of 62.5–125 μg/mL while its MIC against Gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae* and *P. aeruginosa*) was >500 μg/mL and its methanolic extract showed MIC of 500 μg/mL against *S. aureus*.[37] The methanolic extract had MIC of 5000 μg/mL against *S. aureus*, *E. coli*, and *P. aeruginosa*.[28]

CONCLUSION

Two structural isomers bergapten and xanthotoxin were purified efficiently in a high yield from *A. majus*, using preparative TLC and benzene/ethyl acetate 90:10, v/v as the development system. Our results showed that PE has higher extraction power of bergapten compared to EtOH and vice versa for xanthotoxin. Analysis of these isomers by HPLC revealed that the mobile phase formed of 37% of acetonitrile in water was efficient to separate these furanocoumarins by 4 min on a nonpolar column. The synthesis abilities of furanocoumarins in plants of the same family are different which can be attributed to the genetic variation or availability of the building blocks. The antibacterial activity of the plants discussed above from previous researches, showed partial agreement with our results and between each other, and this is probably attributed to the type of extract, part of the plant extracted and abundance of active constituents in that part, time of harvesting, type of method used for evaluation of the antibacterial activities, and virulence factors of the bacterial isolates. The plants extracts, bergapten and methoxsalen, showed weak to intermediate antibacterial activities against several bacterial isolates, prominently against Gram-positive bacteria. These findings support the medicinal use of seeds of seven plants from *Apiaceae* family and quantify the two pharmacologically important furanocoumarins (bergapten and methoxsalen).

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Conflicts of interest

There are no conflicts of interest.

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