PHYTOTHERAPY RESEARCH
Phytother. Res. **26**: 783–786 (2012)
Published online 14 November 2011 in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/ptr.3600

SHORT COMMUNICATION

Biological Activities of Indian Celery, *Seseli diffusum* (Roxb. ex Sm.) Sant. & Wagh

Ahmed Abbaskhan,[1] Muhammed Iqbal Choudhary,[1,2]* Mohammed Nabeel Ghayur,[3] Zeba Parween,[1] Farzana Shaheen,[1] Anwar-ul-Hassan Gilani,[3] Takuro Maruyama,[4] Kiran Iqbal[1] and Yoshisuke Tsuda[1]†

[1]H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan
[2]Department of Chemistry, College of Science, King Saud University, Riyadh-11451, Saudi Arabia
[3]Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi -74800, Pakistan
[4]Division of Pharmacognosy, Phytochemistry and Narcotics, National Institute of Health Sciences, 1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

In continuation of our work on Indian celery (*Seseli diffusum* (Roxb. ex Sm.) Santapau & Wagh; Umbelliferae), the fractionation of the 80% MeOH–H_2O extract of the seeds was performed to identify the principles responsible for its folk use as an antispasmodic and diuretic. Several compounds were isolated as active components: seselin (1) and anthriscinol methyl ether (4) showed a selective cytotoxicity to some yeast strains. Compound 1 also showed spasmolytic activity. On the other hand, isopimpinellin (3) and isorutarin (5) exhibited a spasmogenic effect on the smooth muscle preparations. Compound 5 was also found to have antioxidant activity. Among them, compound 4 was isolated for the first time from this plant.

Keywords: Indian celery; *Seseli diffusum*; cytotoxicity; spasmolytic activity; spasmogenic activity; antioxidant activity.

INTRODUCTION

Previously it was reported that the celery (Indian celery) sold in southern areas of Pakistan is not the common celery, *Apium graveolens*. This was identified as *Seseli diffusum* (Umbelliferae) based on the nuclear rDNA, and ITS sequence analysis, morphological features and phytochemical compositions (Maruyama *et al*., 2009). Seeds of *S. diffusum* are prescribed as an antispasmodic for the treatment of bronchitis, asthma, as well as in liver obstructions, intestinal debility and spleen disorders (Usmanghani *et al*., 1997). In continuation of our work on Indian celery, 80% methanol–water extract of seeds (botanically fruits) showed some interesting biological activities, such as cytotoxicity against the yeast strains, smooth muscle relaxant and antioxidant activities. Bioassay-guided fractionation using yeast cytotoxicity assay has led to the isolation and identification of DNA damaging agents **1–2** in lipophilic extracts of *S. diffusum*. Then fractionation was performed of the extract of Indian celery to identify the compounds responsible for the biological activities, and four coumarin derivatives and one phenylpropanoid isolated as some of the active principles.

* Correspondence to: M. I. Choudhary, H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan.
E-mail: hej@cyber.net.pk
† This publication is dedicated to the memory of the Professor Yoshisuke Tsuda (1932– 2006).

MATERIAL AND METHODS

General. Melting points (m.p.) were determined on a Yanaco MP-S3 apparatus and are uncorrected. The NMR spectra were recorded on Bruker AM-400 and AMX-500 spectrometers using a UNIX data system at 400 and 500 MHz. The ^{13}C-NMR spectra were recorded on the same instruments at 100 and 125 MHz respectively. The ^{1}H- and ^{13}C-NMR spectra were measured using solvents CD_3OD or $CDCl_3$ and referenced with respect to the residual solvent signals. The chemical shift (δ) values were reported in ppm, and coupling constants (J) were measured in Hz. Electron impact mass spectra (EI-MS) were taken at 70 eV on Finnigan MAT-112 or MAT-312 instruments, and major ions are given by m/z (%). Optical rotations were measured on a digital polarimeter Jasco DIP-360 in methanol. Infrared spectra were obtained on a Vector 22 Bruker spectrophotometer, either in KBr pellets or in chloroform, and presented in cm^{-1}. The TLC was performed on pre-coated silica gel cards (E. Merck, Germany) and the spots were observed first under UV (254 nm), and then stained with cerium (IV) sulfate spray reagent, and heated until coloration developed.

Plant material. The seeds of Indian celery were purchased from a local market, Jodia Bazar, Karachi, Pakistan, in July 2000. This was then identified as *S. diffusum* (Maruyama *et al*., 2009). The sample was deposited in the Division of Pharmacognosy, Phytochemistry and Narcotics, National Institute of Health Sciences, Japan.

Received 03 January 2011
Revised 27 May 2011
Accepted 27 May 2011

Extraction and isolation. One hundred grams of Indian celery seeds (*S. diffusum*) was extracted successively with 400 mL of hexane, chloroform, acetone and methanol for 4 h under reflux. The extraction was performed four times for each solvent. The yields of extracts were 1.1, 2.2, 5.0 and 18 g, respectively. The silica gel column chromatography of the hexane extract was carried out with hexane–EtOAc and EtOAc–MeOH gradient elution to afford five fractions. Further chromatographic separation yielded compounds **1–3** (43, 14 and 5 mg, respectively) from the third fraction (Fr. 3, 64 mg), while compounds **1** and **4** (27 and 35 mg, respectively) were isolated from the second fraction (Fr. 2, 740 mg). Compounds **1–3** were also isolated from chloroform–acetone extracts through similar chromatographic processes. The methanol extract (water soluble part) was partitioned into EtOAc and water. The aqueous layer was evaporated and the residue was chromatographed on a silica gel column with an EtOAc–MeOH–BuOH–H_2O (80:10:0.5:0.5) elution to obtain isorutarin (**5**; 50 mg). The DNA damaging activities of fractions and pure compounds are presented in Table 1. Physical and spectral data of **1–5** (Fig. 1) were in agreement with those reported in the literature for seselin (Sattar *et al*., 1978), bergapten (Masuda *et al*., 1998), isopimpinellin (Elgamal *et al*., 1979), anthriscinol methyl ether (Ikeda *et al*., 1998) and isorutarin (Okuyama *et al*., 1989), respectively.

Seselin (**1**). Colorless prisms from hexane, mp. 120-121 °C (119–120 °C, Murray *et al*., 1982).

Bergapten (**2**). Colorless needles from EtOAc–hexane, mp.188–189 °C, (188 °C, Murray *et al*., 1982).

Isopimpinellin (**3**). Colorless needles from EtOAc–hexane, mp. 151–153 °C (lit.149–150 °C, Murray *et al*., 1982).

Anthriscinol methyl ether (**4**). Pale yellow oil. EI-MS (M^+, *m/z* 222). ^{1}H-NMR ($CDCl_3$, 400 MHz) : 6.59 (1H, d, $J_{2',6'} = 1.4$ Hz), 6.51 (1H, d, $J_{6',2'} = 1.4$ Hz), 6.46 (1H, dd, $J_{3,2} = 15.8$ Hz, $J_{3,1(a,b)} = 1.4$ Hz), 6.09 (1H, dt, $J_{2,3} = 15.8$ Hz, $J_{2,1(a,b)} = 6.0$ Hz), 5.92 (2H, s), 4.03 (2H, dd, $J_{1(a,b),2} = 6.0$ Hz, $J_{1(a,b),3} = 1.4$ Hz), 3.86 (3H, s), 3.35 (3H, s).

Isorutarin (**5**). Colorless prisms from EtOH, mp. 265–267 °C (lit. 261–263 °C, Okuyama *et al*., 1989).

Cytotoxicity to DNA repair-deficient (rad 52Y) and repair-proficient (RAD^+) yeast strain. The bioassay was performed according to the method reported by Gunatilaka *et al*. (1992, 1994). This mechanism-based bioassay, employing DNA repair-deficient (*rad* 52Y) and repair-proficient (RAD^+) yeast strains, is a convenient method for the *in vitro* screening of potential antitumor compounds (Gunatilaka and Kingston, 1998). The two types of genetically engineered yeast strains (*Saccharomyces cerevisiae*), which were provided by Mr L. Faucette in SmithKline Beecham Pharmaceuticals (King of Prussia, Pennsylvania USA), were plated on YPD agar plates (9×9 cm; 7 mm layer). Each 96-well plate (6 mm diameter each) was filled for various concentrations of samples (100 μL in (1:1) DMSO–MeOH). The plates were read after 48 h at 30 °C. The activity was expressed as IC_{12} (μg/mL) (concentration required to produce an inhibition zone of 12 mm diameter). The data are presented in Table 1. Streptonigrin was used as the positive control.

Activity on isolated smooth muscle contractility. The experiments on rabbits were performed in accordance with the guidelines of the Institute of Laboratory Animal Resources, Commission on Life Sciences (National Research Council, Washington DC, USA) and were approved by the Aga Khan University's Ethics Committee for Research on Animals. The assay was carried out as described earlier by Ghayur and Gilani (2005). Briefly, segments of rabbit jejunum tissue were suspended in 10 mL tissue bath containing Tyrode's solution, aerated with a mixture of 95% O_2 and 5% CO_2 at 37 °C. A preload of 1 g was applied to each tissue, and then kept undisturbed for an equilibrium period of 30 min. Afterwards, responses to sub-maximal concentrations of acetylcholine (0.3 μM) were obtained. The tissues were presumed stable only after the reproducibility of these responses. Contractions for control and test

Table 1. Results of cytotoxicity bioassays on extracts and compounds of *Seseli diffusum*

Fraction/Compound	*rad* 52Y (mutant)[a]	RAD^+ (Wild)[a]	Muscle contractility	DPPH
80% MeOH–water	1000	> 1500	0.3 ± 0.05[b]	22.51[e]
Hexane extract	100	500	0.03 ± 0.002[b]	-
Chloroform extract	800	> 1000	0.12 ± 0.005	-
Acetone extract	650	> 1000	0.14 ± 0.06[b]	-
MeOH extract (water soluble)	> 1000	> 1000	0.24 ± 0.09[b]	37.55[e]
Seselin (**1**)	25	> 200	0.04 ± 0.005[b]	-
Bergapten (**2**)	50	> 200	-	-
Isopimpinellin (**3**)	100	> 200	0.1 ± 0.01[c]	-
Anthriscinol methyl ether (**4**)	20	50	-	-
Isorutarin (**5**)	40	> 100	0.3 ± 0.05[c]	79.34[d]
Streptonigrin (reference)	0.4	1.0		
Propyl gallate (reference)			92.14[d]	

- , Inactive; NT, not tested.

[a]IC_{12} in μg/mL.

[b]Spasmolytic activity, EC_{50} (mg/mL) mean ± SEM.

[c]Spasmogenic activity, EC_{50} (mg/mL) mean ± SEM.

[d]% inhibition at 1 mg/mL.

[e]200 μg/mL/1 mM.

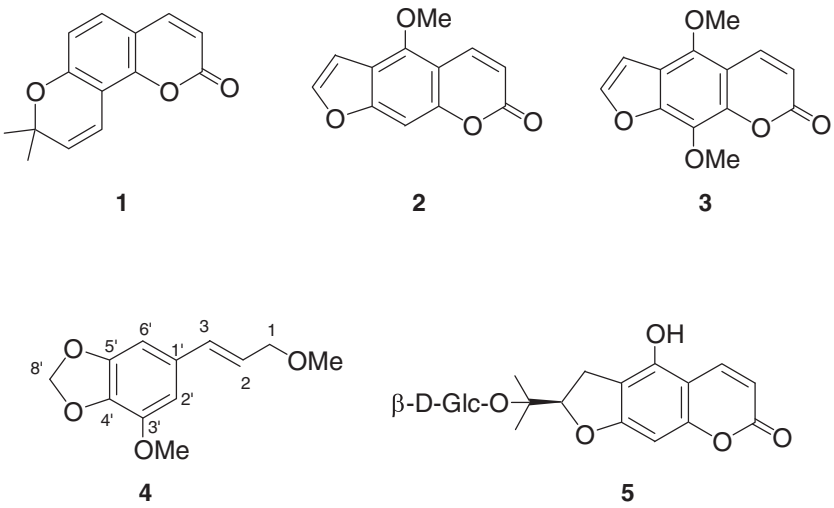

Figure 1. Structures of compounds **1–5**.

were recorded isotonically, using Harvard student oscillographs and transducers. Under these conditions, rabbit jejunum exhibited spontaneous rhythmic contractions, allowing the testing of relaxant (spasmolytic) or stimulant (spasmogenic) activities. Stock solutions of all the test extracts, fractions and compounds were made in saline or if not soluble, in 10% DMSO. All dilutions were made in saline. The final bath concentration was kept at < 0.001 mg/mL of DMSO, which had no effect on the contractions (data not shown).

DPPH Radical scavenging assay. The assay was performed according to the method developed by Lee *et al.* (1998). Briefly, reaction mixtures comprising incremental concentrations of test compounds and 300 mM of DPPH were prepared. After the incubation in a 96-well plate at 37 °C for 30 min, the absorbance at 515 nm was measured by an ELISA reader (SpectraMax plus, Molecular Devices, CA, USA). The percent radical scavenging activity was determined in comparison with the DMSO-treated control (3-*t*-butyl-4-hydroxyanisole).

RESULTS AND DISCUSSION

The 80% methanol–water extract of the Indian celery, *Seseli diffusum* (Roxb. ex. sm.) Santapau & Wagh, showed significant cytotoxicity in the mechanism-based bioassay, employing DNA repair-deficient (*rad* 52Y) and repair-proficient (RAD^+) yeast strains. This fraction also showed spasmolytic and antioxidant activities. The cytotoxicity to yeast strains was found in hydrophobic (hexane and chloroform) extracts of the seeds. In contrast, the water-soluble fraction showed a potent antioxidant activity, but a weak cytotoxic activity. Spasmolytic activity was found in both hydrophobic and hydrophilic fractions (Table 1). The hexane fraction was found to be most active in the cytotoxicity assay. Two compounds, seselin (**1**) and anthriscinol methyl ether (**4**), were mainly isolated from this fraction. The chloroform fraction yielded mostly bergapten (**2**) and isopimpinellin (**3**). The compound **4**, a known constituent from *Anthriscus sylvestris* (Ikeda *et al.*, 1998; Kozawa *et al.*, 1982), is reported for the first time from *Seseli diffusum*. The activities of the isolated compounds were also determined (Table 1). Gunatilaka *et al.* (1994) reported that in the mutant yeast assay, angular pyrano-coumarins, such as seselin (**1**), isolated from Rutaceous plants, were active, while linear furanocoumarins were inactive. Interestingly, our results demonstrated that linear furanocoumarin, bergapten (**2**) and non-cyclized compound **4**, were as active as angular pyrano-coumarins, such as seselin (**1**).

The 80% methanol–water extract of the seeds prepared for the preliminary random screening process demonstrated a dose dependent spasmolytic activity in spontaneously contracting isolated rabbit jejunum. Among the extracts prepared as a result of refluxing with various solvents, the hexane extract showed the most potent activity, followed by methanol, chloroform and acetone extracts. Among the lipophilic compounds isolated from Indian celery, compound **1** was the most potent in its spasmolytic activity, equipotent to that of its parent hexane fraction. Compounds **2** and **4** were inactive in a dose up to 0.5 mg/mL. By contrast, compound **3** showed a stimulant (spasmogenic) effect in the assay. Compound **5**, isolated from the water-soluble fraction, also showed a spasmogenic effect. However, compound **1** was much more potent in its spasmolytic effect than the spasmogenic effects of **3** and **5**. Judging from the content and potency of these compounds in Indian celery, the crude extract of the seed showed domination of spasmolytic activity. Isorutarin (**5**) also showed prominent radical scavenging activity in a DPPH assay, while the others were inactive.

It is worth mentioning here that the isolated compounds **1–5** were the major constituents in various fractions of the crude extract. This study, therefore, does not represent an account of the minor constituents contributing towards the observed bioactivities of the crude extract. A study of the spasmolytic and antioxidant constituent(s) from the water-soluble (hydrophilic) fraction will be made later.

CONCLUSION

In conclusion, the isolation study of Indian celery, guided by its biological activities, revealed that the cytotoxicity to yeast strains was mainly attributed to seselin (**1**) and anthriscinol methyl ether (**4**). Bioassay-guided

fractionation (Table 1), using antispasmodic and spasmogenic assays, have led to the identification of several active constituents. Spasmolytic activity was observed in **1**, while isopimpinellin (**3**) and isorutarin (**5**) exhibited a spasmogenic effect on the smooth muscle preparations. This study therefore rationalizes the folk usage of *Seseli diffusum* as an antispasmodic and diuretic medicine (Datta and Banerjee, 1978; Usmanghani *et al*., 1997). Furthermore, compound **5** was found to have an antioxidant activity.

Dedication

This publication is dedicated to the memory of the Professor Yoshisuke Tsuda (1932–2006). Professor Tsuda made major contributions in the field of carbohydrate, alkaloid and saponin chemistry. He spent his life exploring the fascinating world of organic synthesis and in developing a conceptual foundation of pharmacognosy. He worked tirelessly for the promotion of science in developing countries and dedicated many years of his life to creating a scientific environment across the globe, irrespective of national boundaries. He was a great friend of Pakistan, and an admirer of the rich traditions and heritage of this ancient land.

Acknowledgements

We are thankful to Professor Dr Anwar Ali Siddiqui, Associate Dean Research, Aga Khan University, Karachi, for his help in yeast bioassay.

Conflict of Interest

The authors have declared that there is no conflict of interest.

REFERENCES

Datta SC, Banerjee AK. 1978. Useful weeds of west Bengal rice fields. *Econ Bot* **32**: 297–310.

Elgamal MHA, Elewa NH, Elkhrisy EA, Duddeck H. 1979. ^{13}C NMR chemical shifts and carbon-proton coupling constants of some furocoumarins and furochromones. *Phytochemistry* **18**:139–143.

Ghayur MN, Gilani AH. 2005. Pharmacological basis for the medicinal use of ginger in gastrointestinal disorders. *Dig Dis Sci* **50**: 1889–1897.

Gunatilaka AAL, Kingston DGI. 1998. DNA-Damaging natural products with potential anticancer activity. In *Studies in Natural Product Chemistry*, Atta-ur-Rahman FRS(ed.). Elsevier Science Publishers: Amsterdam; Vol **20**, 457–505.

Gunatilaka AAL, Kingston DGI, Wijeratne EMK, Bandara BMR, Hoffmann GA, Johnson RK. 1994. Biological activity of some coumarins from Sri Lankan Rutaceae. *J Nat Prod* **57**: 518–520.

Gunatilaka AAL, Samaranayake G, Kingston DGI, Hoffman G, Johnson RK. 1992. Bioactive ergost-5-ene-3β,7α-diol derivatives from *Pseudobersama mossambicensis*. *J Nat Prod* **55**: 1648–1654.

Ikeda R, Nagao T, Okabe H *et al*. 1998. Antiproliferative constituents in umbelliferae plants. III. Constituents in the root and the ground part of *Anthriscus sylvestris* Hoffm. *Chem Pharm Bull* **46**: 871–874.

Kozawa M, Baba K, Matsuyama Y, Kido T, Sakai M, Takemoto T. 1982. Components of the root of *Anthriscum sylvestris* Hoffm II. Insecticidal activity. *Chem Pharm Bull* **30**: 2885–2888.

Lee SK, Mbwambo ZH, Chung HS *et al*. 1998. Evaluation of the antioxidant potential of natural products. *Comb Chem High Throughput Screen* **1**: 35–46.

Maruyama T, Abbaskhan A, Choudhary MI *et al*. 2009. Botanical origin of Indian celery seed (fruit). *J Nat Med* **63**: 248–253.

Masuda T, Takasugi M, Anetai M. 1998. Psoralen and other linear furanocoumarins as phytoalexins in *Glehnia littoralis*. *Phytochemistry* **47**: 13–16.

Murray RDH, Mendez J, Brown SA. 1982. *The Natural Coumarins, Occurrence, Chemistry and Biochemistry*. John Wiley & Sons: Bristol, Belgium, 368 and 376.

Okuyama T, Takata M, Shibata S. 1989. Structures of linear furano- and simple-coumarin glycosides of Bai-Hua Qian-hu. *Planta Med* **55**: 64–67.

Sattar A, Ashraf M, Bhatty MK, Chisti NH. 1978. β-Cyclolavandulic acid and seselin in the essential oil of *Carum roxburghianum*. *Phytochemistry* **17**: 559–560.

Usmanghani K, Saeed A, Alam MT. 1997. *Indusyunic Medicine, Traditional Medicine of Herbal Animal and Mineral Origin in Pakistan*. Karachi University Press: Karachi, 104–106.

