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Chemical Composition and Cytotoxic Effect of *Prangos turcica* A. Duran, M. Sagiroglu & H. Duman

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Abstract: In addition to the antiflatulent, emollient, antifungal, antihemorrhoidal, antioxidant, anthelmintic effects, *Prangos* species have been used to stop bleeding and for the treatment of wounds and scars in central Asia and Turkey. In the present study, the compounds were isolated using chromatographic methods, and their structures were identified by ^{1}H NMR and direct comparison with the reference compounds where available. Fifteen known coumarins were isolated from the dichloromethane extract as osthol, murraol, auraptenol, peroxyauraptenol, 4'-senecioiloxyosthol, meranzin hydrate, scopoletin, umbelliferone, isoimperatorin, oxypeucedanin, oxypeucedanin hydrate, oxypeucedanin methanolate, gosferol, psoralen, and marmesin. The cytotoxic activities of all isolated compounds from dichloromethane extract of *P. turcica* roots were evaluated using MTT assay on human adenocarcinoma (prostate PC-3) cells. 4'-senecioiloxyosthol, oxypeucedanin methanolate, gosferol, psoralen, peroxyauraptenol and marmesin were tested for the first time on the PC-3 cell line. Osthol and peroxyauraptenol showed the highest cytotoxic activity with IC_{50} values of 65 and 72 µg/mL, respectively. Additionally, auraptenol, scopoletin, gosferol, psoralen, 4'-senecioiloxyosthol and dichloromethane extract of root part (Pt/R/DCM) demonstrated moderate to low cytotoxic activity. Consequently, the most potent compounds, osthol and peroxyauraptenol, may be used as a lead compound to develop effective drug substances to treat prostate cancer.

Keywords: Coumarin derivatives; *Prangos turcica*; cytotoxic activity.

1. Introduction

Cancer is one of the leading causes of death that still presents a major public health problem worldwide. In 2020, approximately 19.3 million new cases and 9.9 million deaths for all ages were reported globally. The most common types of cancer in men are lung, prostate, and colorectal, while in

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women are breast, colorectal and lung [1]. Furthermore, the estimated number of new cases for 2030 for both sexes and all ages is 24.6 million [2]. The year-to-year increase in cancer cases requires the discovery of new effective compounds as well as new approaches for the treatment. The natural sources containing cytotoxic compounds have been used to prevent or treat cancer for many years.

Genus *Prangos* (Apiaceae) comprises about 43 species that spread throughout the world. *Prangos*, commonly known as "Caksir", is represented by 21 taxa, and nine of them are endemic in Turkey [3-11]. Traditionally, *Prangos* species were used to stop bleeding. In addition, they were used for wound and scar healing and their emollient, tonic, antifungal, antihemorrhoidal, antioxidant, aphrodisiac, antihelmintic and antiflatulent activities [12-14]. According to literature data, they have been subjected to many scientific studies because they have various coumarins, alkaloids, flavonoids, and terpenoid compounds with their cytotoxic, antifungal, antibacterial and anti-HIV activities [15-18]. Previous studies reported that the cytotoxic effects of various *Prangos* species revealed the presence of coumarins, such as osthol, isoimperatorin, isoarnottinin 4'-glucoside, 8-geranyloxy psoralen, aviprin, scopoletin, umbelliferone, murraol, meranzin hydrate, oxypeucedanin, oxypeucedanin hydrate and oxypeucedanin methanolate on many kinds of cell lines [19-30]. Although the natural coumarins isolated from the *Prangos* species have many biological effects, there have been limited studies about their cytotoxic activities.

In recent years, the focus on the effects of coumarins especially on prostate cancer, has increased [25-30]. Since prostate cancer is the second most common cancer type with a 30.7 age-standardized incidence rate worldwide [1], various extracts of *P. turcica* have been evaluated against the PC3 prostate cancer cell line to identify the potential lead compound(s) that has cytotoxic activity on this cell line. Unlike normal cells, carcinoma cells are capable of being *in vitro* models for toxicity tests by preserving some of the cell/tissue-specific functions, which are substantial to mimicking *in vivo* responses [31]. Therefore, human prostate adenocarcinoma (PC-3) cells, which are one of the most commonly used cells as *in vitro* prostate model [32], were utilized to investigate the cytotoxic effects of the extracts.

The focus of this study is *Prangos turcica* A. Duman, M. Sagiroglu & H. Duman, which is the recently described endemic species of Flora of Turkey [5]. The chemical composition of essential oil of its fruits was reported before [33]. Anticholinesterase activity of different extracts of fruits and antimicrobial properties of root extracts were also studied by our group [34-35]. In this study, we report the cytotoxic activities of dichloromethane extract and coumarin derivatives isolated from the dichloromethane extract of the roots of *P. turcica*.

2. Materials and Methods

2.1. Chemicals

Dimethyl sulfoxide (DMSO), sodium dodecyl sulfate (SDS) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). Phosphate buffered saline (PBS), Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM F-12), fetal bovine serum (FBS), penicillin-streptomycin (Pen-Strep) solution and trypsin-EDTA were obtained from Multicell Wisent (Saint-Jean Baptiste, QC, Canada), and all sterile plastic materials were from NEST Biotechnology (Jiangsu, China). All chemicals used in isolation studies were purchased from Merck (Merck Co., Darmstadt, Germany), and all solvents were analytical grades.

2.2. Plant Material

The roots and aerial parts of *Prangos turcica* A. Duran, M. Sagiroglu & H. Duman were collected in August 2015 from Osmaniye between Yarpuz-Yağlıpınar, Turkey. The plant materials were identified by Prof. E. Akalın and vouchers were deposited (ISTE 115740) in the Herbarium of Istanbul University, Faculty of Pharmacy. The plant specimens were dried at room temperature in shadow and were powdered for phytochemical and bioactivity analysis.

2.3. Preparation of Plant Extracts

The air-dried and powdered roots (3000 g) and aerial parts (450 g) were extracted using a Soxhlet apparatus exhaustively with petroleum ether, dichloromethane and methanol.

2.4. Isolation of Compounds

The first chromatographic experiments pointed that the dichloromethane extract of the root part had a richer chemical content when all the root and aerial parts extracts were compared. Thus, the dichloromethane extract of the root part was selected for subsequent isolation procedures. The dichloromethane extract (55.5 g) was dissolved in acetone (2500 mL) and kept in the fridge overnight. The precipitated hydrocarbon mixtures were filtered from the extract by filtration using Gooch crucible, and the filtrate was concentrated in vacuo. 8 g of yielded extract (48 g) was separated using a Sephadex LH-20 column packed in hexane-dichloromethane-methanol (14:9:1). Based on TLC behaviour, similar fractions were combined to yield six main fractions. Compounds **1**, **7**, **9,** and **11** were directly purified with Preparative Thin Layer Chromatography (PTLC) (1-2 mm thickness and using silica gel with mobile phase mixtures of cyclohexane-ethyl acetate, 7:3, 2:3) from fraction I and II. Other fractions were chromatographed on a silica gel column using a step gradient of hexane-ethyl acetate solvent system (in different ratios starting with 100:0 to 0:100) and Preparative TLC (1-2 mm thickness and using silica gel with mobile phase mixtures of cyclohexane-ethyl acetate-benzene, 1:1, 4:2:2, 5:3:2) was used for purification of compound **2, 3, 4, 5, 6**, **8**, **10**, **12**, **13**, **14**, **15**. NMR spectroscopy was used for identification and structure elucidation.

2.5. Cell culture and MTT Cytotoxicity Assay

The PC-3 (CRL-1435) cell line was purchased from the American Type Culture Collection (ATCC, USA) and grown DMEM F-12 supplemented with FBS (10%) and Pen-Strep (100 U-100 µg/mL) at 37°C in a humidified atmosphere with 5% CO_2. When the cells reached 70-80% confluence every 3-4 days, sub-culturing was performed using Trypsin-EDTA.

The MTT assay is based on the conversion of pale-yellow tetrazolium salt into pink/purple formazan crystals by mitochondrial dehydrogenase in living cells. Since the mitochondrial activity is related to viability, the color changes indicate the proportion of viable and/or dead cells [36]. The cells were seeded at 10^4 cells/100 µL medium into each well of 96-well plates and incubated overnight for cell attachment. Then, cells were treated with the serial dilutions of extracts for 24 h. Cell culture medium, 1% DMSO, and 1% SDS were used as growth, negative and positive controls, respectively. 20 µL MTT solution (5 mg/mL in PBS) was added to each well and the plates were incubated for a further three h. Then, the supernatant was removed, and 100 µL DMSO was added to each well for the dissolution of formazan crystals. Cell treatment and all assays were administered in triplicate on three independent days for biological control. The optical densities (ODs) of every well was read at 590 nm (against the reference wavelength of 670 nm) using a microplate spectrophotometer system (Epoch, Germany), and the half-maximal inhibition of enzyme activity (IC_{50}) was calculated as compared to the solvent control by using the equation below [37]. Inhibition of cell growth (%) = 100 - [100 × (sample absorbance)/(negative control absorbance)].

3. Results and Discussion

3.1. Phytochemistry

Fifteen coumarin compounds were isolated from the dichloromethane extract of the roots and their structures were identified by ^{1}H NMR spectroscopy as osthol (**1**) (4.4 mg) [38], murraol (**2**) (11 mg) [39], auraptenol **(3**) (25 mg) [40], peroxyauraptenol (**4**) (3.6 mg) [38], 4'-senecioiloxyosthol (**5**) (2.1 mg) [41], meranzin hydrate (**6**) (31.6 mg) [27], scopoletin (**7**) (21.5 mg) [42], umbelliferone (**8**) (2.2 mg) [43], isomperatorin (**9**) (21 mg) [44], oxypeucedanin (**10**) (9.3 mg) [44], oxypeucedanin

hydrate (**11**) (32.7 mg) [45], oxypeucedanin methanolate (**12**) (10.9 mg) [46], gosferol (**13**) (7.1 mg) [47], psoralen (**14**) (4.5 mg) [48], and marmesin (**15**) (1 mg) [49], respectively. Optical rotation measurements showed that the optical rotation values of the compounds were near zero. Thus, the compounds were identified as optically inactive and racemic compounds. Molecular structures of compounds are presented in Figure 1.

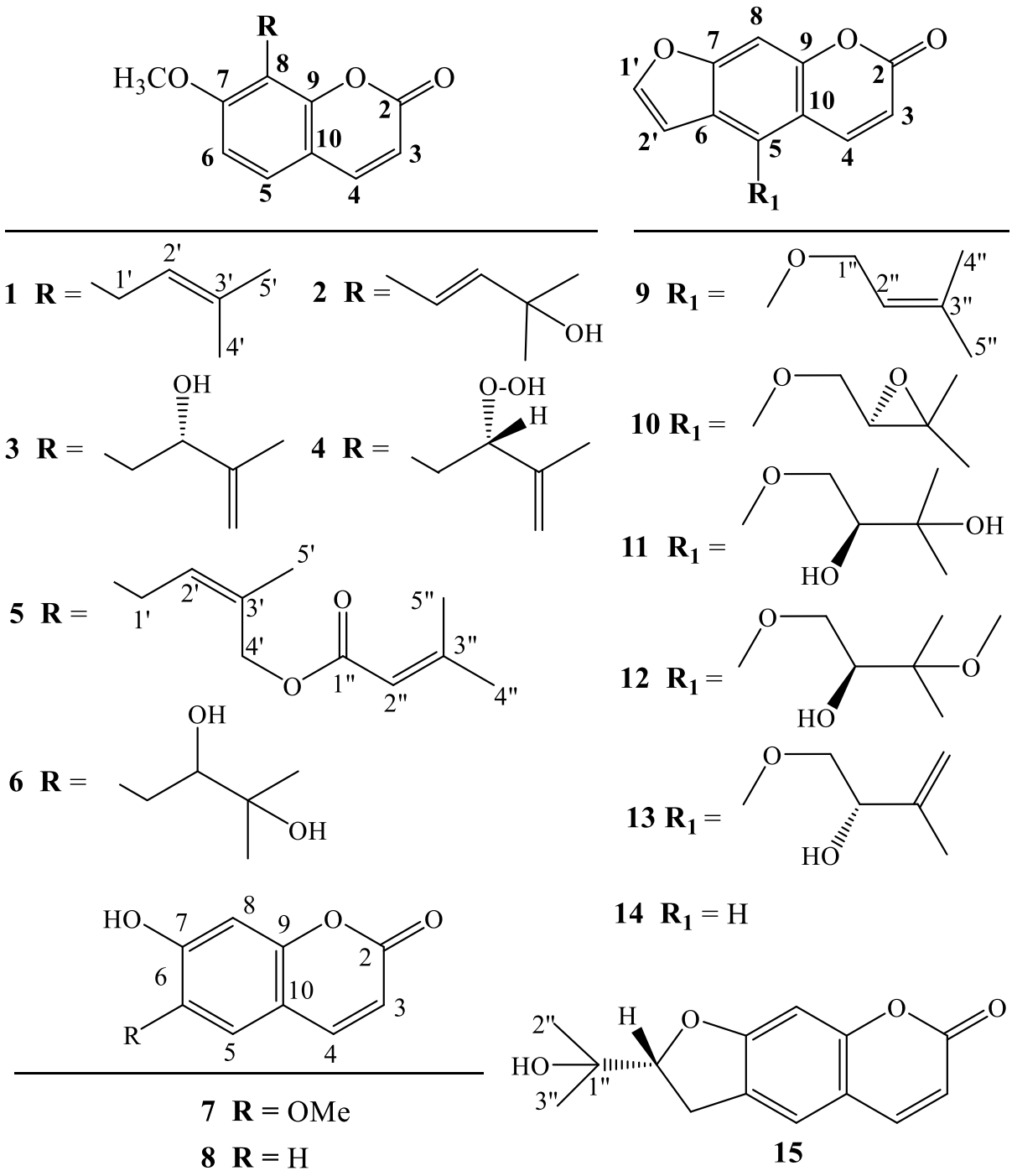

Figure 1. Coumarins isolated from the dichloromethane extract of *P. turcica*

3.2. Cytotoxicity

Cytotoxic activities of the compounds and dichloromethane extract of root part in PC-3 cells for 24h exposure are summarized in Table 1 and Figure 2. Compounds **1** and **4** showed the highest

cytotoxic activity with IC_{50} values of 0.065 and 0.072 mg/mL, respectively. Additionally, compounds **3**, **7**, **13**, and **14** demonstrated modest cytotoxic activity, whereas compound **5** and dichloromethane extract of root part (Pt/R/DCM) demonstrated lower cytotoxic activity. Due to solubility challenges and viability troubles, eight out of the 16 samples could not be tested in higher concentrations; therefore, the percentage of the observed maximal cell death is given in Table 1. Among the compounds, at 0.75 mg/mL concentration, the observed maximal cell death was ≤9.09 for compounds **2**, **6**, **8**, **9, 10**, **11**, **12** and **15**.

Table 1. IC_{50} values or maximum cell death determined of the compounds/extract in PC-3 cells after 24h exposure

Compound/Extract	IC_{50} values (µg/mL) ±SD	Max. cell death (%) ±SD*
1	**65 ±5**	
2	>750	29.24 ±5.86
3	258 ±13	
4	**72 ±3**	
5	421 ±45	
6	>750	9.09 ±1.02
7	194 ±28	
8	>750	21.54 ±3.25
9	>750	40.42 ±2.99
10	>750	39.13 ±9.47
11	>750	26.23 ±5.68
12	>750	38.38 ±5.57
13	148 ±18	
14	129 ±13	
15	>750	15.78 ±3.48
Pt/R/DCM	364 ±38	

*For 750 µg/mL.
Pt/R/DCM: dichloromethane extract of root part, IC_{50}: 50% of inhibitor concentration, SD: standard deviation

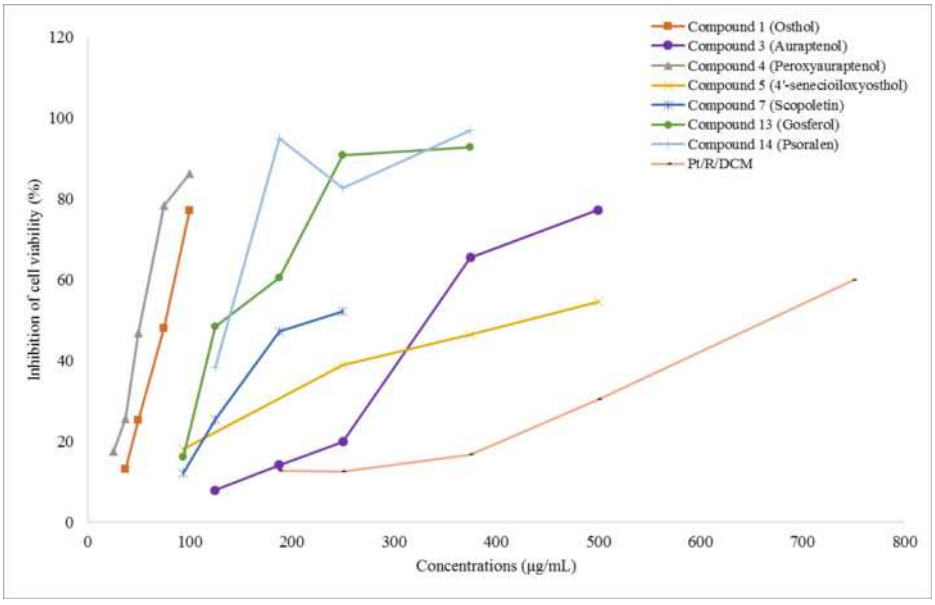

Figure 2. Cytotoxicity effects of pure compounds and Pt/R/DCM on PC-3 cells after 24h incubation

The cytotoxic potentials of the compounds according to IC_{50} values are as follows: **1** (osthol) > **4** (peroxyauraptenol) > **14** (psoralen) > **13** (gosferol) > **7** (scopoletin) > **3** (auraptenol) > **5** (4'-senecioiloxyosthol).

Studies in recent years have focused on natural resources to find novel agents for the treatment of cancer which is one of the most significant causes of death. Studies in literature reported that plants belonging to the *Prangos* genus showed cytotoxic and antiproliferative activities in various cancer cells. The antiproliferative effects of essential oils of *P. orientalis* and *P. asperula* were evaluated in human amelanotic melanoma (C32) and renal adenocarcinoma (ACHN) cells using sulphorhodamine B assay. *P. orientalis* showed higher cytotoxic activity against renal adenocarcinoma cells (IC_{50}=121 µg/mL) rather than amelanotic melanoma cells (IC_{50}=330.04 µg/mL); likewise, *P. asperula* showed cytotoxicity against only renal adenocarcinoma cells with an IC_{50} of 139.17 µg/mL [50]. MTT assay was used to evaluate the cytotoxic activity of *n*-hexane, dichloromethane and methanol extracts of *P. uloptera* roots; and, observed that only the dichloromethane extract reduced the viability of human cervix adenocarcinoma (HeLa) cells with an IC_{50} of 100 µg/mL [51]. Furthermore, in the literature [20], the findings showed that isoarnottinin 4'-glucoside isolated from the leaves of *P. uloptera* had modest cytotoxic activity against HeLa cells with an IC_{50} of 840 µg/mL. Similarly, in HeLa cells, dichloromethane extract of *P. pabularia* displayed cytotoxic activity with an IC_{50} of 526 µg/mL [52].

On the other hand, the *Prangos* genus showed low to moderate or no cytotoxic activity in other cells. The antiproliferative effects of aqueous extracts of *P. platychloena* were evaluated by the Trypan Blue dye [53]. The extract exhibited the highest cytotoxic activity at 1 mg/mL with an inhibition of 72% and 59% in human colorectal adenocarcinoma cells, CCL-221 and Caco-2, respectively, whereas no cytotoxic activity was observed against the human fibroblast cells, which are used as non-carcinoma cells. It was noticed in the literature that the extracts of *P. meliocarpoides* were not displaying cytotoxic activity up to 0.1 mg/mL concentrations in baby hamster normal kidney fibroblast (BHK 21) cells [54]. Similarly, MTT assay was utilized to determine the cytotoxicity of the ethanolic extract of aerial parts of *P. asperula* in monkey normal kidney cells; and showed activity with IC_{50} values of 660 µg/mL [55]. In a previous study of our group, extracts derived from aerial parts and roots of *P. hulusii* were investigated using MTT and LDH assays in rat normal kidney (NRK-52E) cells [56]. None of the extracts showed any cytotoxic activity by MTT assay, whereas only petroleum ether extract displayed lower activity in the LDH assay.

Coumarin derivatives are one of the major groups of natural-based compounds with different biological effects. Natural and synthetic coumarin derivatives attract attention in studies, especially due to their therapeutic properties in various types of cancer. When the effects of coumarin compounds on cell growth are examined, it is shown that they are effective against different cell lines such as human gastric adenocarcinoma (MK-1), human rhinopharynx cancer (KB) and human bronchial epidermoid carcinoma (NSCLC-N6) [57-59].

The mechanism of action of coumarin and coumarin-derived compounds are known to be generally caspase-dependent apoptosis [60-62]. In Phase-I trial studies, the patients with metastatic, hormone-naive, or hormone-refractory prostate cancer, positive results were obtained with 3 g of coumarin daily in patients with low tumor burden [61-62]. In a study, the findings showed that after five days of treatment at concentrations of ≤500 µg/mL, coumarin inhibited the proliferation of two malignant prostate cell lines (DU145 and LNCaP) [57].

In the present study, we evaluated the cytotoxic effects of DCM extract and isolated compounds from *Prangos turcica* roots on prostate adenocarcinoma (PC-3) cell lines. Our results revealed that scopoletin had cytotoxic activity against PC-3 cells with an IC_{50} value of 194 µg/mL. Similar to our results, scopoletin was reported to have an antiproliferative activity on human prostate tumor PC-3 cells (IC_{50}=157 µg/mL) by marked time- and concentration-dependent [25]. Umbelliferone was evaluated against the different prostate cancer cell lines. In addition to inducing apoptosis significantly, it was seen to decrease cell viability in a dose-dependent manner [26]. In contrast, our results revealed that umbelliferone had no cytotoxic activity at 750 µg/mL against the PC-3 cell line.

The cytotoxic activities of osthol, murraol, and meranzin hydrate isolated from *Phellolophium madagascariense* Baker was determined on human prostatic LNCaP, PC-3, and DU145 cells [27]. At 100 µM concentration (equals to 24 µg/mL for osthol, 26 µg/mL for murraol, 27 µg/mL for meranzin hydrate), the compounds showed a weak antiproliferative activity with 25, 32 and 9% inhibition for osthol, murraol, and meranzin hydrate, respectively, on PC-3 cells. In accordance with the study, we

determined the IC_{50} value of osthol as 65 µg/mL; however, similar inhibition of cellular growth was observed at higher concentrations than 750.000 µg/mL for both murraol and meranzin hydrate.

The cytotoxic effects of coumarin derivates isolated from *Prangos ferulacea* (L.) Lindl. were evaluated in prostate adenocarcinoma (PC-3), human neuroblastoma (SKNMC) and non-small cell human lung cancer (H1299 (p53 null)) cells [22]. Among isolated coumarins, osthol (**1**) demonstrated cytotoxic activity against PC-3, SKNMC and H1299 cells. Moreover, osthol (**1**) induced apoptosis by increasing caspase activation, upregulating apoptotic genes, and downregulating anti-apoptotic genes in PC-3 cells, in accordance with a previous study [28] which suggested that osthol had substantial activity against hormone-independent prostate cells. Additionally, in the same study, isoimperatorin (**9**) demonstrated a weaker activity in PC-3 and SKNMC cells with an IC_{50} value of 35.8 and 54.6 µg/mL, respectively, and had no effect against H1299 cells. However, in the present study, compound **9** displayed very weak cytotoxic activity with 38.69% cell death at 750 µg/mL.

According to the literature, [22] and [29], neither oxypeucedanin (**10**) nor oxypeucedanin hydrate (**11**) showed a cytotoxic activity up to 300 µM against PC-3 cells. Likewise, in our study, oxypeucedanin (**10**) and its hydrate and methanolate (**12**) derivatives did not show cytotoxic activity on PC-3 cells. It was shown that oxypeucedanin methanolate displayed cytotoxic activity against mouse T-cell lymphoma cells with an IC_{50} value of 17 µg/mL and against NIH/3T3 mouse fibroblast cells with an IC_{50} value of 15 µg/mL, whereas no cytotoxicity against human ABCB1-transfected subline [30]. To our knowledge, in the present study, for the first time, we observed that oxypeucedanin methanolate (**12**) had no cytotoxic activity on human prostate cancer (PC-3) cells.

Additionally, in the present study, the cytotoxic activity of 4'-senecioiloxyosthol (**5**), gosferol (**13**), psoralen (**14**) and marmesin (**15**) were evaluated for the first time in PC-3 cells. Psoralen and gosferol showed moderate cytotoxic activity with IC_{50} values of 129 and 148 µg/mL, respectively, whereas 4'-senecioiloxyosthol demonstrated low cytotoxic activity with an IC_{50} value of 421 µg/mL compared to the most active compound, osthol (**1**). Marmesin had no activity against the PC-3 cell line.

Based on the observed results, compounds **1** and **4** were the most active compounds in prostate adenocarcinoma (PC-3) cells, with IC_{50} values of 65 and 72 µg/mL, respectively. Furthermore, osthol isolated from *P. pabularia* induced apoptosis in HeLa and human promyelocytic leukemia (HL-60) cells; thus, *P. pabularia* was suggested as a good source for the development of antitumor drugs [24]. Although the same genus is subjected, it is challenging to make a precise prediction due to the severity of selected plants, obtained parts, derived extracts, used assays and cell type. Previous studies have shown that the *Prangos* genus is not cytotoxic against normal cells, so osthol (**1**) and peroxyauraptenol (**4**) could be considered as promising coumarin compounds and could be a naturally sourced alternative to synthetic drugs in the treatment of prostate cancer. Further studies are needed to account for the exact mechanisms of these effects of the active compounds.

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Supporting Information

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