Research Article

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Cytotoxic Activities of Isolated Compounds from *Prangos uechtritzii* Boiss & Hausskn Roots

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ABSTRACT

Prangos uechtritzii Boiss&Hausskn is an endemic plant of Türkiye, and the plant roots are rich in coumarins. This study aims to evaluate the cytotoxic activities of the 16 natural compounds along with the n-hexane (PH), chloroform (PC), and methanol (PM) extracts of P. *uechtritzii.* For this purpose, fourteen coumarin derivatives; umbelliferone(1), 6-formylumbelliferone(2), suberosin(3), 7-demethylsuberosin(4), (+)-ulopterol(5), tamarin(6), psoralen(7), imperatorin(8), (+)-oxypeucedanin(9), (+)-oxypeucedanin hydrate(10), (+)-oxypeucedanin methanolate(11), (+)-marmesin(12), (-)-prantschimgin(13), and (-)-adicardin(14); two polyacetylenes (-)-panaxynol(15), (+)-falcarindiol(16) and three extracts (PH, PC, and PM) were tested for their cytotoxicity against two healthy (HK-2, NIH/3T3), and four cancer (MCF-7, A-549, SH-SY5Y, PC-3) cells by WST-1 method. Doxorubicin was used as a positive control. PH and PC showed cytotoxic effects on all the cell lines with IC50 values of 8.16-91.56 µg/mL. PH displayed a selective effect on SH-SY5Y cells [Selectivity Index (SI)= 2.5] compared to NIH/3T3. PC exhibited cytotoxic effects on PC-3 cells (SI=2) compared to both NIH/3T3, and HK-2. PM didn't display cytotoxicity at 100 µg/mL. (-)-Panaxynol, (+)-falcarindiol, 6-formylumbelliferone, 7-demethylsuberosin, and suberosin exhibited effects with IC50 values of 8.65-87.91 µM, while others didn't work at 100 µM. So, P. uechtritzii could be a promising natural source in the development of new drugs for cancer treatment.

Keywords: *Prangos uechtritzii, coumarin, cytotoxicity.*

1. Introduction

Prangos sp. belonging to the Apiaceae family is distributed in the Irano-Turanian floristic region and is composed of 45 species in the world, 19 of which are in Türkiye [1]. In Anatolia, the plant roots are used for their aphrodisiac properties internally and wound-healing benefits externally, while the aerial parts of the plant have benefitted as a stimulant and carminative [2]. In literature, some studies have been reported such as antioxidant, antibacterial, anticholinesterase, and cytotoxic properties with different members of the genus. *Prangos uechtritzii* Boiss & Hausskn is an endemic plant grown in the Central and Eastern parts of Türkiye. It is a perennial herb and has a vernacular name as 'Deli çakşır' among local people [3].

The phytochemical studies of the plant's aerial parts were reported as chromatographic analysis of essential oils, and LC-MS analysis of *n*-hexane, ethyl acetate, methanol, and water extracts of the plant [4-6]. α-pinene, β-phellandrene, γ-3-carene, *p*-cymene, 7-epi-1,2-dehydro- sesquicineole, caryophyllene oxide are recorded as major volatile compounds [5,7,8] and coumarins, furanocoumarins, flavonoids, and hydroxycinnamic acids are found as major non-volatile compounds in the plant [6]. Polyacetylenes which are known as potential cytotoxic compounds [9] have also been revealed to be distributed in the genus [10]. In our previous study, coumarin and polyacetylene derivatives were obtained from *P. uechtritzii* roots [10].

The bioactivity studies conducted with the plant have been reported as antioxidant, antifungal, antibacterial, acetylcholinesterase, butyrylcholinesterase, tyrosinase, α-amylase, α-glucosidase enzyme inhibition, and vasorelaxant studies in the literature [4-6,11].

However, the plant roots have not been evaluated for cytotoxic activity yet. Therefore, this study aims to screen the cytotoxic activities of the pure compounds along with the *n*-hexane (PH), $CHCl_3$ (PC), and MeOH (PM) extracts of the plant roots.

Data are the mean of three independent samples determined in triplicate. IC50 is presented as mean ± standard deviation from linear regression dose-response curves. Units of concentration are given in terms of [μM]. A dash indicates that there is no activity at 100 μM. Dox means doxorubicin.

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2. Material and Methods

2.1. General experimental materials

Human neuroblastoma cell line (SH-SY5Y), human breast cancer cell line (MCF-7), human prostate cancer cell line (PC-3), adenocarcinomic human alveolar basal epithelial cells (A-549), human kidney cells (HK-2), and murine embryonic fibroblasts (NIH/3T3) were purchased from the American Type Culture Collection (ATCC). The cell culture reagents including fetal bovine serum (FBS), medium, and others were obtained from Biowest (France). DMSO as a vehicle control, was purchased from Carlo Erba (Italy) and Santa Cruz (USA). Doxorubicin was obtained from Sigma-Aldrich (Germany).

2.2. Plant extracts and pure compounds

Plant roots were obtained from the Taskent-Konya region of Türkiye in 2016, authenticated by Prof. S. Gokhan Senol, Biology Department, Ege University. A voucher specimen (IZEF-6050) was stored in the Herbarium of the Faculty of Pharmacy (IZEF), Ege University. In our previous study, air-dried plant roots were extracted with *n*-hexane, $CHCl_3$, and MeOH, sequentially. Furthermore, 16 compounds [6 coumarin derivatives (**1-6**), 7 furanocoumarins (**7-13**), one coumarin glycoside (**14**), and two polyacetylenes (**15-16**)] were isolated from the *P. uechtritzii* root extracts by chromatographic techniques, and their structures were elucidated via spectroscopic methods [10]. PH, PC, PM extracts, and compounds **1-16** (Figure 1) were prepared for further activity studies.

2.3. Cytotoxicity assay

2.3.1. Cell culture

Except for HK-2 cells, all cells were cultivated in DMEM solution. HK-2 cells were cultured in a 1:1 ratio of DMEM/F12 added with 10% FBS and 1% L-glutamine. The cells were incubated at 37°C and gassed with 5% CO_2.

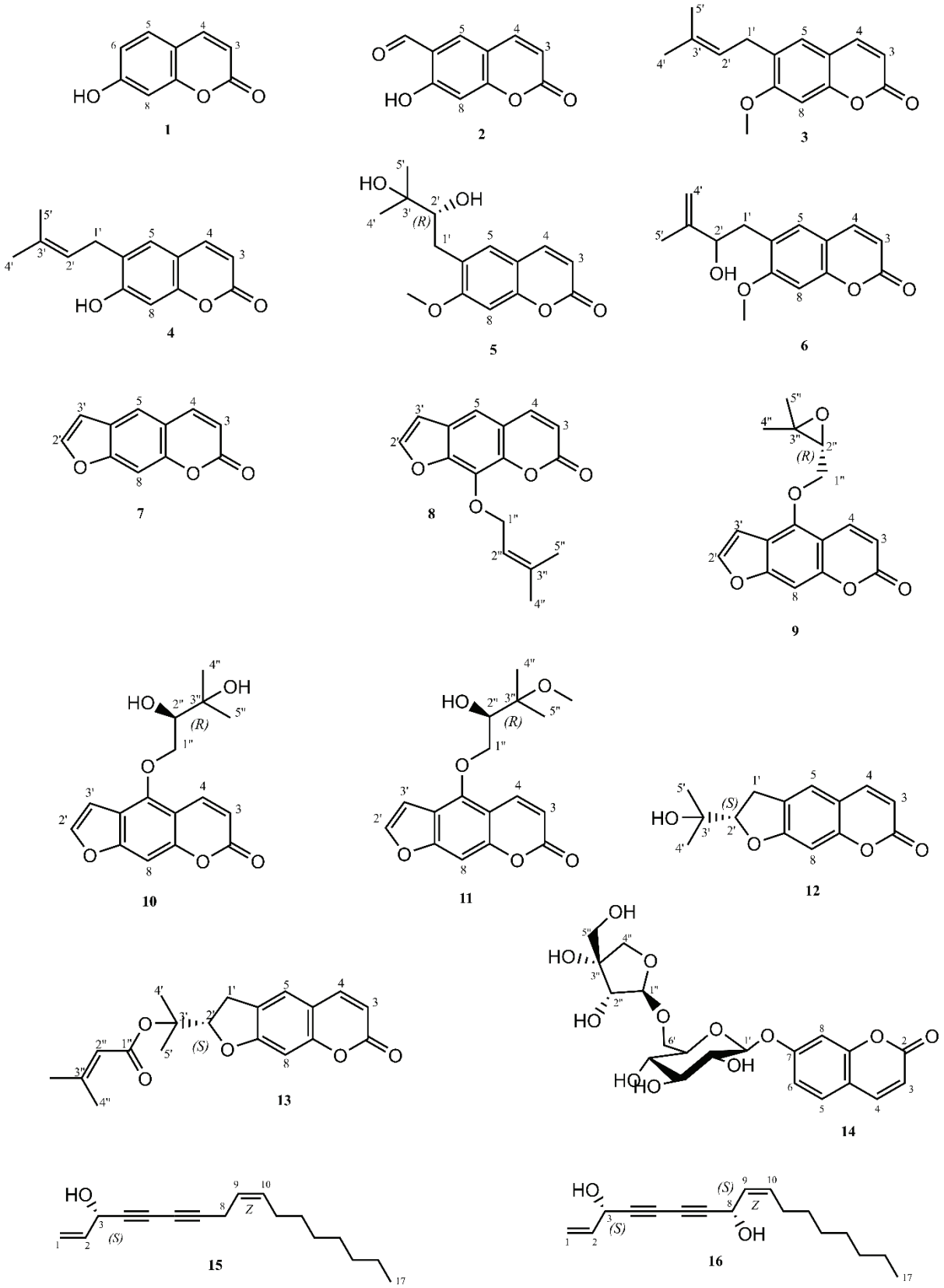

Figure 1. The tested compounds **1-16** from *Prangos uechtritzii* roots.

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Table 1. Cytotoxic activities of *P. uechtritzii* root extracts

	IC50 ± S.D. (μM)					
Comp.	NIH/3T3	HK-2	A549	MCF-7	PC-3	SH-SY5Y
1	-	-	-	-	-	-
2	19.64±1.22	8.65±0.06	24.98±1.46	87.91±3.72	15.89±0.73	-
3	30.3±1.14	9.35±0.21	42.36±1.02	55.12±3.03	49.25±1.05	-
4	9.73±0.36	26.1±1.03	25.94±1.02	-	22.42±0.84	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	-	-	-	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	-	-	-	-	-	-
13	-	-	-	-	-	-
14	-	-	-	-	-	-
15	8.79±0.83	19.3±0.95	19.3±0.95	83.43±2.05	22.73±0.95	-
16	16.75±0.92	20.1±0.97	22.16±2.03	-	19.65±0.65	19.6±0.75
Dox	4.02±0.57	5.07±0.87	0.06±0.001	0.08±0.004	0.48±0.009	0.02±0.001

Table 2. Cytotoxic activities of the compounds **1-16**.

	IC50 ± S.D. (μg/mL)					
Extracts	NIH/3T3	HK-2	A549	MCF-7	PC-3	SH-SY5Y
PH	20.83±2.06	8.69±0.16	14.97±0.65	91.56±3.04	12.88±0.12	8.16±0.06
PC	22.66±1.06	21.77±0.96	31.79±2.06	79.1±4.03	10.91±0.3	35.14±2.10
PM	-	-	-	-	-	-
Dox	2.33±0.329	2.94±0.507	0.03±0.001	0.05±0.002	0.28±0.005	0.01±0.001

2.3.2. Cytotoxic activity assay

The cytotoxicity of PH, PC, PM, and **1-16** on healthy (NIH/3T3, HK-2) and cancer (SH-SY5Y, PC-3, MCF-7, and A-549) cells were determined by WST-1 method [12]. The cells were planted into 96-well plates as 1×10^4 cells/well and incubated for 24 h in an incubator set at 37 °C under 5% CO_2 conditions. After dissolving in DMSO, the test samples were diluted to 100 μg/mL or 100 μM as final concentrations. Each sample was run in triplicate. The control wells were supplemented with 1% DMSO. The cells containing test samples were incubated for 48 hours, then the medium was replaced in each well with 90 μL of fresh medium and 10 μL of WST-1. After that,

the absorbances were recorded at 450 nm and 690 nm at 30 min, 1 h, 2 h, and 4 h, respectively. A microplate reader branded as Thermo-Scientific Varioskan Multi-Mode Flash was used for the assay. Cell viability was determined and displayed as a percentage (%). As a negative control, cells treated with DMSO only were determined. To calculate their IC50 values, the samples (100 µg/mL or 100 µM) that decreased cell viability by more than 60% were assayed at a concentration range of 0, 3.13, 6.25, 12.5, 25, and 50 µg/mL or µM for 48 hours. After incubation, the WST-1 method was performed and the absorbances were read at the specified wavelengths. Doxorubicin HCl was positive control at a concentration range of 0, 0.01, 0.05, 0.1, 0.5, 1.5, and 10 µM.

2.4. Statistical analysis

GraphPad Prism 5.03 (GraphPad Software, San Diego California, USA) was performed for analyzing the data. The results data were provided as '± standard deviation of the mean'. Triplicate analyses were performed, and the results are presented as IC_{50} values of the tested samples. $p< 0.05$ was accepted as the level of significance.

3. Results and Discussion

Cancer is the fourth leading cause of death both in developed and developing countries, according to data from the World Health Organization (WHO) for 2019. It is a significant health problem that varies in diversity, development mechanisms, and treatment options. In addition to synthetic drugs, biotechnological drugs, and vaccines used in the fight against cancer, herbal medicines are also employed. Therefore, researchers are increasingly investigating to detection of potential herbal extracts and molecules with cytotoxicity screenings, aiming to obtain a precursor molecule for the potential active substance or derivatization studies. In our study carried out for this purpose, PH and PC showed cytotoxic effects on all the cell lines with IC_{50} values of 8.16-91.56 µg/mL. PH displayed a selective effect on SH-SY5Y cells [Selectivity Index (SI)= 2.5] compared to NIH/3T3. PC exhibited cytotoxic effects on PC-3 cells (SI=2) compared to both NIH/3T3 and HK-2. PM did not display cytotoxicity at 100 µg/mL (Table 1). Compounds 2, 3, 4, 15, and 16 exhibited effects with IC_{50} values of 8.65-87.91 µM (Table 2). However, their cytotoxic effects were not selective. The other compounds showed no effect on the cell lines at 100 µM. Compounds 2 and 6 were evaluated for their cytotoxicity for the first time through the current study. The sensitivity of the cancer cells to the test compounds was quite variable. For example, 1 and 2 are similar compounds except for small differences and they showed different activity results. The reason why 2 showed a cytotoxic effect and 1 did not may be related to the aldehyde group [13] substituted to 2 and the position where this group is attached. A similar situation occurred between 3 and 4. Considering the structural similarity with 3 and 4, it was considered that the reason why 4 was found to be more effective in some cells, and 3 in others might be due to the difference in the methoxy and hydroxyl groups in the C-7 positions [14]. The cytotoxic activities of compounds 15 and 16 were tested for the first time on NIH/3T3, PC-3, SH-SY5Y, and HK-2 cells in this study. Previously, numerous cytotoxicity studies on different cell lines have been performed with 15 and 16 [15-17]. Studies have reported that 15 has more cytotoxic effects than 16 [18,19]. Compound 16, on the other hand, draws attention to its selectivity in its cytotoxic effect [20,21]. The free hydroxyl group at C-3 and the terminal double bond play an important role in the cytotoxic activities of polyacetylenic compounds [15,19]. In our study, 15 was found to be more effective than 16 in other cell lines except for SH-SY5Y and PC-3 cells. This effect may be due to the high cell permeability of 15 due to its more apolar structure (its lipophilic property is higher). In this respect, our results are consistent with the previous studies [15-21]. It was thought that the difference in activity between these two molecules, whose skeletal structures are very similar, may be due to the hydroxyl (-OH) group attached to the 16 at the C-8 position. According to the results, the activities of the compounds are consistent with the activities of the extracts from which they were isolated. Apolar compounds and extracts were more effective than polar compounds and extracts, respectively. This result was probably due to the existence of glycosidic moiety and multiple hydroxyl units, decreasing the activity.

4. Conclusions

This study is the first cytotoxic study conducted with *P. uechtritzii* roots. The extracts of this endemic species were screened for potential cytotoxic effects along with isolated compounds 1-16. 2 and 6 were

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evaluated for the first time to determine their cytotoxic activity. Moreover, 15 and 16 were tested for the first time against PC-3, HK-2, NIH/3T3, and SH-SY5Y cell lines. In the end, *P. uechtritzii* could be a promising natural source in the development of new drugs for cancer treatment.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Statement of Contribution of Researchers

(Example: Concept – G.A., F.A.K., S.B..; Design – G.A., S.B.; Supervision – S.B.; Resources G.A., S.B.; Materials –G.A., F.A.K., S.B..; Data Collection and/or Processing – G.A., F.A.K.; Analysis and/or Interpretation – G.A., F.A.K.; Literature Search – G.A.; Writing – G.A.; Critical Reviews – G.A.)

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