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RESEARCH ARTICLE

Phytochemical Profile, Antioxidant Capacity and *In Vitro* Cytotoxic Activity of the Water Extract of *Pastinaca erzincanensis* Menemen & Kandemir (Apiaceae)

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ABSTRACT

The present study investigates the phytochemical composition, antioxidant, and anticancer properties of an aqueous extract (AE) obtained from the aboveground parts of *Pastinaca erzincanensis*, an endemic species of Erzincan Province. LC-MS/MS analysis identified 19 phytochemicals among 56 screened standards, with quinic acid, fumaric acid, and gentisic acid as the major components. The extract exhibited a rich profile of phenolic acids and flavonoids, with total phenolic content (TPC) of 80.44 mg GAE/g extract and total flavonoid content (TFC) of 32.62 mg QE/g extract. In the DPPH free radical scavenging assay, AE showed stronger activity than the standard antioxidant trolox (12.80 µg/mL), with an IC_{50} value of 10.99 µg/mL. In the FRAP assay, the extract exhibited high antioxidant capacity (61.49 mg TE/g). Cytotoxicity was evaluated in HeLa (cervical cancer), HT-29 (colon cancer), and LNCaP (prostate cancer) cells using the XTT assay. AE showed a significant, dose-dependent cytotoxic effect against HeLa cells, with an IC_{50} value of 145.04 µM, whereas the effect remained above 500 µM in HT-29 and LNCaP cells. These findings indicate that the aqueous extract of *P. erzincanensis* is a natural source rich in phytochemicals, with potent antioxidant activity and promising cytotoxic potential against HeLa cells. The results provide a basis for further investigation of the extract's molecular mechanisms of action and support evaluation of its potential for developing new therapeutic agents for cancer prevention and treatment.

1. Introduction

Cancer remains one of the most significant global health issues today. According to World Health Organization data, 9.6 million people died of cancer in 2018; cancer ranks as the second leading cause of death worldwide, and an increase in cancer-related deaths has been observed since 2012 [1]. In recent years, the most prevalent types of cancer have included breast, stomach, colon, prostate, lung, ovarian, lymphoma, prostate and cervical. Cervical cancer (HeLa) is a type of cancer that develops in cervical cells and is one of the most common gynecological cancers worldwide. It ranks 14th among all cancer types and 4th among cancers affecting women [2]. It is widely established that various types of the sexually transmitted human papillomavirus (HPV) are the most common biological cause of cervical cancer [3]. HeLa cells have been employed as a model cell line in numerous studies investigating the effects of steroid hormones, estrogen-like compounds, flavonoids, and antioxidants on cancer cell proliferation [4–7]. Prostate cancer is a type of cancer that develops in the prostate gland, which is part of the male reproductive system. It typically

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progresses slowly, but can spread to bones and lymph nodes [1, 8]. Colorectal cancer, in contrast, is the fourth most prevalent cancer worldwide. Despite advances in screening and effective treatment methods, its incidence is reported to be increasing, particularly among individuals under 50 years of age. This necessitates the development of treatment approaches specific to this patient group [9]. Synthetic drugs used in cancer treatment, including cisplatin, doxorubicin and docetaxel, have achieved significant clinical success. Also, 5-Fluorouracil (5-FU) is a classic fluoropyrimidine antimetabolite that targets the thymidylate synthase (TS) enzyme. TS is a folate-dependent enzyme that is essential for DNA synthesis and cell proliferation. It is recognised as one of the most clinically validated targets in anticancer chemotherapy [10]. 5-FU is one of the primary chemotherapeutic agents used in the treatment of many types of cancer; however, its efficacy is limited by chemoresistance [11]. 5-FU is renowned for its cytotoxic effects on numerous types of cancer, most notably breast cancer. Its prevalence in cancer research can be attributed to its extensive clinical utilisation and its role as a model drug for investigating resistance mechanisms. However, these drugs can also cause serious side effects and toxicity to non-cancerous cells. Consequently, numerous research groups worldwide have concentrated on the development of more effective anti-proliferative agents with reduced side effects from secondary metabolites derived from ethnobotanical plants [12].

Due to the advantages of plant-based medicines - such as their limited side effects, low cost, and easy accessibility - there has been a marked increase in the use of medicinal and aromatic plants in recent years [13]. With approximately 3,800 endemic plant species and an endemism rate of 34.5%, Türkiye's flora offers significant research potential in this field [14]. A plethora of studies on biological activity have been conducted on plants belonging to the genus Pastinaca, to which *Pastinaca erzincanensis* - a species endemic to Erzincan and belonging to the Apiaceae family - belongs. *Pastinaca sativa* L., which is also referred to as wild parsnip, is employed in a number of countries as an appetite stimulant, digestive aid, and diuretic. The plant contains a variety of active compounds, including coumarins, furanocoumarins, xanthotoxin, bergapten, imperatorin, polyacetylenes, and flavonoids [15, 16]. In the medical text Al-Qanun fi'l-Tibb (The Canon of Medicine) by Ibn Sina, the topical and oral use of wild parsnip is recommended for the treatment of headaches, stomatitis, eye inflammation, dermatitis, and fever [17]. In traditional Iranian medicine, it is reported to be used as a stomach tonic, laxative, kidney stone dissolver, and diuretic, as well as in the treatment of lung and liver disorders [18, 19].

The species belonging to the genus *Pastinaca* have been recognised by the local population in Anatolia for a considerable time, as they are in other regions of the world. They are referred to by various names, including " wild parsnip" and "karakavza". The harvesting of the fleshy roots takes place, notably in the autumn, and they are utilised for culinary purposes in a variety of ways, including boiling, roasting, and pickling. It has been documented that decoctions and infusions prepared from the roots find application in folk medicine for the treatment of women's health complaints, while a root poultice is employed for the management of wounds and inflamed areas [20, 21]. Despite the absence of direct ethnobotanical records attesting to the traditional use of *P. erzincanensis*, a species endemic to Erzincan Province and comparatively recently described, it is evident that local communities employ related *Pastinaca* taxa, which naturally proliferate in the same region, for analogous purposes. This finding suggests the potential for *P. erzincanensis* to contain bioactive compounds with similar properties, which could lead to therapeutic applications.

This study aimed to characterise the phytochemical profile of the water extract of *Pastinaca Erzincanensis*, a species endemic to Erzincan whose biological properties have only been

studied to a limited extent, using LC–MS/MS analysis. The study also aimed to evaluate the extract's cytotoxic effect on HeLa (cervical), LNCaP (prostate) and HT-29 (colorectal) cancer cell lines, which have different tissue origins. Selecting these cell lines enables a comparative assessment of the general antiproliferative potential of the aqueous extract against different cancer types, while also reinforcing the biological and ethnobotanical significance of the study, particularly given the traditional use of water-based preparations. As the current literature on *P. erzincanensis* is characterised by limited phytochemical and biological data, this study aims to address this by examining both the chemical composition of the plant's water extract and its cytotoxic activity against cancer cells. This will establish a unique basis for understanding the potential biological value of this endemic species.

2. Material and Methods

2.1. Plant Material

Pastinaca erzincanensis specimens utilised in this study were collected in limited quantities from the mountain slopes of Tatlısu Village in Erzincan Province during the flowering period. This collection was conducted in a manner that would not compromise the natural habitats of the specimens or the sustainability of their population. A herbarium collection was prepared from the collected samples, and the plant species identification was performed by Prof. Dr. Ali Kandemir, a faculty member of the Department of Biology, Faculty of Science and Letters, Erzincan Binali Yıldırım University (Kandemir, *P.erzincanensis*, 10616, 2 July 2014).

2.2. Extraction

The aboveground parts of the plant were collected during the flowering period and dried under appropriate conditions (The plant was dried in the dark at room temperature for 7 to 15 days) prior to being subjected to an extraction process. To this end, 250 g of ground plant material was initially shaken five times with n-hexane (300 - 400 mL) for a duration of three hours on each occasion. Thereafter, the hexane fraction was evaporated using a rotary evaporator to remove the nonpolar (oily) components. The residual plant material was then extracted as follows: distilled water (300 - 400 mL) was added to the remaining residue, stirred for 6 hours, and the aqueous fraction was completely dried using a freeze-dryer. Consequently, the water extract was obtained from *P. erzincanensis*.

2.3. Cell Culture

In this study, human cervical cancer (HeLa, ps:17), colorectal adenocarcinoma (HT-29, ps:17), and prostate adenocarcinoma (LNCaP, ps:17) cell lines were used. The cells were obtained from ATCC. For the cytotoxicity studies, 7,500 cells/well were used for each cell type. All three cell lines were processed at passage 17. The study was concluded after a 48-hour period.

2.4. Phytochemical Analysis (LC-MS/MS)

The phytochemical composition of the obtained extract was determined qualitatively and quantitatively using liquid chromatography-mass spectrometry (LC/MS-MS). The analyses were performed on an Agilent 1260 Series LC system coupled to an Agilent G6550A iFunnel Quadrupole Time-of-Flight mass spectrometer equipped with a dual-spray Agilent Jet Stream electrospray ionization source (Agilent Technologies, Inc., CA, USA). The separation process

was executed utilising a Poroshell 120 EC-C18 reverse-phase column (3.0 mm × 150 mm × 2.7 μm) in both positive and negative electrospray ionisation modes, in 2 GHz Extended Dynamic Range mode. The obtained data were then subjected to analysis using Agilent MassHunter software [22].

2.5. Antioxidant Activity Assays

The antioxidant capacity of the watervextract of *P.erzincanensis* was determined using methods for total phenolic content, total flavonoid content, DPPH radical scavenging activity, and ferric (III) ion reduction capacity (FRAP).

2.5.1. Total Phenolic Content

The Folin-Ciocalteu colorimetric method was used for determine the total phenolic content of *P.erzincanensis* [23] with minör modifications [24]. Accordingly, 2% Na_2CO_3 solution (200 μL) was added to a 96-well plate, followed by 10 μL of extracts prepared at different concentrations (0.031–1 mg/mL). The mixture was then incubated at room temperature for 3 minutes. Subsequently, 10 μL of 20% Folin-Ciocalteu reagent was added, and following a 30-minute incubation period, the sample's absorbance was measured at a wavelength of 750 nm. Gallic acid was utilised as the standard, and the results were expressed as milligrams of gallic acid equivalent (GAE) per gram of extract.

2.5.2. Determination of Total Flavonoid Content

The aluminium chloride colourimetric method was utilised for the purpose of determining the total flavonoid content [24]. A volume of 10 μL of extract was added to 128 μL of distilled water, followed by the addition of 6 μL of 5% $NaNO_2$ and 6 μL of 10% $AlCl_3$, respectively. The mixture was then incubated for 45 min., after which 1 M NaOH (40 μL) was added. Absorbance was measured at 415 nm. Quercetin was utilised as the standard, and the results are expressed in milligrams of quercetin equivalent (QE) per gram of extract.

2.5.3. DPPH Radical Scavenging Activity

The assay was performed in accordance with the Blois method [25] with slight modifications [24]. A 0.1 mM DPPH solution (210 μL) prepared in ethanol was mixed with the 15 μL of extract at various concentrations (5-10-20-30-40-60-80-100 μg/mL). The mixture was then left to incubate in the dark for 30 minutes, after which the degree of absorption at 517 nm was measured. The radical scavenging activity was calculated according to the following methodology: The relative standard deviation (RSA %) is calculated using the following formula: [A0 – A1)/A0] x 100, where A0 is the control reaction's (without the test sample) extinction coefficient and A1 is the extinction coefficient in the presence of the extracts or standards. The resulting data were then converted into a percentage activity value, and the IC_{50} concentrations (μg/mL) of the extracts were calculated. The outcomes were then compared with those of Trolox, which was utilised as a standard antioxidant. The procedure required for the Trolox calibration curve was carried out in the following order:

200 μL of reagent is incubated with 20 μL of sample (Trolox). After 1520 seconds, the absorbance is measured at λ = 510 nm. To calculate the antioxidant activity, the values determined prior to the decrease in absorbance (at 224 seconds of the measurement – A_{224}) and the final measurement value (at 1520 seconds of the measurement – A_{1520}) are used. The resulting value is calculated according to the following formula: $A = A_{1520} - A_{224}$ [26].

2.5.4. Iron (III) Reducing Power

The Iron (III) Reducing Power (FRAP) of water extract of *P. erzincanensis* was performed according to the Oyaizu method [27] with slight modifications [24]. Sodium phosphate buffer (62.5 µL, 0.2 M, pH 6.6) and potassium ferricyanide solution (62.5 µL, 1%) were added to the extract (37.5 µL), and the mixture was then incubated at 50 °C for 20 minutes. Following this, trichloroacetic acid (62.5 µL, 10%) and $FeCl_3$ solution (25 µL, 0.1%) were added. Absorbance was then measured at 700 nm. All antioxidant activity experiments were performed in two independent biological experiments with tree technical replicate. The results were presented along with standard deviation (±SD) values.

2.6. Cytotoxicity Assays

The cytotoxic activity of the water extract (WE) of *P. erzincanensis* was evaluated against human cervical cancer (HeLa, ps:17), colorectal adenocarcinoma (HT-29, ps:17), and prostate adenocarcinoma (LNCaP, ps:17) cell lines using the XTT assay [24,28]. HeLa, HT-29, and LNCaP cells were cultured in EMEM, McCoy's 5A, and RPMI-1640 media, respectively, supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin under standard incubation conditions (37 °C, 5% CO_2, and 95% humidity). Cells were treated with the WE at concentrations ranging from 10 to 500 µg/mL, prepared in culture medium. Following treatment, cell viability was determined according to the manufacturer's XTT assay protocol, and absorbance was measured at 470 nm using a microplate reader. IC_{50} values were calculated using GraphPad Prism software. Untreated cells served as a negative control, while 5-fluorouracil (5-FU) at concentrations of 1, 5, 10, 25, and 50 µg/mL was used as a positive control.

2.6. Statistical Analysis

The obtained data were analysed using the GraphPad Prism 6.0 statistical software (GraphPad Software, La Jolla, CA, USA). In the present study, each experiment was replicated triplicate.

3. Results and Discussion

3.1. LC-MS/MS Analysis Results

The results of the LC-MS/MS analysis are presented in detail in Figure 1 and Table 1. The table below provides a detailed breakdown of the phytochemical component contents (in milligrams per gram of extract) of the extract (WE) obtained from *P. erzincanensis*, as determined by liquid chromatography–tandem mass spectrometry analysis.

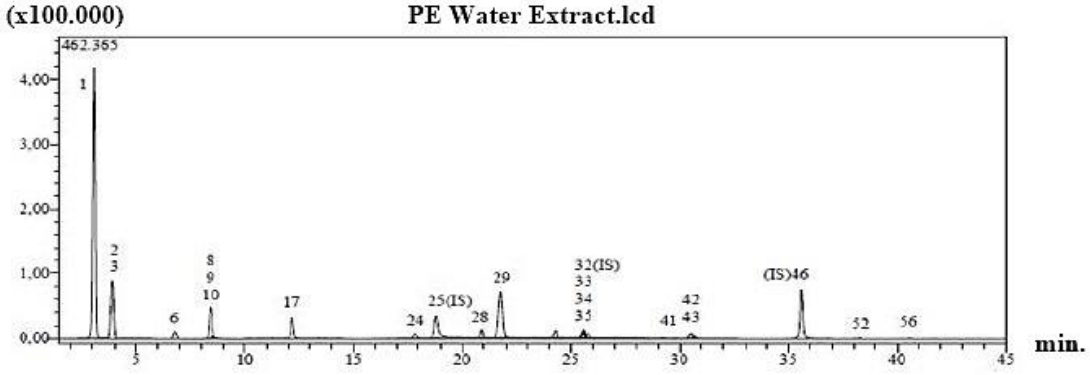


Figure 1. LC-MS/MS chromatogram of the water extract of the species *P. erzincanensis*.

In the chromatogram (see Figure 1), numerous peaks were observed at different retention times during the LC - MS/MS analysis of the water extract, and a peak with the highest signal intensity was identified, particularly in the range of approximately 0.5 - 1 minute. Other peaks observed at later retention times (approximately 5 - 37 minutes) indicate that the extract contains components with different chromatographic properties. X-axis (min.): Retention time—that is, the time it takes for compounds to elute from the column (min.). Y-axis: Signal intensity measured by the detector; in LC–MS/MS chromatograms, ion intensity (counts) is typically displayed [22, 29]. The chemical components identified were listed in Table 1.

Table 1. Phytochemical constituents identified in the water extract of *P. erzincanensis* by LC–MS/MS

No	Compound	Amount (mg/g extract)
1	Quinic acid	25.707
2	Fumaric acid	22.756
3	Aconitic acid	0.014
4	Gallic acid	N.D.
5	Epigallocatechin	N.D.
6	Protocatechuic acid	0.158
7	Catechin	N.D.
8	Gentisic acid	4.009
9	Chlorogenic acid	0.047
10	Protocatechuic aldehyde	0.007
11	Tannic acid	N.D.
12	Epigallocatechin gallate	N.D.
13	Cynarin	N.D.
14	4-Hydroxybenzoic acid	N.D.
15	Epicatechin	N.D.

16	Vanillic acid	N.D.
17	Caffeic acid	0.141
18	Syringic acid	N.D.
19	Vanillin	N.D.
20	Syringaldehyde	N.D.
21	Daidzin	N.D.
22	Epicatechin gallate	N.D.
23	Piceid	N.D.
24	p-Coumaric acid	0.254
25	Ferulic acid-D3-IS	N.A.
26	Ferulic acid	N.D.
27	Sinapic acid	N.D.
28	Coumarin	0.069
29	Salicylic acid	0.362
30	Cynaroside	N.D.
31	Miquelianin	N.D.
32	Rutin-D3-IS	N.A.
33	Rutin	0.096
34	Isoquercitrin	0.489
35	Hesperidin	0.164
36	o-Coumaric acid	N.D.
37	Genistin	N.D.
38	Rosmarinic acid	N.D.
39	Ellagic acid	N.D.
40	Cosmosine	N.D.
41	Quercitrin	0.038
42	Astragalin	0.313
43	Nicotiflorine	0.14
44	Fisetin	N.D.
45	Daidzein	N.D.
46	Quercetin-D3-IS	N.A.
47	Quercetin	N.D.
48	Naringenin	N.D.

49	Hesperetin	N.D.
50	Luteolin	N.D.
51	Genistein	N.D.
52	Kaempferol	0.008
53	Apigenin	N.D.
54	Amentoflavone	N.D.
55	Chrysin	N.D.
56	Acacetin	0.002

N.A.: not applicable
N.D.: not detected

Following the LC- MS/MS analyses, it was determined that 19 of the 56 screened standard phytochemical compounds [22, 29] were detected in the water extract of *P. erzincanensis*. This finding indicates that this species is a phytochemically characterised by quinic acid and fumaric acid, which are both organic acids found in plant, particularly in terms of its phenolic content. Nineteen phytochemicals were identified in the water extract (WE). Analysis of the water extract revealed the presence of quinic acid (25.707 mg/g extract), fumaric acid (22.756 mg/g extract), and gentisic acid (4.009 mg/g extract).

Phenolic acids and flavonoids, which are found in medicinal and aromatic plants, have been shown to exhibit notable biological properties in living systems. In consideration of the established biological activities of the identified phytochemicals, Fumaric acid has a wide range of industrial applications, including in the food, chemical, agricultural, and pharmaceutical sectors [30]. It has been reported that quinic acid exhibits antioxidant, anti-inflammatory, and anticancer activities in various *in vitro* and *in vivo* studies, and that these effects are associated with different molecular mechanisms. With regard to anticancer activity, it has been reported that quinic acid promotes apoptosis, inhibits the protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) signaling pathways and activator protein-1 (AP-1) activity, and consequently reduces MMP-9 expression. [31,32]. Gentisic acid and its derivatives have been reported to possess antioxidant and antimicrobial activities [33], as well as inhibitory activity against phenoloxidase [34] and human carbonic anhydrase [35]. A review of the flavonoids identified in the extracts reveals that isoquercitrin exhibits antioxidant and anti-inflammatory activities [36], antiviral activity [37], antihypertensive activity [38], and anticancer activity, as well as inducing mitochondria-dependent apoptosis in SK-Mel-2 melanoma cells and anti-diabetic activity in type II diabetic rats [39]. Rutin has been shown to possess a broad spectrum of bioactivities, including cardioprotective, cytoprotective, neuroprotective, vasoprotective, anticarcinogenic, and antioxidant activities [40]. It has been reported that astragalin (kaempferol-3-O-glucoside), a natural flavonoid, exhibits various pharmacological activities, including neuroprotective, cardioprotective, anti-inflammatory, anti-obesity, anti-osteoporotic, anti-ulcer, antioxidant, anticancer, and antidiabetic properties [41].

3.2. Antioxidant Activity Results

In this study, the antioxidant activities of *P. erzincanensis* were evaluated based on total phenolic content (TPC), total flavonoid content (TFC), radical scavenging capacity (DPPH), and metal ion reduction (FRAP) capacity. In the course of investigating the antioxidant capacity of medicinal and aromatic plants, the amount and variety of phytochemicals they

contain – particularly their phenolic and flavonoid ratios – are of great importance [24]. A study was conducted to ascertain the TPC value of the extract. The results demonstrated that the TPC of WE was 80.44 mg GAE/g extract. When the total flavonoid content was examined, the results were similar to those of the phenolic content. The TFC of WE was 32.62 mg QE/g extract.

There are a limited number of studies on antioxidant activity conducted with different species of the genus *Pastinaca* in the literature. In their study on *Pastinaca sativa* L. subsp. urens, Tosun et al. (2019) reported the total phenolic content of extracts obtained from the plant's fruits and aboveground parts as 50.40 mg GAE /g and 67.86 mg GAE /g of extract, respectively [42]. Düşgün et al. (2021) conducted a study on *Pastinaca zozimoides* Fenzl, a plant endemic to Niğde Province. Their research determined the total phenolic content of the ethanol extract to be 28.79 mg GAE/g of extract [43]. Despite the presence of certain discrepancies when the TPC and TFC values obtained in this study were compared with the limited number of studies in the literature, the results were largely analogous [42,43]. These disparities are hypothesised to originate from the characteristics of the plant species employed, the temporal context of collection, the specific plant part utilised, the extraction methodology, and the type of solvent employed. In the course of the DPPH radical scavenging assays, WE demonstrated strong activity with an IC_{50} value (IC_{50}: 10.99 µg/mL). It was determined that WE possessed a stronger radical scavenging capacity, with IC_{50} values lower than those of the standard antioxidant Trolox (IC_{50}: 12.80 µg/mL). The metal ion reduction power of a compound is widely regarded as a significant indicator of an antioxidant's capacity [24]. The FRAP results were consistent with the DPPH results; WE had a FRAP value of 61.49 mg TE/g extract. When these data are evaluated in conjunction with the LC-MS/MS results, it can be concluded that the antioxidant capacity of the extract is determined not so much by the quantity of secondary metabolites in its composition as by their diversity and synergistic effects. In conclusion, this study has demonstrated for the first time the potential of *P. erzincanensis* as a powerful antioxidant source due to its significant phytochemical (phenolic and flavonoid) content.

Table 2. Antioxidant activity results of the *P. erzincanensis* water extract.

Extract / Standard	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	FRAP (mg TE/g extract)	DPPH IC50 (µg/mL)
Water	80.44±1.05	32.62±0.82	61.49±0.44	10.99±0.80
Trolox	-	-	-	12.80±0.42

TPC: Total phenolic content
TFC: Total flavonoid content
FRAP: Ferric reducing antioxidant power

3.3. Cytotoxic Activity Results

Cytotoxicity assays are a widely utilized method for investigating the potential toxicity of natural or synthetic compounds [39]. In this study, the cytotoxic activity of *P. erzincanensis* extract (WE) was investigated using the XTT method on HeLa, HT-29, and LNCaP cancer cell lines. It was observed that WE exhibited cytotoxic activity following a 48-hour incubation with HeLa cells. The water extract was found to have expressive cytotoxic activity, exhibiting an IC_{50} value of 145.04 µg/mL on HeLa cells. The other two cell lines exhibited IC_{50} values exceeding 500 µg/mL.

The findings indicate that the water extract of *P. erzincanensis* (WE) exhibits a marked dose-dependent cytotoxic effect on HeLa cells (IC_{50}: 145.04 µg/mL), whereas this effect is weaker in the HT-29 and LNCaP cell lines (IC_{50}> 500 µg/mL). These results suggest that the extract may possess a cell-type-specific cytotoxic potential against cervical cancer cells.

Table 3. IC_{50} values of *P. erzincanensis* water extract in human cervical (HeLa), colorectal (HT-29), and prostate (LNCaP) cancer cell lines. The cytotoxicity study was conducted over a 48-hr period.

Extract / Standard	**HeLa** IC_{50} (µg/mL)	**HT-29** IC_{50} (µg/mL)	**LNCap** IC_{50} (µg/mL)
Water	145.04±2.74	>500	>500
5-FU	35.14±1.94	23.2±0.98	29.60±1.60

5-FU: Fluorouracil (positive control)

While it is acknowledged that other species within the Apiaceae family, to which *P. erzincanensis* belongs, have been shown to possess various biological activities, notably wound healing, in both humans and certain animals, there is a paucity of detailed anticancer studies on this species in the extant literatüre [44].

In a limited number of studies on the cytotoxic activity of the *Pastinaca* genus, Düşgün et al. (2021) reported that the methanol extract of *P. zozimoides* exhibited moderate to high levels of cytotoxic effects on Caco-2 cells, a human colon cancer cell line [43]. In addition, Madari and Jacobs (2004) reported that the *P. sativa* plant exhibited a very strong antiproliferative effect on the HeLa S3 (5µg/mL) cervical cancer cell line [45]. Since *P. sativa* is a different species from *P. erzincanensis*, the results of the cytotoxicity study differed between the two. Upon comprehensive evaluation of the available data, it becomes evident that several factors exert a significant influence on the observed cytotoxic activity. These include the geographical distribution of the *Pastinaca* species, the specific plant part utilised in the study, the extraction method employed, and the cancer cell line that was employed in the testing process. Furthermore, it was determined that *P. erzincanensis*, which was evaluated for the first time in this study, demonstrates significant overlap in cytotoxic activity with other *Pastinaca* species documented in the extant literature. In conclusion, the present study demonstrates that the *P. erzincanensis* species contains secondary metabolites with the potential to be evaluated *in vivo* anticancer studies. Furthermore, the isolation of these metabolites from the plant provides important data for their use in individual or combined anticancer studies.

The significant cytotoxic activity exhibited by *P. erzincanensis* against the HeLa cell line — as demonstrated via the XTT assay — has paved the way for further validation through alternative methods of assessing cell toxicity. Furthermore, the substantial data obtained from these studies have facilitated the investigation of potential anticancer mechanisms, including apoptosis and various signalling pathways such as PI3K/AKT/mTOR and JAK-STAT, at the molecular level through both *in vitro* and *in vivo* research.

4. Conclusion

This study represents the first investigation to characterize the phytochemical composition and biological activities of the water extract (WE) of *P.erzincanensis*, a species endemic to Erzincan Province. LC-MS/MS analyses revealed the presence of 19 phytochemicals in the extract among the 56 standard compounds screened, and quinic acid, fumaric acid, and gentisic acid were identified as the major components. The results indicate that the WE possesses significant antioxidant potential, as evidenced by both its total phenolic (80.44 mg

GAE/g extract) and flavonoid (32.62 mg QE/g extract) content, as well as its DPPH radical scavenging (IC_{50}: 10.99 µg/mL, more potent than Trolox (IC_{50}:12.80 µg/mL) and FRAP (61.49 mg TE/g extract). In the context of a cellular study, the extract was found to demonstrate a more significant and selective impact on HeLa cells (IC_{50}: 145.04 µg/mL) in comparison to the HT-29 and LNCaP cell lines (IC_{50}> 500 µg/mL). When these findings are evaluated in their totality, it can be concluded that the aqueous extract of *P. erzincanensis* constitutes a natural source of compounds due to its rich phytochemical profile and strong antioxidant capacity, and that its selective cytotoxic effect against cervical cancer cells offers a promising starting point for further *in vivo* studies and the isolation of active compounds.

Declarations

Ethics statement

There are no ethical issues regarding the publication of this study.

Author contributions

Conceived and designed the experiments: EKKY, AA. Performed the experiments: EKKY, AA, MAY. Analysed the data: EKKY, AA and MAY. Contributed reagents/materials/analysis tools: EKKY, AA and MAY. Wrote the article: EKKY, AA and MAY. All authors read and approved the final manuscript.

Conflict of interest

Any conflict of interest. If none: "The authors declare that they have no conflict of interest."

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Use of artificial intelligence

No generative AI tool was used in the preparation of this work.

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