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Cultivated Parsnip and Its Two Wild Relatives as Sources of Polyphenols with Antioxidant and Antimicrobial Activities

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Highlights

What are the main findings?

- LC-DAD-QTOF-MS/MS analysis revealed 45 compounds in dry methanol extracts of cultivated and two wild-growing parsnips.
- Cultivated *Pastinaca sativa* subsp. *sativa* leaf extract exhibited the strongest antimicrobial activity, whereas *P. hirsuta* leaf and fruit extracts showed the strongest antioxidant activity.

What are the implications of the main findings?

- Investigated parsnips represent valuable sources of polyphenols for food applications.
- *Pastinaca sativa* subsp. *sativa* leaves have potential as an agricultural by-product.

Abstract

The composition and bioactivities of dry methanol extracts from roots, leaves and fruits of cultivated *Pastinaca sativa* subsp. *sativa* (Ps) and wild-growing *P. sativa* subsp. *urens* (Pu) and *P. hirsuta* (Ph) were investigated. Their compositions were analyzed by LC-DAD-QTOF-MS/MS, total phenolics and antioxidant activity were measured spectrophotometrically, antimicrobial activity was assessed by microdilution (14 strains), and cytotoxicity was evaluated by the MTT assay (four cell lines). Chemical analysis revealed 45 compounds. While Ps leaf extract had the highest rutin content (13.21 mg/g), Ph leaf and fruit extracts were the richest in total quercetin glycosides and their derivatives (103.70 and 163.93 mg/g); Ph leaf extract was also rich in chlorogenic acid (58.08 mg/g). Extract amounts corresponding to established furanocoumarin intake thresholds were calculated (e.g., regarding leaf extracts, up to 5.46 g for Ph). Ph leaf extract showed the highest total phenolic content (70.07 mg quercetin equivalents/g), FRAP value (0.97 mmol Fe^{2+}/g) and anti-DPPH activity (64.56 mg quercetin equivalents/g). The best antimicrobial activity was observed against *Micrococcus luteus* (MIC = 0.156–2.5 mg/mL; MBC = 5–10 mg/mL), a known food spoilage bacterium, with Ps leaf extract being the most active. Ps fruit extract showed weak-to-moderate cytotoxicity; others were generally non-cytotoxic. The investigated parsnips, including Ps agricultural waste, represent potential sources of bioactive polyphenols for food applications.

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Keywords: *Pastinaca sativa* subsp. *sativa*; *Pastinaca sativa* subsp. *urens*; *Pastinaca hirsuta*; LC-DAD-QTOF-MS/MS; polyphenols; furanocoumarins; antioxidant activity; antimicrobial activity; cytotoxic activity

1. Introduction

The Apiaceae family (also known as the parsley, carrot or celery family) includes many aromatic vegetables, as well as spices valued not only for their culinary uses but also for their health-promoting properties. These benefits are primarily attributed to a wide range of bioactive compounds (e.g., polyphenols, coumarins, terpenes and phenylpropanoids) present in different plant parts. Therefore, extracts and compounds isolated from Apiaceae species have been widely investigated for possible applications as functional food ingredients, as well as in food preservation [1,2]. Modern agriculture and food industries generate significant amounts of waste, including biomass from food crops, creating challenges related to disposal costs and environmental impact. Adopting a circular economy approach, agricultural and food residues are increasingly being studied as sustainable sources of bioactive extracts and compounds [3]. Parsnip, *Pastinaca sativa* subsp. *sativa* L., is a biennial Apiaceae species primarily cultivated in temperate regions. Demand for the thick, fleshy root of parsnip has steadily grown due to its versatility as a vegetable in soups, stews, salads and various cooked dishes [4–6]. Although parsnip leaves are occasionally used in soups, they are more commonly regarded as agricultural waste [7]. In addition to its culinary use, parsnip has a long history in traditional medicine. For example, teas from roots, leaves and fruits have been used to promote digestion, improve appetite and as diuretics [4,8,9].

Wild-growing relatives of cultivated crops also present a promising, underexplored resource for obtaining valuable phytochemicals. The genus *Pastinaca* L. encompasses about fifteen wild species native to regions spanning from Europe to Lebanon and Mongolia in the east [10]. Some wild-growing plants from this genus, such as *P. sativa* subsp. *urens* (Req. ex Godr.) Čelak. (a subspecies common in Europe), are traditionally consumed in various forms, including raw, pickled or cooked [11]. In folk medicine, decoction from the roots of this subspecies has been used to alleviate colic [12]. Another member of this genus, *P. hirsuta* Pančić, is endemic to the central Balkan Peninsula, i.e., it can be found in eastern Serbia, south and west Bulgaria, and North Macedonia [13].

It is well established that different plant parts of *Pastinaca* species contain essential oils rich in phenylpropanoids, monoterpenes, sesquiterpenes and/or aliphatic esters, as well as a variety of other metabolites, including coumarins, polyacetylenes, flavonoids and phenolic acids [4,6,7,14]. The most common coumarins identified in parsnips are furanocoumarins [4]. These specialized metabolites are usually found in vegetables belonging to the Apiaceae family and in fruits of the Rutaceae family [15]. Furanocoumarins are well-known photosensitizing agents that can cause erythema, itching, blistering and hyperpigmentation [16]. For example, exposure to 14 mg of xanthotoxin (8-methoxypsoralen, 8-MOP) in combination with UV irradiation has been estimated to result in detectable phototoxicity [17,18]. The Senate Commission on Food Safety of the German Research Foundation (DFG-SKLM) initially estimated the average dietary intake of furanocoumarins to be 1.45 mg/day [17] but revised this estimate to 0.56 mg/day in a subsequent assessment [18]. The Committee on Herbal Medicinal Products of the European Medicines Agency (EMA/HMPC) concluded that a daily intake of up to 1.5 mg of total furanocoumarins is not expected to contribute significantly to the overall risk [16].

Previously, the composition of essential oils obtained from various organs of *P. sativa* subsp. *sativa*, *P. sativa* subsp. *urens* and *P. hirsuta* has been investigated [7,19–25]. Additionally, the essential oils from different parts of all three plants have demonstrated antimicrobial activity [7,19,22,26], while the essential oils from the aerial parts of *P. sativa* subsp. *sativa* and *P. hirsuta* have exhibited insecticidal and repellent effects [22,25]. Additionally, cultivated parsnip contains varying amounts of coumarins, including furanocoumarins, in its different plant parts [6,27,28]. On the other hand, studies on the composition and bioactivity of polar, polyphenol-containing extracts (e.g., methanolic or ethanolic extracts) of *Pastinaca* species remain scarce, although such extracts have attracted more and more attention in recent years [27–30]. For example, for the dry hydroethanolic extract of *P. sativa* subsp. *sativa* roots, in vitro antioxidant and in vitro and in vivo anti-inflammatory activities were revealed [27], while for the root dry water extract, in vivo mitigation of metabolic dysfunction-associated steatotic liver disease via modulation of metabolic endotoxemia was demonstrated [28]. Additionally, the dry ethanol (along with hexane) extracts of *P. sativa* subsp. *urens* leaves and fruits exhibited in vitro enzyme inhibitory (AChE, BChE, tyrosinase, α-amylase and α-glucosidase), antioxidant and antimicrobial activities [29].

Therefore, this study aimed to investigate the chemical composition of the dry methanol extracts prepared from the roots, leaves and fruits of *P. sativa* subsp. *sativa*, *P. sativa* subsp. *urens* and *P. hirsuta*, as well as to evaluate their antioxidant, antimicrobial and cytotoxic activities. The obtained results were used to evaluate the potential of the studied parsnips, including agricultural waste consisting of *P. sativa* subsp. *sativa* leaves, as sources of bioactive polyphenols for food applications.

2. Materials and Methods

2.1. Plant Material

Cultivated *P. sativa* subsp. *sativa* roots, leaves (both from one-year-old plants) and fruits (from two-year-old plants) were collected in Kovačica, Serbia, in August 2018; the mean temperature at this locality during July–August 2018 is 24.1 °C. Wild-growing *P. sativa* subsp. *urens* roots, leaves and fruits were collected at Lake Perućac, in Bajina Bašta, Serbia, in July 2016 (the specimen serving as the voucher was archived at the Natural History Museum's Herbarium (BEO) in Belgrade, registered as No. 20160702/01); the mean temperature at this locality during June–July 2016 is 17.6 °C. Wild-growing *P. hirsuta* roots, leaves and fruits were collected at Vlasina Plateau, Serbia, in July 2019 (voucher specimen number in BEO: 20190707); the mean temperature at this locality during June–July 2019 is 21.7 °C (all mean temperature data were obtained from meteostat.net).

2.2. Extraction

Roots, leaves and fruits of all three parsnip species were first air-dried and ground into powder using an electric grinder. For each sample, 20 g of the powdered material was subjected to extraction, initially using dichloromethane as the solvent. Once dried, the remaining plant residue was further extracted using methanol; both solvents used were of analytical grade. The extractions in both cases followed a bimaceration protocol conducted at room temperature. During the first stage, maceration was carried out for three days and then filtered; the plant material was then macerated for an additional two days in a fresh aliquot of the same solvent. In both maceration steps, the ratio of herbal drug to solvent was maintained at 1:10 (w/v). Following extraction, the solvents were evaporated under reduced pressure using a rotary evaporator (Büchi Rotavapor RII, Flawil, Switzerland). The pre-extraction with dichloromethane was applied to remove most of the fats, as well as potentially phototoxic furanocoumarins. Thus, the dichloromethane extracts were discarded, and the dry methanol extracts were subjected to further analysis.

The extract yield was calculated as the percentage (*w*/*w*) of dry methanol extract obtained relative to the initial dry plant material. The yields of dry methanol extracts were as follows: *P. sativa* subsp. *sativa* roots, leaves and fruits 24.99%, 8.30% and 4.24% *w*/*w*; *P. sativa* subsp. *urens* roots, leaves and fruits 10.53%, 8.47% and 8.46% *w*/*w*; and *P. hirsuta* roots, leaves and fruits 6.59%, 8.45% and 8.42% *w*/*w*. The extracts were stored at −80 °C until analysis.

2.3. Chemical Analysis

Two complementary LC-MS-based methodologies were employed for compound identification and quantification. LC-DAD-QTOF-MS/MS analysis was used for comprehensive qualitative profiling. Compounds were identified based on accurate mass measurements, MS/MS fragmentation patterns, molecular formulae, UV spectra, and database/literature information. A separate LC-DAD-MS method was employed for quantitative analysis using DAD peak areas by employing external standards. In this method, compounds were assigned based on their retention times and UV and mass spectral (obtained by single quadrupole MS) characteristics, and their identities were further confirmed using reference standards when available.

2.3.1. LC-DAD-QTOF-MS/MS Analysis

Qualitative analysis, based on interpretation of spectral data, was performed using an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) coupled with an Agilent 6210 Quadrupole Time-of-Flight (QTOF) mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). A Phenomenex Gemini C18 column (2 mm × 100 mm, 3 µm) (Phenomenex, Torrance, CA, USA) was employed for chromatographic separation. Mobile phase A comprised water with 0.1% formic acid, while mobile phase B consisted of acetonitrile with 0.1% formic acid (both LC-MS grade); the flow rate was maintained at 0.2 mL/min. A gradient elution program was applied: starting at 0% B (0 min), increasing to 60% B by 45 min, and reaching 95% B between 46 and 55 min. Extracts, dissolved in methanol at a concentration of 10 mg/mL, were injected in a volume of 10 µL. Monitoring via diode-array detector (DAD) was carried out at four wavelengths: 210, 254, 320 and 366 nm. Both positive and negative electrospray ionization (ESI) modes were used for mass spectrometric detection, spanning an *m*/*z* range of 50–1700. Regarding ion source settings, the following parameters were applied: nitrogen drying gas flow of 10 L/min at 275 °C, sheath gas flow of 12 L/min at 325 °C, a nebulizer pressure of 35 psi, capillary voltage of 4000 V, nozzle voltage of 1000 V, fragmentor voltage of 140 V, skimmer voltage of 65 V, and an octapole RF peak voltage of 750 V. Collision energies of 10 and 30 V were applied during MS/MS fragmentation. MassHunter software (version B08.00, Agilent Technologies) was used for both data acquisition and subsequent processing. Compound identification relied on tandem mass spectra together with molecular formulas derived from the measured mass, cross-referenced against the KNApSAcK Core System and LOTUS databases [31,32]; UV spectral data supplemented this identification in certain cases.

2.3.2. LC-DAD-MS Analysis

Qualitative and quantitative analysis relying on external standards was carried out using an Agilent 1260 HPLC system equipped with an Agilent 6130 single quadrupole ESI mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was achieved on an Agilent Zorbax SB-Aq column (150 mm × 3.0 mm; 3.5 µm particle size), held at a constant temperature of 25 °C. Phase A of the mobile phase consisted of water containing 0.1% (*V*/*V*) formic acid and 5% (*V*/*V*) isopropanol, whereas phase B was methanol (both LC-MS grade). Gradient elution proceeded as follows: 0% B at 0 min, 40% B at 5 min, 45% B between 25 and 27 min, 55% B at 32 min, and 100% B from 40 to 45 min. With a flow rate of 0.3 mL/min, injection volumes of 5 or 10 µL were used for methanol

solutions of the extracts (5 mg/mL). DAD monitoring was conducted at five wavelengths: 210, 250, 270, 320 and 350 nm. Both positive and negative ionization modes were applied on the mass spectrometer, with the following optimized source parameters: an m/z range of 80–2500, fragmentor voltage of either 100 or 250 V, nebulizing gas flow rate of 10 L/min at 350 °C, nebulizer pressure of 40 psi, and a capillary voltage of 3500 V. Compounds were identified through comparison of retention times, UV spectra and mass spectra against those obtained from available reference standards, all purchased from Sigma-Aldrich (St. Louis, MO, USA). The external standard method, applying data obtained from the DAD, was used for quantification purposes (Table 1). Based on structural similarity and similarity in UV spectra, the phenolic acid content is expressed in chlorogenic acid equivalents (eq.); flavonoids belonging to the quercetin and methylquercetin groups are expressed as rutin eq.; flavonoids of the kaempferol group are expressed as kaempferitrin eq.; and the furanocoumarin content is expressed either as xanthotoxin/8-MOP eq. (for xanthotoxin, sphondin and imperatorin), bergapten eq. (for bergapten, isopimpinellin and phellopterin), or umbelliferone eq. (for the single dihydrofuranocoumarin detected). The results are reported as mg of compound per g of dry extract, with standard deviations derived from three independent experiments. The lowest standard concentration at which the signal-to-noise ratio reached 3.3 was taken as the limit of detection (LOD), whereas the smallest standard concentration included in the calibration curve was defined as the limit of quantification (LOQ).

Table 1. Data from quantitative analysis.

Standard	λ (nm)	Slope	Intercept	r^2	Linear Range (μg)	LOD (μg)	LOQ (μg)
Chlorogenic acid	320	8451.59	35.43	0.9999	0.005–1.000	0.002	0.005
Rutin	350	2773.98	10.80	1.0000	0.005–2.500	0.002	0.005
Kaempferitrin	350	5040.91	6.80	1.0000	0.020–1.000	0.007	0.020
Xanthotoxin (8-MOP)	250	17,811.42	−35.62	0.9999	0.005–1.000	0.002	0.005
Bergapten	270	11,532.90	35.38	0.9998	0.005–1.000	0.002	0.005
Umbelliferone	320	15,602.81	20.14	1.0000	0.010–1.500	0.003	0.010

2.4. Investigation of Total Phenolic Content and Antioxidant Activity

2.4.1. Total Phenolic Content

Determination of the total phenolic content involved combining 20 μL of extract solutions (prepared in methanol at concentrations of 0.2 or 1.0 mg/mL) with 150 μL of Folin–Ciocalteu reagent (10-fold diluted) and 150 μL of sodium carbonate solution (60 g/L), using 96-well microtiter plates. Following a 90 min incubation, absorbance readings were taken at 725 nm on a SPECTROstar Nano microplate reader (BMG Labtech, Ortenberg, Germany). For the blank sample, extract solutions were substituted with an equal volume (20 μL) of methanol [33]. Using a quercetin calibration curve (0.01–0.1 mg/mL), the results are reported as the mean ± standard deviation, based on three independent experiments. Differences between the results obtained were statistically assessed via the non-parametric Kruskal–Wallis test, with subsequent multiple comparisons of mean ranks; all analyses were carried out in Statistica 12 (StatSoft Inc., Tulsa, OK, USA).

2.4.2. Total Antioxidant Activity

Total antioxidant activity was evaluated by means of the ferric reducing antioxidant power (FRAP) assay. In 96-well microtiter plates, 20 μL of extracts (dissolved in methanol at 0.5 or 2.5 mg/mL) was combined with 300 μL of freshly prepared FRAP reagent. This reagent was composed of three components: 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL

of TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution (10 mM, dissolved in 40 mM hydrochloric acid), and 2.5 mL of ferric chloride solution (20 mM). Following a 30 min incubation period, absorbance was measured at 593 nm on a SPECTROstar Nano microplate reader. The blank sample was prepared analogously, substituting the extracts with 20 µL of methanol added to 300 µL of FRAP reagent [33]. As a positive control, quercetin was used at a concentration of 0.016 mg/mL. Based on a ferrous sulfate calibration curve (200–1000 mM), the results from three independent experiments were expressed as the mean values ± standard deviations. As with the total phenolic content assay, statistical evaluation relied on the non-parametric Kruskal–Wallis test with subsequent multiple comparisons of mean ranks, carried out in Statistica 12.

2.4.3. DPPH Scavenging Activity

In 96-well microplates, 0.2 mL of methanol extract solutions (at concentrations between 0.17 and 5 mg/mL) were combined with 0.05 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in methanol (0.5 mM). After a 30 min incubation, the absorbance was recorded at 517 nm on a SPECTROstar Nano microplate reader, using methanol as the blank. For the negative control, 0.05 mL of DPPH solution (0.5 mM) was diluted with 0.2 mL of methanol [33]. A quercetin calibration curve (concentrations of 0.16–10 µg/mL) served as the basis for expressing the results as the mean values ± standard deviations, calculated from three independent experiments. Statistical significance among the results was determined using the non-parametric Kruskal–Wallis test, followed by multiple mean rank comparisons; Statistica 12 was used to perform the analysis.

2.5. Investigation of Antimicrobial Activity

Following EUCAST (European Committee on Antimicrobial Susceptibility Testing) guidelines, the broth microdilution method was applied to determine antibacterial and antifungal properties. Using Mueller–Hinton broth as the growth medium, antibacterial activity was assessed against a panel of Gram-positive strains (*Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC BA1707, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 10240, *Bacillus cereus* ATCC 10876 and *Enterococcus faecalis* ATCC 29212) alongside Gram-negative strains (*Salmonella* Typhimurium ATCC 14028, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 12453, *Klebsiella pneumoniae* ATCC 13883 and *Pseudomonas aeruginosa* ATCC 9027). For antifungal testing, Mueller–Hinton broth enriched with 2% glucose was used against a set of yeast strains (*Candida glabrata* ATCC 90030, *C. albicans* ATCC 102231 and *C. parapsilosis* ATCC 22019). Before testing began, extracts were first dissolved in dimethyl sulfoxide (DMSO), then diluted using the corresponding broth medium, and finally subjected to a series of two-fold dilutions. Sterile 96-well microtiter plates (Nunc, Roskilde, Denmark) received the resulting solutions, yielding final concentrations spanning 10 to 0.156 mg/mL. To prepare microbial inocula, fresh cultures were adjusted in sterile 0.85% NaCl until reaching turbidity matching the 0.5 McFarland standard; these were then added to the wells, producing final densities of 5×10^5 CFU/mL for bacterial strains and 5×10^4 CFU/mL for yeast strains. After 18–20 h of incubation at 35 °C, minimum inhibitory concentration (MIC) values were determined through a combination of visual assessment and spectrophotometric measurement of absorbance at 600 nm, with the MIC defined as the lowest concentration at which no visible microbial growth occurred. To determine the minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC), 5 µL aliquots from each well were plated onto extract-free agar and incubated for a further 24 h at 35 °C; the lowest concentration yielding complete growth inhibition was taken as the MBC/MFC value. Triplicate experiments were performed throughout, and the highest recorded value was reported in each case. Three types of controls were incorporated into

the experimental design: a DMSO control (10% *v*/*v* final concentration), a positive control consisting of inoculum without added extract, and a negative control consisting of extract without inoculum.

2.6. Cell Culturing and the Evaluation of Cytotoxicity

The cytotoxic potential of the extracts was assessed using one non-malignant cell line, VERO (ATCC: CCL-81, monkey kidney), and three human cancer cell lines originating from gastric adenocarcinoma (AGS; ATCC: CRL-1739), hypopharyngeal squamous cell carcinoma (FaDu; ATCC: HTB-43) and colon carcinoma (RKO; ATCC: CRL-2577). While the cancer cell lines were maintained in Minimum Essential Medium (MEM; Corning, Tewksbury, MA, USA), VERO cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Corning). Both culture media received supplementation with fetal bovine serum (FBS; Corning) and Penicillin–Streptomycin Solution (Corning). The extracts were dissolved in cell culture-grade DMSO (PanReac Applichem, Darmstadt, Germany) to obtain stock solutions. The stock solutions were stored frozen (−23 °C) until used.

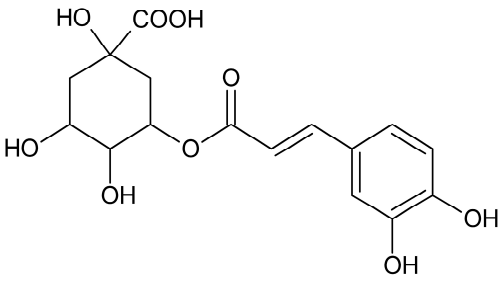
Cell viability was determined by the MTT assay according to the protocol previously reported by Pecio et al. [34]. Briefly, cells were seeded into 96-well microplates and allowed to attach overnight before exposure to serial dilutions of the extracts for 72 h. At the same time, the cytotoxicity of DMSO at concentrations equivalent to those in the stock solution dilutions was evaluated to rule out any impact on the assay. Once the incubation was complete, the cells were rinsed with PBS after the treatment medium had been removed. This was followed by the addition of fresh medium containing MTT; after a 3 h incubation period, the formazan crystals that had formed were dissolved. Following an additional overnight incubation, the absorbance readings were taken at 540 and 620 nm using a Synergy H1 Multi-Mode Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA). These absorbance values were subsequently processed in GraphPad Prism (version 9.0.0) to calculate the CC_{50} values (the 50% cytotoxic concentration), obtained by fitting the data to dose–response curves using non-linear regression.

3. Results and Discussion

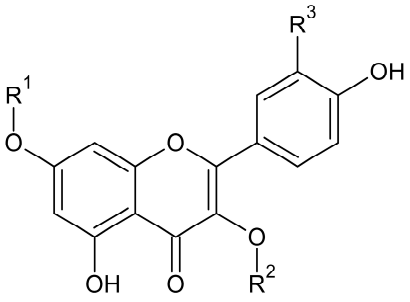
3.1. Chemical Composition of Pastinaca Dry Methanol Extracts

Analysis using high-resolution MS (HRMS) data obtained on a QTOF instrument enabled the detection of a total of 45 compounds in the dry methanol extracts of roots, leaves and fruits from the three parsnip species investigated: 21 flavonoids (**5**, **10**–**13**, **15**, **16**, **18**, **19**, **24**–**28**, **30**–**35** and **39**), 11 phenolic acids (**1**, **2**, **4**, **6**, **8**, **14**, **17**, **20**, **23**, **36** and **38**), 11 coumarins, i.e., one simple coumarin (**3**) and 10 furanocoumarins (**7**, **9**, **21**, **37** and **40**–**45**), and two other benzofuran derivatives (**22** and **29**) (Figure 1; Table 2). The richest in compounds were *P. hirsuta* leaf extract (33 compounds) and fruit extract (31), *P. sativa* subsp. *urens* leaf extract (27) and fruit extract (25), and *P. sativa* subsp. *sativa* leaf extract (23). Flavonoids were detected based on the signals of pseudomolecular ions, as well as fragment ions resulting from the successive losses of sugar units (including signals corresponding to aglycones); negative ion mode was employed. The identities of aglycones were confirmed using UV spectra. This analysis revealed nine quercetin glycosides (**5**, **10**, **11**, **18**, **19**, **27**, **28**, **30** and **39**; aglycone signal at *m*/*z* 299.0188–301.0346), seven glycosides of methylated quercetin (**12**, **13**, **16**, **25**, **26**, **32** and **34**; *m*/*z* 315.0472–315.0511), four kaempferol glycosides (**15**, **24**, **31** and **35**; *m*/*z* 285.0332–285.0372) and one apigenin glycoside (**33**; *m*/*z* 269.0452). Regarding sugar moieties, the signals corresponding to losses of 162, 146, 132 and 308 Da indicated the presence of hexose (**5**, **10**–**13**, **15**, **16**, **18**, **19**, **24**–**26**, **30** and **33**–**35**), deoxyhexose (**5**, **11**, **13**, **15**, **16**, **18**, **19**, **24**–**26**, **28**, **31** and **39**), pentose (**33**) and deoxyhexosylhexose (**27** and **32**), respectively, in the flavonoid structures [34–39]. Among the detected flavonoids, quercetin 7-*O*-rhamnoside 3-*O*-

glucoside (vincetoxicoside A; **18**), quercetin 3-*O*-rutinoside (rutin; **27**), quercetin 3-*O*-glucoside (isoquercitrin; **30**) and kaempferol 3,7-di-*O*-rhamnoside (kaempferitrin; **31**) were identified using standard compounds.



Chlorogenic acid (**6**)



Compound	R^1	R^2	R^3
Vincetoxicoside A (**18**)	α-L-rhamnopyranosyl	β-D-glucopyranosyl	OH
Rutin (**27**)	OH	α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl (rutinosyl)	OH
Isoquercitrin (**30**)	OH	β-D-glucopyranosyl	OH
Kaempferitrin (**31**)	α-L-rhamnopyranosyl	α-L-rhamnopyranosyl	H

Figure 1. Structures of the major polyphenols identified in *Pastinaca* dry methanol extracts by comparison with reference standard compounds.

The detected phenolic acids included three simple hydroxycinnamic acids, i.e., caffeic, coumaric and ferulic acids (**8**, **20** and **23**), two hexosides of protocatechuic and coumaric acids (**1** and **4**), and six depsides of quinic acid with caffeic, coumaric or ferulic acid (**2**, **6**, **14**, **17**, **36** and **38**). The three simple hydroxycinnamic acids (**8**, **20** and **23**) were detected relying on the fragmentation patterns observed in negative ionization mode, all displaying a characteristic signal corresponding to the loss of CO_2 (44 Da; resulting in fragments at *m*/*z* 135.0443, 119.0487 and 149.0605, respectively). Two hexosides of phenolic acids (**1** and **4**) were revealed by the occurrence of a signal indicating hexose loss (162 Da; resulting in fragments at *m*/*z* 152.0105 and 163.0379, respectively) [40]. Depsides were identified based on the signals of their pseudomolecular ions and characteristic fragment ions resulting from the loss of dehydrated units: caffeic acid (162 Da; **2**, **6**, **36** and **38**), quinic acid (179 Da; **2**, **6**, **14**, **17**, **36** and **38**), coumaric acid (146 Da; **14**) and ferulic acid (176 Da; **17**) [39,41–43]. Among the detected phenolic acids, chlorogenic (**6**), caffeic (**8**), ferulic (**23**) and 3,5-di-*O*-caffeoylquinic (**36**) acids were identified using commercial standards, while 1,5-di-*O*-caffeoylquinic acid (**38**) relied on a compound that had earlier been isolated from the dry methanol extract obtained from the underground parts of *Hieracium scheppigianum* Freyn (Asteraceae) [44]. Although most detected flavonoids and phenolic acids can be regarded for ubiquitous compounds, the reports on their presence in *Pastinaca* species, even in cultivated parsnip, are very scarce. In a few rare studies, rutin (**27**) and one deoxyhexosyl hexosyl methylquercetin, i.e., isorhamnetin 3-*β*-D-glucopyranoside 4′-*α*-L-rhamnopyranoside, were isolated from the fruits of *P. sativa* subsp. *sativa*, and *p*-coumaric acid was identified in the roots of this plant [6]. In our study, rutin (**27**) was detected in the leaves and coumaric acid (**20**) isomer in the fruits of cultivated parsnip. Additionally, one hexosyl pentosyl apigenin (**33**) was detected in the fruits of *P. sativa* subsp. *sativa* in our study. It should be noted that a hexosyl pentosyl apigenin, apiin (apigenin 7-*O*-apioglucoside), was previously detected in some other vegetables from the Apiaceae family, such as parsley (*Petroselinum crispum* (Mill.) Fuss) and celery (*Apium graveolens* L.) roots [45].

Among the 11 detected coumarins, seven were furanocoumarin aglycones, which, at positions 5 and/or 8 (linear type) or 5 and/or 6 (angular type), contained one methoxy group (signals corresponding to the loss of a methyl group, i.e., 15 Da in the mass spectra recorded in positive ion mode; **40**, **41** and **43**), two methoxy groups (successive losses of 2 × 15 Da; **42**), one prenyloxy group (loss of 68 Da; **44**), prenyloxy and methoxy group (successive losses of 68 and 15 Da; **45**), or oxygenated prenyloxy group (loss of 102 Da; **37**) [43,46]. Depending on the group and the position of attachment, these furanocoumarins also had characteristic UV spectra [47]. They were identified as xanthotoxin (8-MOP; **40**), isopimpinellin (**42**), bergapten (**43**) and imperatorin (**44**) using commercial standards, whereas sphondin (**41**), phellopterin (**45**) and heraclenol (**37**) were identified by comparison with the LC-MS data of dry dichloromethane extracts of *Heracleum ternatum* Velen. (Apiaceae) roots and fruits, in which these compounds had previously been identified [48]. These furanocoumarins are common compounds for Apiaceae species and were previously detected in different parts of *P. sativa* subsp. *sativa* [6,30], as well as some other vegetables such as celery and parsley [16]. Two further furanocoumarins (both with characteristic UV spectra) were hexosides of psoralen with one or two hydroxyl groups (**7** and **9**). In their mass spectra, in addition to signals from aglycone fragmentation, they displayed signals corresponding to hexose loss (162 Da; resulting in fragments at m/z 219.0267 and 203.0323, respectively; positive ion mode) [49,50]. The basic structures of a detected dihydrofuranocoumarin hexoside (**21**) and a simple hydroxylated coumarin hexoside (**3**) were also determined based on hexose loss (162 Da) and the characteristic fragmentation of aglycones previously reported in the literature [51]. For example, the dihydrofuranocoumarin hexoside previously identified in the aboveground parts of *P. sativa* subsp. *sativa* was apterin [6], while simple umbelliferone 7-*O*-glucoside (skimmin) has been reported in various other Apiaceae species [31]. Compounds **22** and **29** were benzofuran derivatives, detected based on their fragmentation patterns in negative ion mode [48,52,53]. The structure of **22** corresponded, for example, to cnidioside A, while the structure of **29** was consistent with picraquassioside A or cnidioside B, all previously identified in different Apiaceae species, such as *Glehnia littoralis* (J. G. Cooper) F. Schmidt ex Miq. and *Angelica tenuisecta* var. *furcijuga* (Kitag.) H. Ohba [31].

In the quantitative analysis (Table 2), the highest total content of detected compounds was determined in the extracts of *P. hirsuta* leaves and fruits (172.92 and 166.72 mg/g of dry extract), followed by the extract of *P. sativa* subsp. *urens* fruits (57.91 mg/g), as well as the extract of *P. sativa* subsp. *sativa* leaves, which usually represent agricultural waste (31.96 mg/g). The remaining extracts contained less than 30 mg/g of detected metabolites. Both these *P. hirsuta* extracts were dominated by flavonoids (109.29 and 163.93 mg/g) (mainly quercetin/methylquercetin flavonoids), whereas the leaf extract was also rich in phenolic acids (61.08 mg/g). *Pastinaca sativa* subsp. *urens* fruit extract contained noteworthy amounts of quercetin/methylquercetin flavonoids and furanocoumarins (38.57 and 18.21 mg/g), while *P. sativa* subsp. *sativa* leaf extract included 17.11 mg/g of quercetin/methylquercetin flavonoids. The most abundant compound overall was chlorogenic acid (**6**), detected at 58.08 mg/g in *P. hirsuta* leaf extract, whereas *P. hirsuta* leaf and fruit extracts contained notable amounts of one deoxyhexosyl hexosyl methylquercetin (**25**), one deoxyhexosyl deoxyhexosyl hexosyl methylquercetin (**26**), rutin (**27**; minor signal) and one deoxyhexosyl deoxyhexosyl quercetin (**28**) (54.98 and 78.93 mg/g), which were quantified together due to co-elution and similar UV spectra. The most abundant in *P. sativa* subsp. *sativa* leaf extract was rutin (**27**) (13.21 mg/g). It should be noted that in all investigated root extracts, the content of polyphenols and other secondary metabolites was negligible, possibly due to the high proportion of sugars and other primary metabolites.

Table 2. Chemical composition of investigated Pastinaca dry methanol extracts (DMEs).

Rt (min)	Molecular Mass	Calculated Mass	Diff (ppm)	Molecular Formula	Pseudomolecular Ion	Fragments	Compound	*P. sativa* ssp. *sativa* DME (mg/g) [1]			*P. sativa* ssp. *urens* DME (mg/g)			*P. hirsuta* DME (mg/g)			Reference
								Root	Leaf	Fruit	Root	Leaf	Fruit	Root	Leaf	Fruit	
12.66	316.0794	315.0722	1.13	$C_{13}H_{16}O_9$	315.0718 $[M-H]^-$	152.0105, 108.0211	Protocatechuic acid hexoside (**1**)	-	1.32 ± 0.00	-	t	t	t	-	t	-	Ali et al. [40]
14.90	354.0951	353.0878	1.15	$C_{16}H_{18}O_9$	353.0874 $[M-H]^-$	191.0563, 179.0343, 135.0448	Caffeoylquinic acid (**2**)	-	-	-	-	-	-	-	1.99 ± 0.01	-	Dai et al. [41]
15.38	324.0845	369.0827	0.37	$C_{15}H_{16}O_8$	369.0826 $[M+HCOOH-H]^-$ /325.0917 $[M+H]^+$	161.0240/ 163.0375, 119.0477, 107.0487	Hydroxy coumarin hexoside (**3**)	-	-	-	-	-	-	-	t	-	Olennikov [51]
16.67	326.1002	325.0929	−7.08	$C_{15}H_{18}O_8$	325.0952 $[M-H]^-$	163.0379, 119.0470	Coumaroyl hexoside (**4**)	-	t	-	-	t	t	-	-	t	Ali et al. [40]
17.19	772.2121	771.1989	−2.81	$C_{33}H_{40}O_{21}$	771.2011 $[M-H]^-$	610.1492, 609.1520, 463.0834, 301.0258, 300.0210, 271.0241	Deoxyhexosyl hexosyl hexosyl quercetin (**5**)	-	-	-	-	-	-	-	-	t	Wojdyło et al. [39]
17.87	354.0951	353.0878	−7.61	$C_{16}H_{18}O_9$	353.0905 $[M-H]^-$	191.0586	Chlorogenic acid (**6**)	0.09 ± 0.00	3.12 ± 0.01	t	0.36 ± 0.00	4.27 ± 0.02	1.13 ± 0.04	0.32 ± 0.00	58.08 ± 0.00	t	Dai et al. [41]
18.34	380.0743	379.0671	−9.29	$C_{17}H_{16}O_{10}$	379.0706 $[M-H]^-$ /381.0772 $[M+H]^+$	216.0058/ 219.0267, 173.0212	5,8-Dihydroxy psoralene hexoside (**7**)	-	t	-	-	-	-	6.33 ± 0.03	-	t	Farag et al. [49]
18.59	180.0423	179.0350	−5.65	$C_9H_8O_4$	179.0360 $[M-H]^-$	135.0443, 117.0333, 107.0495	Caffeic acid (**8**)	-	-	t	-	t	-	-	-	-	Ali et al. [40]
18.66	364.0794	409.0776	−9.52	$C_{17}H_{16}O_9$	409.0811 $[M+HCOOH-H]^-$ /365.0854 $[M+H]^+$	201.0181/ 203.0323, 175.0373, 147.0426	Hydroxy psoralene hexoside (**9**)	-	-	-	-	-	-	1.19 ± 0.01	t	t	Yang et al. [50]
18.59	626.1483	625.1410	−3.48	$C_{27}H_{30}O_{17}$	625.1432 $[M-H]^-$	463.0854, 301.0302, 300.0195, 299.0189, 271.0189	Hexosyl hexosyl quercetin (**10**)	-	-	-	-	-	-	-	-	t	Ammar et al. [36]

Table 2. *Cont.*

Rt (min)	Molecular Mass	Calculated Mass	Diff (ppm)	Molecular Formula	Pseudomolecular Ion	Fragments	Compound	*P. sativa* ssp. *sativa* DME (mg/g) [1]			*P. sativa* ssp. *urens* DME (mg/g)			*P. hirsuta* DME (mg/g)			Reference
								Root	Leaf	Fruit	Root	Leaf	Fruit	Root	Leaf	Fruit	
19.69	756.2113	755.2040	−8.18	$C_{33}H_{40}O_{20}$	755.2102 $[M-H]^-$	610.1499, 609.1438, 447.0906, 301.0296, 300.0256, 299.0188, 271.0238	Deoxyhexosyl deoxyhexosyl hexosyl quercetin (**11**)	-	-	-	-	-	-	-	10.08 ± 0.00 [2]	23.88 ± 0.01 [2]	Farag et al. [38]
20.06	640.1639	639.1567	−1.14	$C_{28}H_{32}O_{17}$	639.1574 $[M-H]^-$	477.1028, 315.0479, 300.0278, 271.0237	Hexosyl hexosyl methyl-quercetin (**12**)	-	-	-	-	0.45 ± 0.00	1.45 ± 0.05	-			Wojdyło et al. [39]
19.97	786.2219	785.2146	−2.31	$C_{34}H_{42}O_{21}$	785.2164 $[M-H]^-$	624.1688, 623.1651, 477.1080, 315.0511, 300.0277	Deoxyhexosyl hexosyl hexosyl methyl-quercetin (**13**)	-	t	-	-	1.36 ± 0.01	0.20 ± 0.02	-	-	-	Wojdyło et al. [39]
20.55	338.1002	337.0929	3.52	$C_{16}H_{18}O_8$	337.0917 $[M-H]^-$	191.0554, 164.0463, 119.0451	Coumaroyl-quinic acid (**14**)	-	t	-	-	t	-	-	-	-	Wojdyło et al. [39]
20.59	740.2164	739.2091	−1.75	$C_{33}H_{40}O_{19}$	739.2104 $[M-H]^-$	594.1548, 593.1515, 446.0841, 431.0934, 285.0348, 284.0295, 255.0268	Deoxyhexosyl deoxyhexosyl hexosyl kaempferol (**15**)	-	-	-	-	-	-	-	t	t	Farag et al. [38]
20.69	770.2269	769.2197	−3.42	$C_{34}H_{42}O_{20}$	769.2223 $[M-H]^-$	624.1666, 623.1619, 461.1073, 315.0472, 314.0420, 299.0208	Deoxyhexosyl deoxyhexosyl hexosyl methyl-quercetin (**16**)	t	-	-	-	-	2.11 ± 0.00	-	5.35 ± 0.01	24.12 ± 0.01	Farag et al. [38]
21.00	368.1107	367.1035	0.42	$C_{17}H_{20}O_9$	367.1033 $[M-H]^-$	191.0552, 173.0449	Feruloylquinic acid (**17**)	-	0.42 ± 0.01	-	-	t	t	-	1.02 ± 0.00	-	Wojdyło and Nowicka [42]

Table 2. *Cont.*

Rt (min)	Molecular Mass	Calculated Mass	Diff (ppm)	Molecular Formula	Pseudomolecular Ion	Fragments	Compound	*P. sativa* ssp. *sativa* DME (mg/g) [1]			*P. sativa* ssp. *urens* DME (mg/g)			*P. hirsuta* DME (mg/g)			Reference
								Root	Leaf	Fruit	Root	Leaf	Fruit	Root	Leaf	Fruit	
21.47	610.1534	609.1461	−2.28	$C_{27}H_{30}O_{16}$	609.1495 $[M-H]^-$	464.0906, 463.0849, 447.0902, 301.0331, 300.0220, 271.0261	Quercetin 7-*O*-rhamnoside 3-*O*-glucoside (vincetoxicoside A; **18**)	-	1.32 ± 0.03 [2]	-	-	5.37 ± 0.02 [2]	2.00 ± 0.55	-	27.82 ± 0.01	29.52 ± 0.00	Pecio et al. [34]
21.52	756.2113	755.2040	−3.55	$C_{33}H_{40}O_{20}$	755.2067 $[M-H]^-$	610.1498, 609.1466, 447.0921, 301.0315, 271.0247	Deoxyhexosyl deoxyhexosyl hexosyl quercetin (**19**)	-		-	-		-	-	t	t	Farag et al. [38]
21.59	164.0473	163.0401	−5.68	$C_9H_8O_3$	163.0410 $[M-H]^-$	119.0487	Coumaric acid (**20**)	-	-	t	-	-	t	-	-	-	Ali et al. [40]
21.91	424.1369	423.1297	4.88	$C_{20}H_{24}O_{10}$	469.1392 $[M+HCOOH-H]^-$, 423.1276 $[M-H]^-$/447.1249 $[M+Na]^+$	179.0552, 89.0550/ 267.0621, 263.0989, 187.0386	Hydroxypropyl hydroxy 2′,3′-dihydrofuranocoumarin hexoside (**21**)	0.11 ± 0.00	0.45 ± 0.00	0.04 ± 0.00	1.88 ± 0.00	0.72 ± 0.00	0.47 ± 0.02	2.16 ± 0.01	t	t	Olennikov [51]
22.16	368.1107	367.1035	−0.94	$C_{17}H_{20}O_9$	367.1038 $[M-H]^-$	205.0494, 187.0367, 161.0597, 105.0693	Hexosyloxy benzofuran propanoic acid (**22**)	-	n.q.	-	-	n.q.	n.q.	-	n.q.	n.q.	Wubuli et al. [53]
22.74	194.0579	193.0506	−4.49	$C_{10}H_{10}O_4$	193.0516 $[M-H]^-$	178.0266, 149.0605, 134.0371	Ferulic acid (**23**)	-	-	-	t	-	-	t	-	-	Ali et al. [40]
22.82	594.1585	593.1512	−5.40	$C_{27}H_{30}O_{15}$	593.1544 $[M-H]^-$	431.0867, 285.0332, 255.0245	Deoxyhexosyl hexosyl kaempferol (**24**)	-	-	-	-	-	-	-	t	t	Allambergenova et al. [35]

Table 2. *Cont.*

Rt (min)	Molecular Mass	Calculated Mass	Diff (ppm)	Molecular Formula	Pseudomolecular Ion	Fragments	Compound	*P. sativa* ssp. *sativa* DME (mg/g) [1]			*P. sativa* ssp. *urens* DME (mg/g)			*P. hirsuta* DME (mg/g)			Reference
								Root	Leaf	Fruit	Root	Leaf	Fruit	Root	Leaf	Fruit	
22.84	624.1690	623.1618	−3.59	$C_{28}H_{32}O_{16}$	623.1640 [M-H]$^-$	478.1054, 461.1048, 315.0495, 300.0333, 271.0217	Deoxyhexosyl hexosyl methyl-quercetin (**25**)	-	-	t	-			-			Wojdyło et al. [39]
22.94	770.2269	769.2197	−4.33	$C_{34}H_{42}O_{20}$	769.2230 [M-H]$^-$	624.1664, 623.1621, 461.1084, 315.0498, 299.0229	Deoxyhexosyl deoxyhexo-syl hexosyl methyl-quercetin (**26**)	-	1.77 ± 0.08	-	-	10.96 ± 0.19 [2]	27.22 ± 0.76 [2]	-	54.98 ± 0.04 [2]	78.93 ± 0.04 [2]	Wojdyło et al. [39]
23.69	610.1534	609.1461	−7.85	$C_{27}H_{30}O_{16}$	609.1509 [M-H]$^-$	301.0329, 300.0274, 271.0244, 255.0275	Quercetin 3-*O*-rutinoside (rutin; **27**)	-	13.21 ± 0.04	-	-			-			Wojdyło et al. [39]
22.88	594.1585	593.1512	−5.40	$C_{27}H_{30}O_{15}$	593.1544 [M-H]$^-$	447.0788, 301.0189, 271.0125	Deoxyhexosyl deoxyhexo-syl quercetin (**28**)	-	-	-	-	-	-	-			Pecio et al. [34]
23.18	398.1213	397.1140	5.08	$C_{18}H_{22}O_{10}$	397.1120 [M-H]$^-$	235.0586, 217.0475, 202.0231, 191.0685, 176.0451, 161.0240	Hexosyloxy methoxy benzofuran propanoic acid (**29**)	n.q.	n.q.	n.q.	-	n.q.	n.q.	n.q.	n.q.	n.q.	Aloo et al. [52]
24.25	464.0955	463.0882	−1.94	$C_{21}H_{20}O_{12}$	463.0891 [M-H]$^-$	301.0326, 300.0287, 271.0275, 255.0320	Quercetin 3-*O*-glucoside (iso-quercitrin; **30**)	-	0.81 ± 0.04	-	-	0.09 ± 0.00	1.45 ± 0.01	-	4.08 ± 0.01	3.35 ± 0.00	Ammar et al. [36]
24.29	578.1636	577.1563	−1.07	$C_{27}H_{30}O_{14}$	577.1569 [M-H]$^-$	432.1001, 431.0936, 285.0372, 255.0263	Kaempferol 3,7-di-*O*-rhamnoside (kaempfer-itrin; **31**)	-	-	-	-	0.17 ± 0.00	-	-	5.58 ± 0.09	-	Farag et al. [38]
25.26	624.1690	623.1618	−1.99	$C_{28}H_{32}O_{16}$	623.1630 [M-H]$^-$	315.0488, 300.0276, 272.0247	Deoxyhexosyl hexosyl methyl-quercetin (**32**)	-		-	-	0.06 ± 0.00	1.24 ± 0.06	-	0.43 ± 0.46	t	Wojdyło et al. [39]
25.39	564.1479	563.1406	−4.38	$C_{26}H_{28}O_{14}$	563.1431 [M-H]$^-$	431.1015, 269.0452	Hexosyl pentosyl apigenin (**33**)	-	-	t	-	-	-	-	-	-	Emad et al. [37]

Table 2. *Cont.*

Rt (min)	Molecular Mass	Calculated Mass	Diff (ppm)	Molecular Formula	Pseudomolecular Ion	Fragments	Compound	*P. sativa* ssp. *sativa* DME (mg/g) [1]			*P. sativa* ssp. *urens* DME (mg/g)			*P. hirsuta* DME (mg/g)			Reference
								Root	Leaf	Fruit	Root	Leaf	Fruit	Root	Leaf	Fruit	
25.71	478.1111	477.1038	−5.12	$C_{22}H_{22}O_{12}$	477.1063 $[M-H]^-$	315.0475, 314.0433, 300.0274, 299.0211, 271.0250	Hexosyl methyl-quercetin (**34**)	-	-	-	-	-	2.91 ± 0.08	-	0.97 ± 0.66	4.12 ± 0.00	Wojdyło et al. [39]
25.79	448.1006	447.0933	−2.49	$C_{21}H_{20}O_{11}$	447.0944 $[M-H]^-$	285.0371, 284.0317, 255.0296, 227.0359, 151.0050	Hexosyl kaempferol (**35**)	-	-	-	-	-	-	-	t	t	Allamber-genova et al. [35]
25.97	516.1268	515.1195	−5.81	$C_{25}H_{24}O_{12}$	515.1225 $[M-H]^-$	353.0857, 191.0553, 179.0318, 173.0450, 135.0423	3,5-Di-*O*-caffeoylquinic acid (**36**)	-	t	-	-	t	-	-	t	-	Dai et al. [41]
26.81	304.0947	327.0839	5.62	$C_{16}H_{16}O_6$	327.0822 $[M+Na]^+$	224.0083, 173.0956	Heraclenol (**37**)	-	-	t	-	-	t	t	-	-	Li et al. [46]
26.92	516.1268	515.1195	3.10	$C_{25}H_{24}O_{12}$	515.1179 $[M-H]^-$	353.0843, 191.0493, 179.0351, 173.0422, 135.0407	1,5-Di-*O*-caffeoylquinic acid (**38**)	-	t	-	-	-	-	-	t	-	Dai et al. [41]
27.78	448.1006	447.0933	−3.16	$C_{21}H_{20}O_{11}$	447.0947 $[M-H]^-$	301.0346, 300.0246, 151.0030	Deoxyhexosyl quercetin (**39**)	-	-	-	-	t	-	-	-	t	Ammar et al. [36]
31.84	216.0423	217.0495	4.79	$C_{12}H_8O_4$	217.0485 $[M+H]^+$	202.0251, 174.0306, 161.0590	Xanthotoxin (8-MOP; **40**)	0.19 ± 0.00	4.70 ± 0.01	7.66 ± 0.07	0.55 ± 0.00	2.26 ± 0.00	6.46 ± 0.09	0.77 ± 0.03	0.76 ± 0.07	0.41 ± 0.00	Li et al. [46]
34.40	216.0423	217.0495	4.79	$C_{12}H_8O_4$	217.0485 $[M+H]^+$	202.0246, 174.0300, 161.0589	Sphondin (**41**)	t	0.93 ± 0.00	0.71 ± 0.01	0.27 ± 0.00	0.93 ± 0.00	1.34 ± 0.01	0.39 ± 0.01	1.47 ± 0.00	1.12 ± 0.00	Li et al. [46]
34.60	246.0528	247.0601	3.66	$C_{13}H_{10}O_5$	247.0592 $[M+H]^+$	232.0352, 217.0113, 189.0178, 161.0226	Isopimpinellin (**42**)	t	3.49 ± 0.00 [2]	8.31 ± 0.06 [2]	0.20 ± 0.01 [2]	1.51 ± 0.00 [2]	7.95 ± 0.05 [2]	0.23 ± 0.00 [2]	0.13 ± 0.06 [2]	1.25 ± 0.07 [2]	Li et al. [46]
35.22	216.0423	217.0495	4.79	$C_{12}H_8O_4$	217.0485 $[M+H]^+$	202.0247, 174.0301, 161.0581	Bergapten (**43**)										Li et al. [46]
43.18	270.0892	271.0965	6.98	$C_{16}H_{14}O_4$	271.0946 $[M+H]^+$	203.0326, 175.0380, 159.0432	Imperatorin (**44**)	-	0.42 ± 0.00	3.61 ± 0.06	t	0.25 ± 0.00	1.99 ± 0.00	0.35 ± 0.00	0.19 ± 0.00	t	Li et al. [46]

Table 2. *Cont.*

Rt (min)	Molecular Mass	Calculated Mass	Diff (ppm)	Molecular Formula	Pseudomolecular Ion	Fragments	Compound	*P. sativa* ssp. *sativa* DME (mg/g) [1]			*P. sativa* ssp. *urens* DME (mg/g)			*P. hirsuta* DME (mg/g)			Reference
								Root	Leaf	Fruit	Root	Leaf	Fruit	Root	Leaf	Fruit	
44.85	300.0998	301.1071	5.83	$C_{17}H_{16}O_5$	301.1053 $[M+H]^+$	233.0430, 218.0202, 190.0249, 162.0300	Phellopterin (**45**)	-	t	0.19 ± 0.00	t	t	t	0.42 ± 0.00	t	t	Li et al. [46]
							Phenolic acids	0.09	4.86	t	0.36	4.27	1.13	0.32	61.08	t	
							Quercetin/ methyl-quercetin flavonoids	t	17.11	t	-	18.28	38.57	-	103.70	163.93	
							Kaempferol flavonoids	-	-	-	-	0.17	-	-	5.58	t	
							Total flavonoids	t	17.11	t	-	18.45	38.57	-	109.29	163.93	
							Furano-coumarins (FCs)	0.30	9.99	20.52	2.90	5.67	18.21	11.84	2.56	2.79	
							Total quantified compounds	0.39	31.96	20.52	3.26	28.39	57.91	12.16	172.92	166.72	
							Extract amount (g) = 14 mg FCs	46.81	1.40	0.68	4.82	2.47	0.77	1.18	5.46	5.03	
							Extract amount (mg) = 1.5 mg FCs	5082.38	152.19	74.06	523.57	268.18	83.48	128.38	592.74	545.68	
							Extract amount (mg) = 0.56 mg FCs	1872.46	56.07	27.29	192.90	98.80	30.76	47.30	218.38	201.04	

[1] Phenolic acids are expressed as chlorogenic acid equivalents (eq.), quercetin and methylquercetin flavonoids as rutin eq., kaempferol flavonoids as kaempferitrin eq., and furanocoumarins (FCs) as 8-MOP eq. (8-MOP, sphondin, imperatorin), bergapten eq. (bergapten, isopimpinellin, phellopterin) or umbelliferone eq. (dihydrofuranocoumarin); t = trace (bellow LOQ); n.q. = not quantified. [2] Co-eluting compounds quantified together due to structural and UV spectral similarities.

Given that furanocoumarins were detected in all extracts, the extract amounts corresponding to three exposure scenarios were calculated: the estimated average dietary intake of furanocoumarins, the EMA/HMPC-proposed intake level not expected to significantly contribute to the overall risk, and the intake level potentially associated with phototoxicity (Table 2). The highest total furanocoumarin content was found in the fruit extract of *P. sativa* subsp. *sativa* (20.52 mg/g), whereas the lowest was found in the root extract of the same plant (0.30 mg/g). Based on these values, the extract amounts corresponding to the estimated daily dietary intake of furanocoumarins, as defined in the DFG-SKLM assessment (0.56 mg/day) [18], ranged from 27.29 to 1872.46 mg. The extract amounts corresponding to the EMA/HMPC-proposed daily intake level of 1.5 mg of furanocoumarins, which is not expected to significantly contribute to the overall risk [16], ranged from 74.06 to 5082.38 mg. The calculated extract amounts corresponding to an intake of 14 mg of furanocoumarins, which may result in detectable phototoxicity [18], ranged from 0.68 to 46.81 g. It should be noted that the threshold of 14 mg refers specifically to 8-MOP, whose high phototoxicity is well established [18]. In the absence of sufficient data on the phototoxicity of individual furanocoumarins, a worst-case scenario was assumed in which all detected furanocoumarins were considered to exhibit phototoxicity comparable to that of 8-MOP. The same conservative approach is recommended by the EMA/HMPC for the daily intake level of 1.5 mg [16].

Linear monosubstituted methoxy-furanocoumarins, such as 8-MOP and bergapten (5-methoxypsoralen, 5-MOP), are considered among the most phototoxic furanocoumarins. The introduction of an additional substituent or replacement of the methyl group with a bulkier substituent generally reduces phototoxicity. Angular furanocoumarins also exhibit lower phototoxicity. Thus, comparative in vitro studies of the phototoxicity of the extracts relative to 8-MOP could help establish more realistic, potentially higher safety thresholds for these herbal preparations [16,18].

The preliminary dichloromethane washing step of the plant material was applied to reduce furanocoumarin levels prior to methanolic extraction. This non-polar solvent proved effective for the separation of furanocoumarins from polyphenolic constituents. For translating this laboratory procedure to larger-scale food applications, further studies would be useful to evaluate the furanocoumarin extraction selectivity of some more sustainable solvents, e.g., supercritical carbon dioxide.

It should be noted that the studied taxa were collected at different localities and in different years, and differences in their metabolite profiles may therefore reflect both taxonomic and environmental variation. These findings provide a useful basis for further studies aimed at disentangling the effects of genetic and environmental factors on the metabolite composition of *Pastinaca* taxa.

3.2. Total Phenolic Content and Antioxidant Activity of Pastinaca Dry Methanol Extracts

The total phenolic content (Table 3) varied among the investigated *Pastinaca* dry methanol extracts, with the highest value observed in *P. hirsuta* leaf extract (70.07 mg quercetin equivalents (QE)/g of dry extract), followed by *P. hirsuta* fruit extract (54.69 mg QE/g), *P. sativa* subsp. *urens* leaf and fruit extracts (44.02 and 51.27 mg QE/g), and *P. sativa* subsp. *sativa* agricultural waste-derived leaf extract (41.02 mg QE/g). The lowest total phenolic content was found in *P. sativa* subsp. *sativa* root extract (12.90 mg QE/g). The antioxidant activity, assessed using the FRAP and DPPH assays (Table 3), correlated with the total phenolic content, in line with the antioxidant/radical scavenging role of polyphenols. The highest total antioxidant activity (FRAP value) was determined for *P. hirsuta* leaf extract (0.97 mmol Fe^{2+}/g of dry extract), followed by *P. hirsuta* fruit extract (0.60 mmol Fe^{2+}/g), *P. sativa* subsp. *urens* leaf and fruit extracts (0.46 and 0.56 mmol Fe^{2+}/g),

and *P. sativa* subsp. *sativa* leaf extract (0.37 mmol Fe^{2+}/g), while the lowest FRAP value was revealed for *P. sativa* subsp. *sativa* root extract (0.07 mmol Fe^{2+}/g). Anti-DPPH activity followed a similar trend, with *P. hirsuta* leaf extract showing the highest scavenging ability (64.56 mg quercetin equivalents (QE)/g of dry extract), followed by *P. hirsuta* fruit extract (26.04 mg QE/g), *P. sativa* subsp. *urens* leaf and fruit extracts (16.52 and 15.92 mg QE/g), and *P. sativa* subsp. *sativa* leaf extract (11.88 mg QE/g); the lowest activity was revealed again for *P. sativa* subsp. *sativa* root extract (1.83 mg QE/g).

Table 3. Total phenolic content (TPC), total antioxidant activity (TAA; FRAP values) and anti-DPPH activity of investigated *Pastinaca* dry methanol extracts (DMEs).

	***P. s.* subsp. *sativa* DME**			***P. s.* subsp. *urens* DME**			***P. hirsuta* DME**		
	Root	**Leaf**	**Fruit**	**Root**	**Leaf**	**Fruit**	**Root**	**Leaf**	**Fruit**
TPC (mg quercetin equivalents/g of DME)	12.90 ± 0.15 ^{a}	41.02 ± 1.35 ^{a,b}	13.66 ± 0.25 ^{a}	24.76 ± 1.00 ^{a,b}	44.02 ± 3.65 ^{a,b}	51.27 ± 3.69 ^{a,b}	27.11 ± 1.92 ^{a,b}	70.07 ± 7.39 ^{b}	54.69 ± 3.18 ^{a,b}
TAA (mmol Fe^{2+}/g of DME) [1]	0.07 ± 0.01 ^{a}	0.37 ± 0.05 ^{a,b}	0.15 ± 0.01 ^{a}	0.30 ± 0.02 ^{a,b}	0.46 ± 0.03 ^{a,b}	0.56 ± 0.00 ^{a,b}	0.30 ± 0.00 ^{a,b}	0.97 ± 0.23 ^{b}	0.60 ± 0.05 ^{a,b}
Anti-DPPH activity (mg quercetin equivalents/g DME) [1]	1.83 ± 0.30 ^{a}	11.88 ± 0.77 ^{a,b,c}	5.67 ± 0.10 ^{a,b,c}	5.23 ± 0.18 ^{a,b}	16.52 ± 0.39 ^{a,b,c}	15.92 ± 0.59 ^{a,b,c}	8.04 ± 0.50 ^{a,b,c}	64.56 ± 7.47 ^{c}	26.04 ± 1.84 ^{b,c}

[1] Positive control: quercetin TAA = 35.70 ± 3.98 mmol Fe^{2+}/g; DPPH IC_{50} = 4.29 ± 0.16 µg/mL. $^{a–c}$ Different letters in superscript indicate significant differences ($p < 0.05$) between the values.

The literature data suggest that the observed antioxidant activity can be largely attributed to the polyphenols identified in this work. For example, notable FRAP values (SC_{50} = 18.47 and 5.71 µg/mL) and anti-DPPH activity (SC_{50} = 4.50 and 5.89 µg/mL) were previously observed for quercetin 7-*O*-rhamnoside 3-*O*-glucoside (vincetoxicoside A; **18**) and quercetin 3-*O*-glucoside (isoquercitrin; **30**) [54,55]. Similar results were obtained for chlorogenic acid (**6**; FRAP value = 18.04 mmol Fe^{2+}/g, DPPH SC_{50} = 4.42 µg/mL) [56]. Accordingly, the highest amounts of these compounds were detected in *P. hirsuta* leaf and fruit extracts, which also exhibited the strongest antioxidant activity. Previously, the dry ethanol extracts of the leaves and fruits of *P. sativa* subsp. *urens* demonstrated DPPH and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activities and iron-chelating ability [29], whereas the dry hydroethanolic extract of *P. sativa* subsp. *sativa* roots showed anti-DPPH activity [27].

Given the observed antioxidant properties, the investigated *Pastinaca* species may represent potential sources of natural additives and functional food ingredients for the food industry [57–59]. For example, chlorogenic acid (**6**), one of the major phenolic compounds identified in the investigated extracts, has been reported to have various applications in the food industry, including use in food preservation and storage, the development of active food packaging materials, and as a functional food ingredient [58]. Rutin (**30**) is a valuable flavonoid with potential applications in the food industry due to its antioxidant properties and health-promoting effects. Its incorporation into active packaging systems may contribute to the development of antioxidant food packaging materials, while its use in food fortification strategies may enhance the functional value of food products. However, its limited aqueous solubility requires approaches such as encapsulation to improve its bioavailability [59]. Nevertheless, foods rich in quercetin glycosides, such as microgreens, are increasingly investigated for their potential functionality, for example, due to their antioxidant and anti-inflammatory properties [57].

3.3. Antimicrobial Activity of Pastinaca Dry Methanol Extracts

The tested *Pastinaca* dry methanol extracts exhibited varying activity against a range of Gram-positive and Gram-negative bacteria, as well as yeast species, including foodborne pathogens and food contaminants (Table 4). The strongest activity was observed against Gram-positive bacteria. A food spoilage bacterium, *Micrococcus luteus*, was generally the most susceptible (MIC = 0.156–2.5 mg/mL, MBC = 5–10 mg/mL), with *P. sativa* subsp. *sativa* agricultural waste-derived leaf extract being the most active. Additionally, the extracts richest in polyphenols—*P. hirsuta* and *P. sativa* subsp. *urens* leaf and fruit extracts and *P. sativa* subsp. *sativa* leaf extract—demonstrated notable activity against one or both tested *Staphylococcus aureus* strains and/or *S. epidermidis* (MIC and MBC values of 5 mg/mL). Against *Bacillus cereus*, the strongest inhibitory effect was observed for *P. sativa* subsp. *urens* fruit extract at 2.5 mg/mL. Previously, Eruygur et al. [29] reported a notable MIC value (0.625 mg/mL) for the ethanol extract of the fruits of this plant from Turkey against another species of this genus, *B. subtilis*. For Gram-negative bacteria, the inhibitory and bactericidal concentrations were generally higher (in most cases, 10 mg/mL). Regarding yeast species, *Candida albicans* was the most susceptible (MIC = 5 mg/mL, MFC = 10 mg/mL). For the other two tested *Candida* species, both inhibitory and fungicidal effects were predominantly observed at 10 mg/mL. DMSO, used as a solvent for stock solutions, did not show antimicrobial activity at the concentrations used in this study.

Table 4. Antimicrobial activity of investigated *Pastinaca* dry methanol extracts (DMEs) expressed as MICs and MBCs/MFCs (mg/mL).

Microorganisms	*P. sativa* subsp. *sativa* DMEs						*P. sativa* subsp. *urens* DMEs						*P. hirsuta* DMEs					
	Root		Leaf		Fruit		Root		Leaf		Fruit		Root		Leaf		Fruit	
Gram-positive bacteria	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus ATCC 25923	10	>10	5	5	>10	ND [1]	10	>10	5	5	5	5	10	10	5	5	>10	>10
S. aureus ATCC BA1707	>10	ND	5	5	>10	ND	>10	>10	10	10	10	10	10	>10	5	5	>10	>10
S. epidermidis ATCC 12228	10	>10	5	5	>10	ND	10	>10	10	10	5	10	10	10	5	5	10	10
M. luteus ATCC 10240	2.5	10	0.156	5	0.313	10	1.25	10	2.5	5	1.25	5	1.25	5	1.25	5	0.625	5
B. cereus ATCC 10876	10	>10	>10	>10	>10	ND	5	>10	5	>10	2.5	>10	5	>10	10	>10	10	>10
E. faecalis ATCC 29212	>10	ND	>10	>10	>10	ND	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
Gram-negative bacteria	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. typhimurium ATCC 14028	10	>10	10	10	>10	ND	>10	>10	10	10	10	10	>10	ND	10	10	10	>10
E. coli ATCC 25922	10	>10	10	10	>10	ND	10	>10	10	10	10	10	>10	ND	10	10	10	10
P. mirabilis ATCC 12453	>10	ND	10	10	>10	ND	>10	ND	10	10	10	10	>10	ND	10	10	10	>10
K. pneumoniae ATCC 13883	10	10	10	10	>10	ND	10	10	10	10	10	10	10	10	5	10	10	10
P. aeruginosa ATCC 9027	10	10	10	10	>10	ND	10	10	10	10	10	10	10	10	10	10	10	10
Yeasts	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
C. glabrata ATCC 90030	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
C. albicans ATCC 102231	5	10	5	10	5	10	5	10	5	10	5	10	5	10	5	10	5	10
C. parapsilosis ATCC 22019	10	10	10	10	10	10	10	10	10	10	10	10	5	10	10	10	10	10

[1] ND—activity not determined.

In a recent study, notable anticandidal and antibacterial activities of essential oils obtained from roots, leaves, stems and fruits of these three parsnips were also demonstrated [7].

Among the polyphenols identified in the extracts, chlorogenic acid, quercetin and methylquercetin glycosides are known for their ability to disrupt bacterial membranes and inhibit key enzymes [58,60]. For example, notable activity against *S. aureus* was previously revealed for rutin (**27**; MIC = 0.2–0.4 mg/mL), isoquercitrin (**30**; MIC = 0.1–0.2 mg/mL) [61], and chlorogenic acid (**6**; MIC = 0.5 mg/mL) [62], which were all detected in the most active

extracts. Rutin (**27**) also showed activity against *M. luteus* (MIC = 0.1 mg/mL), *B. cereus* (MIC = 0.25 mg/mL) and *S. epidermidis* (MIC = 0.1–0.5 mg/mL) in previous studies [63–65]. Chlorogenic acid (**6**) has been reported to exert antibacterial activity against *M. luteus* through disruption of cell membrane integrity, reduction of intracellular ATP levels and leakage of cellular contents. Moreover, this phenolic acid at a concentration of 2.5 mg/mL significantly inhibited the growth of *M. luteus* in pork [66]. Against *C. albicans*, rutin (**27**; MIC = 0.04 mg/mL), isoquercitrin (**30**; MIC = 0.04 mg/mL) [67] and chlorogenic acid (**6**; MIC = 0.1 mg/mL) [68] were previously found to be active as well.

The observed antimicrobial potential highlights the applicability of the tested parsnips as sources of bioactive compounds and extracts with potential applications in the food industry. Importantly, the most susceptible microorganism, *M. luteus*, is a food spoilage bacterium commonly associated with fresh and processed foods and is capable of deteriorating the sensory quality of meat, fish, soy products and other food commodities [66]. The activity against this bacterium, as well as against *S. aureus* and *B. cereus*, two major foodborne pathogens, suggests the potential use of *Pastinaca* extracts or their bioactive constituents as natural preservatives. Additionally, the observed activity against different microorganisms may enhance the functional value of food products containing *Pastinaca*-derived ingredients [58,60,69]. For example, among the identified compounds, chlorogenic acid (**6**) and rutin (**27**) have been reported to exhibit antimicrobial activity and show potential for applications in the food industry, particularly in active food packaging and other food preservation strategies [58,59].

3.4. Cytotoxic Activity of Pastinaca Dry Methanol Extracts

The determined cytotoxicity of the extracts (Table 5; Figures S1–S3) was evaluated according to the recommendations of the National Cancer Institute (NCI) [70] and previous studies [71,72]. The highest cytotoxicity was exhibited by *P. sativa* subsp. *sativa* fruit extract. Its effect was moderate ($20 < CC_{50} < 200$ µg/mL) against the FaDu (hypopharynx cancer) cell line and weak ($200 < CC_{50} < 500$ µg/mL) against the AGS (stomach cancer) and RKO (colon cancer) cell lines, as well as the non-cancerous VERO (monkey kidney) cell line. Other extracts showed no cytotoxicity ($CC_{50} > 500$ µg/mL) to VERO cells and most of the tested cancer cells. The exceptions were *P. sativa* subsp. *sativa* leaf extract against FaDu and RKO cells, *P. sativa* subsp. *urens* fruit extract against RKO cells, and *P. hirsuta* leaf extract against FaDu cells, where weak cytotoxicity was recorded. DMSO, used as a solvent for stock solutions, was non-toxic at the concentrations used in this study.

Table 5. Cytotoxic activity and cancer cell selectivity of investigated *Pastinaca* dry methanol extracts (DMEs) expressed as 50% cytotoxic concentration (CC_{50}; µg/mL; mean ± SD).

DMEs	VERO CC_{50}	AGS CC_{50}	FaDu CC_{50}	RKO CC_{50}
Pastinaca sativa subsp. *sativa* root	>1000	>1000	699.78 ± 25.83	534.70 ± 21.78
Pastinaca sativa subsp. *sativa* leaf	>1000	721.45 ± 44.14	492.50 ± 40.33	489.67 ± 30.96
Pastinaca sativa subsp. *sativa* fruit	321.28 ± 28.19	318.25 ± 22.26	192.33 ± 18.75	250.80 ± 33.23
Pastinaca sativa subsp. *urens* root	>1000	1151.50 ± 122.06	805.45 ± 56.93	>1000
Pastinaca sativa subsp. *urens* leaf	813.03 ± 32.36	615.50 ± 20.22	539.38 ± 27.76	583.95 ± 26.53
Pastinaca sativa subsp. *urens* fruit	>1000	516.60 ± 13.64	556.90 ± 6.19	366.95 ± 4.31
Pastinaca hirsuta root	>1000	928.55 ± 84.23	553.30 ± 45.60	652.10 ± 18.68
Pastinaca hirsuta leaf	>1000	705.50 ± 35.89	466.88 ± 16.37	573.48 ± 16.03
Pastinaca hirsuta fruit	>1000	922.30 ± 82.84	504.23 ± 38.58	744.23 ± 33.33

It should be noted that *P. sativa* subsp. *sativa* fruit extract, which was the most cytotoxic, contained the highest quantities of furanocoumarins (mostly xanthotoxin **40**, isopimpinellin **42** and bergapten **43**), with only traces of polyphenols. These results are in line with the

previously demonstrated antiproliferative activity of xanthotoxin (**40**), isopimpinellin (**42**) and bergapten (**43**) on AGS cells [73] and the moderate cytotoxicity of xanthotoxin (**40**) and bergapten (**43**) against VERO cells (CC_{50} = 40.63 and 42.93 µg/mL) [74]. On the other hand, rutin (**27**), isoquercitrin (**30**) and chlorogenic acid (**6**) were not cytotoxic to non-cancerous VERO cells [75,76], in agreement with the current research. These results indicate that furanocoumarins notably contributed to the cytotoxic activity of *Pastinaca* extracts, even in the absence of UV irradiation. According to the literature, the potential chemopreventive activity associated with Apiaceae vegetables is likely related to their complex phytochemical composition, which includes polyphenols, furanocoumarins and polyacetylenes [77–80].

The VERO cells were selected to assess the cytotoxicity of the extracts due to their widespread use in various cytotoxicity studies and *in vitro* efficacy testing [81–83]. VERO cells are also endorsed by ISO experts for *in vitro* cytotoxicity studies, in accordance with ISO 10993-5:2009 [ISO 10993-5:2009] [84]. All of the extracts, with the exception of *Pastinaca sativa* subsp. *sativa* fruit extract, were non-cytotoxic (CC_{50} > 500 µg/mL) to VERO cells, indicating potential safety as food additives and functional food ingredients. In addition to VERO cells, the cell lines derived from hypopharyngeal, gastric, and colon carcinomas were used to assess cytotoxicity toward gastrointestinal-derived cells. Because these cells are malignant, this approach is not ideal, but the results also suggest that most tested plant extracts lack cytotoxicity, supporting their safety for use in food products. Notably, since this report is based on *in vitro* studies, future studies should include *in vivo* cytotoxicity.

4. Conclusions

This study significantly expands the current knowledge of the polyphenolic composition of the investigated *Pastinaca* species. While all studied leaf extracts and wild parsnip fruit extracts were generally rich in these compounds, all the root extracts and cultivated parsnip fruit extract contained negligible amounts of polyphenols. Overall, the obtained results indicate that the investigated parsnip species represent valuable sources of bioactive polyphenols with potential applications in food systems. Likewise, the crude methanol extracts of these plants may serve as potential natural bioactive ingredients, provided that their application considers established furanocoumarin intake thresholds. Importantly, this study highlights the potential value of parsnip (*P. sativa* subsp. *sativa*) byproducts as a sustainable source of bioactive compounds and provides a rationale for the valorization of widespread wild parsnip (*P. sativa* subsp. *urens*) populations, as well as for the cultivation of the endemic species *P. hirsuta*. In addition, the research performed supports the widespread use of cultivated parsnip root as a healthy vegetable. Finally, the bioactivity of the extracts was evaluated exclusively using in vitro models; therefore, their in vivo bioavailability and metabolic stability, as well as their sensory effects in real food matrices, remain to be established before practical and commercial applications can be considered.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae12091174/s1, Figure S1. Dose–response effect of dry methanol (MeOH) extracts of *P. sativa* subsp. *sativa* roots (a), leaves (b) and fruits (c) on a panel of cell lines; Figure S2. Dose–response effect of dry methanol (MeOH) extracts of *P. sativa* subsp. *urens* roots (a), leaves (b) and fruits (c) on a panel of cell lines; Figure S3. Dose–response effect of dry methanol (MeOH) extracts of *P. hirsuta* roots (a), leaves (b) and fruits (c) on a panel of cell lines.

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Abbreviations

The following abbreviations are used in this manuscript:

AGS	Human gastric adenocarcinoma cells
ATCC	American Type Culture Collection
CC_{50}	50% cytotoxic concentration
CFU	Colony-forming unit
DAD	Diode-array detector
DFG-SKLM	Senate Commission on Food Safety of the German Research Foundation
DMEM	Dulbecco's modified Eagle's medium
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EMA/HMPC	Committee on Herbal Medicinal Products of the European Medicines Agency
ESI	Electrospray ionization
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FaDu	Human hypopharyngeal squamous cell carcinoma cells
FBS	Fetal bovine serum
FRAP	Ferric reducing antioxidant power
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MBC	Minimum bactericidal concentration
MEM	Minimum essential medium
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
MS	Mass spectrometry
MTT	3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide
NCI	National Cancer Institute
PBS	Phosphate-buffered saline
QTOF	Quadrupole time-of-flight
RKO	Human colon cancer cells
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine
VERO	Monkey kidney cells

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