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LC-DAD-QTOF-MS/MS-Based Chemical Profiling and Bioactivity Evaluation of *Prangos trifida* Dry Methanol Extracts

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Abstract

The chemical composition and bioactivities of dry methanol extracts from roots, leaves and fruits of *Prangos trifida* (Apiaceae), collected in Serbia, were investigated. LC-DAD-QTOF-MS/MS analysis revealed 30 compounds, primarily polyphenols and coumarins. The root and leaf extracts were rich in chlorogenic and/or 3,5-di-*O*-caffeoylquinic acid (18.20–26.14 mg/g extract), and the fruit extract in oxypeucedanin hydrate and prantschimgin (46.50 and 71.64 mg/g). The leaf extract exhibited the highest total phenolic content (62.86 mg quercetin equivalents/g), total antioxidant activity (FRAP = 0.71 mmol Fe^{2+}/g) and DPPH radical scavenging ability (44.08 mg quercetin equivalents/g). Antimicrobial activity testing (11 bacteria and three yeasts, microdilution method) showed that the most active were the root and leaf extracts against *Micrococcus luteus*, *Staphylococcus aureus*, *S. epidermidis* and *Candida albicans* (MIC = 0.625–5 mg/mL). The fruit extract showed the strongest cytotoxicity against tested stomach, colon and hypopharynx cancer cell lines (MTT test), with the highest selectivity toward hypopharynx cancer FaDu cells (selectivity index 4.71; determined in relation to non-cancerous VERO cells). No antiviral activity against herpesvirus type 1 was found. The results indicate that *P. trifida* represents a promising source of polyphenols and coumarins, notably expanding current knowledge on its chemical composition and supporting its potential relevance for pharmaceutical and food industry applications.

Keywords: *Prangos trifida*; chemical composition; LC-DAD-QTOF-MS/MS; antioxidant; antimicrobial; antibacterial; anticandidal; cytotoxic; antiviral

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1. Introduction

For the analysis of herbal extracts rich in polar constituents, reversed-phase (RP) liquid chromatography, including high-performance liquid chromatography (RP-HPLC) and ultra-high-performance liquid chromatography (RP-UHPLC), is widely employed owing to its high separation efficiency and broad applicability to hydrophilic metabolites.

When coupled with conventional detection systems, such as diode-array detection and mass spectrometry, these approaches enable robust untargeted profiling and quantification of phenolic acids, flavonoids and related polar constituents. The integration of high-resolution mass analyzers, particularly quadrupole time-of-flight (QTOF) and orbitrap mass analyzers, has further enhanced the structural elucidation of compounds in complex plant extracts [1,2].

The Apiaceae plant family, with 455 accepted genera, includes many well-known vegetables, e.g., carrot, celery and parsley; and spices, e.g., caraway, coriander and cumin. Many of them, such as fennel, angelica and asafetida, are used as medicinal plants [3,4]. The genus *Prangos* Lindl. (Apiaceae) encompasses 50 species distributed across Eurasia, ranging from Portugal to Tibet, with the Irano-Turanian region being the center of diversity [3,5]. Both aerial and underground parts of various species from this genus have been employed in folk medicine. For example, these plants have been used internally as tonics, digestive aids (due to carminative, laxative and anti-flatulent effects), antidiabetics, antihypertensives, hemostatics, diuretics, aphrodisiacs, liver tonics and menstrual cycle regulators, as well as for kidney and bladder disorders. Externally, they have been applied for treating wounds, scars, vitiligo, oral mucosal ailments, tooth pain, parasitic diseases and hemorrhoids [5,6]. Previous phytochemical studies on *Prangos* species have been primarily focused on essential oils and coumarins, whereas other compounds—such as phenolic acids, flavonoids, phytosterols, polyacetylenes and carotenoids—have received less attention [5]. Extracts of varying polarity and essential oils obtained from different parts of *Prangos* species have demonstrated a wide range of bioactivities, including in vitro antimicrobial, cytotoxic, antioxidant and enzyme inhibitory effects (targeting acetyl- and butyrylcholinesterase, tyrosinase, α-amylase, α-glucosidase, lipase and angiotensin-converting enzyme), as well as in vivo hypoglycemic and analgesic activities [5,6].

Prangos trifida (Mill.) Herrnst. & Heyn is sporadically distributed on rocky slopes across the European sub-Mediterranean region, from Portugal to Crimea [7,8]. Recent molecular studies revealed two well-supported clades (Western and Eastern, the latter including Balkan and Crimean populations), with no clear morphological differentiation [9,10]. Some authors have therefore suggested that Eastern populations may represent a separate species, *Cachrys alpina* Bieb. [3]. Previous studies of *P. trifida* were predominantly focused on essential oils. Namely, the composition of essential oils was investigated in the case of fruits from Turkey (plant studied under the name *C. alpina*) [11], fruits, flowers, stems with leaves and roots from Spain (plant examined as *C. trifida* L.) [12], fruits from Crimea [13], and aerial parts (collected before flowering) from Italy [14,15]. In addition, antioxidant [14,15] and antimicrobial activities [14] were demonstrated for the essential oil from Italy. Furthermore, three coumarins—imperatorin, isoimperatorin and prantschimgin—isolated from *P. trifida* (studied as *C. trifida*) have been shown to influence macrophage functions, suggesting their anti-inflammatory potential [16]. In our previous study on *P. trifida* from Serbia, the chemical composition of volatile constituents (essential oils and headspace volatiles) and dichloromethane extracts of roots, leaves, stems and/or fruits was investigated. Moreover, the antimicrobial and antibiofilm activities of selected essential oils and coumarin-rich methanol fractions of dichloromethane extracts were examined [17]. This work was conceptualized based on the fact that polar constituents of *Prangos* species have previously been only sparsely investigated and that, to the best of our knowledge, no data on these components are available for *P. trifida*. Therefore, the aim of this study was to investigate the chemical composition of dry methanol extracts of *P. trifida* roots, leaves and fruits (obtained from the residual plant material after dichloromethane extraction) using high-resolution mass spectrometry (HRMS), as well as to evaluate their antioxidant, antibacterial, antifungal, antiviral and cytotoxic activities.

2. Materials and Methods

2.1. Plant Material

Prangos trifida roots, leaves and fruits were collected in Sićevo Gorge (Kusača), Serbia, in 2020 (22.0810369° E, 43.315107° N). The plant was identified by Dr. Marjan Niketić, curator/botanist at the Natural History Museum, Belgrade (Serbia). A voucher specimen has been deposited in the herbarium of the Natural History Museum, Belgrade (BEO), under the number 20200603. The plant material was air-dried before analysis.

2.2. Extraction

Roots, leaves and fruits were powdered and extracted with dichloromethane (analytical grade). After drying, residual plant material was extracted using methanol (analytical grade). A bimaceration process (at room temperature) was applied in the case of both solvents. The first maceration lasted for three days, after which the extract was filtered, and the plant material was subjected to a second maceration for two days with a fresh portion of solvent (an herbal drug-to-solvent ratio of 1:10 w/v was used in both steps). The solvents were evaporated under reduced pressure using a rotary evaporator (Büchi Rotavapor RII, Flawil, Switzerland). In the case of dichloromethane extraction, the extracts rich in coumarins (investigated in our previous study) were obtained [17]. Obtained dry methanol extracts were studied in this work; yields: 7.04%, 9.24% and 5.48% w/w for roots, leaves and fruits, respectively.

2.3. Chemical Analysis

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

An Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA), coupled to an Agilent 6210 Quadrupole Time-of-Flight (QTOF) mass spectrometer, was used in qualitative analysis based on spectral data interpretation. Chromatographic separation was conducted on a Phenomenex Gemini C18 column (2 × 100 mm, 3 µm) (Phenomenex, Torrance, CA, USA). The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) (LC-MS grade), with a flow rate of 0.2 mL/min. The gradient elution was programmed as follows: 0 min 0% B, 45 min 60% B, 46–55 min 95% B. The injection volume was 10 µL, with extracts dissolved in methanol at a concentration of 10 mg/mL. A diode-array detector (DAD) was set at wavelengths of 210, 254, 320 and 366 nm. Mass spectrometric analysis was performed in both positive and negative electrospray ionization (ESI) modes, scanning within the m/z range of 50–1700. The ion source parameters were as follows: drying gas (N_2) flow rate of 10 L/min at 275 °C, sheath gas flow rate of 12 L/min at 325 °C, nebulizer pressure set to 35 psi, capillary voltage of 4000 V, nozzle voltage of 1000 V, fragmentor voltage at 140 V, skimmer voltage at 65 V, and octapole RF peak voltage of 750 V. MS/MS fragmentation was carried out with collision energies of 10 and 30 V. Data processing was performed using MassHunter software (version B08.00, Agilent Technologies, Santa Clara, CA, USA). Compounds were characterized based on the obtained tandem mass spectra and by matching the molecular formula, calculated from the measured mass, with data from the LOTUS and KNApSAcK Core System databases [18,19], and in some instances based on the obtained UV spectra.

2.3.2. LC-DAD-MS Analysis

An Agilent 1260 HPLC system, equipped with an Agilent 6130 single quadrupole ESI mass spectrometer (Agilent Technologies, Santa Clara, CA, USA), was used in qualitative and quantitative analysis based on employing external standards. In the separation process, an Agilent Zorbax SB-Aq column (150 × 3.0 mm; 3.5 µm particle size) set at 25°C was used. The mobile phase comprised 0.1% (v/v) formic acid and 5% (v/v) isopropanol in water

(phase A) and methanol (phase B) (all LC-MS grade). The gradient elution program was as follows: 0 min 0% B, 5 min 40% B, 25–27 min 45% B, 32 min 55% B, 40–45 min 100% B. The flow rate was maintained at 0.3 mL/min, with injection volumes of either 5 or 10 µL of 5 mg/mL solutions of the extracts in methanol. DAD was set to monitor wavelengths of 250, 270, 320 and 350 nm. The mass spectrometer was operated in both positive and negative ion modes under the following optimized conditions: m/z range of 80–2500, fragmentor voltage of either 100 or 250 V, nebulizing gas flow of 10 L/min at 350°C, nebulizer pressure of 40 psi, and capillary voltage of 3500 V. Identification of compounds was performed by comparing their retention times and UV and mass spectra to those of standard compounds. Quantification was done using the external standard method based on DAD-recorded data (Table 1; Figures S1–S3). Reference standards were obtained from Sigma-Aldrich or isolated previously from the dry dichloromethane extracts of *P. trifida* roots and fruits (prantschimgin and oxypeucedanin) [17] and the dry methanol extract of the underground parts of *Hieracium scheppigianum* Freyn, Asteraceae (1,5-di-*O*-caffeoylquinic acid) [20]. Phenolic acids were quantified using a calibration curve of chlorogenic acid; quercetin glycosides using a calibration curve of isoquercitrin; dihydrofuranocoumarins using a calibration curve of prantschimgin; and other furanocoumarins using calibration curves of either xanthotoxin (xanthotoxin, heraclenin, heraclenol), oxypeucedanin (oxypeucedanin, oxypeucedanin hydrate) or imperatorin. Calibration curves were constructed using seven concentration levels for each standard compound. The quantification results were expressed as mean mg of compounds per g of dry extracts, along with standard deviations derived from three separate experiments. The limits of detection (LODs) were determined as the lowest standards concentrations at which the signal-to-noise (S/N) ratio reached 3.3, while the limits of quantification (LOQs) were defined as the minimum standards concentrations used for calibration curve preparation.

Table 1. Data from quantitative analysis.

Standard	λ (nm) [1]	Slope (a)	Intercept (b)	r^2	linear Range (µg)	LOD (µg)	LOQ (µg)
Chlorogenic acid	320	8451.59	35.43	0.9999	0.005–1	0.002	0.005
Isoquercitrin	350	4043.77	0.38	0.9999	0.01–1	0.003	0.01
Xanthotoxin	250	17,811.42	−35.62	0.9999	0.005–1	0.002	0.005
Imperatorin	250	14,013.00	39.91	0.9998	0.01–1.5	0.003	0.01
Oxypeucedanin	250	3354.10	2.38	0.9996	0.002–10.165	0.0007	0.002
Prantschimgin	350	4271.70	269.95	0.9987	0.0019–9.355	0.0006	0.002

[1] Wavelengths used in quantitative analysis.

2.4. Total Phenolic Content and Antioxidant Activity

2.4.1. Total Phenolic Content

Extracts solutions in methanol (0.2 mg/mL; 20 µL) were mixed with a 10-fold diluted Folin–Ciocalteu reagent (150 µL) and sodium carbonate solution (60 g/L; 150 µL) in 96-well microtiter plates. Following incubation for 90 min, absorbances were measured at 725 nm using a SPECTROstar Nano microplate reader (BMG Labtech, Ortenberg, Germany). A blank sample was prepared by substituting methanol (20 µL) for the extract solutions. The results, expressed as the mean values ± standard deviations from three independent experiments, were calculated based on a quercetin calibration curve (0.01–0.1 mg/mL) [21].

2.4.2. Total Antioxidant Activity

Total antioxidant activity was determined using the FRAP (ferric reducing antioxidant power) test. Extracts solutions in methanol (0.5 mg/mL; 20 µL) were combined with 300 µL of freshly prepared FRAP reagent in 96-well microtiter plates. The FRAP reagent was prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of 2,4,6-tris(2-

pyridyl)-s-triazine (TPTZ) solution (10 mM in 40 mM hydrochloric acid) and 2.5 mL of ferric chloride solution (20 mM). After incubating for 30 min, absorbances were recorded at 593 nm using a SPECTROstar Nano microplate reader. A blank sample was prepared by substituting the extract solutions with 20 µL of methanol in 300 µL of FRAP reagent. Quercetin (0.016 mg/mL) served as a positive control. The results, presented as the means ± standard deviations from three independent trials, were determined using a ferrous sulfate calibration curve (200–1000 mM) [21].

2.4.3. DPPH Scavenging Activity

Extract solutions in methanol (0.2 mL; concentrations ranging from 0.17 to 1 mg/mL) were combined with a DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in methanol (0.5 mM; 0.05 mL) in 96-well microplates. After a 30 min incubation period, absorbances were recorded at 517 nm using a SPECTROstar Nano microplate reader, with methanol serving as the blank. The negative control consisted of DPPH solution (0.5 mM; 0.05 mL) mixed with 0.2 mL of methanol [21]. The results, presented as the means ± standard deviations from three independent experiments, were determined using a quercetin calibration curve (concentration range 0.16–10 µg/mL).

2.5. Antimicrobial Activity

The antibacterial and antifungal activities were evaluated using the broth microdilution method according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The antibacterial activity was tested against Gram-positive (*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) and Gram-negative bacteria (*Salmonella* Typhimurium ATCC 14028, *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 12453, *Klebsiella pneumoniae* ATCC 13883 and *Pseudomonas aeruginosa* ATCC 9027) in Mueller–Hinton broth. The antifungal activity was evaluated against yeasts (*Candida glabrata* ATCC 90030, *C. albicans* ATCC 102231 and *C. parapsilosis* ATCC 22019) in Mueller–Hinton broth supplemented with 2% glucose. The extracts were first dissolved in dimethyl sulfoxide, then diluted in the appropriate broth medium, and subsequently subjected to serial two-fold dilutions in the same medium. The diluted extracts were then added to the wells of sterile 96-well microtiter plates (Nunc, Roskilde, Denmark) to obtain final concentrations ranging from 10 to 0.313 mg/mL. Inocula were prepared by adjusting fresh microbial cultures in sterile 0.85% sodium chloride to a turbidity equivalent to a 0.5 McFarland standard and were added to the wells of sterile 96-well microtiter plates to reach final densities of 5×10^5 CFU/mL for bacteria and 5×10^4 CFU/mL for yeasts. The minimum inhibitory concentration (MIC) was determined after incubation at 35 °C for 18–20 h by both visual inspection and spectrophotometric measurement of absorbance at 600 nm, with MIC defined as the lowest concentration of the extract that inhibited visible microbial growth. To assess the minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC), 5 µL from each well was transferred onto extract-free agar plates and incubated at 35 °C for 24 h, with the lowest dilution that prevented microbial growth recorded as MBC/MFC. All experiments were performed in triplicate, and the highest observed value was reported. Appropriate controls were included: a dimethyl sulfoxide control (final concentration of 10%, *v*/*v*), a positive control (inoculum without the tested extract) and a negative control (tested extract without inoculum).

2.6. Cell Culturing and the Evaluation of Cytotoxicity

The cytotoxicity of extracts was tested against non-cancerous VERO (ATCC: CCL-81, monkey kidney) cells and selected cell lines originating from cancers of the stomach (AGS; ATCC: CRL-1739, human gastric adenocarcinoma), hypopharynx (FaDu; ATCC: HTB-43, human hypopharyngeal squamous cell carcinoma) and colon (RKO; ATCC: CRL-2577, human colon cancer). The VERO cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Corning, Tewksbury, MA, USA) and cancer cell lines were maintained in Minimum Essential Medium (MEM; Corning). Antibiotics (Penicillin–Streptomycin Solution, Corning) and fetal bovine serum (FBS; Corning) were added to all cell media. Cytotoxicity was evaluated using the previously described MTT test methodology [22]. In summary, the cells were transferred into 96-well plates and incubated overnight. Following this, they were treated with serial dilutions of the tested extracts in cell media for 72 h. After treatment, the cell media was discarded, and the monolayers were washed with phosphate-buffered saline (PBS). MTT-supplemented media were added, and after 3 h, the formazan product was dissolved. Following overnight incubation, the absorbance was measured using the Synergy H1 Multi-Mode Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA) at 540 and 620 nm. The collected data was then exported to GraphPad Prism (version 9.0.0) to determine the CC_{50} values (50% cytotoxic concentration) from the dose–response curves using non-linear regression analysis. Lastly, based on the CC_{50} values, selectivity indexes (SIs) were calculated (SI = CC_{50}VERO/CC_{50}Cancer; SI > 1 indicates anticancer selectivity) to evaluate the specificity towards cancer cell lines.

2.7. Antiviral Screening

Antiviral screening was performed against the human herpesvirus type 1 (HHV-1, ATCC, VR-260) propagated in VERO cells. The experiments were conducted in the BSL-2 laboratory following previously described experimental procedures [23]. The infectious titers ($CCID_{50}$/mL—50% cell culture infectious concentration) were assessed using an endpoint dilution assay. The antiviral screening evaluated the influence of extracts on the formation of HHV-1-induced cytopathic effect (CPE) in virus-infected VERO cells. In summary, VERO cells were cultured in 48-well Falcon plates (clear, flat-bottom, TC-treated, Corning) and exposed to a suspension of HHV-1 (100-fold $CCID_{50}$/mL) in cell media (500 µL/well) for 1 h, with at least two wells remaining uninfected as controls. After the viral infection, the media were removed, the monolayers were rinsed with PBS, and the non-toxic concentrations of extracts were diluted in the cell media and added. Control wells containing uninfected VERO cells (cell control) were supplied with media holding 2% FBS, as were the virus-infected wells that did not receive treatment (virus control). The plates were incubated until cytopathic effects (CPEs) appeared in the virus control wells. Afterwards, the plates were observed through an inverted microscope (CKX41, Olympus Corporation, Tokyo, Japan) fitted with a camera (Moticam 3+, Motic, Hong Kong, China) and assessed for any inhibition of CPE by the tested extracts in comparison to the virus control, and the results were documented (Motic Images Plus 2.0, Motic).

3. Results and Discussion

3.1. Chemical Composition of P. trifida Dry Methanol Extracts

Based on high-resolution mass spectrometry (HRMS) data acquired using a LC-DAD-QTOF-MS/MS instrument (Table 2; Figures S4 and S5), a total of 30 specialized metabolites were detected in the dry methanol extracts of *P. trifida* roots, leaves and fruits: nine phenolic acids (**1**, **2**, **4**–**6**, **8**, **9**, **16** and **18**), three flavonoids (**13**–**15**), 16 coumarins, including one simple coumarin (**3**), four dihydrofuranocoumarins (**10**, **19**, **28** and **29**) and 11 other furanocoumarins (**7**, **17**, **20**–**27** and **30**), as well as two additional benzofuran derivatives (**11** and **12**). Both the

root and fruit extracts contained 19 compounds, whereas 16 compounds were detected in the leaf extract. Phenolic acids included one hydroxycinnamic acid—caffeic acid (**6**)—along with two hexosides of protocatechuic and coumaric acids (**1** and **4**) and six depsides of quinic acid with caffeic, coumaric or ferulic acid (**2**, **5**, **8**, **9**, **16** and **18**). Caffeic acid (**6**) was detected based on its fragmentation pattern, with characteristic ions corresponding to the successive losses of CO_2 and H_2O (at m/z 135.0445 and 117.0352). The hexosides of phenolic acids **1** and **4** were detected based on the presence of characteristic fragment ions of protocatechuic acid at m/z 108.0212 and coumaric acid at m/z 119.0478, along with the ones resulting from the losses of hexose units at m/z 152.0120 and 163.0368, respectively [24]. Depsides were characterized by the fragment ions resulting from the loss of their dehydrated units of caffeic acid (**2**, **5**, **16** and **18**; 162 Da), quinic acid (**2**, **5**, **8**, **9**, **16** and **18**; 179 Da), coumaric acid (**8**; 146 Da) and/or ferulic acid (**9**; 176 Da) [25–27]. Among these compounds, chlorogenic (**5**), caffeic (**6**) and 3,5-di-*O*-caffeoylquinic (**16**) acids were identified using commercial standards. 1,5-Di-*O*-caffeoylquinic acid (**18**) was previously isolated from the dry methanol extract of the underground parts of *Hieracium scheppigianum* [20] and used as a standard compound in the current research.

Flavonoids (**13**–**15**) were characterized by the fragment ions, which resulted from the sugar losses, and by fragmentation patterns of the aglycones. Aglycone identities were further confirmed through UV spectra. Two quercetin glycosides (**14** and **15**) exhibited the following: (1) fragment ions corresponding to the aglycone at *m/z* 301.0316 and 301.0362, formed by the loss of a hexose (**14**; 162 Da) or hexuronic acid unit (**15**; 176 Da); and (2) characteristic fragmentation patterns of quercetin at m/z 271.0232 and 255.0297. The remaining flavonoid (**13**) was deoxyhexosyl hexosyl kaempferol, with an aglycone signal at m/z 285.0397, resulting from the loss of a deoxyhexosylhexose unit (308 Da) [28,29]. Using a standard compound, **14** was revealed to be quercetin 3-*O*-glucoside (isoquercitrin). Chlorogenic (**5**), caffeic (**6**) and 1,5-di-*O*-caffeoylquinic (**18**) acids, as well as isoquercitrin (**14**), were previously identified in the dry methanol extract of flowering aerial parts of *P. heyniae* H. Duman & M. F. Watson [30].

Among 16 coumarins, 11 were furanocoumarins (**7**, **17**, **20**–**27** and **30**) that contained one or more substituents at positions 5 and/or 8 (linear type) or 5 and/or 6 (angular type). These included one methoxy group (characterized by a loss of a 15 Da fragment; **21**, **22** and **24**), two methoxy groups (successive loss of two fragments of 15 Da; **23**), one hexosyloxy group (loss of a 162 Da fragment; **7**), one prenyloxy group (loss of a 68 Da fragment; **27** and **30**), or an oxygenated prenyloxy group with either one (loss of an 84 Da fragment; **25** and **26**) or two oxygen atoms (loss of a 102 Da fragment; **17** and **20**) [31,32]. The furanocoumarins also exhibited distinct UV spectra, depending on the type and number of oxygen atoms attached to the aromatic ring [33]. Xanthotoxin (**21**), isopimpinellin (**23**), bergapten (**24**) and imperatorin (**27**) were identified using commercial standards, while oxypeucedanin (**26**) was previously isolated from the dry dichloromethane extract of *P. trifida* fruits [17] and used as a standard compound in the current research. Additionally, heraclenol (**17**), oxypeucedanin hydrate (**20**), sphondin (**22**), heraclenin (**25**) and isoimperatorin (**30**) were identified previously in dry dichloromethane extracts of *P. trifida* roots and fruits [17], consistent with findings from this study. These furanocoumarins are common for plants from the Apiaceae family, including those from the *Prangos* genus [17].

Four further detected furanocoumarins can be classified as dihydrofuranocoumarins (**10**, **19**, **28** and **29**). One hydroxypropyl 2′,3′-dihydrofuranocoumarin (**19**) was detected based on characteristic fragment ions in positive ion mode, including a fragment ion at *m*/*z* 229.0845, corresponding to the loss of a water molecule from the hydroxypropyl unit (18 Da), and another at *m*/*z* 175.0379, indicating the loss of the entire hydroxypropyl unit along with two carbons from the dihydrofuran ring (72 Da). This structure corresponded to marmesin or nodakenin, previously reported in the dry methanol extracts of the aerial parts of *P. ferulacea* (L.) Lindl. and *P. peucedanifolia* Fenzl [34]. Another dihydrofuranocoumarin (**29**) was detected based on a characteristic fragment ion at *m*/*z* 229.0826, corresponding to the loss of the isopropylidenylacetyl unit (100 Da), along with fragmentation ions identical to those observed for compound **19**. This dihydrofuranocoumarin was identified as prantschimgin (isopropylidenylacetyl-marmesin), consistent with our findings from a previous study [17]. The compound **28** exhibited the same spectral characteristics as prantschimgin, indicating that it is an isomer of prantschimgin. A dihydrofuranocoumarin hexoside (**10**) and a simple hydroxycoumarin hexoside (**3**) were revealed based on the characteristic loss of hexose (162 Da) and the fragmentation patterns (*m*/*z* 187.0453 for **10** and 119.0479 and 107.0482 for **3**) of their respective aglycones in positive ion mode, as previously described in the literature [35]. For example, a dihydrofuranocoumarin hexoside—apterin and a hydroxycoumarin hexoside—umbelliferone 7-*O*-glucoside (skimmin), were previously reported in various Apiaceae species [19].

The remaining two compounds were benzofuran derivatives (**11** and **12**), detected based on their fragmentation patterns in negative ion mode, with fragment ions at *m*/*z* 205.0499, 187.0396, 161.0606 and 105.0707 for **11** and at *m*/*z* 235.0608, 217.0488, 191.0697 and 161.0201 for **12** [36,37]. The structure of **11** was consistent with cnidioside A, previously isolated from the dry methanol extract of the roots of *P. heyniae* [38], while **12** corresponded to either picraquassioside A or cnidioside B, previously reported in the dry methanol extracts of the aerial parts of *P. ferulacea* and *P. peucedanifolia* [34].

The highest amounts of detected compounds were determined (using external standards) for the fruit extract (189.82 mg/g), followed by the root and leaf extracts (75.68 and 68.09 mg/g) (Table 2). However, the root and leaf extracts were notably richer in polyphenols: phenolic acids (60.70 mg/g root extract and 26.45 mg/g leaf extract) and flavonoids (37.13 mg/g leaf extract). In contrast, the fruit extract was mainly composed of coumarins: dihydrofuranocoumarins and other furanocoumarins (89.60 and 92.70 mg/g). Dominant compounds were chlorogenic and 3,5-di-*O*-caffeoylquinic acids (**5** and **16**) in the root extract (26.14 and 20.84 mg/g), chlorogenic acid and hexuronyl quercetin (**5** and **15**) in the leaf extract (18.20 and 28.04 mg/g), and oxypeucedanin hydrate and prantschimgin (**20** and **29**) in the fruit extract (46.50 and 71.64 mg/g). Compared to previously investigated dry dichloromethane extracts of the roots and fruits of this plant [17], it can be concluded that dichloromethane extracts contained a greater proportion of coumarins. However, due to the abundance of coumarins in fruits, they are still present in considerable amounts in the fruit extract obtained by successive extraction with methanol. Namely, in the root dichloromethane and methanol extracts, coumarins were present in amounts of 199.4 and 14.98 mg/g, and in the fruit dichloromethane and methanol extracts in amounts of 479.3 and 182.3 mg/g (preliminary investigation of the dry dichloromethane extract of leaves revealed very low amounts of coumarins).

Table 2. Chemical composition of investigated *P. trifida* dry methanol extracts (DMEs).

Rt (min)	Molecular Mass	Calculated Mass	Diff (ppm)	Molecular Formula	Pseudomolecular Ion	Fragment Ions	Compound	Root DME [3]	Leaf DME	Fruit DME
12.82	316.0794	315.0722	−8.37	$C_{13}H_{16}O_9$	315.0748 $[M-H]^-$	152.0120, 108.0212	Protocatechuic acid hexoside (**1**) [24] [1]	-	t	t
14.98	354.0951	353.0878	−7.61	$C_{16}H_{18}O_9$	353.0905 $[M-H]^-$	191.0553, 179.0346, 135.0442	Caffeoylquinic acid (**2**) [25]	3.01 ± 0.02	4.39 ± 0.52	-
15.42	324.0845	369.0827	−6.73	$C_{15}H_{16}O_8$	369.0849 $[M+HCOOH-H]^-$ /325.0910 $[M+H]^+$	161.0230/163.0377, 119.0479, 107.0482	Hydroxycoumarin hexoside (**3**) [35]	-	t	t
16.68	326.1002	325.0929	−7.08	$C_{15}H_{18}O_8$	325.0952 $[M-H]^-$	163.0368, 119.0478	Coumaroyl hexoside (**4**) [24]	-	t	-
17.83	354.0951	353.0878	−7.61	$C_{16}H_{18}O_9$	353.0905 $[M-H]^-$	191.0545	Chlorogenic acid (**5**) [25] [2]	26.14 ± 0.54	18.20 ± 0.03	0.45 ± 0.00
18.58	180.0423	179.035	−5.65	$C_9H_8O_4$	179.0360 $[M-H]^-$	135.0445, 117.0352, 107.0473	Caffeic acid (**6**) [24] [2]	t	-	-
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	Hydroxypsoralene hexoside (**7**) [32]	t	-	-
20.48	338.1002	337.0929	−2.10	$C_{16}H_{18}O_8$	337.0936 $[M-H]^-$	191.0557, 164.0459, 119.0476	Coumaroylquinic acid (**8**) [27]	t	t	-
21.20	368.1107	367.1035	−3.11	$C_{17}H_{20}O_9$	367.1046 $[M-H]^-$	191.0541, 173.0404	Feruloylquinic acid (**9**) [26]	3.04 ± 0.36	1.76 ± 0.07	-
21.48	424.1369	469.1351	−8.30	$C_{20}H_{24}O_{10}$	469.1355 $[M+HCOOH-H]^-$, 423.1292 $[M-H]^-$ /447.1324 $[M+Na]^+$	179.0539, 89.0247/267.0693, 263.0970, 187.0453	Hydroxypropyl hydroxy 2′,3′-dihydrofuranocoumarin hexoside (**10**) [35]	-	-	t

Table 2. *Cont.*

Rt (min)	Molecular Mass	Calculated Mass	Diff (ppm)	Molecular Formula	Pseudomolecular Ion	Fragment Ions	Compound	Root DME [3]	Leaf DME	Fruit DME
22.00	368.1107	367.1035	−4.47	$C_{17}H_{20}O_9$	367.1051 $[M-H]^-$	205.0499, 187.0396, 161.0606, 105.0707	Hexosyloxy benzofuran propanoic acid (**11**) [36]	n.q.	n.q.	n.q.
23.14	398.1213	397.114	−8.49	$C_{18}H_{22}O_{10}$	397.1174 $[M-H]^-$	235.0608, 217.0488, 202.0242, 191.0697 176.0472, 161.0201	Hexosyloxy methoxy benzofuran propanoic acid (**12**) [37]	-	n.q.	n.q.
23.84	594.1585	593.1512	−7.92	$C_{27}H_{30}O_{15}$	593.1559 $[M-H]^-$ /595.1653 $[M+H]^+$	285.0397/449.1060, 287.0531	Deoxyhexosyl hexosyl kaempferol (**13**) [28]	-	t	-
24.21	464.0955	463.0882	−7.76	$C_{21}H_{20}O_{12}$	463.0918 $[M-H]^-$	301.0316, 300.0263, 271.0232, 255.0297	Quercetin 3-*O*-glucoside (isoquercitrin; **14**) [29] [2]	-	9.09 ± 0.52	0.55 ± 0.08
25.39	478.0747	477.0675	−3.00	$C_{21}H_{18}O_{13}$	477.0689 $[M-H]^-$	301.0362, 255.0257	Hexuronyl quercetin (**15**) [29]	-	28.04 ± 1.03	2.07 ± 0.20
25.98	516.1268	515.1195	−6.39	$C_{25}H_{24}O_{12}$	515.1228 $[M-H]^-$	353.0849, 191.0560, 179.0348, 173.0445, 135.0453	3,5-Di-*O*-caffeoylquinic acid (**16**) [25] [2]	20.84 ± 2.33	2.10 ± 1.42	1.54 ± 0.56
26.65	304.0947	327.0839	4.96	$C_{16}H_{16}O_6$	327.0824 $[M+Na]^+$	224.0062, 203.0328, 173.0247, 159.0777	Heraclenol (**17**) [31]	0.26 ± 0.04	-	1.08 ± 0.00
26.93	516.1268	515.1195	−6.39	$C_{25}H_{24}O_{12}$	515.1228 $[M-H]^-$	353.0863, 191.0555, 179.0343, 173.0447, 135.0437	1,5-Di-*O*-caffeoylquinic acid (**18**) [25] [2]	7.67 ± 0.19	t	t

Table 2. *Cont.*

Rt (min)	Molecular Mass	Calculated Mass	Diff (ppm)	Molecular Formula	Pseudomolecular Ion	Fragment Ions	Compound	Root DME [3]	Leaf DME	Fruit DME
26.98	246.0892	247.0965	−3.19	$C_{14}H_{14}O_4$	247.0957 $[M+H]^+$	229.0845, 213.0534, 175.0379, 147.0433	Hydroxypropyl 2′,3′-dihydrofuranocoumarin (**19**) [17]	t	t	t
28.57	304.0947	305.1020	8.11	$C_{16}H_{16}O_6$	305.0995 $[M+H]^+$	203.0330, 175.0368, 159.0434	Oxypeucedanin hydrate (**20**) [31]	2.97 ± 0.57	-	46.50 ± 6.18
31.99	216.0423	217.0495	8.96	$C_{12}H_8O_4$	217.0476 $[M+H]^+$	202.0246, 174.0301, 161.0587	Xanthotoxin (**21**) [31] [2]	3.82 ± 0.76	-	-
34.48	216.0423	217.0495	8.96	$C_{12}H_8O_4$	217.0476 $[M+H]^+$	202.0246, 174.0301, 161.0587	Sphondin (**22**) [31]	t	-	-
34.66	246.0528	247.0601	6.10	$C_{13}H_{10}O_5$	247.0586 $[M+H]^+$	232.0351, 217.0119, 189.0172, 161.0224	Isopimpinellin (**23**) [31] [2]	t	-	-
35.31	216.0423	217.0495	8.96	$C_{12}H_8O_4$	217.0476 $[M+H]^+$	202.0252, 174.0301, 161.0575	Bergapten (**24**) [31] [2]	t	-	-
35.57	286.0841	287.0914	3.50	$C_{16}H_{14}O_5$	287.0904 $[M+H]^+$	269.0761, 203.0336, 175.0395, 159.0433	Heraclenin (**25**) [31]	-	-	1.98 ± 0.00
37.72	286.0841	287.0914	3.50	$C_{16}H_{14}O_5$	287.0904 $[M+H]^+$	203.0327, 175.0362, 159.0434	Oxypeucedanin (**26**) [31] [2]	-	-	34.56 ± 4.59
43.14	270.0892	271.0965	8.83	$C_{16}H_{14}O_4$	271.0941 $[M+H]^+$	203.0326, 175.0374, 159.0429	Imperatorin (**27**) [31] [2]	1.92 ± 0.08	-	8.58 ± 0.34

Table 2. *Cont.*

Rt (min)	Molecular Mass	Calculated Mass	Diff (ppm)	Molecular Formula	Pseudomolecular Ion	Fragment Ions	Compound	Root DME [3]	Leaf DME	Fruit DME
44.48	328.1311	329.1384	3.20	$C_{19}H_{20}O_5$	329.1373 $[M+H]^+$	229.0826, 213.0530, 175.0373	Prantschimgin isomer (**28**) [17]	-	-	17.96 ± 0.50
45.55	328.1311	329.1384	8.38	$C_{19}H_{20}O_5$	329.1356 $[M+H]^+$	229.0848, 213.0539, 175.0380	Prantschimgin (**29**) [17] [2]	6.02 ± 0.17	t	71.64 ± 1.99
46.01	270.0892	271.0965	8.83	$C_{16}H_{14}O_4$	271.0941 $[M+H]^+$	203.0326, 175.0379, 159.0435	Isoimperatorin (**30**) [31]	t	-	t
							Phenolic acids	60.70	26.45	1.99
							Flavonoids	-	37.13	2.62
							Dihydrofuranocoumarins	6.02	t	89.60
							Other furanocoumarins	8.96	-	92.70
							Total quantified compounds	75.68	68.09	189.82

[1] References supporting recorded fragmentation. [2] Identity confirmed using commercial standard or previously isolated compound. [3] Results are expressed as mg/g dry extract; phenolic acids were quantified using calibration curve of chlorogenic acid, quercetin glycosides using calibration curve of isoquercitrin, furanocoumarins using calibration curves of xanthotoxin (xanthotoxin, heraclenin, heraclenol), oxypeucedanin (oxypeucedanin, oxypeucedanin hydrate) or imperatorin, and prantschimgin and its isomer using calibration curve of prantschimgin; t = trace (below LOQ); n.q. = not quantified.

3.2. Total Phenolic Content and Antioxidant Activity of *P. trifida* Dry Methanol Extracts

The total phenolic content (determined using Folin–Ciocalteu reagent) varied among the investigated *P. trifida* dry methanol extracts (Table 3), with the highest value found in the leaf extract (62.86 mg quercetin equivalents/g of dry extract), followed by the root extract (55.70 mg quercetin equivalents/g) and the fruit extract (39.31 mg quercetin equivalents/g). Since phenolics are known for their antioxidant properties, the extracts were further evaluated using the FRAP (ferric reducing antioxidant power) test and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (Table 3). Accordingly, the leaf extract exhibited the highest FRAP value (0.71 mmol Fe^{2+}/g of dry extract) and anti-DPPH activity (44.08 mg quercetin equivalents/g), followed by the root extract (FRAP: 0.60 mmol Fe^{2+}/g; anti-DPPH: 41.49 mg quercetin equivalents/g) and the fruit extract (FRAP: 0.35 mmol Fe^{2+}/g; anti-DPPH: 14.67 mg quercetin equivalents/g).

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

Assay	Root DME	Leaf DME	Fruit DME
TPC (mg quercetin equivalents/g DME)	55.70 ± 4.71	62.86 ± 7.66	39.31 ± 0.89
TAA (mmol Fe^{2+}/g DME) [1]	0.60 ± 0.08	0.71 ± 0.06	0.35 ± 0.04
Anti-DPPH activity (mg quercetin equivalents/g DME) [1]	41.49 ± 1.69	44.08 ± 0.74	14.67 ± 0.64

[1] Quercetin (positive control)—TAA = 35.70 ± 3.98 mmol Fe^{2+}/g, DPPH IC_{50} = 4.29 ± 0.16 µg/mL.

According to the literature data, chlorogenic and related caffeoylquinic acids, as well as quercetin glycosides detected in the leaf and/or root extracts, are well-known for their antioxidant activity and ability to scavenge free radicals [39–41]. For example, chlorogenic acid previously exerted antioxidant and cytoprotective effects associated with reduced reactive oxygen species (ROS) and malondialdehyde (MDA) levels, activation of nuclear factor erythroid 2-related factor 2 (Nrf2) signaling, and enhancement of endogenous antioxidant defenses, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione (GSH), heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase-1 (NQO-1) [41]. Thus, *P. trifida* roots and leaves may have practical applications in the food industry as a source of natural preservatives and functional ingredients. Additionally, detected bioactive polyphenols hold potential for pharmaceutical formulations aimed at preventing oxidative damage-related disorders [39].

3.3. Antimicrobial Activity of *P. trifida* Dry Methanol Extracts

The antimicrobial activity of *P. trifida* dry methanol extracts was tested using a microdilution assay on a range of Gram-positive and Gram-negative bacteria, as well as yeasts (Table 4). The best antibacterial activity was observed for the root and leaf extracts against a Gram-positive bacterium *Micrococcus luteus* (MIC = 0.625 and 1.25 mg/mL, MBC = 5 mg/mL), followed by *Staphylococcus aureus* (MIC = MBC = 5 mg/mL), *S. epidermidis* (MIC = 5 mg/mL, MBC = 10 mg/mL) and *Bacillus cereus* (MIC = 1.25 and 10 mg/mL, MBC > 10 mg/mL). The fruit extract exhibited the strongest activity against *S. epidermidis* (MIC = 0.625 mg/mL). The leaf extract was generally the only one showing activity against Gram-negative bacteria, with MIC and MBC values of 10 mg/mL. Regarding yeasts, the best activity in the case of all extracts was observed against *Candida albicans* (MIC = 1.25–5 mg/mL, MFC = 10 mg/mL), followed by *C. parapsilosis* (MIC = 2.5–10 mg/mL, MFC = 10 mg/mL) and *C. glabrata* (MIC = MFC = 10 mg/mL) in terms of sensitivity, with the fruit extract exhibiting slightly better activity compared to the root and leaf extracts.

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

Microorganisms	Root DME		Leaf DME		Fruit DME	
Gram-positive bacteria	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus ATCC 25923	5	5	5	5	>10	ND [1]
S. aureus ATCC BA1707	>10	>10	10	10	>10	ND
S. epidermidis ATCC 12228	5	10	5	10	0.625	5
Micrococcus luteus ATCC 10240	0.625	5	1.25	5	>10	ND
Bacillus cereus ATCC 10876	1.25	>10	10	>10	2.5	>10
Enterococcus faecalis ATCC 29212	1.25	>10	>10	>10	>10	ND
Gram-negative bacteria	MIC	MBC	MIC	MBC	MIC	MBC
Salmonella Typhimurium ATCC 14028	>10	ND	10	10	>10	ND
Escherichia coli ATCC 25922	>10	ND	10	10	>10	ND
Proteus mirabilis ATCC 12453	>10	ND	10	10	>10	ND
Klebsiella pneumoniae ATCC 13883	>10	ND	10	10	10	>10
Pseudomonas aeruginosa ATCC 9027	>10	ND	10	10	>10	ND
Yeasts	MIC	MFC	MIC	MFC	MIC	MFC
Candida glabrata ATCC 90030	10	10	10	10	10	10
C. albicans ATCC 102231	2.5	10	5	10	1.25	10
C. parapsilosis ATCC 22019	10	10	10	10	2.5	10

[1] ND—activity not determined.

The results obtained for the fruit methanol extract, which mainly contained coumarins, are consistent with our previous research on the antimicrobial activity of coumarin-rich dichloromethane extracts of *P. trifida* roots and fruits, which likewise exhibited stronger antifungal than antibacterial activity [17]. The literature data suggest that dominant polyphenols, such as chlorogenic acid in the root and leaf extracts and quercetin glycosides in the leaf extract, contribute to the observed activity against bacteria and yeasts [40,41], whereas the dominant coumarin in the fruit extract—prantschimgin in the first place—affects the activity against yeasts [42]. For example, prantschimgin previously exhibited notable activity against *C. albicans* (MIC = 0.03 mg/mL), whereas its activity against *S. aureus*, *E. coli* and *P. aeruginosa* was at least eightfold weaker [42]. Given the observed antimicrobial properties, particularly against foodborne pathogens (such as *S. aureus* and *B. cereus*) and yeasts, *P. trifida* roots, leaves and fruits could be regarded as potential sources of natural preservatives for the food industry, as well as of active ingredients in pharmaceuticals, offering a natural alternative to synthetic chemicals [40,41,43].

3.4. Cytotoxicity, Anticancer Selectivity and Antiviral Screening of P. trifida Dry Methanol Extracts

The cytotoxicity results obtained in the MTT assay were evaluated in accordance with the recommendations of the National Cancer Institute (NCI) [44] and previous studies [45,46]. Considering the proposed classification and the results presented in Table 5, it can be concluded that *P. trifida* extracts were not cytotoxic (CC_{50} > 500 µg/mL) to non-cancerous VERO (monkey kidney) cells. *Prangos trifida* leaf methanol extract was also not cytotoxic to cancer-derived cells—AGS (stomach cancer), FaDU (hypopharynx cancer) and RKO (colon cancer). Similar observations were made for *P. trifida* root methanol extract, except for FaDu cells, where weak cytotoxicity (CC_{50} between 200 and 500 µg/mL) was found. *Prangos trifida* fruit methanol extract was weakly cytotoxic to AGS and RKO, while moderate cytotoxicity (20 < CC_{50} < 200 µg/mL) was found for the FaDu cell line. Interestingly, the fruit extract selectively inhibited the proliferation of FaDu cells compared to non-cancerous VERO cells with an SI of 4.71. The selectivity was lower against AGS and RKO, with SI of 1.85 and 2.67, respectively. Although the CC_{50} for the root extract on VERO could not be evaluated in the tested concentration range, the viability of these

cells was dose-dependently decreased by this extract (Figure 1A), and even at 125 µg/mL, the cellular viability was below 90%. Therefore, for antiviral screening using VERO cells, the dose of 60 µg/mL was chosen. In the case of the leaf extract used at 1000 µg/mL, the average VERO viability was approx. 90% (Figure 1B), but microscopic evaluation revealed changes in cellular morphology, and for subsequent antiviral assays, a lower dose of 500 µg/mL was selected. Figure 1C confers selective anticancer cytotoxicity of the fruit extract against all cancer-originating cell lines, compared to non-cancerous VERO cells. For antiviral screening, the dose of 250 µg/mL was applied.

Table 5. Cytotoxicity and anticancer selectivity of investigated *P. trifida* dry methanol extracts (DMEs).

DME	VERO	AGS		FaDu		RKO	
	CC_{50} [1]	CC_{50}	SI [2]	CC_{50}	SI	CC_{50}	SI
Root	>1000	769.80 ± 76.22	>1.30	394.93 ± 10.82	>2.53	580.58 ± 23.98	>1.72
Leaf	>1000	1173.50 ± 221.92	>0.85	594.37 ± 28.16	>1.68	957.65 ± 28.55	>1.04
Fruit	626.28 ± 14.48	338.10 ± 34.23	1.85	132.85 ± 4.48	4.71	234.23 ± 18.55	2.67

[1] CC_{50}—50% cytotoxic concentration (µg/mL; mean ± SD). [2] SI—selectivity index.

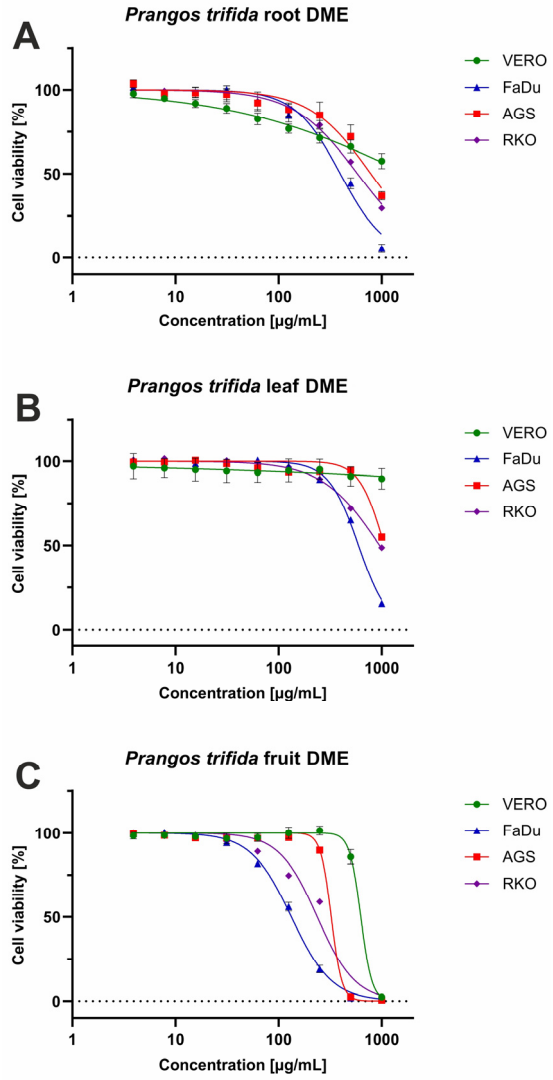

Figure 1. Dose–response effect of dry methanol extracts (DMEs) obtained from *P. trifida* roots (**A**), leaves (**B**) and fruits (**C**) on a panel of cell lines.

The typical cytopathic effect (CPE) induced by human herpesvirus type 1 (HHV-1) in VERO cells (HHV-1 virus control) is shown in Figure 2A, while non-infected VERO cell control is presented in Figure 2B. The incubation of *P. trifida* extracts did not influence the formation of HHV-1-induced CPE in virus-infected VERO cells (Figure 2C,E,F). That is why no further antiviral tests, such as endpoint titration or viral load reduction assays, were carried out. Acyclovir, used as a control antiviral substance, successfully suppressed the formation of CPE in virus-infected cells.

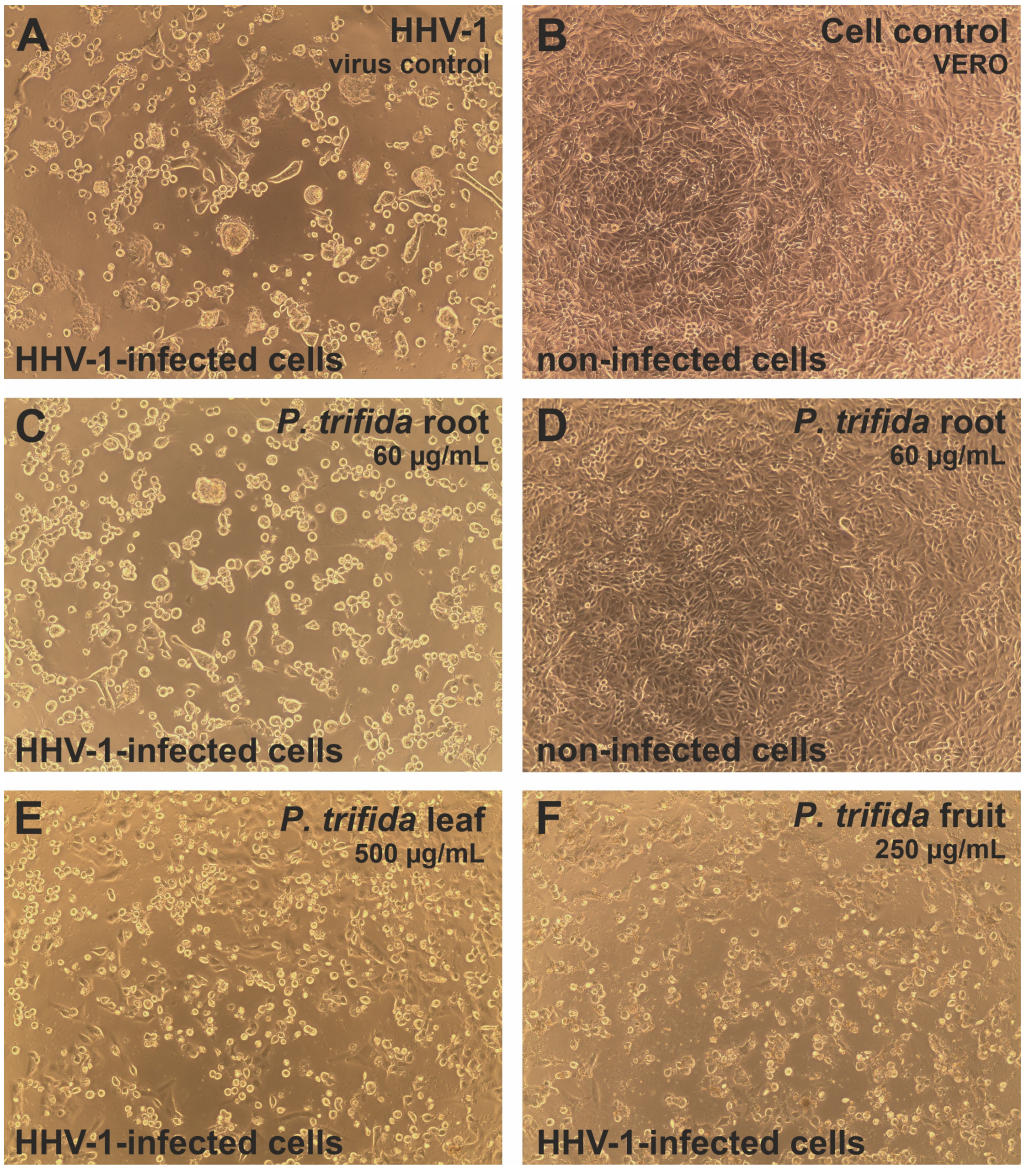

Figure 2. Influence of *P. trifida* extracts on the HHV-1-induced cytopathic effect in virus-infected VERO cells ((**A**)—HHV-1-induced cytopathic effect in VERO cells, virus control; (**B**)—non-infected VERO cells, cell control; (**C**)—influence of the root extract 60 µg/mL on HHV-1-infected VERO cells; (**D**)—influence of the root extract 60 µg/mL on non-infected VERO cells; (**E**)—influence of the leaf extract 500 µg/mL on HHV-1-infected VERO cells; (**F**)—influence of the fruit extract 250 µg/mL on HHV-1-infected VERO cells); magnification 40×.

The obtained cytotoxicity results are in agreement with the previously reported literature data [47,48]. For example, oxypeucedanin (**26**) has previously demonstrated cytotoxic activity against gastric cancer AGS cells, which were also used in our study. Additionally, it exhibited activity against HT-29 colorectal adenocarcinoma cells [48], whereas the current study utilized PKO colon cancer cells. The literature data also indicate that prantschimgin

(**29**) has shown cytotoxicity against two colorectal cancer cell lines, HCT 116 and HT-29 [47]. Overall, these findings suggest that *P. trifida* fruits may represent a potential source of cytotoxic compounds of interest for further investigation in pharmaceutical research.

4. Conclusions

This study provides new insights into the chemical composition of *P. trifida*, reporting for the first time its polar constituents, including phenolic acids and flavonoids. Performed chemical analysis and demonstrated bioactivities of the tested dry methanol extracts from roots, leaves and fruits revealed that *P. trifida* could serve as a new natural source of compounds relevant to the food and pharmaceutical industries. In particular, the roots and leaves are potential reservoirs of natural antioxidants and antimicrobial agents for use as food preservatives and functional food ingredients, as well as pharmaceutical components. Meanwhile, the fruits appear to be a possible source of compounds that are promising candidates for further research into antifungal and anticancer activity.

Additionally, it should be noted that caution should be exercised regarding the medicinal use of plants rich in furanocoumarins due to their potential phototoxicity and other adverse effects. Therefore, furanocoumarin-rich extracts may be more suitable as sources for isolation and further pharmacological and toxicological evaluation of individual bioactive compounds rather than for direct use as crude herbal preparations. In contrast, polyphenol-rich extracts may have greater potential for direct application, provided that their safety and the absence of undesirable constituents are confirmed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/analytica7020040/s1, Figure S1. Representative DAD chromatograms of *P. trifida* root (320 nm), leaf (350 nm) and fruit (250 nm) extracts. Numbered peaks correspond to the quantified compounds listed in Table 2. In addition, a benzofuran derivative (11) is also indicated; Figure S2. Representative UV spectra of the quantified phenolic acids and flavonoids. Compound numbers correspond to those given in Table 2; Figure S3. Representative UV spectra of the quantified coumarins, as well as of a benzofuran derivative (11). Compound numbers correspond to those given in Table 2; Figure S4. QTOF-MS/MS spectra of the detected compounds obtained in the negative ionization mode using collision energies of 10 V (left) and 30 V (right). Compound numbers correspond to those given in Table 2; Figure S5. QTOF-MS/MS spectra of the detected compounds obtained in the positive ionization mode using collision energies of 10 V (left) and 30 V (right). Compound numbers correspond to those given in Table 2.

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Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Materials. Further inquiries can be directed to the corresponding authors.

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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
$CCID_{50}$	50% cell culture infectious concentration
CFU	Colony-forming unit
CPE	Cytopathic effect
DAD	Diode-array detector
DMEM	Dulbecco's modified Eagle's medium
DPPH	2,2-diphenyl-1-picrylhydrazyl
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
HHV-1	Human herpesvirus type 1
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
SI	Selectivity index
TPTZ	2,4,6-tris(2-pyridyl)-s-triazine
VERO	Monkey kidney cells

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