Anticancer potential of acetone extracts from selected *Potentilla* species against human colorectal cancer cells

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Cinquefoils have been widely used in local folk medicine in Europe and Asia to manage various gastrointestinal inflammations and/or infections, certain forms of cancer, thyroid gland disorders, and wound healing. In the present paper, acetone extracts from aerial parts of selected *Potentilla* species, namely *P. alba* (PAL7), *P. argentea* (PAR7), *P. grandi flora* (PGR7), *P. norvegica* (PN7), *P. recta* (PRE7), and the closely related *Drymocalis rupestris* (syn. *P. rupestris*) (PRU7), were analysed for their cytotoxicity and antiproliferative activities against human colon adenocarcinoma cell line LS180 and human colon epithelial cell line CCD841 CoN. Moreover, quantitative assessments of the total polyphenolic (TPC), total tannin (TTC), total proanthocyanidins (TPrC), total flavonoid (TFC), and total phenolic acid (TPAC) were conducted. The analysis of secondary metabolite composition was carried out by LC-PDA-HRMS. The highest TPC and TTC were found in PAR7 (339.72 and 246.92 mg gallic acid equivalents (GAE)/g extract, respectively) and PN7 (332.11 and 252.3 mg GAE/g extract, respectively). The highest TPrC, TFC, and TPAC levels were found for PAL7 (21.28 mg catechin equivalents (CAT)/g extract, 71.85 mg rutin equivalents (RE)/g extract, and 124.18 mg caffeic acid equivalents (CAE)/g extract, respectively). LC-PDA-HRMS analysis revealed the presence of 83 compounds, including brevifolincarboxylic acid, ellagic acid, pedunculagin, agrimoniin, chlorogenic acid, astragalin, and tiliroside. Moreover, the presence of tri-coumaroyl spermidine was demonstrated for the first time in the genus *Potentilla*. Results of the MTT assay revealed that all tested extracts decreased the viability of both cell lines; however, a markedly stronger effect was observed in the colon cancer cells. The highest selectivity was demonstrated by PAR7, which effectively inhibited the metabolic activity of LS180 cells (IC₅₀ = 3.8 μg/ml), while at the same time causing the lowest unwanted effects in CCD841 CoN cells (IC₅₀ = 1.134 μg/ml). BrdU assay revealed a significant decrease in DNA synthesis in both examined cell lines in response to all investigated extracts. It should be emphasized that the tested extracts had a stronger effect on colon cancer cells than normal colon cells, and the most significant antiproliferative properties were observed in the case of PAR7 (IC₅₀ LS180 = 174 μg/ml) and PN7 (IC₅₀ LS180 = 169 μg/ml). The results of
Introduction

The modern world struggles with the increasing problem of cancer, a significant cause of death worldwide. In 2020, the third most commonly diagnosed type of cancer after breast and lung cancers was colorectal cancer, estimated to represent 10.0% of total cancer cases and the second leading cause of cancer death (9.4% of total cancer deaths) (Sung et al., 2021). However, due to the Western lifestyle, which is closely associated with low physical activity, a high-fat diet, and high red meat consumption, the projected number of global new colorectal cancer cases will rise from 1.93 million in 2020 to 3.15 million cases in 2040 (Xi and Xu, 2021). Therefore, the economic burden of treatment and the high mortality rate of patients resulting from cancer recurrence after chemotherapy suggests a significant need for more efficient and safer drug candidates. However, access to the most effective and modern diagnostic methods and treatments is limited for a large proportion of people. Especially in rural areas, people predominantly still depend on phytotherapy (Edgar et al., 2007). Notably, Potentilla species, known as cinquefoils, are widely used, since they are well known in traditional medicine throughout the Asian and European continents as valuable phytomedicines in a remedy inter alia against diarrhoea, ulcers, fever, jaundice, oral inflammations, topical infections, and thyroid gland disorders (Tomczyk and Latte, 2009). Moreover, ancient Chinese medical works, in particular Compendium of Chinese Materia Medica and Mingyi Bielu mentioned that aerial parts of two Potentilla species, namely P. indica and Duchesnea chrysantha were used as anticancer agents in monotherapy or as a main ingredient of complex formulas against unspecified types of cancers (Peng et al., 2009). A number of studies have reported on the abundance of secondary metabolites in Potentilla species, which determine their anti-inflammatory, antimicrobial, and antioxidative properties (Augustynowicz et al., 2021b). Moreover, earlier studies on several Potentilla species have shown their anti-cancer potential against various cell lines, e.g., triterpenoids isolated from P. chinensis were cytotoxicity against MCF7 (human breast cancer), Hep G2 (human hepatocellular carcinoma) and T84 (human colonic adenocarcinoma), while extracts and fractions from aerial parts of P. alba decreased proliferation and viability of HT-29 (human colon adenocarcinoma) (Zhang et al., 2017; Kowalik et al., 2020).

We hypothesized that aerial parts of selected Potentilla species, similarly to other species from this genus, would exhibit broad pharmacological potential. Therefore, the primary aim of our study was to assess their cytotoxicity and antiproliferative activities against human colon adenocarcinoma cell line LS180 and human colon epithelial cell line CCD841 CoN. Moreover, we identified the marker metabolites present in extracts through LC-PDA-HRMS analysis to uncover correlations between the qualitative chemical composition of extracts and their possible mechanism of action.

Materials and methods

Reagents

The reference substances, including procyanidin B1, procyanidin B2 and procyanidin C1 were obtained from Cayman Chemical (Ann Arbor, MI, United States). Quercetin 3-O-glucuronide, kaempferol 3-O-glucuronide andisorhamnetin 3-O-glucoside were obtained from Extrasynthese (Genay, France) (±)-Catechin, (±)-epicatechin and gallic acid were the products of Carl Roth (Karlsruhe, Germany). Quercetin 3-O-glucoside, quercetin 3-O-rutinoside, kaempferol 3-O-glucoside (purity >96%) were isolated from flowers of Ficaria verna L. Hud (Ranunculaceae) (Gudej and Tomczyk, 1999). Quercetin 3-O-galactoside (purity >96%) was isolated from aerial parts of Rubus saxatilis L. (Rosaceae) (Tomczyk and Gudej, 2005) and pedunculagin was isolated from leaves of Rubus caesius L. (Rosaceae) (Grochowski et al., 2020). Quercetin 3-O-arabinofuranoside, ellagic acid and tiliroside (purity >96%)

LDH assay revealed that all tested extracts were not cytotoxic against normal colon epithelial cells, whereas in the cancer cells, all compounds significantly damaged cell membranes, and the observed effect was dose-dependent. The highest cytotoxicity was observed in LS180 cells in response to PAR7, which, in concentrations ranging from 25 to 250 μg/ml, increased LDH release by 110%–1,062%, respectively. Performed studies have revealed that all Potentilla species may be useful sources for anti-colorectal cancer agents; however, additional research is required to prove this definitively.

KEYWORDS

Potentilla, Rosaceae, LC-PDA-HRMS, polyphenols, colorectal cancer, LS180 cells, cytotoxicity
were isolated from aerial parts of *Drymocolis rupestris* (L.) Sojak (Rosaceae) (Tomczyk, 2011). Agrimoniin and ellagic acid 3,3′-di-O-methyl ether 4-O-xyloside (purity >96%) were isolated from aerial parts of *P. recta* (Tomczyk, 2011; Bazyliko et al., 2013). Apigenin and 3-O-cafeoylquinic acid (purity >96%) were isolated from leaves and inflorescences of *Arctium tomentosum* Mill. (Asteraceae) (Strawa et al., 2020). All other chemicals of analytical grade used in the study were purchased from Sigma-Aldrich (St. Louis, MO, United States). A POLWATER DL3-100 Labopol (Kraków, Poland) assembly was used to obtain ultra-pure water. Working solutions of investigated compounds were prepared by dissolving an appropriate stock solution in a culture medium. The final concentration of DMSO in all working solutions used in the studies was the same including control and it was 0.25%.

### Plant materials and procedure of plant extracts preparation

Seeds of five species, namely *P. alba* (ind. sem. 354), *P. grandiﬂora* (ind. sem. 758), *P. norvegica* (ind. sem. 303), *P. recta* (ind. sem. 1549) and *P. rupestris* (ind. sem. 763) were kindly provided by the Botanical Garden of Vilnius University (Vilnius, Lithuania), Giardino Botanico Alpino (Cogne, Italy), Hortus Botanicus Universitatis Masarykianae (Brno, Czech Republic) and Hortus Botanicus University of Tartu (Tartu, Estonia). Plants were cultivated in common plots at the Medicinal Plant Garden at the Medical University of Białystok (Białystok, Poland), and aboveground materials were collected in June-August 2016–2019. Aerial parts of *P. argentea* were collected in June–July 2017–2019 from natural habitat, at Puszcza Knyżyska (Poland, 53°15′6″N, 23°27′58″E). The taxonomic identification of plant material was carefully authenticated by one of the authors (M.T.). Voucher specimens of *P. alba* (PAL-17039), *P. argentea* (PAR-02009), *P. grandiﬂora* (PGR-06020), *P. norvegica* (PNO-08024), *P. recta* (PRE-06019) and *P. rupestris* (PRU-06021) have been deposited at the Herbarium of the Department of Pharmacognosy, Medical University of Białystok (Poland). Collected dried materials were subsequently finely grounded with an electric grinder and stored in air-tight containers at ambient temperature. Powdered dry plant materials (2.0 g each time) were separately submitted to ultrasound-assisted extraction with 70% acetone (3 × 50 ml) using an ultrasonic bath (Sonic-5, Polasonic, Warszawa, Poland) at a controlled temperature (40 ± 2 °C) for 45 min in a 1:75 (w:v) solvent ratio. The obtained raw extracts after solvent evaporation were diluted with water (50 ml) and subsequently portioned with chloroform (10 × 20 ml). The acetone extracts were obtained using this method for *P. alba* (PAL7), *P. argentea* (PAR7), *P. grandiﬂora* (PGR7), *P. norvegica* (PN7), *P. recta* (PRE7) and *P. rupestris* (PRU7).

### Determination of total phenolic content

The total phenolic content (TPC) was measured by the Folin-Ciocalteu assay with some modifications (Slinkard and Singleton, 1977). Briefly, 25 µl of tested solution (1 mg/ml) was mixed with 100 µl of diluted Folin-Ciocalteu reagent (1:9, v/v) and the mixture was allowed to react for 3 min. Thereafter, 75 µl of 1% Na2CO3 solution was added and the prepared mixture was incubated for 2 h at ambient temperature. The absorbance was measured at 760 nm using a microplate reader EPOCH2 BioTech (Winooski, VT, United States). The TPC determination was repeated at least three times for each sample solution. Obtained results were expressed as milligrams of gallic acid equivalents per Gram of dry extract (mg GAE/g extract).

### Determination of total tannin content

The total tannin content (TTC) of each extract was measured by the employment of the protein-binding method and Folin-Ciocalteu assay described in the European Pharmacopoeia 10th ed (European Pharmacopoeia, 2019), with modifications. Briefly, each extract dissolved in water (1 mg/ml) was partitioned into two parts. For the first part of extracts total polyphenols were determined for each aliquot (25 µl) by mixing with 100 µl of diluted Folin-Ciocalteu reagent (1:9, v/v). After 3 min 75 µl of 1% Na2CO3 was added and the mixture was allowed to stand for 2 h at room temperature. Thereafter the absorbance of each sample (A1) was recorded at 760 nm using a EPOCH2 microplate reader. Subsequently, the second part of aliquots of 0.5 ml each were mixed with 10 mg of hide powder. These preparations were shaken for 1 h without light and then centrifugated. A 25 µl of supernatants were assayed for total polyphenolics as described above and the absorbance of each sample (A2) was recorded at 760 nm. Afterwards, the total tannin content was determined by subtraction of absorbances of total polyphenols (A1) from total non-tannin polyphenols (A2) and the obtained absorbance values were referred to a gallic acid calibration curve to obtain their values as milligrams of gallic acid equivalents per Gram of dry extract (mg GAE/g extract). The determination of TTC was repeated at least three times for each sample solution.
Determination of total proanthocyanidin content

The total proanthocyanidin content (TPrC) was analysed with the employment of a 4-dimethylamino-cinnamaldehyde (DMCA) reagent (Feliciano et al., 2012). The analysis was carried out in a microplate reader. A 50 µl of sample solution (1 mg/ml) dissolved in methanol was mixed with 250 µl of 0.1% DMCA in 6 M HCl in methanol. The mixture was incubated at ambient temperature for 15 min, and thereafter, the absorbance was recorded at 635 nm. The TPrC determination was repeated at least five times for each sample solution and was expressed as milligrams of catechin equivalents per Gram of dry extract (mg CE/g extract).

Determination of total flavonoid content

The total flavonoid content (TFC) of each extract was determined using the previously described aluminium chloride (AlCl₃) colorimetric method (Augustynowicz et al., 2021a) with slight modifications. In brief, 100 µl of tested solution or 100 µl of blank sample (methanol) was mixed with 250 µl of 0.1% DMCA in 6 M HCl in methanol. The mixture was incubated at ambient temperature for 15 min, and thereafter, the absorbance was recorded at 635 nm. The TFC determination was repeated at least at least five times for each sample solution and was expressed as milligrams of catechin equivalents per Gram of dry extract (mg CE/g extract).

Determination of total phenolic acid content

The total phenolic acid content (TPAC) determination was carried out using the procedure with the use of Arnov’s reagent (1 g of sodium molybdate and 1 g of sodium nitrate dissolved in 10 ml of distilled water) (Polumackanycz et al., 2019). A 30 µl of the tested solution, 180 µl of water, 30 µl of 0.5 M HCl, 30 µl of Arnov’s reagent and 30 µl of 1 M NaOH were sequentially added to the microplate well. After incubation of mixture at room temperature for 20 min, the absorbance was measured at 490 nm. The TPAC determination was repeated at least three times for each sample solution and the obtained values were expressed as milligrams of caffeic acid equivalents per Gram of dry extract (mg CAE/g extract).

Estimation of qualitative composition with the employment of LC-PDA-HRMS

Evaluation of the secondary metabolite composition of each extract was conducted using an Agilent 1260 Infinity LC chromatography system coupled to a photo-diode array (PDA) detector and 6230 time-of-flight (TOF) mass spectrometer (Santa Clara, CA, California). The MS conditions were as follows: electrospray ionization (ESI) source in both negative and positive ionization mode, drying and sheath gas flow 11 L/min and temperature of 350 °C, nebulizer pressure of 60 psi, capillary voltages of 2,500 and 4500 V for negative and positive ion modes, respectively and fragmentor experiments at 60, 180 and 320 V. The data were collected in the 120–3,000 m/z range. The separation was performed using a Kinetex XB-C18 column (150 × 2.1 mm, 1.7 µm, Phenomenex, Torrance, CA, United States). The mobile phases were ultra pure water (A) and acetonitrile (B) with 0.2% formic acid. The separation was achieved by a gradient of 0–3 min 65% B; 3–35 min 1% B, 35–80 min 12% B, 80–113 min 45% B, extended by 7 min of equilibrating time. The flow rate was 0.2 ml/min, and the column temperature was maintained at 35 ± 0.8°C. The UV-vis spectra were recorded in the range of 190–540 nm with selective wavelength monitoring at 280 and 360 nm. Data were processed with the employment of MassHunter Qualitative 10.0. Analysis software. Compounds were characterized based on UV–Vis and MS spectra and retention time of standards.

### Table 1

| Samples | TPC (mg GAE/g extract) | TTC (mg GAE/g extract) | TPrC (mg CE/g extract) | TFC (mg RE/g extract) | TPAC (mg CAE/g extract) |
|---------|------------------------|------------------------|------------------------|-----------------------|------------------------|
| PAL7    | 159.87 ± 1.79          | 84.89 ± 1.40           | 21.28 ± 0.04           | 71.85 ± 1.40          | 124.18 ± 1.18          |
| PAR7    | 339.72 ± 5.29          | 246.92 ± 4.64          | 6.95 ± 0.07            | 56.79 ± 0.98          | 78.95 ± 0.90           |
| PGR7    | 228.36 ± 3.40          | 156.53 ± 3.71          | 3.80 ± 0.06            | 47.61 ± 0.35          | 58.61 ± 0.34           |
| PN7     | 332.11 ± 1.40          | 252.30 ± 1.70          | 1.14 ± 0.02            | 38.06 ± 0.79          | 92.78 ± 1.03           |
| PRE7    | 257.68 ± 2.95          | 170.45 ± 2.86          | 2.70 ± 0.08            | 43.37 ± 0.84          | 75.20 ± 1.23           |
| PRU7    | 304.08 ± 2.51          | 209.43 ± 2.57          | 1.11 ± 0.02            | 47.74 ± 0.73          | 55.45 ± 0.59           |

*GAE, gallic acid equivalent; CE, catechin equivalent; RE, rutin equivalent; CAE, caffeic acid equivalent.

Augustynowicz et al. 10.3389/fphar.2022.1027315
| No. | Compounds                        | Rt [min] | UV spectra [Å max nm] | Observed* λ\_max [nm] | Δ [ppm] | Formula          | Fragmentation  | Presence in extracts | Ref                        |
|-----|----------------------------------|----------|-----------------------|------------------------|---------|------------------|------------------|---------------------|----------------------------|
|     |                                  |          |                       |                         |         |                  | Negative | Positive | PAL7 | PAR7 | PGR7 | PN7 | PRE7 | PRU7 |
| 1   | Gallic acid                      | 5.70     | 270                   | 169.01370              | -3.44   | C\(_6\)H\(_6\)O\(_5\) | 169, 125 | +       | +    | +    | +    |     |       | (s)  |
| 2   | 2-Pyrene-4,6-dicarboxylic acid  | 6.65     | 316                   | 182.99276              | -3.46   | C\(_6\)H\(_6\)O\(_6\) | 366, 182, 139 | +       | +    | +    | +    |     |       |      |
| 3   | Pedunculagin α or β              | 15.35    | 260sh                 | 783.06883              | 0.38    | C\(_{34}\)H\(_{24}\)O\(_{22}\) | 783, 481, 301 | +       | +    | +    | +    |     |       |      |
| 4   | Polyphenol derivative            | 15.70    | 280                   | 337.11359              | -1.03   | C\(_{13}\)H\(_{22}\)O\(_{10}\) | 193, 125 | +       | +    | +    | +    |     |       |      |
| 5   | 5-O-Caffeoylquinic acid          | 20.07    | 295sh, 325            | 353.08747              | -0.96   | C\(_{34}\)H\(_{18}\)O\(_{10}\) | 353, 191, 179 | +       | +    | +    | +    |     |       | (s)  |
| 6   | Galloyl-HHDP-glucose             | 22.26    | 250h                  | 633.07245              | -0.24   | C\(_{22}\)H\(_{12}\)O\(_{14}\) | 633, 301 | +       | +    | +    | +    |     |       |      |
| 7   | Unknown                          | 22.45    | 276                   | 345.11788              | -2.82   | C\(_{15}\)H\(_{22}\)O\(_{9}\) | 345, 299, 161 | +       |       |     |     |     |     |       |      |
| 8   | Pedunculagin α or β              | 23.30    | 260sh                 | 783.06929              | 0.85    | C\(_{34}\)H\(_{24}\)O\(_{22}\) | 783, 481, 301 | +       | +    | +    | +    |     |       |      |
| 9   | Galloyl-HHDP-glucose             | 24.07    | 280h                  | 633.07359              | 0.36    | C\(_{27}\)H\(_{22}\)O\(_{18}\) | 633, 481, 301 | +       | +    | +    | +    |     |       |      |
| 10  | p-Coumaroylquinic acid isomer    | 24.41    | 308                   | 337.09247              | -0.92   | C\(_{34}\)H\(_{18}\)O\(_{10}\) | 337, 191, 163 | +       |       |     |     |     |     |       |      |
| 11  | p-Coumaroylquinic acid isomer    | 25.27    | 312                   | 353.09218              | -1.59   | C\(_{34}\)H\(_{18}\)O\(_{10}\) | 337, 191, 163 | +       |       |     |     |     |     |       |      |
| 12  | Procyanidin B1                   | 26.20    | 280                   | 577.13507              | 0.81    | C\(_{34}\)H\(_{18}\)O\(_{12}\) | 577, 289 | 579, 291, 139 | +   |       |     |     |     |     |       |      |
| 13  | Catechin                         | 27.10    | 280                   | 289.07136              | -1.42   | C\(_{15}\)H\(_{14}\)O\(_{6}\) | 289, 245 | 291, 139 | +   | +    | +    |     |       | (s)  |
| 14  | 3-O-Caffeoylquinic acid          | 28.21    | 295sh, 325            | 353.08729              | -1.45   | C\(_{16}\)H\(_{18}\)O\(_{9}\) | 353, 191, 179 | +       | +    | +    | +    |     |       |      |
| 15  | Digalloyl-HHDP-glucose           | 28.56    | 275                   | 785.08369              | -0.63   | C\(_{34}\)H\(_{26}\)O\(_{22}\) | 785, 301 | +       |       |     |     |     |     |       |      |
| 16  | Feruloylquinic acid isomer       | 29.86    | 295sh, 325            | 367.10365              | 0.61    | C\(_{17}\)H\(_{18}\)O\(_{10}\) | 367, 193 | 369, 177 | +   |       |     |     |     |     |       |      |
| 17  | Caffeoylquinic acid isomer       | 30.91    | 295sh, 325            | 353.08779              | -0.08   | C\(_{17}\)H\(_{18}\)O\(_{10}\) | 353, 191, 179 | 355, 163 | +   |       |     |     |     |     |       |      |
| 18  | Galloyl-HHDP-glucose             | 31.61    | 275                   | 633.07306              | -0.27   | C\(_{34}\)H\(_{18}\)O\(_{14}\) | 633, 463, 301 | +       | +    | +    | +    |     |       |      |
| 19  | Brevifolin carboxylic acid       | 32.11    | 278, 360              | 291.01385              | -2.02   | C\(_{13}\)H\(_{8}\)O\(_{8}\) | 291, 247 | 293 | +   | +    | +    | +    |     |       |      |
| 20  | Procyanidin B2                   | 33.73    | 278                   | 577.13556              | 0.82    | C\(_{28}\)H\(_{14}\)O\(_{12}\) | 577, 289 | 579, 291, 139 | +   |       |     |     |     |     |       |      |
| 21  | Ellagic acid derivative          | 33.89    | 285sh                 | 898.13313              | -1.54   | C\(_{36}\)H\(_{32}\)O\(_{18}\) | 898, 633, 301 | +       |       |     |     |     |     |       |      |
| 22  | Ellagic acid derivative          | 34.13    | 325sh                 | 632.06474              | -1.85   | C\(_{22}\)H\(_{24}\)O\(_{14}\) | 632, 463, 301 | +       |       |     |     |     |     |       |      |
| 23  | Quercetin O-hexosyl O-uronic acid derivative | 34.20 | 255, 350 | 639.12125 | 1.20 | C\(_{22}\)H\(_{24}\)O\(_{14}\) | 639, 300 | 641, 478, 303 | + |       |     |     |     |     |       |      |
| 24  | Quercetin O-di-uronic acid derivative | 34.40 | 255, 355 | 653.09958 | -0.45 | C\(_{22}\)H\(_{24}\)O\(_{14}\) | 653, 477, 301 | 655, 479, 303 | + | +    | +    | +    |     |     |       |      |
| 25  | p-Coumaroylquinic acid isomer    | 35.13    | 312                   | 337.09285              | 0.02    | C\(_{16}\)H\(_{18}\)O\(_{9}\) | 337, 191 | 339, 147 | +   |       |     |     |     |     |       |      |

(Continued on following page)
| No. | Compounds                                                                 | Rt [min] | UV spectra [λ max nm] | Observed* Δ [ppm] | Formula Fragmentation | Presence in extracts | Ref |
|-----|---------------------------------------------------------------------------|---------|----------------------|-------------------|----------------------|---------------------|-----|
| 26  | Quercetin O-hexoso O-uronic acid derivative                               | 35.35   | 270, 350             | 639.12105 -0.61   | C_{27}H_{30}O_{16}  | 639, 463, 301       | +   |
|     |                                                                          |         |  |                   | 465, 303           |                     |     |
| 27  | Epicatechin                                                              | 35.74   | 280                  | 289.07133 -1.43   | C_{15}H_{14}O_{6}  | 289, 245            | +   |
|     |                                                                          |         |  |                   | 291, 139           |                     | (s) |
| 28  | 2-Caffeoylisocitric acid                                                 | 36.30   | 300b, 328            | 353.05046 -2.69   | C_{15}H_{18}O_{9}  | 353, 191, 155       | +   |
|     |                                                                          |         |  |                   | 339, 147           |                     |     |
| 29  | p-Coumaroylquinic acid isomer                                            | 36.52   | 312                  | 337.09314 -1.43   | C_{16}H_{18}O_{7}  | 337, 163            | +   |
|     |                                                                          |         |  |                   | 339, 147           |                     |     |
| 30  | Caffeoylmalic acid                                                       | 38.51   | 295b, 326            | 295.04541 -3.26   | C_{15}H_{14}O_{7}  | 295, 295, 179, 153  | +   |
|     |                                                                          |         |  |                   |                  |                     |     |
| 31  | Kaempferol O-di-uronic acid derivative                                    | 38.97   | 265, 350             | 637.10470 -0.62   | C_{27}H_{26}O_{16} | 637, 463, 287       | +   |
|     |                                                                          |         |  |                   | 463, 287           |                     |     |
| 32  | Trigalloylglucose isomer                                                 | 39.10   | 276                  | 635.08918 0.20    | C_{27}H_{28}O_{18} | 635, 463, 303       | +   |
|     |                                                                          |         |  |                   | 300, 169           |                     |     |
| 33  | Brevifolin                                                               | 39.4    | 275, 350             | 247.02433 -1.83   | C_{12}H_{18}O_{6}  | 247, 191            | +   |
|     |                                                                          |         |  |                   | 249               |                     | +   |
| 34  | Procyanidin A-type trimer                                                | 40.51   | 280                  | 863.18353 1.19    | C_{45}H_{36}O_{18} | 863, 573, 289       | +   |
|     |                                                                          |         |  |                   | 865, 287           |                     |     |
| 35  | Ellagic acid O-hexoside derivative                                        | 41.20   | 252, 365             | 463.05162 -0.79   | C_{26}H_{16}O_{13} | 463, 301            | +   |
|     |                                                                          |         |  |                   |                  |                     |     |
| 36  | Isohamnetin O-di-uronic acid derivative                                   | 41.78   | 254, 352             | 667.11555 1.29    | C_{26}H_{18}O_{11} | 667, 315, 300       | +   |
|     |                                                                          |         |  |                   | 669               |                     | +   |
| 37  | Procyanidin C1                                                           | 41.88   | 276                  | 863.20796 0.68    | C_{46}H_{36}O_{18} | 865, 577, 289       | +   |
|     |                                                                          |         |  |                   | 867, 579, 291     |                     | (s) |
| 38  | Galloyl-bis-HHDP-glucose                                                 | 43.98   | 255                  | 935.07947 0.30    | C_{41}H_{28}O_{26} | 935, 300, 271       | +   |
|     |                                                                          |         |  |                   | 935, 467, 301     |                     |     |
| 39  | Laevigatin isomer                                                        | 44.46   | 255                  | 1,567.14331 -1.15 | C_{26}H_{16}O_{13} | 1,567, 783, 301     | +   |
|     |                                                                          |         |  |                   | 1,567, 301        |                     |     |
| 40  | Laevigatin isomer                                                        | 45.94   | 255                  | 1,567.14331 -1.15 | C_{26}H_{16}O_{13} | 1,567, 783, 301     | +   |
|     |                                                                          |         |  |                   | 1,567, 301        |                     |     |
| 41  | Quercetin O-hexoso O-deoxyhexoside isomer                                | 46.98   | 255, 352             | 609.16415 0.73    | C_{30}H_{16}O_{13} | 609, 446, 299       | +   |
|     |                                                                          |         |  |                   | 611, 499, 303     |                     |     |
| 42  | Galloyl-bis-HHDP-glucose                                                 | 47.33   | 276b                 | 935.07900 -0.79   | C_{46}H_{36}O_{26} | 935, 300, 271       | +   |
|     |                                                                          |         |  |                   | 935, 467, 301     |                     |     |
| 43  | Quercetin O-hexoso O-deoxyhexo-pentoside isomer                          | 47.90   | 255, 355             | 741.18912 -0.64   | C_{26}H_{16}O_{13} | 741, 447, 300       | +   |
|     |                                                                          |         |  |                   | 743, 465, 303     |                     |     |
| 44  | Quercetin O-deoxyhexo-O-hexoso-deoxyhexoside isomer                      | 48.66   | 256, 356             | 755.20326 -0.67   | C_{26}H_{16}O_{13} | 755, 464, 299       | +   |
|     |                                                                          |         |  |                   | 757, 449, 303     |                     |     |
| 45  | Quercetin O-hexoso-pentoside isomer                                      | 49.55   | 255, 355             | 595.13019 -0.10   | C_{26}H_{16}O_{13} | 595, 300, 271       | +   |
|     |                                                                          |         |  |                   | 597               |                     | +   |
| 46  | Quercetin O-hexoso-pentoside isomer                                      | 50.48   | 255, 355             | 595.13046 -0.72   | C_{26}H_{16}O_{13} | 595, 300, 271       | +   |
|     |                                                                          |         |  |                   | 597               |                     | +   |

*(Continued on following page)*
### Table 2 (Continued) MS and UV-Vis data of compounds detected in acetone extracts prepared from aerial parts of selected Potentilla species.

| No. | Compounds                        | Rt [min] | UV spectra [λ max nm] | Observed $\Delta$ [ppm] | Formula | Fragmentation | Presence in extracts | Ref                  |
|-----|----------------------------------|----------|-----------------------|--------------------------|---------|---------------|----------------------|----------------------|
|     |                                  |          |                       |                          |         |               | PAL7 | PAR7 | PGR7 | PN7 | PRE7 | PRU7 |
| 47  | Laevigatin isomer                | 51.09    | 255                   | 1.567.14239             | C$_{68}$H$_{48}$O$_{44}$ | 1,567, 783, 301 | +     |     |     |     | +     |     |
| 48  | Quercetin O-pentosyl-O-uronic acid derivative | 52.30    | 255, 354              | 609.11075               | C$_{69}$H$_{53}$O$_{47}$ | 609, 301, 611, 479, 303 | +     |     |     |     | +     |     |
| 49  | Ellagic acid 3′-O-methyl ether O-uronic acid derivative | 54.06    | 254, 360              | 491.04709               | C$_{62}$H$_{48}$O$_{44}$ | 491, 315, 301 | +     | +   | +   |     | +     |     |
| 50  | Ellagic acid O-pentoside          | 55.72    | 252, 360              | 433.04108               | C$_{62}$H$_{48}$O$_{44}$ | 433, 301                     | +     |     |     |     | +     |     |
| 51  | Ellagic acid                      | 56.74    | 254, 370              | 300.99841               | C$_{62}$H$_{48}$O$_{44}$ | 301, 271, 303 | +     | +   | +   |     | +     |     |
| 52  | Quercetin 3-O-glucoside           | 59.30    | 255, 355              | 463.08816               | C$_{62}$H$_{48}$O$_{44}$ | 463, 300, 271, 465, 303 + | +     | +   | +   |     | +     |     |
| 53  | Unknown                           | 59.80    | 290                   | 435.09238               | C$_{62}$H$_{48}$O$_{44}$ | 871, 435, 285, 151 | +     |     |     |     | +     |     |
| 54  | Ellagic acid 3′-O-methyl ether 4-O-pentoside | 60.40    | 252, 362              | 447.05600               | C$_{62}$H$_{48}$O$_{44}$ | 447, 301                     | +     |     |     |     | +     |     |
| 55  | Quercetin 3-O-rutinoside          | 63.38    | 256, 354              | 609.14571               | C$_{62}$H$_{48}$O$_{44}$ | 609, 300, 271, 465, 303 | +     | +   | +   |     | +     |     |
| 56  | Quercetin 3-O-galactoside         | 64.03    | 255, 355              | 463.08816               | C$_{62}$H$_{48}$O$_{44}$ | 463, 300, 271, 465, 303 | +     | +   | +   |     | +     |     |
| 57  | Quercetin 3-O-glucuronide         | 64.83    | 255, 355              | 477.06649               | C$_{62}$H$_{48}$O$_{44}$ | 477, 300, 271, 479, 303 | +     | +   | +   |     | +     |     |
| 58  | Kaempferol O-hexosylpentoside     | 66.55    | 265, 350              | 579.13594               | C$_{62}$H$_{48}$O$_{44}$ | 579, 284, 581, 449, 287 | +     |     |     |     | +     |     |
| 59  | Quercetin 3-O-arabinofuranoside   | 66.95    | 256, 354              | 477.06713               | C$_{62}$H$_{48}$O$_{44}$ | 477, 301, 479, 303                     | +     |     |     |     | +     |     |
| 60  | Kaempferol O-hexosylpentoside     | 66.84    | 265, 350              | 579.13520               | C$_{62}$H$_{48}$O$_{44}$ | 577, 284, 581, 449, 287 | +     |     |     |     | +     |     |
| 61  | Kaempferol O-hexosylpentoside     | 66.85    | 265, 350              | 579.13520               | C$_{62}$H$_{48}$O$_{44}$ | 577, 284, 581, 449, 287 | +     |     |     |     | +     |     |
| 62  | Kaempferol O-hexosylpentoside     | 67.40    | 252, 350              | 447.09365               | C$_{62}$H$_{48}$O$_{44}$ | 447, 284, 449, 287                     | +     |     |     |     | +     |     |
| 63  | Galloyl-bis-HHDP-glucose          | 69       | 260d                  | 935.07978               | C$_{62}$H$_{48}$O$_{44}$ | 935, 467, 301                     | +     | +   | +   |     | +     |     |
| 64  | Isorhamnetin O-hexosylpentoside   | 73.99    | 255, 352              | 609.14611               | C$_{62}$H$_{48}$O$_{44}$ | 609, 314, 271, 611, 479, 317 | +     |     |     |     | +     |     |
| 65  | Quercetin 3-O-arabinofuranoside   | 85.20    | 254sh, 350            | 433.07665               | C$_{62}$H$_{48}$O$_{44}$ | 433, 300, 435, 303 | +     | +   | +   |     | +     |     |
| 66  | Kaempferol 3-O-glucoside          | 88.33    | 265, 350              | 447.09298               | C$_{62}$H$_{48}$O$_{44}$ | 447, 284, 449, 287 | +     | +   | +   |     | +     |     |
| 67  | Kaempferol 3-O-glucuronide        | 89.30    | 265, 346              | 461.07171               | C$_{62}$H$_{48}$O$_{44}$ | 461, 285, 463, 287 | +     | +   | +   |     | +     |     |

(Continued on following page)
| No. | Compounds                                 | Rt [min] | UV spectra [λ max nm] | Observed$^a$ Δ [ppm] | Formula | Fragmentation | Presence in extracts | Ref                                           |
|-----|------------------------------------------|---------|-----------------------|----------------------|---------|---------------|---------------------|-----------------------------------------------|
|     |                                          |         |                       |                      |         |               | PAL7    | PAR7 | PGR7 | PN7 | PRE7 | PRU7 |               |
| 68  | Apigenin O-hexoside                      | 90.04   | 266, 340              | 431.09754            | -0.71   | C_{20}H_{16}O_{10} | 431, 268, 433, 271  | +                                            |
| 69  | Agrimonin                                | 90.30   | 250sh, 365            | 447.05604            | -0.72   | C_{20}H_{10}O_{12} | 447, 315, 301        | +                                            |
| 70  | Ellagic acid 3'-O-methyl ether 4-O-pentoside | 90.42   | 280sh, 365            | 477.10387            | 0.49    | C_{22}H_{22}O_{12} | 477, 314, 479, 317  | +                                            |
| 71  | Isorhamnetin 3-O-glucoside               | 91.43   | 265, 355              | 477.10387            | 0.49    | C_{20}H_{10}O_{12} | 939, 769, 469, 169  | +                                            |
| 72  | Pentagalloyloglucose isomer              | 91.68   | 280                   | 939.11105            | 0.49    | C_{41}H_{32}O_{26} | 939, 769, 469, 169  | +                                            |
| 73  | Isorhamnetin O-deoxyhexosyl-hexosyl-O-pentoside isomer | 91.92   | 254sh, 355            | 753.18766            | -0.45   | C_{22}H_{18}O_{20} | 753, 314, 299        | +                                            |
| 74  | Isorhamnetin O-uronic acid derivative    | 92.81   | 255, 354              | 491.08356            | 0.77    | C_{22}H_{18}O_{12} | 491, 315, 301        | +                                            |
| 75  | Ellagic acid 3,3'-di-O-methyl ether 4-O-xylloside | 94.14   | 245, 370              | 461.07148            | -1.26   | C_{22}H_{18}O_{12} | 461, 328, 297        | +                                            |
| 76  | Quercetin O-uronic acid derivative       | 94.80   | 270sh, 370            | 477.06758            | 0.10    | C_{22}H_{18}O_{12} | 477, 301            | +                                            |
| 77  | Kaempferol derivative                    | 94.90   | 266sh, 340            | 533.09391            | 0.27    | C_{20}H_{16}O_{14} | 533, 489, 284        | +                                            |
| 78  | Apigenin                                 | 98.04   | 268, 338              | 269.04538            | -2.04   | C_{15}H_{10}O_{5}  | 269, 227            | +                                            |
| 79  | trans-Tiliroside                         | 101.48  | 268, 315              | 593.12979            | -0.55   | C_{20}H_{18}O_{13} | 593, 284, 595, 287  | +                                            |
| 80  | Kaempferol derivative                    | 101.87  | 268, 330              | 623.14131            | 1.02    | C_{20}H_{18}O_{14} | 623, 284            | +                                            |
| 81  | cis-Tiliroside                           | 102.37  | 268, 315              | 593.12928            | 0.44    | C_{20}H_{18}O_{13} | 593, 284            | +                                            |
| 82  | Unknown                                  | 105.54  | 280                   | 445.18621            | -1.13   | C_{20}H_{18}O_{13} | 445, 385            | +                                            |
| 83  | N', N', N''-tricoumaroyl spermidine      | 104.47  | 295, 310sh            | 582.26072            | -0.06   | C_{14}H_{11}N_{4}O_{4} | 582, 462, 584, 438, 292, 147 | +                                            |

$^a$Exact mass of [M-H]$^-$ ion; sh—peak shoulder; bold—most abundant ion; (s)—reference substance; HHDP, hexahydroxydiphenoyl group.
48 h of cell treatment, the compounds’ antiproliferative effect was determined using Cell Proliferation ELISA BrdU, following the manufacturer’s instructions (Roche Diagnostics GmbH, Penzberg, Germany), while the compounds’ cytotoxicity was examined by the In Vitro Toxicology Assay Kit Lactate Dehydrogenase Based according to the manufacturer’s instructions (Sigma-Aldrich, St. Louis, MO, United States). Furthermore, cell viability in response to 48 h of exposure to investigated compounds was determined by MTT assays. A detailed description of the execution of the above-mentioned assays was presented by Langner and co-authors (Langner et al., 2019).

### Cell cultures

Human colonic epithelial cell line CCD841 CoN was purchased from the American Type Culture Collection (ATCC, Manassas, VA, United States). Human colon adenocarcinoma cell line LS180 was obtained from the European Collection of Cell Cultures (ECACC, Centre for Applied Microbiology and Research, Salisbury, United Kingdom). Cell cultures were conducted in accordance with the guidelines of the collections in which they were purchased.

### Examination of the anticancer potential of extracts

Both colon epithelial, as well as colon adenocarcinoma cells, were seeded on 96-well microplates at a density of 5 × 10⁴ cells/mL. The following day, the culture medium was exchanged for fresh medium supplemented with investigated extracts or 25 μM 5-fluorouracil (5-FU). After 48 h of cell treatment, the compounds’ antiproliferative effect was determined using Cell Proliferation ELISA BrdU, following the manufacturer’s instructions (Roche Diagnostics GmbH, Penzberg, Germany), while the compounds’ cytotoxicity was examined by the In Vitro Toxicology Assay Kit Lactate Dehydrogenase Based according to the manufacturer’s instructions (Sigma-Aldrich, St. Louis, MO, United States). Furthermore, cell viability in response to 48 h of exposure to investigated compounds was determined by MTT assays. A detailed description of the execution of the above-mentioned assays was presented by Langner and co-authors (Langner et al., 2019).

### Statistical analysis

The analysed data were presented as the mean ± SEM. Statistical analyses were performed using One way-ANOVA with the Tukey post-hoc test and column statistics. Statistical significance was accepted at p < 0.05. The IC₅₀ value (concentration leading the 50% inhibition of proliferation compared to the control) was calculated using GraphPad PRISM.

### Results

In the first set of experiments, the studied extracts were examined for their TPC, TTC, TPrC, TFC, and TPAC using colorimetric methods. The obtained results are presented in Table 1. PAR7 and PN7, followed by PRU7, were found to contain the highest TPC (339.72, 332.11, and 304.08 mg GAE/g extract, respectively) and TTC (246.92, 252.30, and 209.43 mg GAE/g extract, respectively). Moreover, the highest TPAC values were revealed for PAL7 and PN7 (124.18 and 78.95 mg CAE/g extract, respectively).

To unveil the secondary metabolite composition, acetone extracts of selected Potentilla species were analysed via LC-PDA-HRMS. The analysis demonstrated the presence of 83 compounds, predominately polyphenolic compounds, ascribed to hydrolysable and condensed tannins, flavonoids, and phenolic acids. Hydrolysable tannins were present in all

### Table 3 IC₅₀ values (concentration causing viability/proliferation inhibition by 50% compared to control) of acetone extracts isolated from the aerial parts of Potentilla L and 5-fluorouracil (5-FU). IC₅₀ values were calculated for human colon epithelial cell line CCD841 CoN and human colon adenocarcinoma cell line LS180 based on results of MTT and BrdU assays performed after 48 h of cells treatment with investigated compounds.

| Sample | MTT assay | BrdU assay |
|--------|-----------|------------|
|        | IC₅₀ (µg/ml) | Trust range (µg/ml) | R² | IC₅₀ (µg/ml) | Trust range (µg/ml) | R² |
| L5180  |            |              |     |            |              |     |
|        | IC₅₀ (µg/ml) | Trust range (µg/ml) | R² | IC₅₀ (µg/ml) | Trust range (µg/ml) | R² |
| PAL7   | 182       | 169-196      | 0.983 | 233       | 209-261      | 0.971 |
| PAR7   | 38        | 32-44        | 0.974 | 1,134     | 575-2,235    | 0.902 |
| PGR7   | 58        | 50-67        | 0.957 | 982       | 498-1,938    | 0.890 |
| PN7    | 32        | 28-37        | 0.981 | 757       | 459-1,248    | 0.903 |
| PRE7   | 35        | 30-42        | 0.969 | 918       | 449-1,879    | 0.882 |
| PRU7   | 36        | 30-42        | 0.974 | 846       | 481-1,489    | 0.916 |
| 5-FU   | 31        | 28-33        | 0.977 | 113       | 81-157       | 0.884 |
|        | 12,008    | 2096-68,805  | 0.752 | 4,164     | 1759-9,859   | 0.867 |
|        | 174       | 165-183      | 0.982 | 217       | 203-231      | 0.977 |
|        | 372       | 338-409      | 0.968 | 570       | 488-666      | 0.965 |
|        | 169       | 159-179      | 0.974 | 217       | 202-233      | 0.958 |
|        | 237       | 223-251      | 0.966 | 268       | 248-289      | 0.943 |
|        | 360       | 311-416      | 0.95  | 538       | 425-681      | 0.926 |
|        | 15        | 13-16        | 0.956 | 94        | 80-111       | 0.933 |
extracts with the exception of PAL7, primarily represented by ellagitannins, such as pedunculagin α and β (3,8), agrimoniin (69), laevigatin isomers (39, 40, 47), galloyl-HHDP-glucose isomers (6, 9, 18), galloyl-bis-HHDP-glucose isomers (38, 42, 63), ellagic acid (51), ellagic acid 3,3′-di-O-methyl ether 4-O-xylloside (75), ellagic acid 3-O-methyl ether 4-O-xylloside (54), and ellagic acid 3-O-methyl ether 4-O-arabinoside (70). PRU7 showed the presence of gallotannins, such as tri-, tetra-, and pentagalloylgucose isomers (32, 55, 72). Moreover, the degradation products of hydrolysable tannins, namely brevifolin-carboxylic acid (19) and brevifolin (33), were found. On the other hand, PAL7 and PAR7 were rich in the condensed tannins, such as (+)-catechin (13) and (-)-epicatechin (27), and oligomeric procyanidins, such as procyanidin B1 (12), B2 (20), and C1 (37). A number of flavonoids were detected and characterized, including apigenin (78) and its 6-hexoside (68), isorhamnetin (36, 64, 71, 73, 74), kaempferol (31, 59, 61, 62, 66, 67, 77, 79–81), and quercetin (23, 24, 26, 41, 43–48, 52, 56–58, 60, 65, 76) derivatives. In addition, phenolic acids were also present in extracts, such as gallic acid (1), caffeic acid derivatives (5, 14, 17, 28, 30), p-coumaroylquinic acid isomers (10, 11, 25, 29), and N1, N5, N10-tricoumaroyl spermidine (83). The detailed chromatographic data are shown in Table 2 and in Supplementary Figures S1–S6.

In the next set of experiments, the viability of both human colon epithelial cell line CCD841 CoN and human colon adenocarcinoma cell line LS180 was examined in response to the investigated *Potentilla* extracts. Cells were exposed to either culture medium (control) or extracts (25–250 μg/ml) for 48 h and, afterward, an MTT test was performed. As presented in Figure 1 and Table 3, all investigated acetone extracts inhibited, in a dose-dependent manner, the metabolic activity of both the normal and cancer colon cell lines. The most significant anticancer effect was achieved by extract PN7, which at the
The highest tested concentration decreased LS180 cell proliferation by 87.3% (IC$_{50}$ PN7 LS180 = 32 $\mu$g/ml), while the weakest effect was noted for PAL7, which at a concentration of 250 $\mu$g/ml inhibited cancer cells division by 57.5% (IC$_{50}$ PAL7 LS180 = 182 $\mu$g/ml). In the case of colon epithelial cells, the strongest reduction (49.5%) of their metabolic activity was observed after exposure to 250 $\mu$g/ml PAL7 (IC$_{50}$ PAL7 CCD841 CoN = 233 $\mu$g/ml), while the weakest effect, as reflected by the IC$_{50}$ value, was for PAR7 (IC$_{50}$ PAR7 CCD841 CoN = 1,134 $\mu$g/ml). Although all investigated extracts affected both normal and cancer colon cells, LS180 cells were more sensitive to the tested compounds. Comparing the metabolic activity in both analysed cell lines in response to extracts at the corresponding concentrations, greater sensitivity of cancer cells was observed in the entire range of analysed concentrations in the case of PAR7, PRE7, PRU7, and PN7, while PGR7 showed statistically significant differences in concentrations from 50 $\mu$g/ml to 250 $\mu$g/ml. Even PAL7 at the highest tested concentrations (150 and 250 $\mu$g/ml) strongly inhibited the viability of cancer cells than colon epithelial cells. As a positive control of the experiment, 5-fluorouracil (5-FU) at a concentration of 25 $\mu$M was used (Figure 1). The metabolic activity of CCD841 CoN and LS180 cells decreased in response to 5-FU by 22.2% and 46.2%, respectively. Comparing data obtained from the extracts with cell responses to 5-FU revealed that four of six investigated fractions at higher concentrations inhibited the metabolic activity of CCD841 CoN cells more strongly than 25 $\mu$M 5-FU: PAL7 (100, 150, 250 $\mu$g/ml); PRE7 (150, 250 $\mu$g/ml); and both PAR7 and PRU7 (250 $\mu$g/ml). In the case of colon cancer cells, PGR7, PAR7, PRE7, PRU7, and PN7 at FIGURE 2
Antiproliferative effect of acetone extracts isolated from the aerial parts of Potentilla L. on human colon epithelial cell line CCD841 CoN and human colon adenocarcinoma cell line LS180. The cells were exposed for 48 h to the culture medium alone (control), or the extracts at concentrations of 25, 50, 100, 150, and 250 $\mu$g/ml, or 25 $\mu$M 5-fluorouracil (5-FU; positive control). The antiproliferative impact of the investigated compounds was measured by immunoassay based on BrdU incorporation into newly synthesized DNA. Results are presented as mean ± SEM of at least six measurements. **p < 0.01; ***p < 0.001 vs. control, #p < 0.05; ###p < 0.001 vs. positive control, p < 0.05; p < 0.01; p < 0.001 colon cancer cells treated with extract/5-FU vs. colon epithelial cells exposed to the extract/5-FU at the corresponding concentration; one-way ANOVA test; post-test: Tukey.
concentrations from 50 to 250 μg/ml and PAL7 at a concentration of 250 μg/ml showed a stronger anti-metabolic effect than the analysed cytostatic.

In the next step, the antiproliferative activity of Potentilla extracts was assessed in the abovementioned cell lines using BrdU assay (Figure 2; Table 3). A significant decrease of DNA synthesis in colon cancer cells was observed in response to all investigated extracts at concentrations ranging from 100 μg/ml to 250 μg/ml, and simultaneously in the case of PAR7 a statistically significant antiproliferative effect was also noted at the concentration 50 μg/ml. Furthermore, PAR7 and PN7 showed the strongest inhibition of cancer cell proliferation, as reflected by the lowest IC50 values (IC50 PAR7 LS180 = 174 μg/ml and IC50 PN7 LS180 = 169 μg/ml) and the greatest decrease of DNA synthesis in LS180 cells in response to the extracts at a concentration of 250 μg/ml (cell proliferation was reduced by 63.1% (PAR7) and 71.1% (PN7)). Colon cancer cell division was least inhibited by PAL7 (IC50 PAL7 LS180 = 12 mg/ml), which at the highest tested concentration decreased DNA synthesis by only 9.1%. The investigated extracts also affected the proliferation of colon epithelial cells and statistically significant inhibition of DNA synthesis was noted in response to all compounds at concentrations of 150 and 250 μg/ml, while in the case of PN7, PAR7, PRU7, and PGR7 the antiproliferative effect was observed also at a concentration of 100 μg/ml. Similar to the colon cancer cells, epithelial cells were the most sensitive to PN7 and PAR7, which at a concentration of 250 μg/ml reduced their proliferation by 58.4% and 53.4%, respectively (IC50 PN7 CCD841 CoN = 217 μg/ml and IC50 PAR7 CCD841 CoN = 217 μg/ml). The weakest

FIGURE 3
Influence of acetone extracts isolated from the aerial parts of Potentilla L. on cell membrane integrity of human colon epithelial CCD841 CoN cells and human colon adenocarcinoma LS180 cells. The cells were exposed for 48 h to the culture medium alone (control), or extracts at concentrations of 25, 50, 100, 150, and 250 μg/ml, or 25 μM 5-fluorouracil (5-FU; positive control). Compound cytotoxicity (level of LDH released into the cell culture medium from damaged cell membranes) was measured using LDH assay. Results are presented as mean ± SEM of at least six measurements. ***p < 0.001 vs. control, #p < 0.05; ##p < 0.01; ###p < 0.001 vs. positive control, p < 0.001 colon cancer cells treated with extract/5-FU vs. colon epithelial cells exposed to the extract/5-FU at the corresponding concentration; one-way ANOVA test; post-test: Tukey.
antiproliferative effect in CCD841 CoN cells was observed after exposure to PAL7, which at the highest tested concentration inhibited cell division by only 6.7%. Studies have revealed the antiproliferative abilities of Potentilla extracts in both normal and cancer colon cells, nevertheless PGR7 at the highest tested concentration, as well as PAR7, PRU7, and PN7 at concentrations 150 and 250 µg/ml, inhibited DNA synthesis significantly more strongly in LS180 cells than CCD841 CoN cells. As presented in Figure 2, 25 µM 5-fluorouracil (5-FU) decreased DNA synthesis in the investigated cell lines by 90.7% (CCD841 CoN) and 29.7% (LS180). The antiproliferative effect of 5-FU observed in colon cancer cells was significantly stronger than changes induced by examined extracts. On the contrary, data collected from colon epithelial cells revealed that five out of six investigated extracts in higher concentrations inhibited DNA synthesis more strongly than 25 µM 5-FU: both PAR7 and PN7 (100, 150, 250 µg/ml); PRE7 (150, 250 µg/ml); and both PGR7 and PRU7 (250 µg/ml). The obtained data indicated a higher selectivity of the analysed cytostatic compared with examined extracts in the case of influence on DNA synthesis.

In the last step of the in vitro studies, extracts cytotoxicity was examined in CCD841 CoN cells and LS180 cells using LDH-based assay. As presented in Figure 3, the tested extracts were not cytotoxic against human colon epithelial cells, while they significantly damaged the cell membranes of colon cancer cells, and the observed effect was dose-dependent. The strongest release of LDH was noted in LS180 cells in response to PAR7, which in concentrations ranging from 25 to 250 µg/ml increased the LDH level by 110% and 1,062%, respectively. Very similar results were obtained after LS180 cell exposure to PRU7, which in the mentioned range of concentrations increased LDH release by 161% (25 µg/ml) and 956% (250 µg/ml). The weakest cytotoxic effect was noted in colon cancer cells treated with PAL7, which at the highest tested concentration caused an increase in the LDH level of 68%. Used as a positive control, 5-FU at a concentration of 25 µM was not cytotoxic against colon epithelial or colon cancer cells (Figure 3). The LDH levels of the cells were 100.7% (CCD841 CoN) and 113.4% (LS180). All investigated extracts damaged colon cancer cell membranes more effectively than 5-FU, and this difference was especially evident in the case of PRU7, PAR7, PN7, and PRE7, which even at the lowest tested concentration (25 µg/ml) increased the LDH level to 261, 210, 185, and 156%, respectively.

**Discussion**

Many studies have shown that Potentilla species are a source of a wide spectrum of secondary metabolites, mainly polyphenols, such as hydrolysable and condensed tannins, flavonoids and their glycosides, and phenolic acids, which show a variety of biological activities (Augustynowicz et al., 2021b). Potentilla species have a long history of use to treat intestinal problems, such as diarrhoea, inflammatory bowel disease (Tomeczyk and Latte, 2009). Selected species for the present study are common in the Europe and were rarely selected as a subject of anticancer evaluation. Basing on the literature search we hypothesized that similarly to other species from this genus would exhibit anticancer potential. The quantitative identification of polyphenolic classes present in extracts using colorimetric methods offers information on their general contents. In our study, high TPC, TFC, and TTC values were observed in all tested samples. The TPC and TFC results were significantly higher compared to previous studies on aerial parts of P. argentea, P. grandiflora, P. recta, and P. norvegica (Tomeczyk et al., 2010; Sut et al., 2019; Augustynowicz et al., 2021a). The differences in results can be partially explained by the type of solvent used in the extraction process. Aqueous acetone during the extraction process inhibits the interaction between tannins and proteins, and decreases the cleaving of depside bonds in hydrolysable tannins in comparison to aqueous alcohols. These mechanisms may lead to higher contents of high-molecular tannins in acetone extracts (Hagerman, 1988; Mueller-Harvey, 2001). Moreover, on several occasions, acetone has been reported as a good solvent for the extraction of flavonoids with higher contents, in comparison to water and alcoholic solvents (Dirar et al., 2019; Patel and Ghane, 2021). Furthermore, LC-PDA-HRMS analysis revealed a number of polyphenols, such as ellagitannins and products of their degradation, flavonoids, and phenolic acids, that were present in all extracts. Ellagitannins are plant secondary metabolites that tend to form relatively high molecular weight dimers and oligomers, ranging from 300 to 20,000 Da. Plants from the Rosaceae family accumulate a series of oligomeric, macrocyclic oligomeric, and C-glycosidic ellagitannins that can be used as chemophenetic markers (Grochowski et al., 2017; Gesek et al., 2021). On several occasions the presence of dimeric ellagittannin - agrimonin in aerial parts of Potentilla species, in particular P. anserina and P. kleiniana, P. recta, have been reported (Okuda et al., 1982; Fecka, 2009; Bazyliko et al., 2013). The chromatographic analysis reported herein indicates that agrimonin is the most abundant ellagittannin in all extracts except PAL7. Moreover, several other phenolic compounds, such as pedunculagin, laevigatin, brevifolincarboxylic acid, and ellagic acid, an artifact, are released as a product of hydrolysis of ellagitannins. These compounds are widely present in the aerial parts of different species belonging to the genus Potentilla, including P. indica, P. freyniana, Duchesnea chrysanththa, and P. anserina, and therefore could be considered significant in the chemophenetics of this genus (Okuda et al., 1992; Lee and Yang, 1994; Fecka, 2009; Luo et al., 2020). Furthermore, flavonol derivatives, such as querectin 3-O-rutoside, querectin 3-O-galactoside, querectin 3-O-glucuronide, querectin 3-O-arabinoside, kaempferol 3-O-glucoside, kaempferol 3-O-glucuronide, and tiliroside, were found in at least one of the
17 investigated *Potentilla* species (Tomczyk and Latte, 2009; Augustynowicz et al., 2021b). More interestingly, N-acylated biogenic amine derivative, N1, N5, N10-tricoumaroyl spermidine, was reported for the first time in the genus *Potentilla*. This compound accumulates exclusively in the pollen coat and has been detected in several other genera in the Rosaceae family (Elejalde-Palmett et al., 2015).

The anticancer potential of acetone extracts isolated from selected *Potentilla* species was examined in both colon cancer LS180 cells as well as normal colon epithelial CCD841 CoN cells by investigation compounds influence on cell viability (MTT assay), proliferation (BrdU assay), and cytotoxicity (LDH assay). All investigated extracts decreased viability of both normal and cancer colon cells in a dose-dependent manner; however, LS180 cells were more sensitive to the tested compounds. The results of MTT assay indicated that the tested extracts effectively decreased the mitochondrial metabolism of human colon cancer cells, which could be associated with the presence of hydrolysable tannins in all extracts except the PAL7, which revealed the weakest anticancer effect. Moreover, the highest impact in decrease of cancer cells viability by PAR7, PN7 and PRU7 correlate with their highest TPC and TTC values. Agrimonini was shown to have prominent antioxidant, anti-inflammatory, and anticancer effects. Hoffman et al. (2016) found that agrimoninium-enriched fractions from rhizomes of *P. erecta* directly inhibit UVB-induced cyclooxygenase-2 (COX-2) expression and production of PGE2 in human keratinocytes (HaCaT), as well as in an *in vivo* model, and inhibit epidermal growth factor receptor (EGFR) phosphorylation. Shi et al. (2015) demonstrated that lyophilized strawberries (*Fragaria x ananassa*, Rosaceae) containing 16.2% agrimoninin downregulated the mRNA expression of COX-2, IL-1β, IL-6, TNF-α, and iNOS in AOM/DSS-induced colon cancer in mice. BrdU assay revealed a significant decrease of DNA synthesis in both colon cancer and non-cancer cells in response to all investigated extracts. The strongest antiproliferation effect in cancer cells was observed after treatment with PAR7 and PN7. Those extracts revealed to possess the highest total polyphenol and tannin contents. Notably, the antiproliferative effect of 5-FU observed in colon cancer cells was significantly stronger than that of the examined extracts. Similarly, data collected from colon epithelial cells revealed that five out of six investigated extracts in higher concentrations inhibited DNA synthesis stronger than the positive control. The *in vivo* bioavailability of high weight ellagitannins is relatively low. Ellagitannins at neutral or alkaline pH are hydrolysed with the release of free ellagic acid, which exerts a number of biological activities (Ismael et al., 2016). Whitley et al. (2003) found that the human colorectal adenocarcinoma cell line Caco-2 strongly accumulated ellagic acid and, furthermore, 93% of it was irreversibly bounded to cellular DNA and proteins. Moreover, ellagic acid significantly decreased the expression of genes involved in the p53, PI3K-Akt, mitogen-activated protein kinase (MAPK), and TGF-β signaling pathways in human colorectal carcinoma cell line HCT 116 (Zhao et al., 2017). Ellagic acid also reduced the viability of human nasopharyngeal carcinoma cell line NPC-BM1 via activation of caspase-3 and inhibition of Bcl-2 and telomerase (Huang et al., 2009). Our results are in agreement with the studies by Kowalik and co-authors (2020), showing that selected extracts and fractions from aerial parts of *P. alba* significantly decreased proliferation of human colon cancer HT-29 cells. Additionally, the authors found that selected extracts and fractions from *P. alba* increased proliferation of human normal epithelial CCD 841 CoTr cells. Moreover, the tested samples damaged cell membranes and decreased their viability (Kowalik et al., 2020). Kaempferol 3-O-glucoside, present in all investigated extracts, exhibits anti-inflammatory, antioxidant, and anticancer effects. A recent study conducted on human colon cancer HCT 116 cells revealed that kaempferol 3-O-glucoside induces cell apoptosis by increasing expression of pro-apoptotic caspases (caspase 3, caspase 6, caspase 7, caspase 8, and caspase 9), protein p53, and Bax, and decreasing expression of anti-apoptotic proteins, cleaved caspase 3, and Bcl-2. Moreover, the investigated compound causes G0/G1 arrest, inhibits the expression of metalloproteinases MMP-2 and MMP-9, and decreases the activity of the NF-κB signalling pathway (Yang et al., 2021). Notably, tiliroside isolated from *P. argentea* exerted inhibitory activity against topoisomerase I and II and showed moderate cytotoxicity against human breast carcinoma cell line MCF-7 (Tomczyk et al., 2008). Finally, the LDH assay showed that the tested extracts even at the lowest concentration (25 μg/ml) significantly damaged the cell membranes of investigated colon cancer cells, releasing the high doses of LDH into the cell culture medium. The weakest effect was observed for PAL7, which may be due to the absence of hydrolysable tannins, which modify the permeability of cell membranes. However, strong observed effect of rest of tested extracts can be explained by high TTC. Moreover, the exerted the strongest cytotoxic effects of PAR7 and PRU7 among all extracts can be explained by their higher TPC and TTC values. At the same time, all tested samples were not cytotoxic against normal colon cells. In a recent paper, Borisowa and co-authors (2019) found that hydrolysable tannins selectively block calcium-activated chloride channels and form selective pores in the cell membrane (Borisova et al., 2019). Moreover, pedunculagin increased cytotoxicity of 5-FU against human liver cancer cells QGY-7703, probably through increased permeability of the cancer cell membrane, as observed by the authors through a microscope (Xiao et al., 2012). A recent study revealed that agrimoninin stimulates apoptosis via the mitochondria pathway, inducing activity of the mitochondrial permeability transition pore (MTPP), which leads to mitochondria swelling and a decrease in energy production. Moreover, the authors found that agrimoninin is cytotoxic against K562 and HeLa cell lines (Fedotcheva et al., 2021). The *in vivo* effects of tannin-rich acetone extracts from
selected *Potentilla* species may vary from obtained *in vitro* results. A recent study on aerial parts of *P. anserina* and rhizomes of *P. erecta* revealed that human intestinal microbiota convert ellagitannins to urolithins, which possess potent anti-inflammatory and anticancer activities (Piwowarski et al., 2014). Moreover, several studies suggest that the chronic application of tannin-rich extracts may lead to iron-deficiency anemia. Hydrolysable tannins possess antinutritional properties, due to their potential to complex iron ions and reduce their absorption (Petry et al., 2010). However, those effects may be offset by the development of formulations with modified release of extract or by the inclusion in diet of other bioactive compounds, such as ascorbic acid, which prevents the inhibitory effect of polyphenols on iron absorption (Petroski and Minich, 2020). The acute complications of advanced stages of colorectal cancers includes a number of complications, such as bleeding, perforation and/or obstruction (Yang and Pan, 2014). Hydrolysable tannins are well known for their anti-bleeding properties. The tannin-rich extracts from *Potentilla* species may be used as potent, plant-based styptic agents as a complementary therapy in advanced stages of colorectal cancers.

**Conclusion**

In conclusion, this study reports, for the first time, analysis of the LC-PDA-HRMS profile of acetone extracts of selected *Potentilla* species. The analysis revealed the presence of several phenolic compounds, such as agrimoniin, pedunculagin, brevifolincarboxylic acid, ellagic acid, tiliroside, and tricoumaroyl spermidine. These secondary metabolites can be considered as chemophenetic markers for the genus *Potentilla*. Four of six investigated extracts (PAR7, PRE7, PRU7, PN7) showed great chemopreventive potential, manifested by the effective elimination of colon cancer cells, causing both damage to their cell membranes and inhibition of their proliferation and metabolic activity, with a simultaneous lack of a cytotoxic effect on normal colon epithelial cells and a significantly weaker effect on their metabolism and DNA synthesis compared to cancer cells. While it is impossible to specify the extract with the greatest therapeutic potential, these studies unequivocally showed that PAL7 had the lowest anti-cancer potential in a cellular model of colon cancer.

**Data availability statement**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

**Author contributions**

DA and MT designed the research, DA performed experiments, analysed the data, and wrote the draft of the manuscript. MKL and JWS performed experiments, analysed the data, and revised the manuscript. AW and MT supervised the research and revised the manuscript. All authors approved the submitted version.

**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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**Supplementary material**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.1027315/full#supplementary-material

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