THE INFLUENCE OF AQUEOUS EXTRACTS OF SELECTED *POTENTILLA* SPECIES ON NORMAL HUMAN COLON CELLS

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Abstract: *Potentilla* L. (Rosaceae) species have been used in traditional medicine in Asia, Europe and Northern America. This study analyzed the biological activity of aqueous extracts of *Potentilla* species (Rosaceae): *Dasiphora fruticosa* (syn. *P. fruticosa*), *P. norvegica, P. pensylvanica, P. thuringiaca, P. crantzii* and *P. nepalensis*. The activities were tested using MTT, NR and DPPH assays on normal human colon epithelium (CCD 841 CoTr) and colon myofibroblast (CCD-18Co) cells. Moreover, cell morphology using the May-Grünwald-Giemsa method, IL-6 by ELISA, and nitric oxide (NO) analysis with the Griess method in culture supernatants were performed after 24 h. Extracts were tested at dose levels between 25 and 250 µg/mL. For ELISA, 15 µg/mL was chosen. All extracts suppressed the metabolism of myofibroblasts, while epithelial cells' mitochondrial dehydrogenase activity decreased after incubation with extracts. All extracts showed a free radical scavenging (DPPH) effect in a concentration-dependent manner. The most potent was the extract from *D. fruticosa*, while the least action was observed for *P. thuringiaca*. *Potentilla* extracts stimulated IL-6 production in tested cells but the level of the cytokine was found to decrease in epithelial cells. Pre-incubation of cells with LPS resulted in increased IL-6 secretion. Modulation of NO production after extract addition and cell pre-incubation with LPS was also observed. *Potentilla* extracts may be interesting natural factors modulating the main features of cells forming the colon wall, and thus may be potentially useful in the prophylaxis or healing of colon disorders

Keywords: *Potentilla* L., Rosaceae, cytotoxicity, normal human colon cells

Natural products, especially plant extracts, have been used for millennia in the treatment of many diseases. More attention is now being devoted to the curative properties of plant extracts and the search for new natural substances which can be used in the treatment of different diseases.

Potentilla L. (Rosaceae) species have been used for a long time in traditional medicine in Asia, Europe and Northern America. Many of them are known for their therapeutic properties and are considered to be among the safest natural astringents in the treatment of diarrhoea, dysentery and sore throat. Recent investigations have also shown that some extracts of different parts of plants from *Potentilla* species exhibit potential antioxidant, hypoglycemic, anti-inflammatory, antitumor and anti-ulcerogenic properties (1). Phytochemical studies, of both the aerial and underground parts of plants, have concentrated on the isolation of many different classes of compounds. Among them, triterpenoids, condensed and hydrolyzable tannins, and flavonoids, as well as other ingredients including a series of organic acids, phenol carboxylic acids, sterols, sugars, amino acids and fatty acids have been detected in *Potentilla* species (1, 2). Recent pharmacological *in vitro* studies on *Potentilla* species have confirmed their anti-secretory, anti-inflammatory, antimicrobial and antioxidative effects. Application in *in vivo* test models also showed their different biological activities (1, 3, 4).

The aim of our study was to analyze the influence of aqueous extracts from selected *Potentilla*

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species: *Dasiphora fruticosa* (L.) Rydb. (syn. *Potentilla fruticosa* L.)*, Potentilla norvegica* L.*, Potentilla pensylvanica* L.*, Potentilla thuringiaca* Bernh. Ex Link*, Potentilla crantzii* (Crantz) J. Beck ex Fritsch and *Potentilla nepalensis* Hook. Var. 'Miss Willmott' on the viability and proliferation of normal human colon cells, lines: CCD 841 CoTr (normal colon epithelium) and CCD-18Co (colon myofibroblasts). Additionally, the subject of this study was the determination whether tested extracts have immunostimulatory, anti-inflammatory and reactive oxygen radical-scavenging properties. Therefore, the principal objective was to assess in *in vitro* studies whether *Potentilla* species used as food supplements may influence (and eventually to what degree may affect) normal human colon cell wall lining, and to evaluate their potential health benefits in humans.

Plant material

Seeds of six species (*D. fruticosa* – index seminum /ind. sem./ 2566, *P. norvegica* – ind. sem. 303, *P. thuringiaca* – ind. sem. 1551, *P. pensylvanica* – ind. sem. 1546, *P. crantzii* – ind. sem. 1534, *P. nepalensis* – ind. sem. 1542) were requested from the Hortus Botanicus Universitatis Masarykianae, Brno, Czech Republic; the Botanical Garden of Vilnius University, Lithuania; Hortus Botanicus Universitatis Posnaniensis, Poznań, Poland, and the Giardino Botanico Alpino, Cogne, Italy. Plants were cultivated in common plots at the Medicinal Plants Garden, near the Medical University of Białystok, Poland. Voucher specimens of plants were deposited in the Herbarium of the Department of Pharmacognosy, Medical University of Białystok, Poland and carefully authenticated by one of the authors (MT), based on morphological characteris-

tics by comparison with literature data. Collected plant materials (aerial parts of plants including leaves, stem and flowers), during June-July 2007-2010, were air-dried under shade at room temperature and then ground with an electric grinder into fine powders which were stored in air tight containers at room temperature. Processing of the dried plant material (preparation of aqueous extracts) was carried out after four years of harvest in 2010. The list of taxons studied is given in Table 1.

Preparation of extracts

Powdered plant material (2.0 g) was separately extracted with water (2 × 150 mL) in an ultrasonicator bath (Sonic-5, POLSONIC, Poland) at a controlled temperature (40 ± 2°C) for 45 min. Supernatants were filtered through a funnel with glass wool, which was washed with 5 mL of solvent and concentrated to dryness under vacuum (Büchi System, Switzerland) at a controlled temperature (40 ± 2°C) and subjected to lyophilization using a Lymph-Lock 1.0 (Labconco, USA) vacuum concentrator until a constant weight was obtained. Yields: (1, 2.05%; 2, 2.46%; 3, 5.03%; 4, 3.01%; 5, 2.69%; 6, 1.98%) – numbering according to Table 1.

Phytochemical profile

Total phenolic contents in extracts were determined spectrophotometrically at 765 nm (Specord 40, Analytik Jena, Germany) after the reaction with Folin-Ciocalteu's phenol reagent as gallic acid equivalents GAE/100 g in mg/g of dry weight (d.w.) according to the manual colorimetric method described by Tawaha et al. (5). Total phenolic acids content in plant material was determined by using the spectrophotometric method with Arnov's reagent according to the procedure described in the

Table 1. Plant material from the selected *Potentilla* L. species.

Sample no.	Plant sources		Parts used	Voucher specimen no.
	Scientific name	Common name		
1.	*Dasiphora fruticosa* (L.) Rydb. (syn. *Potentilla fruticosa* L.)[a]	Shrubby cinquefoil	herbs	PFR-06018
2.	*Potentilla norvegica* L.	Norwegian cinquefoil	herbs	PNO-08024
3.	*Potentilla thuringiaca* Bernh. ex Link	European cinquefoil	herbs	PTH-06022
4.	*Potentilla pensylvanica* L.	Pennsylvania cinquefoil	herbs	PPS-08025
5.	*Potentilla crantzii* (Crantz) J. Beck ex Fritsch	Alpine cinquefoil	herbs	PCR-09026
6.	*Potentilla nepalensis* Hook. var. 'Miss Willmott'	Nepal cinquefoil	herbs	PNE-06023

[a] botanical nomenclature in accordance with the taxonomy of USDA Natural Resources Conservation Service (NRCS).

European Pharmacopoeia 6.0 (6). The total content of flavonoids was determined by the spectrophotometric method according to Christ and Müller and followed the procedure described in the European Pharmacopoeia 6.0 (6). The total tannin content was determined by the weight method with hide powder according to the DAB 10 (7). The total proantocyanidin content was measured according to the European Pharmacopoeia 6.0 (6).

Cell cultures

Normal human colon myofibroblasts CCD-18Co (ATCC No. CRL-1459) and normal human colon epithelial cells CCD 841 CoTr (ATCC No. CRL-1807) were cultured in RPMI 1640 medium (Sigma) supplemented with 10% FCS at 37°C (CCD-18Co) or 34°C (CCD 841 CoTr) in a 5% CO_2/95% air atmosphere. For experiments, cells concentration at 1×10^5 cells/mL was used.

1,1-Diphenyl-2-picrylhydrazyl (DPPH•) test

Free radical scavenging activity of extracts was measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH•) assay. This method is based on the ability of antioxidants to reduce the stable dark violet radical DPPH• (Sigma) to the yellow colored diphenylpicrylhydrazine. Briefly, 100 µL of DPPH• solution (0.2 mg/mL in ethanol) was added to 100 µL of extract concentrations (25-250 µg/mL) and standards. Trolox (Sigma) at increasing concentrations (1-50 µg/mL) was used as a standard for the free radical scavenging activity. After 20 min of incubation at room temperature, the absorbance of the solution was measured at 515 nm. The lower the absorbance, the higher the free radical scavenging activity of the extracts. The activity of each extract was determined by comparison of its absorbance with that of a control solution (reagents without extract) and standard.

The capability to scavenge DPPH• radical was calculated by the following formula:

$$\text{DPPH• scavenging effect (\%)} =$$
$$= [(X_{control} - X_{extract})/X_{control}] \times 100$$

$X_{control}$ is the absorbance of the control and $X_{extract}$ is the absorbance in the presence of extracts (8).

Neutral red (NR) uptake assay

The NR cytotoxicity assay is based on the uptake and lysosomal accumulation of the supravital dye, neutral red. Dead or damaged cells do not take up the dye (9).

Cells were grown in 96-well multiplates in 100 mL of RPMI 1640 culture medium with 2% FCS (fetal calf serum) and *Potentilla* extracts (25-250 µg/mL). Subsequently, the medium was discarded and 0.4% NR (Sigma) in culture medium was added to each well. The plate was incubated for 3 h at 37°C in humidified 5% CO_2. After incubation, the dye containing medium was removed, cells were fixed with 1% $CaCl_2$ in 4% paraformaldehyde, and thereafter, the incorporated dye was solubilized using 1% acetic acetate in 50% ethanol solution (100 mL). The plates were gently shaken for 20 min at room temperature and the extracted dye absorbance was measured spectrophotometrically at 540 nm using a microplate reader (Molecular Devices Corp., Emax, Menlo Park, CA, USA).

MTT assay

Cells sensitivity to *Potentilla* extracts was determined in a standard spectrophotometric 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay according to Mosmann (10). The MTT test is based on the conversion of yellow tetrazolium salt by viable cells to purple crystals of formazan. The reaction is catalyzed by mitochondrial succinyl dehydrogenase.

Cells grown in 96-well multiplates in 100 µL of culture medium were incubated for 3 h with MTT solution (5 mg/mL, 25 µL/well) (Sigma). The yellow tetrazolium salt was metabolized by viable cells to formazan purple crystals. The reaction was catalyzed by mitochondrial succinyl dehydrogenase. The crystals were solubilized overnight in a 10% sodium dodecyl sulfate (SDS) in 0.01 M HCl mixture. The product was quantified spectrophotometrically by absorbance measurement at 570 nm wavelength using an E-max Microplate Reader (Molecular Devices Corporation, Menlo Park, CA, USA).

Nitric oxide (NO) measurement

Nitrite, a stable end product of NO, was determined in culture supernatants by a spectrophotometric method based on the Griess reaction. The level of nitrite reflects NO production (11). Briefly, cells were incubated for 24 h with the 15 µg/mL of *Potentilla* extract concentration and then culture supernatants were collected. The cell cultures, before the addition of extracts, were also pre-incubated with LPS (10 µg/mL) for 2 h. Next, 100 µL of supernatant was plated in 96-well flat-bottomed plates in triplicate and incubated with 100 µL of Griess reagent (1% sulfanilamide/0.1% N-(1-naphthyl)ethylenediamine dihydrochloride) (Sigma) in 3% H_3PO_4 (POCH Gliwice, Poland) at room temperature for 10 min. The optical density was measured at 550 nm using a microplate reader (Molecular

Devices Corp., Emax, Menlo Park, CA, USA). A standard curve was plotted using 0.5-25 μM sodium nitrite (NaNO$_2$) for calibration.

ELISA

The level of human IL-6 was tested immunoenzymatically (ELISA) in culture supernatants using commercially available kits (Diaclone) according to the manufacturer's instructions. The optical density at 450 nm with the correction wavelength of 570 nm of each ELISA sample was determined using a microplate reader. The IL-6 concentration was calculated on the basis of a standard curve: 2 pg/mL (IL-6) was the detection limit.

Statistical analysis

Results are presented as the means ± SD from three experiments. Data were analyzed using a one-way ANOVA test. Differences of p ≤ 0.05 were considered significant. Only the results with a significance of p ≤ 0.05 are reported. All experiments were repeated three times.

RESULTS AND DISCUSSION

The total polyphenol content in aqueous extracts was determined from the regression equation of the calibration curve and expressed in gallic acid equivalent. The content of total polyphenols ranged from 49.9 ± 1.5 mg GAE/g d.w. for aerial parts of *P. pensylvanica* to 116.3 ± 3.9 mg GAE/g d.w. for *D. fruticosa*. All these differences in the values of total phenolic content in all the analyzed extracts can be attributed to the differences in the composition of these six extracts. It was obvious that the total phenolic compounds determined by Folin-Ciocalteu's method had not given a full char-

acterization of the quality and quantity of the various groups of polyphenolic compounds. The presence of these groups of compounds encouraged us to determine them in plant material by more precise methods (weight, spectrophotometrical and chromatographic). Our data (Table 2) show that the aerial parts of *D. fruticosa* had relatively very high concentrations of tannins (167.3 ± 2.0 mg/g d.w.), proanthocyanidins (4.6 ± 0.2 mg/g d.w.), and phenolic acids (16.4 ± 0.8 mg/g d.w.), as well as flavonoid compounds (7.0 ± 1.1 mg/g d.w.) and (10.1 ± 0.9 mg/g d.w.), calculated as quercetin and hyperoside, respectively. Earlier communication proved a new, rapid and simple HPTLC method to authenticate the aerial parts of selected *Potentilla* species for the confirmation of the presence of different types of polyphenolics, including flavonoid derivatives in both free and glycoside-bound forms, and hydrolyzable tannins in the analyzed plant material. In this study, ellagic acid, kaempferol, quercetin, tiliroside, isoquercitrin and quercetin 3-glucuronide were detected in all the tested extracts. In all analyzed samples consistent profiles were observed, but the qualitative differences of the most analyzed compounds within species were significant (2). However, the aerial parts of *D. fruticosa* have been studied extensively and have been found to contain a wide variety of polyphenolics, such as ellagic acid, catechins and flavonols (quercetin, kaempferol and rhamnetin glycosides) (1, 12, 13). Our chromatographical fingerprinting also confirmed that *D. fruticosa* is rich in polyphenolic compounds, and they are consistent with earlier studies which have been reported by Shikov et al. and Miliauskas et al. (14, 15).

Currently, polyphenolic compounds are of great interest in nutrition and medicine for their

Table 2. Extraction yield (%)*, total phenolic (TPC), total phenolic acids (TPA), total flavonoids (TFC), total tannins (TTC) and total proanthocyanidins (TPDC) content of *Potentilla* species.

Sample no.	Yield (wt. %)	Polyphenols content (mg/g d.w.) [a]					
		TPC	TPA	TFC		TTC	TPDC
				Quercetin	Hyperoside		
1.	2.05	116.3 ± 3.9	16.4 ± 0.8	7.0 ± 1.1	10.1 ± 0.9	167.3 ± 2.0	4.6 ± 0.2
2.	2.46	82.9 ± 2.2	8.5 ± 0.6	2.8 ± 0.6	4.1 ± 0.3	72.1 ± 0.8	4.3 ± 0.1
3.	5.03	58.4 ± 3.1	4.5 ± 0.2	5.1 ± 0.3	7.2 ± 0.8	72.5 ± 1.2	4.1 ± 0.2
4.	3.01	49.9 ± 1.5	6.8 ± 0.5	5.5 ± 0.3	6.8 ± 0.4	59.7 ± 2.1	3.3 ± 0.2
5.	2.69	94.8 ± 2.8	5.3 ± 0.2	4.8 ± 0.4	7.9 ± 0.3	72.2 ± 0.9	4.5 ± 0.3
6.	1.98	73.9 ± 3.7	7.7 ± 0.4	2.1 ± 0.5	2.9 ± 0.6	65.7 ± 1.9	3.7 ± 0.1

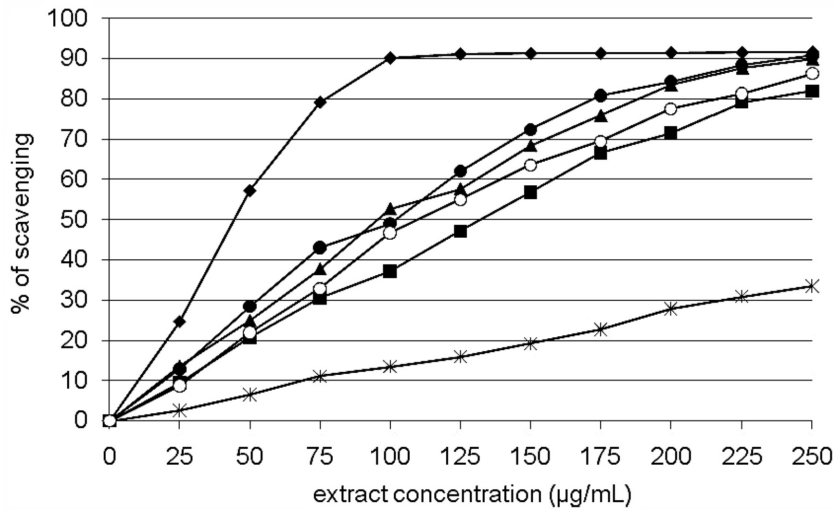[a] Results are the means ± SD of three different experiments.

Figure 1. Free radicals scavenging (DPPH) effect demonstrated by *Potentilla* sp. extracts (♦ – *D. fruticosa*, ■ – *P. pensylvanica*, ▲ – *P. norvegica*, ● – *P. crantzii*, ✳ – *P. thuringiaca*, o – *P. nepalensis*). The most potent free radical reducing effect was observed for *D. fruticosa*, but the lowest effect was connected with *P. thuringiaca*. The control value (no free radical scavenging ability) was established as 0%
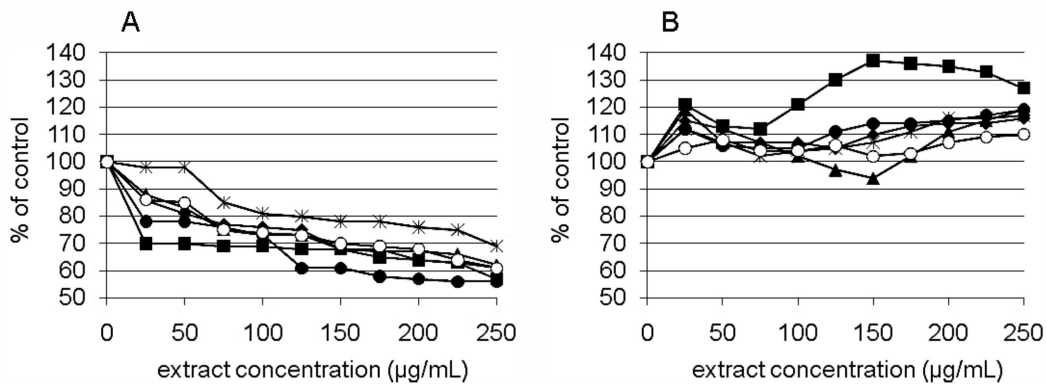

Figure 2. Viability of CCD 841 CoTr (**A**) and CCD-18Co (**B**) cells analyzed by MTT method after 24 h incubation with *Potentilla* sp. extracts (♦ – *D. fruticosa*, ■ – *P. pensylvanica*, ▲ – *P. norvegica*, ● – *P. crantzii*, ✳ – *P. thuringiaca*, o – *P. nepalensis*). The results are presented as % of control determined as 100%
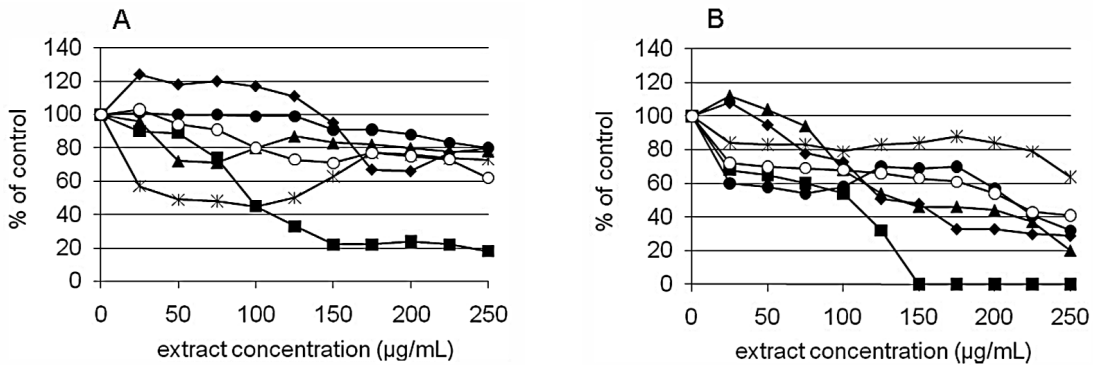

Figure 3. Viability of CCD 841 CoTr (**A**) and CCD-18Co (**B**) cells analyzed by NR method after 24 h incubation with *Potentilla* sp. extracts (♦ – *D. fruticosa*, ■ – *P. pensylvanica*, ▲ – *P. norvegica*, ● – *P. crantzii*, ✳ – *P. thuringiaca*, o – *P. nepalensis*). The results are presented as % of control determined as 100%

potent antioxidant capacity and protective effects on human health and diseases with free radical etiologies, including cardiovascular diseases, neoplastic diseases and blood clotting diseases. As a result, a number of studies on antioxidative substances, isolated from aerial and underground parts of various genus *Potentilla* species, have been published. The DPPH method enables the testing of whether *Potentilla* extracts scavenge oxygen free radicals. Our results show that all tested extracts possess a strong ability to reduce oxygen free radicals. As displayed in Figure 1, the most active was *D. fruticosa* extract, which at a concentration of 100 µg/mL reduced 90% of radicals as compared to the control. A strong antioxidant effect was also observed for extracts from *P. nepalensis, P. pensylvanica, P. crantzii* and *P. norvegica.* Conversely, *P. thuringiaca* extracts had significantly weaker radical reducing activity, which was only 35% lower than in the control (pure methanol established as 0% of reactive oxygen species-reducing activity) when the highest extract concentration was used. Our antioxidant bioassay results, employing the DPPH method, were also similar to those previously published. Several authors investigated the antioxidant effect of isolated compounds and extracts obtained from different parts of plants from *Potentilla* species against organic radicals, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS$^+$) (4, 16-18). In another study, the content of polyphenols including flavonoids in 50% aqueous ethanol extracts from aerial parts of *P. atrosanguinea* exhibited strong antioxidant activity measured in terms of Trolox equivalent antioxidant capacity (TEAC) with ABTS$^+$, DPPH$^{\cdot}$, and the ferric reducing antioxidant potential FRAP assay (19). Similar antioxidant activity was reported on extracts and isolated compounds obtained from the flowers of *D. fruticosa* (14, 20).

The following tests were performed using two cell lines: normal human colon myofibroblasts (CCD-18Co) and normal human colon epithelial cells (CCD 841 CoTr). The selection was based on the idea suggesting potential health benefits on alimentary tract when *Potentilla* was considered in diet (1). These tests were, therefore, completed to answer the question concerning influence of *Potentilla* components on human colon wall lining in view of pro-health benefits and safety. Using the MTT method the cytotoxic effect of the extracts was tested (Fig. 2). The results revealed the strong inhibitory activity of the extracts on the CCD 841 CoTr human colon epithelial cell line. The lowest inhibitory action was found when the extract from *P. thuringiaca* was used (about a 30% decrease in comparison to the control). On the other hand, the extract from *P. crantzii* showed the strongest cytotoxic action, which at an extract concentration of 125 µg/mL decreased cell viability by about 40%. *Potentilla* species exerted a significant stimulatory effect when the CCD-18Co human colon myofibroblast metabolism was analyzed. The strongest was the extract from *P. pensylvanica,* whose maximum activity was at a concentration of 150 µg/mL and increased induced cell proliferation by about 40%. The exception was *P. norvegica*, whose extracts slightly inhibited cell proliferation at concentrations of 100-175 µg/mL.

Using the NR method the integrity of cellular membranes after cell (CCD 841 CoTr and CCD-18Co) incubation with *Potentilla* extracts was tested (Fig. 3). The results indicate that the tested extracts disturb the integrity of human colon epithelial cells. Significant changes in cellular metabolism were found only when *D. fruticosa* extracts at concentrations of 25-125 µg/mL were used. There was a significant increase in cellular metabolism, but when higher concentrations were added, an inhibitory effect was observed. *P. crantzii* extracts at concentrations ranging from 25 to 125 µg/mL were neutral in relation to CCD 841 CoTr cells, but at higher concentrations were cytotoxic. Cellular integrity was most disturbed after cell incubation with *P. pensylvanica* and *P. thuringiaca* extracts. Moreover, our results showed that *Potentilla* extracts also disturbed CCD-18Co cell membrane integrity. *P. norvegica* and *D. fruticosa* at the lowest concentrations stimulated myofibroblast proliferation. On the basis of our results, extracts from *P. pensylvanica* were the most potent in the inhibition of cell viability and proliferation. We showed 100% cytotoxicity at a concentration of 150 µg/mL. On the other hand, *P. thuringiaca* extract in the slightest degree disturbed myofibroblast membrane integrity.

The level of nitric oxide (NO) after cell (CCD 841 CoTr and CCD-18Co) incubation with LPS (10 µg/mL) (*E. coli*), and/or plant extracts was analyzed (Fig. 4). The LPS usage aimed at the analysis of extracts' action on cells growing in inflammatory conditions. All extracts decreased NO production in comparison to the control. Incubation of cells with LPS was associated with increased NO production. The most potent in the inhibition of NO production by epithelial cells were extracts from *P. pensylvanica* and *P. crantzii*. In the case of myofibroblasts, tested extracts also led to a decrease in NO production. The most significant changes compared to the con-
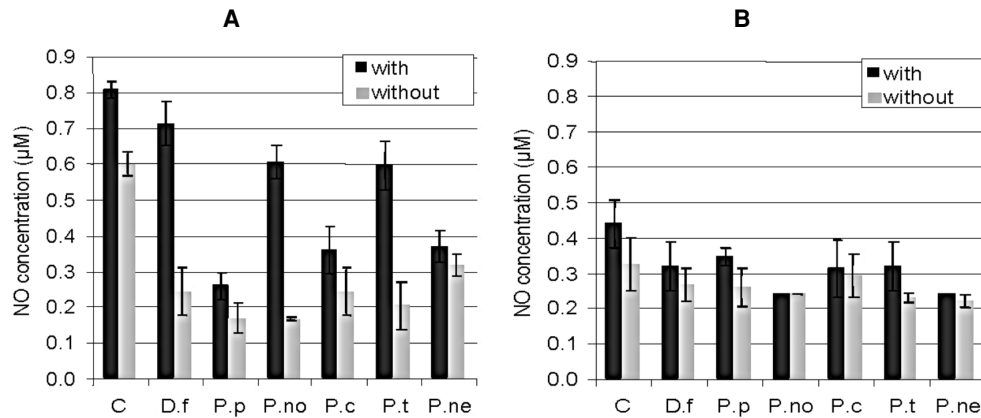
Figure 4. The influence of *Potentilla* sp. extracts on NO level produced by CCD 841 CoTr (**A**) and CCD-18Co (**B**) cells. (C – control, D.f – *D. fruticosa*, P.t – *P. thuringiaca*, P.p – *P. pensylvanica*, P.no – *P. norvegica*, P.c – *P. crantzii*, P.ne – *P. nepalensis*)
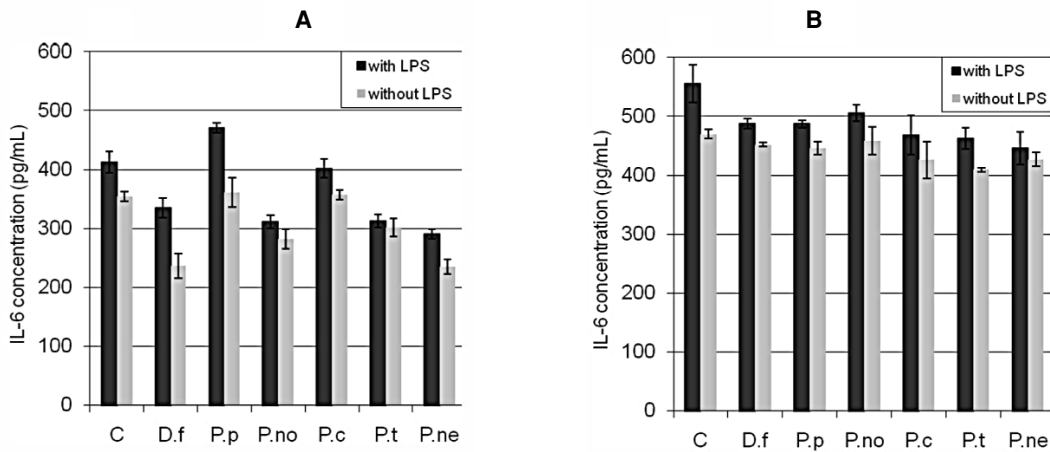

Figure 5. The influence of *Potentilla* sp. extracts on IL-6 production by CCD 841 CoTr (**A**) and CCD-18Co (**B**) cells. (C – Control, D.f – *D. fruticosa*, P.t – *P. thuringiaca*, P.p – *P. pensylvanica*, P.no – *P. norvegica*, P.c – *P. crantzii*, P.ne – *P. nepalensis*)

trol were observed in the case of *P. norvegica* and *P. nepalensis* extracts. As a result of their action, a 30% decrease in NO level was observed.

Lipopolysaccharide is a strong stimulator of adaptive immune mechanisms, among others, the stimulation of inflammatory reactions. The *Potentilla* extracts decreased NO production, which in some cases can be recognized as an anti-inflammatory mechanism. This activity is closely correlated with antioxidant presence in the tested extracts. This hypothesis is confirmed by Wang and Mazza (21), who revealed that gallic and chlorogenic acids, catechins, isoflavones, and also anthocyanins reduce

NO production by macrophages after LPS stimulation. With the use of the immunoenzymatic ELISA test the ability of IL-6 induction by *Potentilla* sp. on CCD 841 CoTr and CCD-18Co cells was analyzed (Fig. 5). Similar to NO analysis, pre-incubation with LPS (10 µg/mL) was performed. *Potentilla* extracts limited IL-6 production by human colon epithelial cells. The exception was *P. pensylvanica* extract, which insignificantly increased cytokine production. In each experiment, cell pre-incubation with LPS led to an increase in IL-6 production. However, the strongest inhibition in cytokine production was observed after cell incubation with *P. nepalensis* and
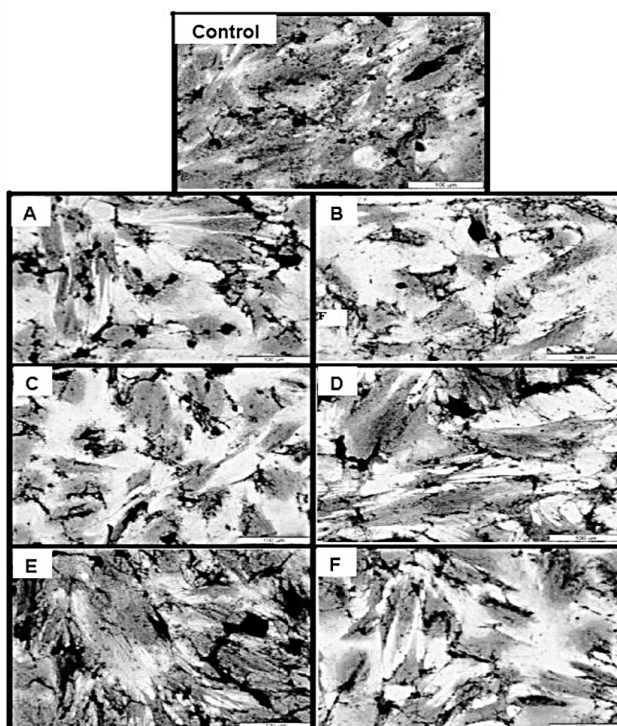
Figure 6. Morphology of cells: line CCD 841 CoTr after incubation with *Potentilla* sp. extracts (May-Grünwald-Giemsa staining). (**A** – *D. fruticosa*, **B** – *P. thuringiaca*, **C** – *P. pensylvanica*, **D** – *P. norvegica*, **E** – *P. crantzii*, **F** – *P. nepalensis*)

D. fruticosa extracts. The studied extracts had a very weak inhibitory effect on IL-6 production by myofibroblasts. The most potent were extracts from *P. thuringiaca* and *P. nepalensis*. Interleukin-6 (IL-6) is a pro-inflammatory cytokine with diverse activity. It takes part in immune reactions, or induces an inflammatory state. Moreover, this cytokine is very important in the pathogenesis, development and dissemination of tumors (22). Lipopolysaccharide activates the membrane complex of the differentiation CD14 and TLR4 receptors on the outer surface of the cell membrane. Thereafter, signal transduction by MAPK and NF-κB is undergone. The effect is that many genes are activated, among them genes for IL-6 (23). In the presented experiments, we showed that *Potentilla* extracts limited IL-6 production by tested cells after LPS stimulation. This activity may be connected with antioxidant activity, which is present in large amounts in this plant. Based on available data, antioxidants influence inflammatory factor production by changing cellular redox potential. Some of the factors which belong to this group inhibit NF-κB translocation to the nucleus, which implies limitation of the gene expression, e.g., for IL-6 (24).

Similar results were presented by Fang and colleagues (25), who tested extracts from *Phyllanthus urinaria*. They showed free radical-scavenging activity produced by macrophage-inhibited NO, TNF-α and IL-6 production. Moreover, extracts from *Cinammomum camphora* and *Opuntia humifusa* reduced free radicals and inhibited IL-6 production by RAW264.7 cells pre-incubated with LPS (26, 27).

In the May-Grünwald-Giemsa (MGG) method, the morphology of cells was observed (Fig. 6). In the images the cytotoxic action of the extracts on human colon epithelial cells can be seen. The results are in agreement with those obtained in the NR method. The extract from *D. fruticosa* demonstrated the strongest action on CCD 841 CoTr cell morphology. On the other hand, the lowest activity on cells was found when extracts from *P. crantzii* was applicated. As compared to the control, cells treated with *Potentilla* extracts begun to separate away. Moreover, depending on extract used, cells tended

rather to contract (after *P. thuringiaca, P. pensylvanica, P. nepalensis*) or to streatch (mainly after *D. fruticosa*, D – *P. norvegica,* E – *P. crantzii*). It can be supposed that this effect may be closely linked with extract components influence on cytoskeleton elements like actin filaments. However, more exact tests should be performed to find out which components are responsible for such different influence on cells growth. Moreover, it could be also possible that these components influence on adhesive molecules enabling close contact among cells and cells adhere with solid layer. These suppositions need further tests. Myofibroblasts staining also revealed the disadvantageous, similar to epithelial cells, effect of the extracts on cells morphology and viability.

CONCLUSIONS

The results of the study suggest that aqueous extracts from selected *Potentilla* species contain high concentrations of polyphenols, such as tannins (proanthocyanidins) and phenolic acids, as well as flavonoids. All analyzed extracts from *Potentilla* species express strong free radical-reducing effects. The influence of extracts on cell viability varies depending on the *Potentilla* species they were obtained from and the experimental method used. Tested extracts reduce IL-6 and NO production by colon cells. Analyzed extracts influence the morphology of tested colon cells. In limited amounts *Potentilla* may be used in human diet demonstrating pro-health benefits.

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