Crude Polysaccharide Fraction from Rosa rugosa Thunb. Root—Chemical Characterisation, Enzyme Inhibitory, Antioxidant and Antiproliferative Activity

Agnieszka Łubek-Nguyen 1, Marta Olech 1,* 1, Natalia Nowacka-Jechalke 1, Aleksandra Martyna 2, Konrad Kubiński 2, Maciej Masłyk 2, Marcin Moczulski 3 and Sebastian Kanak 1

1 Department of Pharmaceutical Botany, Medical University of Lublin, ul. Chodzki 1, 20-093 Lublin, Poland
2 Department of Molecular Biology, The John Paul II Catholic University of Lublin, ul. Konstantynów 1, 20-708 Lublin, Poland
3 Sciex Europe (c/o Hach Lange Sp. z.o.o.), ul. Krakowska 119, 50-428 Wrocław, Poland
* Correspondence: marta.olech@umlub.pl; Tel./Fax: +48-81-448-70-60

Abstract: Rosa rugosa Thunb. (Rosaceae) plantations can provide industrial amounts of roots, which contain many biologically active metabolites. Here, we report the first studies of the chemical composition and pharmacological potential of a crude polysaccharide fraction obtained from R. rugosa roots (CPL-Rx). It was found that the roots contained water-soluble and -insoluble sugars, including a large amount of β-glucans (12.95 ± 0.30 g/100 g). The water-soluble fraction was found to be mostly composed of sugars (28.94 ± 0.01%), uronic acids (2.61 ± 0.41%), and polyphenols (4.55 ± 0.12%). High-performance capillary electrophoresis analysis showed that glucose was the prevalent monosaccharide (64.31%), followed by a smaller amount of galactose (11.77%), fructose (11.36%), and arabinose (4.27%). SDS-PAGE followed by liquid chromatography mass spectrometry revealed the presence of unbound protein identified as protein plastid transcriptionally active 14-Like. CPL-Rx was found to inhibit the activity of pro-inflammatory enzymes (cyclooxygenase and hyaluronidase) and to have antioxidant potential in the Trolox equivalent antioxidant capacity assay (0.302 ± 0.01 mmol Trolox/g), DPPH radical scavenging effect (EC50 3.92 ± 0.12 mg/mg DPPH•) and oxygen radical absorbance capacity assay (0.134 ± 0.10 mmol Trolox/g). It also exerted a slight antiproliferative effect on SW480 (colon) and A549 (lung) cancer cell lines. Our research provides the first insights into the composition and pharmacological application of crude polysaccharides from rugosa rose roots. It suggests that CPL-Rx may potentially be used for cosmetic and bio-medical purposes, especially as an antihyaluronidase or chemopreventive agent.

Keywords: plant polysaccharide; plant protein; anti-inflammatory effect; COX inhibition; hyaluronidase inhibition; antiradical activity; Japanese rose

1. Introduction

Algae, plants, fungi, animals and microorganisms can be potential sources of polysaccharides valuable in terms of medicinal, food, and industrial applications. These biomacromolecules represent various structures, physicochemical properties, and bioactivity [1]. Polysaccharides are polymers composed of monosaccharides that are largely interconnected by glycosidic bonds. Depending on the type of monomers linked, there are monosaccharides or heterosaccharides, which can be linear or branched [2]. Many studies have been conducted showing a wide range of health-promoting biological activities of polysaccharides (e.g., wound healing, antioxidative, hepatoprotective, neuroprotective, immunostimulatory, or anti-proliferative activity), which are strongly correlated with their
chemical composition, configuration, and molecular weight [3–5]. Some of the mechanisms of these activities are still under research. The involvement of immune cells, especially macrophages, is suspected in the immunostimulatory effect of polysaccharides. It has been shown that glycan may enhance macrophage functions through stimulation of phagocytosis and an increase in the secretion of cytokines and chemokines. Moreover, it may enhance cytotoxic activity against cancer cells and free oxygen radicals [1].

Roots of different rose species are a relatively poorly known plant material. Rose roots are used in Chinese herbal medicine to treat enuresis, diarrhoea and redness [6]. They have been also used in folk medicine in the treatment of pain, chronic inflammatory diseases or diabetes [7]. They contain many valuable components representing multiple phytochemical groups. *Rosa laevigata* Michx. and *Rosa cymosa* Tratt. roots were shown to contain triterpenes (e.g., betulinic acid), triterpenoid saponins (e.g., kajiichigoside and rosamutin), flavonoids and tannins [6,8]. In the root of *Rosa acicularis* Lindl., high levels of polyphenolic compounds, e.g., proanthocyanidins, epigallocatechin, catechin, and ellagic acid, were found [9]. The analysis of the root of *Rosa rugosa* Thunb. of European origin showed a high content of polyphenols, including a high proportion of phenolic acids (e.g., gallic, and caffeic) and tannins. Additionally, a remarkable amount of flavonoids (e.g., quercetin, and isoquercetin) was detected [10]. The phytochemical composition strongly affects the biological activity and therapeutic use of these organs. Triterpenoids from *Rosa laevigata* Michx. roots have been shown to exert anti-inflammatory, antifungal and acetylcholinesterase-inhibiting properties [6]. Extracts from rugosa rose roots possess antifungal, antibacterial, and hyaluronidase inhibitory activity as well as high antioxidant potential [10,11]. Moreover, teas from rugosa rose roots were found to strongly affect the proliferation of breast (T47D), ovarian (TOV-112D), cervical (HeLa) and lung cancer (A549) cell lines [7].

Rose polysaccharides are the least studied rose metabolites. Some previous studies revealed the chemopreventive potential of polysaccharide-rich fractions prepared from rose petals, pseudo-fruits, leaves, and achenes. The samples were also shown to have radical scavenging, strong hyaluronidase inhibitory and anti-inflammatory activity [12,13]. However, polysaccharides from rose roots have not been investigated to date. Meanwhile, rose roots can be obtained in industrial amounts after renovation of rose cultivations. One of the most commonly grown rose cultivars is rugosa rose (*Rosa rugosa* Thunb.; Japanese rose). It is known mostly for its large pseudo-fruits and fragrant petals. At present, its roots are rarely used, as they are regarded rather as post-harvest residue and discarded [10]. This is quite surprising and seems to be a waste, considering the value of the ingredients contained in this plant material. Perhaps the knowledge of this issue is insufficiently promoted, or further investigations on the phytochemistry of rose roots and their possible new applications are needed.

Therefore, the aim of the present study was to determine the composition of the polysaccharide fraction obtained from *R. rugosa* roots and evaluate its anti-inflammatory, antioxidant, and antiproliferative activity. Since other macromolecules (e.g., proteins or polysaccharide-peptide conjugates) often co-elute with polysaccharides and can be found in the final extract (even despite the de-ballasting stages) we also wanted to examine the presence of proteins and their conjugates in the prepared samples.

2. Materials and Methods

2.1. Plant Material

Roots (Rx) of *Rosa rugosa* Thunb. were collected in Łukówiec (Lubelskie region, Eastern Poland; 51°35′ N 22°30′ E) in October in 2019. Botanical identification was performed by the authors. A voucher specimen was deposited at the Department of Pharmaceutical Botany, Medical University of Lublin, Poland (No. R-017/19). The roots were air dried at ambient temperature and powdered in the cutting mill SM 100 (Retsch GmbH; Haan, Germany).
2.2. Chemicals

Redistilled phenol, Bradford reagent, Bromophenol Blue, epigallocatechin gallate, Thiazolyl Blue Tetrazolium Blue (MTT), bovine serum albumin (BSA), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), ascorbic acid, galacturonic acid, gallic acid, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), linoleic acid, potassium persulphate, dihydrochloride (AAPH), m-hydroxydiphenyl, sodium phosphate dibasic solution, sodium phosphate monobasic solution, hyaluronic acid soybean 15-lipooxygenase, and hyaluronidase were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). Dithiothreitol (DTT) was from Serva (Heidelberg, Germany), and Coomassie Brilliant Blue from Fluka (Buchs, Switzerland). β-D-(+)-glucose was provided by ChromaDex (Irvine, CA, USA). Foetal bovine serum (FBS), DMEM (Dulbecco’s Modified Eagle Medium, high glucose + GlutaMAX), and Dulbecco’s phosphate-buffered saline (DPBS) were from Gibco (Life Technologies, Paisley, UK). SDS (sodium dodecyl sulfate) was provided by BioShop (Burlington, ON, Canada). Disodium hydrogen phosphate dehydrate (Na₂HPO₄ × 2H₂O) was purchased from Chempur (Piekary Śląskie, Poland). Standards of monosaccharides were provided by Dr. Ehrenstorfer GmbH (Augsburg, Germany). Isoamyl alcohol, sulphuric acid, and solvents were purchased from Avantor Performance Materials Poland (Gliwice, Poland). A Millipore Direct-Q3 purification system (Bedford, MA, USA) was used to prepare ultrapure water.

Spectrophotometric analyses were performed using an Infinite Pro 200F microplate reader (Tecan Group Ltd., Männedorf, Switzerland) with the use of 96-well microplates (Nunclon, Nunc; Roskilde, Denmark). An IKA RV 10 evaporator (Staufen, Germany) was used for evaporation. Lyophilisation was performed in the Free Zone 1 apparatus (Labconco, Kansas City, KS, USA).

2.3. Extraction of Crude Polysaccharide (CPL-Rx)

Powdered roots of *R. rugosa* were subjected to preliminary maceration two times at room temperature with 95% ethanol (1:15 g/mL) for 24 h. Then, ultrasound-assisted extraction with 95% ethanol (1:15 g/mL) was performed twice at 35 °C for 30 min. The ethanolic supernatants were removed, and the plant residue was air dried at room temperature before the next step of the double extraction procedure with water. First, unballasted roots were macerated for 15 min with water (1:20 g/mL) at room temperature; then, ultrasound assisted extraction was conducted at 90 °C for 1 h. The aqueous extracts were combined and concentrated. Purification of the extract was performed with Sevag reagent (2% isoamyl alcohol in chloroform) in order to remove proteins. The resulting aqueous fraction was subjected to precipitation with cold anhydrous ethanol (1:4 *v/v*) and kept overnight at 4 °C. After centrifugation (8000 rpm for 10 min), the precipitated polysaccharides were dissolved in deionised water, and precipitated again in the aforementioned conditions. The final precipitate was centrifuged and lyophilised to obtain crude polysaccharides from the *R. rugosa* roots (CPL-Rx).

2.4. Analyses of the Chemical Composition

2.4.1. Sugar Content

The content of sugars in root dry weight (d.w.) and in CPL-Rx was assayed with the phenol-sulphuric acid method [14] using glucose as a standard. The results were expressed as water soluble sugars (g/100 g d.w.), % of CPL-Rx weight, and as mean ± standard deviation (SD).

2.4.2. Uronic Acid Content

The content of uronic acids was evaluated with the method described previously [14] using galacturonic acid as a standard. The results were expressed as % of CPL-Rx and as mean ± SD.
2.4.3. Total Phenolic Content (TPC)

The total phenolic content in CPL-Rx was determined according to the method described in detail by Olech and Nowak [15] using gallic acid as a standard. The results were expressed as % of CPL-Rx and as mean ± SD.

2.4.4. Content of Proteins

The amount of protein was evaluated with the Bradford method [14] using bovine serum albumin as a standard. The results were expressed as % of CPL-Rx weight and as mean ± SD.

2.4.5. Determination of the Glucan Content

The contents of total glucans, α- and β-glucans were determined using an Megazyme Ltd. assay kit (Bray, Ireland) according to the manufacturer’s instructions described previously in detail [12]. Results were expressed as g/100 g d.w. and as mean ± SD.

2.4.6. High-Performance Capillary Electrophoresis (HPCE) Analysis of the Monosaccharide Composition

Before evaluation of the carbohydrate composition of CPL-Rx, the sample (50 g) was hydrolysed with 25 mL of 2 M TFA (trifluoroacetic acid) for 2 h at 100 °C. The TFA was then removed in a rotary evaporator. The hydrolysate was dissolved in water and centrifuged at 11,000 × g for 2 min. The supernatant was collected, and the sugar composition was analysed by high-performance capillary electrophoresis using a PA 800 plus (Sciex, Framingham, MA, USA) instrument with a photodiode array detector according to a previously described method [16] with some modifications. Detection was performed at a wavelength of 270 nm. A fused silica capillary (Polymicro, Phoenix, AZ, USA) with ID 25 µm and a total length of 60.2 cm (50.0 cm effective length) was used. The capillary and samples were thermostatted at 15 °C. The capillary was conditioned before analysis by rinsing with 130 mM sodium hydroxide, Mili-Q water, and separation buffer (130 mM NaOH and 360 mM Na₂HPO₄ × 2H₂O; pH 12.5–12.7). The samples were injected with a pressure of 1.0 psi for 15 s and then a small portion of water was injected with a pressure of 0.5 psi for 10 s. The separation was performed with a voltage of 30 kV for 25 min. Electropherograms were acquired and evaluated using 32 Karat Software Version 9.1. The carbohydrates were identified by comparing the migration times with corresponding standards (e.g., arabinose, fucose, galactose, fructose, glucose, mannose, and xylose) tested in the same conditions, both with the standard addition method.

2.4.7. SDS-PAGE

The sample was mixed with a 4X electrophoresis sample buffer (0.125 M Tris–HCl; pH 6.8; 20% v/v glycerol, 4% SDS, 0.2 M dithiothreitol, 0.01% bromophenol blue). Electrophoresis was performed in 10% polyacrylamide gels using a BioRad vertical electrophoresis system (Hercules, CA, USA) according to Laemmli’s method [17] at 120 V for the stacking gel and 180 V for the resolving gel. Following the electrophoresis, the proteins were visualised using Coomassie Brilliant Blue staining (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Perfect™ Colour Protein Ladder (EurX, Gdańsk, Poland) was used to assess the molecular weight of the protein in a range from 7 kDa to 240 kDa.

2.4.8. Identification of Protein in CPL-Rx—Liquid Chromatography Mass Spectrometry and Analysis of Mass Spectrometry Data

Proteomic analysis was preceded by a sample preparation procedure. The protein band revealed during the SDS-PAGE analysis was excised from the gel and analysed by liquid chromatography mass spectrometry (LC-MS). The sample was subjected to trypsin digestion, during which proteins were reduced (using DTT 10 mM; 30 min at 56 °C), alkylated with iodoacetamide (for 45 min at room temperature in darkness), and digested overnight with 10 ng/µL trypsin. The obtained peptide mixture was concentrated and
desalted on a RP-C18 pre-column (Waters, Milford, MA, USA). Peptide separation was carried out on a nano-Ultra Performance Liquid Chromatography (UPLC) RP-C18 column (Waters, Milford, MA, USA, BEH130 C18, 75 µm i.d., 250 mm long), using a linear gradient of acetonitrile with 0.1% formic acid (45 min). The analysis of the sample was preceded by a blank run to ensure the absence of cross contaminations. LC was coupled to the ion source of the Orbitrap Velos mass spectrometer (Thermo Electron Corp., San Jose, CA, USA) working in the regime of the data-dependent MS to MS/MS switch.

The analysis of the mass spectrometry data was performed using Mascot Distiller software (v. 2.6, MatrixScience, London, UK) and the Mascot Search Engine (MatrixScience, London, UK, Mascot Server 2.5) against the NCBInr database (155,200,242 sequences; 56,856,634,127 residues) with a Rosaceae filter (304,414 sequences). The mass tolerance settings for the peptide and fragment were determined separately for individual LC-MS/MS runs after a measured mass recalibration to reduce mass errors. The remaining search parameters were as follows: enzyme, trypsin; missed cleavages, 1; fixed modifications, carbamidomethyl (C); variable modifications, oxidation (M); and instrument, HCD. Analyte identification was carried out using the Mascot search engine with the probability-based algorithm. The expected value threshold of 0.05 was used (all peptide identifications had a less than 1 in 20 chance of being a random match) [18].

2.5. Biological Activity of Crude Polysaccharide from R. rugosa Roots

2.5.1. Inhibition of Cyclooxygenase (COX) Activity

The inhibitory activity of CPL-Rx in relation to cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) was evaluated using the COX (ovine) Colorimetric Inhibitor Screening Assay Kit (Cayman, Ann Arbor, MI, USA) according to the manufacturer’s instructions. A total amount of 160 µL of assay buffer (0.1 M Tris-HCl, pH-8 with 5 mM EDTA and 2 mM phenol) was mixed with 10 µL of heme and 10 µL of the enzyme (either COX-1 or COX-2) prior to adding 10 µL of the CPL-Rx samples (0.25 to 5 mg/mL, in 5% DMSO). After 10 min incubation at 37 °C, 10 µL of arachidonic acid was added in order to initiate a reaction. The reaction mixtures were mixed and incubated again for 2 min at 37 °C. Then, the reaction was stopped by adding 30 µL of a saturated SnCl₂ solution in 1 M HCl. The prostanoid product was measured via enzyme immunosorbent assay (ELISA) using a broadly specific antiserum that binds to all the major prostaglandin compounds. The blank consisted of 10 µL of 5% DMSO instead of the sample and 10 µL of the inactivated enzyme instead of its active form. Acetylsalicylic acid (1 mM) was used as a positive control in the assay. The percent COX inhibition was calculated as shown below:

\[
\text{COX inhibition activity (\%) = } \frac{(\text{AIAS} - \text{ASP})}{\text{AIAS}} \times 100
\]

where ASP = absorbance of the inhibitor sample, and AIAS = absorbance of the 100% initial activity sample.

2.5.2. Inhibition of Hyaluronidase (HYAL) Activity

The ability of CPL-Rx to inhibit hyaluronidase activity was determined using the protocol proposed by Yahaya and Don [19] with some modification described previously in detail [20]. The reaction mixture contained 20 µL of the CPL-Rx sample, 20 µL of assay buffer and the hyaluronidase enzyme (20 µL; 40–100 U/mL in the assay buffer). After 10 min of incubation at 37 °C, 20 µL of hyaluronic acid (0.5 mg/mL in 300 mM sodium phosphate buffer; pH 5.35; 37 °C) were added. The mixture was incubated for 45 min at 37 °C. Then, undigested hyaluronic acid was precipitated with 100 µL of an acid albumin solution (2 mg/mL in 79 mM acetic acid with 24 mM sodium acetate; pH 3.75; 25 °C). The mixture was shaken (at 25 °C; 10 min), and transmittance was measured at 600 nm. The percent HYAL inhibition was calculated as shown below:

\[
\% \text{ inhibition} = \frac{(\text{TS} - \text{TBLK})}{(\text{TC} - \text{TBLK})} \times 100\%
\]
where TS is the transmittance of the sample, TBLK is the transmittance of the negative control (containing buffer instead of the sample) and TC is the transmittance of the mixture containing the buffer instead of the enzyme. Epigallocatechin gallate (EGCG) was used as a positive control. A dose–response curve for at least six prepared dilutions of the CPL-Rx and EGCG was plotted to determine their IC\textsubscript{50} values.

2.5.3. TEAC (Trolox Equivalent Antioxidant Capacity) Assay

The TEAC of the CPL-Rx was evaluated using ABTS\textsuperscript{•+} (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical, according to a previously described method [12]. The reaction mixture consisted of 20 µL of the CPL-Rx (at different concentrations) and 180 µL of an ABTS solution (0.096 mg/mL) in methanol. After shaking and 6 min incubation at 30 °C, the absorbance was determined at 734 nm. The results were given as mM of Trolox equivalent per gram of dry extract (DE).

2.5.4. DPPH Radical Scavenging Activity

The scavenging activity of the CPL-Rx was determined using a previously described method with 2,2-diphenyl-1-picrylhydrazyl (DPPH) [12]. The reaction mixture consisted of 20 µL of the CPL-Rx at different concentrations and 180 µL of a DPPH solution in methanol (0.07 mg/mL). After shaking and 30 min incubation, the absorbance was measured at 517 nm. A dose–response curve for at least five prepared dilutions of the CPL-Rx was plotted to determine the EC\textsubscript{50} values. The results were also presented as standard equivalents using Trolox (TE) and ascorbic acid (AsAE) based on their EC\textsubscript{50} values obtained according to the same procedure.

2.5.5. Oxygen Radical Absorbance Capacity (ORAC) Assay

The ORAC assay was performed according to the method proposed by Dienaité et al. [21] with some modifications described previously in detail [22]. A total of 25 µL of the tested sample or standard solution and 150 µL of fluorescein (working solution 10 nM) were added to the wells of a 96-well black microplate and pre-incubated at 37 °C for 20 min. The reaction was initiated by adding 25 µL of 240 mM 2,2′-azobis (2-methylpropionamide) dihydrochloride (AAPH), and the fluorescence was measured every 90 s for 120 min at an excitation wavelength of 485 nm and an emission wavelength of 515 nm. The buffer was used as a blank. A phosphate buffer (75 mM, pH 7.4) was used for all sample dilutions and reagent preparations. The sample activity was expressed as mM of Trolox equivalents per g DE and as mean ± standard deviation.

2.5.6. Antiproliferative Activity

Cell Culture

The A549 (lung carcinoma) and SW480 (colorectal adenocarcinoma) cell lines were purchased from ATCC (Manassas, VA, USA). The cells were grown in DMEM (Dulbecco’s Modified Eagle Medium, high glucose) + GlutaMAX with the addition of penicillin (50 U/mL), streptomycin (50 U/mL), and 10% heat-inactivated FBS. The cells were maintained in a CO\textsubscript{2} incubator at 37 °C with adequate humidity and passaged twice before running the assay.

Antiproliferative Assay

In order to determine the antiproliferative activity, the cells were seeded in 96-well microplates at a density of 2.5 × 10\textsuperscript{4} cells/mL in 100 µL DMEM + GlutaMAX with 10% heat-inactivated FBS in two sets for different times of the exposure to the tested compound. After 24 h, the plates were rinsed with 100 µL/well of Dulbecco’s phosphate buffered saline (DPBS) and the cells were exposed to 10, 100 and 200 µg/mL final concentration of the compound prepared in fresh medium (without FBS) for 24 and 48 h. The compound was dissolved in sterile water and filtered with a 0.22 µm syringe filter (Wuxi Nest Biotechnology, Wuxi, China) in order to prepare a stock solution. Culture medium containing sterile water was used as a negative control. Each concentration was tested in triplicate. The
antiproliferative activity of the compound was evaluated using the MTT assay as further described. Following 24 and 48 h of exposure to the compound, the medium was removed, the cells were rinsed with DPBS, and 100 µL of fresh medium containing 0.5 mg/mL of MTT was added to each well. Next, the plates were incubated for 3 h at 37 °C in a 5% CO2 incubator. After the incubation, the medium was aspirated, the cells were rinsed with 100 µL of DPBS, and 100 µL of DMSO was added to the wells to extract the dye. Then, the plate was placed on a shaker for 10 min, and the absorbance was quantified at 570 nm using a Synergy H1 microplate reader (BioTek Instruments, Winooski, VT, USA). Viability was estimated as the ratio of the mean of the absorbance reads obtained for each condition to the control one.

2.6. Statistical Analysis

All results were expressed as mean ± standard deviation (SD) from at least three replications. Calculations were performed in STATISTICA 10.0 (StatSoft Poland, Cracow, Poland).

3. Results and Discussion

3.1. Preliminary Chemical Characterisation of R. rugosa Root Polysaccharides

Crude rugosa rose root polysaccharide (CPL-Rx) was obtained using the previously optimised extraction methodology [12]. Its chemical composition was characterised using spectrophotometric, electrophoretic (CE, SDS-PAGE), and chromatographic (LC) techniques and mass spectrometry (MS).

CPL-Rx constitutes a water-soluble fraction of root polysaccharides obtained after hot-water extraction of plant material. The preliminary analyses of the CPL-Rx composition revealed that it contains 28.94 ± 0.01% of sugars, followed by a lower amount of phenolics (4.55 ± 0.12%), uronic acids (2.61 ± 0.41%), and proteins (Table 1). Besides this water-soluble fraction obtained with the yield of 0.80 ± 0.02%, the rose root was found to contain water-insoluble carbohydrates. This was revealed during the determination of the total glucan content, where the procedure started with dry plant material and involved its extraction combined with acidic and enzymatic hydrolysis. The determined amount of glucans (16.65 ± 0.39% of root dry weight; Table 1) exceeded the CPL-Rx yield and the content of soluble sugars (Table 1). This carbohydrate fraction was composed mostly of β-glucans constituting 12.95 ± 0.30% of root d.w. (Table 1). This result is particularly interesting since β-glucans are regarded as one of the most valuable bio-active carbohydrates showing, for example, immunomodulatory, antiviral, antitumour, hypoglycaemic and hypolipidemic activity [23,24]. However, recent research has shed new light also on α-glucans, revealing their bio-medical (e.g., prebiotic and immunostimulatory), nutraceutical, and technological potential [25]. The content of α-glucans in the rose roots was found to be a few times lower than that of β-glucans (3.70 ± 0.09% d.w.). However, their amount was still relatively high. The results are in agreement with previous reports showing remarkable amounts of glucan-type polysaccharides in rose organs [12,26].

Table 1. Yield and chemical composition of crude polysaccharide obtained from R. rugosa root; results given in g/100 g of dry weight (d.w.) or in % of crude polysaccharide. Values are presented as mean ± standard deviation (n ≥ 3).

| Result                              | Result               |
|-------------------------------------|----------------------|
| Yield (g/100 g d.w.)                | 0.80 ± 0.02          |
| Water soluble sugars (g/100 g d.w.)| 0.23 ± 0.00          |
| Total glucans (g/100 g d.w.)       | 16.65 ± 0.39         |
| α-Glucans (g/100 g d.w.)           | 3.70 ± 0.09          |
| β-Glucans (g/100 g d.w.)           | 12.95 ± 0.30         |
| Sugar content (% of CPL-Rx)        | 28.94 ± 0.01         |
| Uronic acids (% of CPL-Rx)         | 2.61 ± 0.41          |
| Phenolic content (% of CPL-Rx)     | 4.55 ± 0.12          |
| Protein content (% of CPL-Rx)      | 0.88 ± 0.03          |
3.2. Monosaccharide Composition Analysis

High-performance capillary electrophoresis was used to perform monosaccharide analysis of rose root polysaccharides. This technique has many advantages e.g., high resolution or low consumption of reagents and requires relatively limited sample pretreatment [27].

The carbohydrate analysis of the hydrolysed CPL-Rx revealed the presence of 7 different carbohydrate peaks (Figure 1).

![Electropherogram of components of crude polysaccharide from R. rugosa root (CPL-Rx). Peak designations: EOF—electro-osmotic flow; 1—Unknown I; 2—Unknown II; 3—Unknown III; 4—galactose; 5—glucose; 6—fructose; 7—arabinose.](image)

Their percent ratio calculated based on the corresponding peak area is shown in Table 2. Glucose was identified as the major monosaccharide. Galactose, fructose, and arabinose were determined in smaller amounts. Moreover, signals from 3 other unidentified carbohydrates were observed (marked as Unknown I, II and III). These carbohydrates constitute a relatively small part of the analysed sample. Based on their migration times as well as our previous observations and experiments, these peaks may represent oligo- or disaccharides. It was also reported by other authors that oligo- or disaccharides migrate before monosaccharides during HPCE analysis [16].

| Unknown I | Unknown II | Unknown III | Galactose | Glucose | Fructose | Arabinose |
|-----------|------------|-------------|-----------|---------|----------|-----------|
| 1.34      | 2.65       | 4.29        | 11.77     | 64.31   | 11.36    | 4.27      |

To the best of our knowledge, the composition of root polysaccharides of any rose species has not been studied to date. CPL-Rx was found to have a different monosaccharide profile from those previously reported for *Rosa roxburghii* Tratt. or *Rosa laevigata* Michx. fruits [28,29]. A study of Kushui Rose (*Rosa setate x Rosa rugosa*) petal wastes has, however, indicated that glucose and galactose constituted the major components of its neutral polysaccharide fraction [30].

3.3. Detection of Proteins and Their Complexes in CPL-Rx

Previous studies have indicated that rose organs contain polysaccharide-peptide complexes [12,13]. The presence of other types of protein conjugates or unbound proteins in
natural crude polysaccharides may be also observed [31]. Therefore, we decided to examine the presence of such macromolecules using SDS-PAGE analysis. This technique facilitates an easy and fast analysis of proteins and their complexes. As a result, a clearly separated band was revealed on the line of CPL-Rx (Figure 2). It indicated the presence of an unbound protein within the range of 15–20 kDa. This observation was quite surprising since the CPL-Rx was obtained using a previously optimised procedure, including a deproteinisation step. Therefore, we decided to identify this macromolecule. For this purpose, the separated protein band was cut from the gel and subjected to further analysis (LC-MS).

![SDS-PAGE analysis of rugosa rose root crude polysaccharide (CPL-Rx). Lanes: M—standard marker; Rx—crude root polysaccharide.](image)

**Figure 2.** SDS-PAGE analysis of rugosa rose root crude polysaccharide (CPL-Rx). Lanes: M—standard marker; Rx—crude root polysaccharide.

### 3.4. Result of LC-MS Analysis—Protein Identification

The SDS-PAGE analysis of CPL-Rx revealed the presence of a clear protein band (Figure 2). In order to identify its content, the band was subjected to MS/MS analysis. The obtained results showed the evident presence of one polypeptide, namely “Protein Plastid Transcriptionally Active 14-Like” (Figure S1 in the Supplementary material). The identified protein appears as “predicted” in the NCBI database (LOCUS XP_024155957). Therefore, the sequence found was aligned to an analogue from *Arabidopsis thaliana* identified at the protein level (UniProtKB-Q84JF5) in order to confirm the similarity using the SIM alignment tool from [www.expasy.org](http://www.expasy.org), accessed on 30 Augusts 2022. The identified sequence showed 70.68% identity when compared to Protein Plastid Transcriptionally Active 14 from *A. thaliana* (Supplementary material; Figure S2). The protein is a methyltransferase engaged in regulation of the transcription of chloroplast development, especially for thylakoid formation. This component of transcriptionally active chromosome complexes is mostly expressed in leaves, flowers and seedlings and, to a lesser extent, in stems and roots. It was found that the protein interacts with PTAC12 and PTAC7, i.e., expression factors that are essential for the initiation of photomorphogenesis mediated by phytochromes (PHYS), by mediating PHY localisation to photobodies and thylakoid formation, respectively [32]. It can be concluded that the detected protein is probably an element of the transcription machinery regulating the expression of proteins responsible for the development of chloroplasts by modulating the activity of RNA polymerase.
3.5. Bio-Medical Potential of CPL-Rx

3.5.1. Inhibition of Pro-Inflammatory Enzymes

Crude polysaccharides from other *R. rugosa* organs were previously reported to have anti-inflammatory potential [12,33]. Therefore, we decided to examine CPL-Rx in terms of its influence on the activity of pro-inflammatory enzymes—cyclooxygenases (COX-1 and COX-2) and hyaluronidase (HYAL).

Several concentrations (0.25–5 mg/mL) were tested to evaluate the COX-1 and COX-2 inhibitory potential (Table 3). The CPL-Rx activity was compared with acetylsalicylic acid, which is commonly used in pharmacotherapy as a non-selective COX inhibitor. It was observed that the rose root crude polysaccharide influenced both COX isoenzymes in a dose-dependent manner. However, a stronger impact on COX-2 was shown, particularly at the highest concentrations tested, where 45.64 to 71.98% of COX-2 inhibition was observed. COX-1 was much less affected by CPL-Rx. At the highest concentrations tested, the COX-1 activity decreased by 18.91–23.26%. In terms of selectivity and potency, CPL-Rx was found to be similar to the crude polysaccharide from rose leaves [12].

Table 3. Inhibition of COX-1, COX-2, and hyaluronidase (Hyal) activity in the presence of *R. rugosa* root crude polysaccharide (CPL-Rx).

| Sample | Concentration (mg/mL) | COX-1 Inhibition (%) | COX-2 Inhibition (%) | HYAL Inhibition (IC$_{50}$; µg/mL) |
|--------|------------------------|-----------------------|----------------------|-----------------------------------|
| CPL-Rx | 0.25                   | n.a.                  | n.a.                 |                                   |
| CPL-Rx | 0.5                    | n.a.                  | n.a.                 |                                   |
| CPL-Rx | 0.75                   | 4.67 ± 0.26           | 6.96 ± 0.36          | 335.23 ± 4.16                     |
| CPL-Rx | 1                      | 10.64 ± 0.59          | 8.19 ± 0.39          |                                   |
| CPL-Rx | 2                      | 18.91 ± 0.83          | 45.64 ± 1.95         |                                   |
| CPL-Rx | 5                      | 23.26 ± 0.88          | 71.98 ± 1.82         |                                   |
| ASA    | 1 mM                   | 65.26 ± 1.84          | 75.03 ± 4.07         | 280.50 ± 3.52                     |
| EGCG   | 4                      | 70.50 ± 3.52          |                      |                                   |

Positive controls: acetylsalicylic acid (ASA)—for the COX inhibitory assay; epigallocatechin gallate (EGCG)—for the HYAL inhibitory assay; Abbreviation: n.a.—no activity observed.

Hyaluronidase is responsible for degradation of hyaluronic acid; therefore, it is associated with the proper functioning of connective tissue and the course of inflammatory and other physiological and pathological processes (e.g., invasion or metastasis). The HYAL inhibitory assay revealed strong anti-hyaluronidase potential of CPL-Rx. Its IC$_{50}$ value (concentration that reduced the activity of the enzyme by 50%) was 335.23 ± 4.16 µg/mL, which was even lower than the value previously reported for other highly active rose polysaccharides [12]. Moreover, the CPL-Rx activity was comparable to the potential of EGCG (IC$_{50}$ = 280.50 ± 3.52 µg/mL). This result suggests the possibility of the use of CPL-Rx as an anti-hyaluronidase agent for pharmaceutical or cosmetic purposes and encourages further research, e.g., on in vivo models.

3.5.2. Antioxidative Activity

Various natural polysaccharides exhibit antioxidative or radical scavenging potential [4,34]. Therefore, we aimed to examine the CPL-Rx using three antioxidant tests, i.e., TEAC, DPPH$^*$ radical scavenging activity, and oxygen radical absorbance capacity assays. The results were compared with the activity of well-known and highly active antioxidant molecules (Trolox or ascorbic acid) and presented as equivalents of the standards (Table 4). All protocols revealed high potential of the samples. In the Trolox equivalent antioxidant capacity (TEAC) assay, the activity of the rose root crude polysaccharide was found to be 0.302 ± 0.01 mmol/g of dry extract (DE). The result of the DPPH$^*$ radical scavenging assay was presented as the half maximal effective concentration (EC$_{50}$ value) as well as Trolox (TE) and ascorbic acid equivalents (AsAE). The ability to scavenge DPPH$^*$ radical (TE = 0.092 ± 0.01 mmol/g DE) was found to be lower than the activity against ABTS$^{**}$ radical cation (used in the TEAC protocol).
Table 4. Antioxidant activity of R. rugosa root crude polysaccharide determined with Trolox equivalent antioxidant capacity (TEAC), DPPH• radical scavenging activity, and oxygen radical absorbance capacity (ORAC) assays.

| TEAC (mmol/g DE) | DPPH Scavenging Activity | ORAC (mmol/g DE) |
|------------------|--------------------------|------------------|
| EC$_{50}$ (mg DE/mg DPPH•) | TE (mmol/g DE) | AsAE (mmol/g DE) | TE (mmol/g DE) |
| 0.302 ± 0.01 | 3.92 ± 0.12 | 0.092 ± 0.01 | 0.139 ± 0.08 | 0.134 ± 0.10 |

Abbreviations: DE—dry extract; TE—Trolox equivalent; AsAE—ascorbic acid equivalent. Values are presented as mean ± standard deviation ($n \geq 3$).

ORAC is a somewhat more advanced and accurate assay since it engages peroxy radicals and takes into account antioxidant reaction kinetics [35]. It was demonstrated that CPL-Rx was an efficient peroxy radical scavenger (0.134 ± 0.10 mmol Trolox/g DE).

Our results are in agreement with previous studies of the antioxidant potential of rose polysaccharides [12,34,36].

3.5.3. Antiproliferative Potential

The antiproliferative effect of CPL-Rx was tested on SW480 (colon) and A549 (lung) cancer cell lines. CPL-Rx exhibited slight activity against both cell lines (Figure 3). The maximum concentration tested (200 µg/mL) caused a decrease in viability by 19.24% and 20.01% after 24 and 48 h of incubation, respectively, in the case of the A549 cells. In turn, the values for the SW480 cells were 18.13% and 14.1%, respectively. In addition, higher proliferation was observed at the extended incubation time (48 h), which may be a result of the reduced effect of the substance. In this case, this may be caused by the gradual adaptation of the cells to the content of CPL-Rx in the medium or the possible degradation of the compound in the cell culture conditions, and thus its lower stability. Nevertheless, the chemopreventive potential of this compound is worth exploring further, especially due to the fact that it is a compound of natural origin, which may require a prolonged use, an increase in the dose, or maintenance of its concentration at a constant level through regular use.

4. Conclusions

The presented study provides the first report on the composition and biological activity of crude polysaccharides obtained from rose roots. It indicates new possibilities of use of this plant material as a source of water-soluble and insoluble carbohydrates, including glucans. Our study revealed the presence of protein that has previously not been reported in this species. At this stage, it is difficult to assess the contribution of the protein to the activity of the sample. However, due to the total low protein content in the CPL-Rx, this impact is likely to be inconsiderable. To sum up, our research provides the first insights...
into the bio-active macromolecules of rugosa rose roots. Since *R. rugosa* is one of the rose cultivars and industrial amounts of roots can be obtained from its plantations, its root polysaccharides deserve further attention and research. Our results suggest that CPL-Rx may potentially be used in cosmetics, food and biomedical industries, especially as an anti-hyaluronidase and chemopreventive agent. We believe that our paper will contribute to the valorisation of post-harvest wastes and the more efficient utilisation of rose crops.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app121910126/s1, Figure S1: Result of identification of protein revealed in CPL-Rx; Figure S2: Graphical representation of the alignment of Protein Plastid Transcriptionally Active 14 from *Rosa chinensis* and *Arabidopsis thaliana*. Figure S3: Morphology of *Rosa rugosa* Thunb.

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