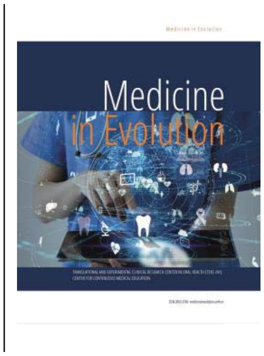
Phytochemical Characterization and Biological Evaluation of Ribes Nigrum Leaf Extracts: A Study of Aqueous and Butanolic Fractions



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Abstract

Background: Ribes nigrum (black currants) are recognized for their outstanding biological properties, including antioxidant, anti-inflammatory, and anticancer effects. Colorectal cancer (CRC) is a type of cancer with a high mortality, for which various botanical products have been investigated as alternatives that have emerged as potential candidates in the oncological domain. Methods: The present work involved the development of two types of Ribes nigrum leaves extracts (in aqueous and butanolic fractions), which were evaluated by the HPLC method and subsequently assessed for the antioxidant capacity, the antimicrobial activity, and in vitro cytotoxicity on the DLD-1 cell line. Results: The results indicated that both types of extracts contain amounts of polyphenols, the antioxidant activity of the extracts supports their potential as natural sources of free radical scavengers, antimicrobial evaluation showed that the extract in aqueous fraction produced a selective antimicrobial effect on gram-positive bacteria, and the MTT assay suggested that the extracts produced a dose-dependent cytotoxicity, with a superior effect noted for the extract in the butanolic fraction. Conclusion: These findings emphasized the biological potential of Ribes nigrum extracts due to their versatile composition and opened new research directions regarding their anticancer potential in CRC.

Keywords: blackcurrants, Ribes nigrum, colorectal cancer, natural alternative, antimicrobial activity, antioxidant activity.

INTRODUCTION

Nature has offered for thousands of years an inexhaustible source of substances that have framed multiple therapeutic remedies. Plants have been used in traditional medicine for centuries to treat various diseases. Today, many herbal compounds are being studied for their potential in drug development. These plant-based medicines offer a cost-effective, effective, and safer alternative to conventional treatments [1]. Blackcurrant (Ribes nigrum L., Grossulariaceae) is a shrub generally found in temperate climates, and its fruits are a great source of vitamin C and other beneficial substances (e.g., essential oils, micro- and macronutrients). The fruits of the shrub also contain polyphenolic constituents that display versatile properties, including antioxidant, antimicrobial, antibacterial, and antiviral properties. In vitro, in cell cultures, it was observed that polyphenols show anti-tumour activity, inducing apoptosis of cancer cells [2]. Beside this, anthocyanins are known dietary antioxidants, valued for their potential to restore balance between oxidative and antioxidant factors in living organisms. Based on the specialized literature, blackcurrants hold significant amounts of anthocyanins (250 mg/100 g of fresh fruit) and are acknowledged in traditional medicine in Europe and Asia for treating various conditions. In addition, a fundamental consideration is that blackcurrant extract has recently been identified as the second most effective of nine different berry extracts studied for their free radical scavenging properties [3].

Black currants have demonstrated therapeutic potential in managing hypertension and other cardiovascular diseases, as well as neurodegenerative, neoplastic, ocular conditions, and diabetic neuropathy [4]. In cancer research, black currant extracts have shown anticancer effects in vitro against several cancer types, including gastric cancer and esophageal squamous cell carcinoma [5]. Additionally, black currant juice and extracts have been found to inhibit the proliferation of breast, prostate, and colon cancer cells [3].

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of cancer-related deaths. The lifetime risk of developing CRC is estimated at 4–5% and is influenced by factors such as medical history, age, and lifestyle. CRC arises from mutations in oncogenes, tumor suppressor genes, and DNA repair genes. Based on the origin of these mutations, CRC is classified as sporadic, hereditary, or familial [6]. In vivo studies have shown that long-term black currant supplementation can influence the gut microbiome in female mice, with effects varying by age [7]. Additionally, blackcurrant-based products have also been noted for their hypocholesterolemic and anti-inflammatory actions (whole berry and juice), as well as antioxidant actions that are specific to the leaves [8].

Given the growing interest in botanical compounds for medical applications, and considering the proven health benefits of black currants (e.g., antioxidant, anti-inflammatory, antitumour properties), the objective of the present work is to develop two types of Ribes nigrum leaf extracts containing polar constituents (aqueous and butanolic fractions) and to evaluate their composition by HPLC method, followed by assessment of their antioxidant and antimicrobial activities and to subsequently investigate their cytotoxic potential on a CRC cell line, namely DLD-1 cells. These investigations pave the foundation for future research on the potential of Ribes nigrum L. natural extracts as therapeutic alternatives in CRC.

MATERIAL AND METHODS

Ribes nigrum Extract Preparation

Ribes nigrum leaves were procured from S.C. Hypericum Impex S.R.L. (Baia Sprie, Romania), lot 0164, maintained under appropriate conditions at a temperature of 22 ± 2 °C until processing. The extraction procedure started by obtaining a crude ethanolic extract, that was subjected to liquid-liquid partition in order to yield two polar fractions soluble in butanol and water. The non-polar compounds were eliminated from the crude extract by pre-treatment with petroleum ether, diethyl ether and ethyl acetate. The solvents were purchased as follows: ethanol (99.8%) from Riedel-de Haen (Seelze, Germany), petroleum ether, diethyl ether, ethyl acetate, and n-butanol were purchased from Sigma Aldrich (Steinheim, Germany). 200 g dried leaves were crushed to powder with the IKA A11 basic and mixed with 1000 mL absolute ethanol. The mixture was ultrasonicated for 30 minutes using an ultrasonic water bath (ELMA S120 Elmasonic from Elma Schmidbauer GmbH, Singen, Germany), followed by a filtration procedure using Whatman grade 4 filter paper. The extract was subjected to solvent evaporation using a rotary evaporator (Heidolph, Schwabach, Germany). The plant material was subsequently extracted with additional 1000 mL ethanol (99.8%). After solvent evaporation, 9.7 g crude extract were obtained.

A portion (8.0 g) of the crude extract was suspended in 50 ml of distilled water and then subjected to repeated liquid-liquid separation in a separating funnel, using solvents in order of increasing polarity. To obtain the desired fractions with polar phytochemicals, the suspension was pretreated with organic solvents that depleted the non-polar compounds (petroleum ether, diethyl ether, and ethyl acetate, 600 ml per solvent). In the end, the remaining aqueous suspension collected from the separation funnel was extracted using, successively, 3 times 200 mL of n-butanol. The fraction containing butanol-soluble phytochemicals, and the fraction containing water-soluble constituents were both subjected to solvent evaporation using a rotary evaporator, followed by lyophilisation. The two polar fractions were obtained from the crude extract with the following yields: 11.37% for the n-butanol soluble fraction, and 11.00% for the water-soluble fraction.

HPLC/LC–MS Identification and Quantification of Polyphenolic Compounds

The phytochemical composition of the *Ribes nigrum* extracts was analyzed using LC–MS/MS, based on two previously validated analytical methods described [9–11]. Analyses were conducted on an Agilent 1100 HPLC Series system (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 1100 SL Ion Trap mass spectrometer (LC/MSD Ion Trap VL) [12,13].

The first method focused on the identification and quantification of polyphenolic compounds. It employed a Zorbax SB-C18 reversed-phase column (100 mm × 3.0 mm i.d., 3.5 µm particle size) and a binary mobile phase consisting of methanol and 0.1% acetic acid (v/v), delivered in gradient mode. A total of twenty-eight polyphenolic standards were included; however, only a subset was detected and quantified in the analyzed extracts, as detailed in the Results section. Chromatographic conditions included: (1) a column temperature of 48 °C; (2) a flow rate of 1 mL/min; and (3) an injection volume of 5 µL. UV detection was performed at 330 nm for polyphenolic acids up to 17 minutes and at 370 nm for flavonoids and their aglycones up to 38 minutes, with MS detection in negative electrospray ionization (ESI) mode [14,15]. A second LC–MS method was optimized to identify eight additional polyphenols (e.g., epicatechin, catechin, syringic acid, gallic acid, vanillic acid, protocatechuic acid, epigallocatechin, epigallocatechin gallate), using the same column and instrumentation with a modified gradient. Detection was carried out under the same ESI conditions [11,15].

Compound identification was based on comparison of mass spectra and chromatographic retention times with reference standards, while quantification was completed using UV detection and external calibration curves. Finally, data processing was performed using DataAnalysis (v5.3) and ChemStation (vB01.03) software (Agilent Technologies). Results are expressed as micrograms of bioactive compound per milliliter of extract.

Antioxidant activity of polar *Ribes nigrum* fractions (aqueous and butanolic)

The DPPH assay was carried out to evaluate the antioxidant activity of *Ribes nigrum* extracts, following a previously reported method [16], with minor modifications adapted to our laboratory conditions. Briefly, 0.2 mL of *Ribes nigrum* extracts (at concentrations ranging from 100 to 1000 µg/mL) was mixed with 1.8 mL of 0.1 mM DPPH solution prepared in ethanol. The mixture was incubated in the dark at room temperature for 30 minutes and the ascorbic acid was used as a reference standard. The absorbance was measured at 517 nm using a UV–VIS spectrophotometer (PG Instruments Ltd., Lutterworth, UK).

***In vitro* Antimicrobial Activity**

The antimicrobial activity of the tested samples was checked against five reference microbial strains from ThermoScientific (USA): Staphylococcus aureus ATCC 25923, Streptococcus pyogenes ATCC 19615, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, and Candida parapsilosis ATCC 22019. The evaluation was made in accordance with EUCAST [17] and CLSI [18] guidelines, as well as the procedures described in our previous studies. Gram-positive and Gram-negative bacterial strains were cultured on Columbia agar supplemented with 5% defibrinated sheep blood, while the yeast C. parapsilosis was grown on Sabouraud dextrose agar containing chloramphenicol (Oxoid, Wesel, Germany). Microbial suspensions were prepared in 0.85% NaCl solution and adjusted to a turbidity equivalent to 0.5 McFarland standard, corresponding to approximately 1–2 × 10^8 CFU/mL.

Antimicrobial activity was initially screened using the disk diffusion method. Mueller–Hinton (MH) agar or MH supplemented with 5% sheep blood and β-NAD (MHF medium) for Streptococcus pyogenes (Oxoid, Wesel, Germany) was used for inoculation with microbial suspensions of each tested strain. After inoculation and drying, sterile 6 mm blank paper disks (BioMaxima, Poland) were placed on the agar surface and loaded with 5 µL of each test compound at a concentration of 20 mg/mL. Gentamicin (for Gram-positive and Gram-negative bacterial strains) and fluconazole (for yeast Candida parapsilosis) served as positive controls, while disks impregnated with solvent (DMSO or EtOH/H_2O, depending on the sample) were used as negative controls. Plates were incubated at 35 °C for 24 hours, after which the diameters of the inhibition zones were measured. Strains exhibiting inhibition zones between 6–15 mm were deemed resistant to the tested compounds and were not further evaluated. For strains with inhibition zones exceeding 15 mm, minimum inhibitory concentration (MIC) testing was performed [19].

Chemicals and reagents

Roswell Park Memorial Institute 1640 (RPMI-1640) basal medium, fetal bovine serum (FBS), L-glutamine, penicillin-streptomycin, and trypsin-EDTA 0.25x were obtained from Biowest (Nuaille, France). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was procured from Alfa Aesar.

Cell lines and culture conditions

The human colorectal adenocarcinoma cell line (DLD-1) employed in this research was acquired from the European Cell Culture Center and was cultivated under standard conditions in RPMI-1640 basal medium supplemented with 10% fetal bovine serum (FBS), 1% glutamine, and 1% penicillin -streptomycin. The cells were maintained at 37°C in a humidified atmosphere containing 5% CO2. The medium was refreshed every two days, and the cells were regularly subcultured at 80% confluence by trypsinization (trypsin-EDTA).

Cytotoxicity Assay

The cytotoxicity of the plant extract obtained was evaluated on the DLD-1 human colorectal adenocarcinoma cell line using the classic 3- (4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) method. In summary, the cells were seeded in 96-well microplates at a density of 1 x 10^4 cells/mL per well and incubated for 24 hours. Then, the medium was replaced with each plant extract in eight successive concentrations ranging from 0 to 1000 μg/mL in six technical replicates, followed by incubation for 24 hours

Plant extract samples were previously dissolved in dimethyl sulfoxide (DMSO), and diluted with culture medium as the concentration of DMSO did not exceed 1% v/v. Then 100 μL MTT solution (5 mg/mL in PBS) was added to each well and incubated for 2 h at 37 ºC. The medium containing the MTT solution was aspired without disturbing the formazan crystals formed in the wells. Finally, 150 μL of DMSO was added to each well to dissolve the MTT crystals until the appearance of the specific purple color.

Finally, the absorbance was measured at 570 nm using a microplate reader (BMG Labtech, Germany). The cells without treatment (untreated) were considered as negative control. The cell viability (%) was calculated compared with untreated control, based on the absorbance values, as follows: viability (%) = O.D. (experimental value)/O.D. (control value) x 100. The half maximal inhibitory concentration (IC_{50}) values for each plant extract was calculated using GraphPad Prism 5.0 (San Diego, CA, USA), applying non-linear regression with log concentration vs. normalized response.

RESULTS

Identification and Quantification of Phenolic Compounds in *Ribes nigrum* Extracts

The *Ribes nigrum* extracts studied in this work exhibited varying concentrations of phenolic compounds (Table 1). The aqueous extract contained only rutoside and isoquercitrin, with rutoside being the most abundant. In contrast, the n-butanol (n-BuOH) extract presented a broader polyphenolic profile, including rutoside, isoquercitrin, quercetin, and hyperoside. Chlorogenic acid was detected in both fractions, although only in low concentrations or trace amounts.

Table 1. Identification and Quantification of Polyphenolic Compounds from Ribes nigrum by HPLC/LC–MS

No.	Compounds	Aqueous extract	n-BuOH extract
		Results (μg/mL)	
1	Hyperoside	<LOQ	0.305±0.024
2	Isoquercitrin	0.329±0.029	2.718±0.407
3	Rutoside	0.503±0.005	4.139±0.331
4	Quercetin	<LOQ	1.398±0.167
5	Chlorogenic acid	<LOQ	<LOQ

< LOQ (below limit of quantification). Concentrations are expressed as mean ± SD (n = 3).

The antioxidant activity

The antioxidant activity of the extracts was analyzed using the DPPH test, and the results are shown as the percentage of free radical inhibition at different extract concentrations (100–1000 μg/mL). For both extracts, a significant increase in antioxidant activity was observed with increasing concentration, indicating a dose-dependent relationship (Figure 1).

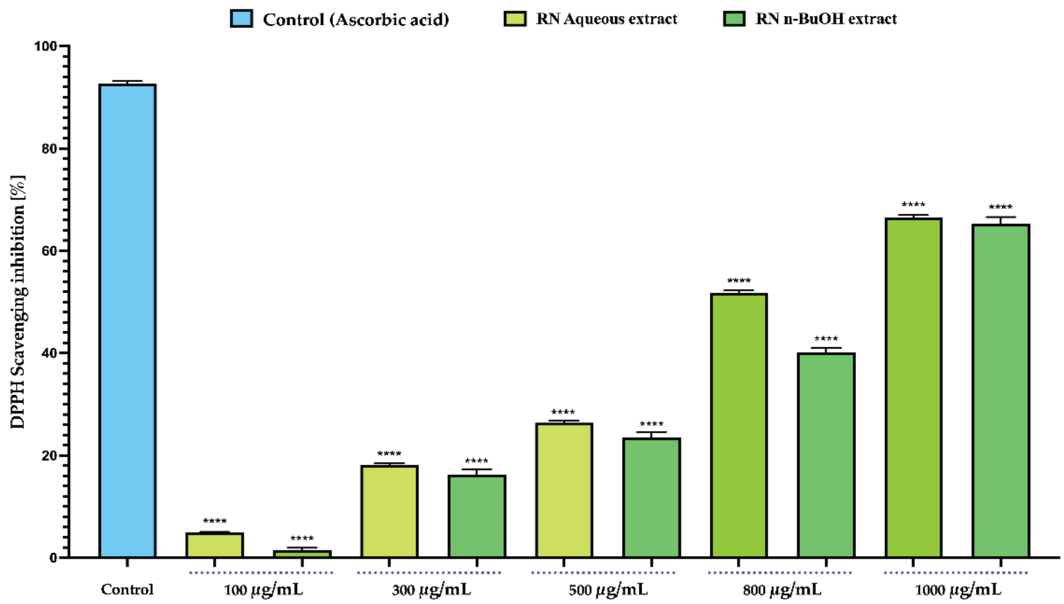

Figure 1. DPPH radical scavenging activity of *Ribes nigrum* aqueous and n-butanol (BuOH) extracts compared with ascorbic acid. Data are presented as mean ± SD (n = 3). One-way ANOVA test followed by a Dunnett's multiple comparison test was used to compare groups (****$p < 0.0001$)

The antimicrobial activity

The antimicrobial activity of *Ribes nigrum* (RN) extracts was investigated using the disk diffusion method. The inhibition zone diameters (mm) for each microbial strain and extract are summarized in the table below:

Table 2. Antimicrobial efficacy of Ribes nigrum extracts expressed as inhibition zone diameters

Microbial strains	Test compound	Inhibition zone (mm)
Staphylococcus aureus ATCC 25923	RN n-BuOH extract RN Aqueous extract	8 15
Streptococcus pyogenes ATCC 19615	RN n-BuOH extract RN Aqueous extract	8 18
Escherichia coli ATCC 25922	RN n-BuOH extract RN Aqueous extract	8 13
Pseudomonas aeruginosa ATCC 27853	RN n-BuOH extract RN Aqueous extract	8 8
Candida parapsilosis ATCC 22019	RN n-BuOH extract RN Aqueous extract	8 8

Extracts producing inhibition zones ≤ 15 mm were considered to have weak or no significant antimicrobial activity. Based on this criterion, only the *Ribes nigrum* aqueous extract showed measurable antimicrobial effects against *S. aureus* (15 mm), *S. pyogenes* (18 mm), and *E. coli* (13 mm).

Cell Viability Evaluation

The two extracts from *Ribes nigrum* leaves (the aqueous fraction and the butanolic fraction) were assessed for their cytotoxicity against the human colorectal adenocarcinoma cell line (DLD-1) in 8 successive concentrations (0-1000 µg/mL) 24 hours following treatment application. Both extracts exerted a dose-dependent inhibitory effect on the cell viability of the DLD-1 cell line, as shown in Figure 2 and Figure 3. Increasing concentrations of the tested plant extracts induced suppression of cell proliferation and reduction of cell viability. The half maximal inhibitory concentration (IC_{50}) for the aqueous fraction was established at 278.4 µM, while in the case of the butanol fraction, the IC_{50} was found to be 137.9 µM. The treatment of the cells with *Ribes nigrum* leaves extract, aqueous fraction, with the highest concentration evaluated, i.e., 1000 µM, caused a reduction in the viability of the cells to about 14%. In the case of the butanol fraction, the treatment with 1000 µM induces a decrease to approximately 3%.

Thus, the *Ribes nigrum* leaves extract obtained in butanol fraction showed superior cytotoxic activity on the DLD-1 cell line compared to the aqueous fraction.

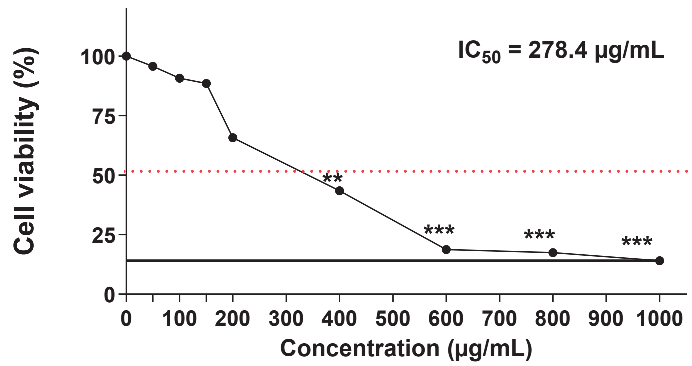

Figure 2. Graphical representation of the DLD-1 cell viability after treatment with the aqueous fraction of *Ribes Nigrum* L. extract in concentrations 0-1000 µg/mL for 24 hours

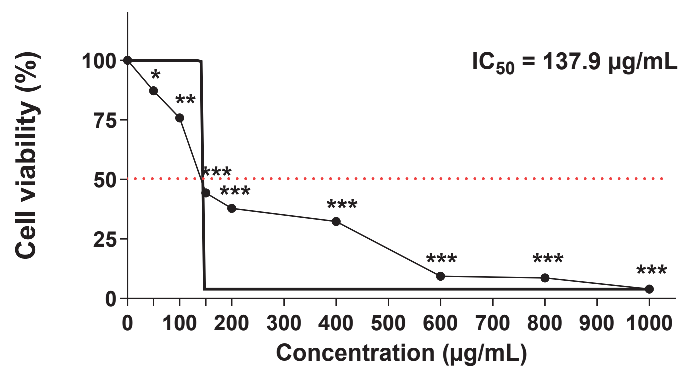

Figure 3. Graphical representation of the DLD-1 cell viability after treatment with the butanolic fraction of Ribes Nigrum L. extract in concentrations 0-1000 µg/mL for 24 hours

DISCUSSIONS

In present, the current conventional therapy for patients diagnosed with CRC constitutes a major challenge, with high toxicity and poor response. However, it was observed that natural components might be able to have antiproliferative and apoptosis-inducing capacity in CRC, as supported by *in vitro, in vivo,* and clinical studies. Many natural compounds also offer the benefit of being well tolerated by patients, with no toxic actions, even at high doses [20]. In this regard, this paper covers HPLC analysis, the antioxidant and antimicrobial activity of polar extracts from Ribes nigrum leaves, as well as the evaluation of cell viability after treating DLD-1 cells (colorectal adenocarcinoma) with the two types of extracts (aqueous and butanolic fraction), taking into account the potential already mentioned in the literature that arouses increasing curiosity about *Ribes nigrum.*

HPLC represents one of the most popular methods for the identification of phenolic compounds contained in plants. The LC–MS/MS phytochemical analysis revealed the presence of several polyphenolic compounds in the *Ribes nigrum* extracts, with notable differences in composition depending on the extraction solvent used. These findings confirm that solvent polarity plays a critical role in determining the qualitative and quantitative profile of the extracted bioactive compounds [20–22]. In the aqueous extract, only two major polyphenols (e.g., rutoside and isoquercitrin) were detected. This suggests a preferential extraction of polar flavonoid glycosides by water. Rutoside or rutin was the most abundant compound, consistent with literature data reporting *Ribes nigrum* as a rich source of glycosylated flavonoids [23–25]. In contrast, the n-butanol extract exhibited a more complex polyphenolic profile, additionally containing quercetin and hyperoside. This broader spectrum is likely due to the intermediate polarity of n-butanol comparative to water, which facilitates the extraction of both polar and moderately polar flavonoids, including aglycones.

Besides, current ongoing research is focused on exploring compounds of botanical origin that can treat microbial diseases, since the increasing occurrence of multidrug resistance in microorganisms across the globe. For these purposes, evaluation of antimicrobial activity was one of the objectives of the study [26]. The antimicrobial screening of *Ribes nigrum* extracts revealed differential activity depending on the solvent used and the microbial strain tested. Notably, the aqueous extract demonstrated superior antimicrobial effects compared to the n-butanol extract, as evidenced by larger inhibition zones observed in the disk diffusion assay. Among the tested strains, *Streptococcus pyogenes* was the most susceptible, showing an inhibition zone of 18 mm for the aqueous extract, which justified further evaluation through minimum inhibitory concentration (MIC) testing. *Staphylococcus aureus* also exhibited moderate sensitivity (15 mm), while *Escherichia coli* showed a weaker response (13 mm). In contrast, both *Pseudomonas aeruginosa* and yeast *Candida parapsilosis* were resistant to the tested extracts, with inhibition zones not exceeding 8 mm, with the same value recorded for the negative control, suggesting a lack of efficacy against these more resilient pathogens. The enhanced antimicrobial activity of the aqueous extract may be attributed to its higher content of polar phenolic compounds, particularly rutoside and isoquercitrin, both of which have been previously reported to possess antimicrobial properties through mechanisms such as membrane disruption and inhibition of nucleic acid synthesis [27–30]. The absence of additional aglycones in the aqueous extract may suggest that glycosylated flavonoids are primarily responsible for the observed bioactivity, at least in the case of Gram-positive bacteria.

In this study, both aqueous and butanolic extracts of *Ribes nigrum* exhibited dose-dependent antioxidant activity, as evidenced by increasing inhibition values with rising extract concentrations. These findings are consistent with previous reports highlighting the antioxidant potential of *Ribes nigrum* due to its high content of polyphenols, anthocyanins, and flavonoids [31–33]. For the *Ribes nigrum* aqueous extract**,** an inhibition of 66.44% was recorded at 1000 μg/mL, with a calculated EC_{50} value of 790 ± 0.18 μg/mL. In comparison, the *Ribes nigrum* ***n***-butanol (BuOH) extract showed a maximum inhibition of 64.17**%** at 1000 μg/mL, with an estimated EC_{50} of 880.7 ± 0.43 μg/mL. Although the antioxidant activity of the BuOH extract was slightly lower, it followed the same dose–response pattern, supporting the reproducibility and consistency of the antioxidant effect.

Further, the actual study aimed to determine the cytotoxic capacity of the two types of extracts on DLD-1 cells. Recently, it has been identified that certain compounds used in standard CRC treatment (e.g., 5-fluorouracil) induce multiple adverse effects, and the tumours show resistance to their administration. Consequently, natural compounds are the target of several research projects intended to identify their potential effect as therapeutic alternatives in the fight against cancerous pathologies, including colorectal cancer, one of the most lethal neoplasms [34]. Regarding the evaluation of the antitumor potential, the two types of *Ribes nigrum* leaf extracts were investigated in DLD-1 cells. After treatment of the cells, the extract in the butanol fraction showed a superior anticancer effect compared to that in the aqueous fraction. Thus, comparing the highest concentrations tested (i.e., 1000 μM) at the same treatment interval, the extract in the butanol fraction reduced the viability of cancer cells up to 3%, while the extract in the aqueous fraction reduced the viability of cancer cells up to 13%. Moreover, the IC_{50} results support the same finding, the IC_{50} being 278.4 μM for the treatment with the aqueous fraction of the *Ribes nigrum* extract and 137.9 μM for the treatment with the n-butanol fraction of the leaf extract. Another group of researchers showed that ethanolic extract from *Ribes nigrum* leaves has the ability to inhibit the growth of HT-29 (human colon adenocarcinoma) and MCF7 (human breast cancer) cancer cells. Also, their results were more significant as the treatment period was longer [35]. Today, current clinical treatments of CRC, including surgical resection, radiotherapy, and chemotherapy, face several barriers among which can be listed the adverse effects, the risk of recurrence, and the drug resistance. Against this background, the development of drugs with low toxicity and reduced potential for the generation of drug resistance is an increasingly major challenge. Therefore, natural compounds have become researched since they have acquired a reputation for their potential anti-tumour activity, a function that is receiving growing interest, in addition to many other therapeutic properties. Several natural compounds have been shown to have the ability to modulate apoptosis, different signaling pathways, and cell differentiation; further establishing their status as low-toxicity compounds [36].

According to the results of the present study on DLD-1 cells, the analyzed extracts showed cytotoxic potential. However, future research directions should be directed towards the identification of the exact mechanism of action underlying the antitumor capacity, verification of the biosafety profile, and further evaluation in complementary experimental models such as *in ovo* and *in vivo*.

CONCLUSIONS

In this study, two extracts from *Ribes nigrum* L. were prepared in butanolic and aqueous fractions and subsequently evaluated. LC–MS/MS profiling revealed that both aqueous and n-butanol extracts contain notable polyphenolic compounds, with rutoside and isoquercitrin as dominant constituents. The aqueous extract showed a simpler, but more selective polyphenolic composition, whereas the n-butanol extract included a broader range of flavonoids, including aglycones (*e.g.*, quercetin, hyperoside). The antioxidant activity of the extracts, attributed to the presence of these phenolic compounds, supports their potential as natural sources of free radical scavengers. Antimicrobial assays demonstrated that the aqueous extract exhibits selective activity against Gram-positive bacteria, with significant inhibition observed against *Streptococcus pyogenes* and moderate effects against *Staphylococcus aureus*. No significant activity was recorded against *Pseudomonas aeruginosa, Escherichia coli*, or the yeast *Candida parapsilosis*. These findings suggest that polar flavonoid glycosides may contribute to antimicrobial activity, especially against more permeable bacterial cell walls. In terms of cytotoxic activity against human colorectal adenocarcinoma DLD-1 cells, the butanolic fraction proved to be more active, reducing cell viability to a greater extent than the aqueous fraction. Future studies focusing on determining the mechanisms of action underlying biological effects represent important milestones in the development and progress of scientific research on *Ribes nigrum*.

Conflicts of Interest

The authors declare no conflict of interest.

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