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Antidiabetic and Anti-Inflammatory Potential of *Sorbus aucuparia* Fruits (Rowanberries) from Romania

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Abstract

This study aimed to obtain extracts concentrated in polyphenolic compounds from *Sorbus aucuparia* fruits and evaluate their antioxidant, antidiabetic, anti-inflammatory, and cytotoxic potential. Two modern extraction methods were used, ultrasound-assisted extraction (UAE) and accelerated solvent extraction (ASE), to obtain hydroalcoholic extracts (50% EtOH *v*/*v*, 15% mass), then the extracts were purified and concentrated by membrane technologies and analyzed spectrophotometrically and chromatographically. HPLC analysis revealed the predominant polyphenolic compounds as chlorogenic acid (526.08 ± 23.35 µg/mL), rutin (36.07 ± 1.23 µg/mL), and caffeic acid (34.41 ± 1.21 µg/mL). The antidiabetic and anti-inflammatory potential of the extracts was analyzed spectrophotometrically by testing their capacity to inhibit α-amylase and α-glucosidase, and, respectively, hyaluronidase (HYA) and lipoxygenase (LOX). The cytotoxic potential of the extracts was tested on the mouse fibroblast NCTC clone L929 cell line. The concentrated ASE extracts showed a pronounced inhibitory activity on the tested enzymes: IC_{50}α-glucosidase was 13.50 ± 0.96 µg/mL, (IC_{50}acarbose was 20.19 ± 1.67 µg/mL), IC_{50}α-amylase was 23.74 ± 1.32 µg/mL (IC_{50}acarbose was 22.65 ± 1.27 µg/mL), and IC_{50LOX} was 24.30 ± 1.54 µg/mL (IC_{50}ibuprofen was 26.91 ± 1.27 µg/mL), IC_{50}HYA was 43.04 ± 2.19 µg/mL (IC_{50}ibuprofen was 51.54 ± 3.67 µg/mL). Also, the concentrated UAE extracts presented inhibitory activity superior to or close to that of the standard used, as follows: IC_{50}HYA was 48.49 ± 3.15 µg/mL (IC_{50}ibuprofen was 51.54 ± 3.67 µg/mL) and IC_{50}α-glucosidase was 21.53 ± 1.25 µg/mL (IC_{50}acarbose was 20.19 ± 1.67 µg/mL). The results obtained showed that *Sorbus aucuparia* fruits could be used in products for diabetes and inflammatory diseases.

Keywords: *Sorbus aucuparia*; antidiabetic; anti-inflammatory; antioxidant; cytotoxic capacity

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1. Introduction

Recent years have seen a marked increase in the focus on bioactive compounds of natural origin. This trend is driven, in part, by the fact that around 80% of the global population relies on medicinal plants for various health concerns, with over 40% of currently available synthetic medications deriving from botanical or microbial sources [1].

Natural bioactive compounds have potential applications in the pharmaceutical, cosmetic, and food industries [2,3], as well as in the field of nutraceuticals and dietary supplements, a growing area of interest [4]. Natural compounds have the potential to be utilized

in food products, with the aim of enhancing their quality and nutritional value. They can be incorporated into food additives, with the objective of extending the shelf life of products [2] or can be used in food supplements and various other formulations [5,6].

Natural bioactive compounds offer significant benefits due to their anti-inflammatory, anti-aging, antioxidant, and anticancer properties [7], as well as their immunostimulatory effects [8], cardiovascular protection [9], and other advantages [10].

Sorbus aucuparia (the Rosaceae family), also known as European rowanberry or mountain-ash, is recognized and used in Europe and Asia in folk medicine as a remedy for various ailments, including respiratory, gastrointestinal, cancer [11,12], hypertension [13], gout, rheumatism, and respiratory infections [14], as well as diuretic, anti-inflammatory, vasorelaxant, and anti-diabetic properties, and also as a source of vitamin C [15].

Preparations from rowanberry fruits have been used to treat hemorrhoids and gout, as well as for wound cleansing in Lithuania [16]. Tea prepared from the bark of *S. aucuparia* has been successfully used to treat cancer [17]. Leaf preparations have been used to treat cancer, gastrointestinal diseases, and prostate diseases [18]. The flowers have been used as a diuretic and anti-inflammatory agent [19].

Phytochemical studies of the plant have described the presence of polyphenolic compounds including flavonoids, flavanols, quercetin, and kaempferol derivatives [20,21], as well as triterpenes, sterols, coumarins, carboxylic acids, and cyanogenic glycosides [11], and carotenoids, organic acids, microelements, and ascorbic acid [22,23].

Numerous studies have demonstrated that berries are a rich source of polyphenolic compounds that exhibit a wide range of biological properties, including anti-diabetic, anti-inflammatory, and anticancer effects [24,25]. These compounds also have important antioxidant properties, as they can capture and neutralize free radicals, chelate heavy metals, and inhibit pro-inflammatory enzymes [26,27]. They are therefore considered promising agents for protecting against, and even treating, pathologies mediated by oxidative stress [23].

Reactive oxygen species (ROS) generate oxidative stress, which causes the denaturation of macromolecules in the body, including nucleic acids, lipids, and proteins. Oxidative stress has been identified as a contributing factor in a number of chronic diseases, including cancer, diabetes, neurodegenerative diseases, and cardiovascular diseases [28], and is also involved in the inflammatory process [29].

The beneficial effects of berry polyphenols on the regulation of type 2 diabetes have attracted increasing attention recently [30]. Studies have primarily focused on red cranberries and blueberries and, to a lesser extent, on *Sorbus* fruits and have shown antidiabetic activity in vitro and in vivo as well as in clinical studies [31,32].

Berry polyphenols act in insulin-independent type 2 diabetes by inhibiting digestive enzymes (α-amylase and α-glucosidase), which are involved in carbohydrate hydrolysis, as well as by inhibiting glucose absorption in the gastrointestinal tract [32]. Drugs such as acarbose, miglitol, and voglibose are commonly used as inhibitors of these enzymes; however, they often cause unpleasant side effects, including discomfort, diarrhea, and flatulence [33]. Therefore, finding new enzyme inhibitors is desirable, and natural compounds are a viable alternative.

The body's first response to external stimuli that cause trauma is the inflammatory process. This can lead to various diseases, including allergies, cardiovascular diseases, autoimmune diseases, and even cancer [34]. Lipoxygenase and hyaluronidase are enzymes involved in the inflammatory process, among others, and inhibitors of their activity are the basis of treatments for allergies and inflammatory diseases [35,36].

Lipoxygenase (LOX) is involved in the catalysis of a fatty acid, producing metabolites that are implicated in various diseases, such as type 1 diabetes, type 2 diabetes, neurodegenerative diseases, and kidney diseases [37], and catalyzes arachidonic acid, generating

metabolites such as leukotrienes, which are released by leukocytes and mast cells [38]. Leukotrienes are involved in the occurrence of the inflammatory process [39]. Inhibitors on the market, e.g., zileuton, have limited use due to unwanted side effects [40]; thus, it is therefore necessary to find new, safe lipoxygenase inhibitors with high clinical efficacy.

Hyaluronidase (HYA) acts on hyaluronic acid (HA), a component of the extracellular matrix in soft connective tissues, including the skin, synovial fluid, and umbilical cord [41]. It has been demonstrated that HYA is implicated in a variety of physiological and pathological processes such as cell migration, embryogenesis, wound healing, malignant transformation [42,43], and inflammation [36]. Excessive degradation of HA by HYA, along with ROS, causes a reduction in both the amount and quantity of HA, as observed in rheumatoid arthritis [39]. Biologically active plant compounds, including flavonoids, tannins, and curcumin, act as hyaluronidase inhibitors [41]. These compounds have the potential to serve as anti-inflammatory and antiallergic agents, as well as being utilized in the complementary therapy of arthritis [41,44,45].

Ultrasound-assisted extraction (UAE) is an efficient extraction technique for a wide range of analytes from various sample types. Bioactive components can be extracted rapidly, at low temperatures, with reduced energy and solvent requirements, making them a promising alternative to traditional methods. The UAE is also useful for extracting thermolabile and unstable compounds while preserving their functionality [46,47].

Accelerated solvent extraction (ASE) is an automated, eco-friendly extraction technique that has been shown to offer a number of advantages, including its speed and ease. It operates at high temperatures and pressures and utilizes a reduced number of solvents, thereby reducing analysis time and increasing extraction yield [48].

Membrane technologies—including microfiltration and ultrafiltration—offer effective alternatives to traditional methods for concentrating biologically active compounds. Membrane technologies enable selective separation and can be efficiently used for the purification and concentration of plant extracts to obtain high-quality products [49,50].

The present study analyzed the antidiabetic, anti-inflammatory, and cytotoxic activities of hydroalcoholic extracts enriched with polyphenols from *S. aucuparia* fruits collected in Romania. These fruits were selected due to their potential as a source of bioactive compounds with therapeutic and nutraceutical properties that have not been fully explored.

2. Materials and Methods

2.1. Chemicals and Reagents

Standards for chromatographic determinations (caffeic acid, coumaric acid, gallic acid, ellagic acid, chlorogenic acid, luteolin, kaempferol, quercetin, p-hydroxybenzoic acid, myricetin, quercitrin, quercetin 3-β-D-glucoside, rutin, and ascorbic acid) were purchased from Sigma-Aldrich (Darmstadt, Germany). Epicatechin, myricetin, and gallic acid were purchased from Fluka (Buchs, Switzerland). HPLC-grade reagents (methanol, ethanol, acetonitrile, and acetic acid (MeCN)) were purchased from Riedel-de Haen (GmbH, Seelze, Germany).

2.2. The Obtaining of the Extracts

To obtain the extracts, two modern, ecological, "green", environmentally friendly methods were used: UAE and ASE.

Hydroalcoholic extracts, EtOH 50% (*v*/*v*) and 15% mass, were obtained from rowanberries collected in October 2024 from Ciungetu, Valcea County, at the geographical coordinates 45°23′14″ N 23°57′13″ E.

The fruits were well dried for several weeks in a convection oven (POLEKO, SWL 115, Wodzisław Śląski, Poland) at 40 °C. This temperature was chosen in order to minimize

the impact on heat-sensitive bioactive compounds. After that, the dried material was ground using a Grindomix GM100 mill until a fine powder was obtained, with particle size < 300 µm (Retsch, Haan, Germany). The extracts were obtained through three methods: a traditional method (maceration) and two modern, ecological, and environmentally friendly methods, UAE and ASE.

The optimal conditions for carrying out the experiment and extracting high-yield polyphenols were established based on our previous studies [51].

2.2.1. Ultrasound-Assisted Extraction

For UAE, an Elma Transsonic T460 ultrasonic bath (frequency 35 kHz), made in Germany (Elma Schmidbauer GmbH & Co. KG, Singen, Germany), was used. A 50% (*v*/*v*) ethanol solution was added to the plant material, and the mixture was stirred for one hour at room temperature, before being ultrasonicated for 40 min at 60 °C. The extract was then filtered and stored at 4 °C.

2.2.2. Accelerated Solvent Extraction

An accelerated solvent extractor (ASE) system (Dionex ASE 350, Thermo Scientific, Waltham, MA, USA) was used for ASE extraction. Cellulose filters were placed in 100 mL stainless steel cells, into which 15 g of dried, ground plant material, pre-mixed with diatomaceous earth, was loaded.

The parameters used in the experiment were as follows: ethanol (50%, *v*/*v*), 60 °C, static time of 10 min, and three cycles. The extracts were collected in 200 mL glass vials and stored at 4 °C. The final concentration of the extracts was 15% (*w*/*v*).

The concentration of bioactive compounds was achieved using membrane technologies, micro- and ultrafiltration, using the KMS Laboratory Cell CF-1 laboratory facility. For microfiltration, membranes with a pore size of 0.45 µm were used, and for ultrafiltration, membranes with a pore size of 1000 Da and a pressure of 3–4 bar were used.

2.3. Analytical Methods

2.3.1. Total Polyphenols Determination (TPF) and Flavonoids Content (TFC)

The content of polyphenolic compounds in the extracts was analyzed using spectrophotometric and chromatographic methods.

The total polyphenol content of the obtained extracts was determined using the Folin–Ciocalteu method [52]. Calculations were performed using a chlorogenic acid calibration curve ($y = 0.0016x + 0.013$, $R^2 = 0.9945$), and the results were expressed as chlorogenic acid equivalents (CAE) µg/mL.

The aluminum chloride spectrophotometric method was used to analyze the total flavonoid content (TFC) of the obtained extracts [53]. Calculations were performed using a rutin calibration curve ($y = 0.0025x + 0.009$, $R^2 = 0.9987$), and the results were expressed as rutin equivalents (RE), µg/mL.

The following formula was used to calculate the extraction yield:

yield (%) = $m_1/m_2 \times 100$, where m_1 = the dry extract'mass and m_2 = the sample'mass.

2.3.2. HPLC Analysis of Individual Phenolic Compounds

An HPLC Shimadzu system was used for the HPLC analysis. This system comprised a SIL-40CXR autosampler, a DGU-405 degasser, an LC-40DXR pump, a CTO-40C column oven (Shimadzu, Kyoto, Japan), and LC Solution software var. 5.1 Shimadzu. The HPLC was coupled to an LCMS-2050 mass spectrometer detector with a DUIS interface, with the following parameters: nebulizing gas flow, 2 L/min; drying gas flow, 5 L/min; heating gas flow, 7 L/min; desolvation line temperature, 200 °C; detector voltage, 0.7 kV; desolvation

temperature, 450 °C; interface voltage, −2 kV; and nitrogen generator, Peak Scientific Genius XE Nitrogen.

An optimized method, previously reported by our team [54], was used in the chromatographic analysis. To perform these determinations, a C18 column, Kromasil 100–3.5-C18 2.1 × 100 mm, was used, with a mobile phase at pH = 3, consisting of 2 solvents: solvent A, water with formic acid, and solvent B, MeCN with formic acid. The mobile phase had a flow rate of 0.15 mL min-1 and was varied during the determinations, following a gradient elution program: 5–30% solvent B, 0–20 min; 30% solvent B, 20.01–35 min; 30–50% solvent B, 35.01–37 min; 50% solvent B, 42.01–45 min 50-5%; and 5% solvent B, 45–55 min, 5%. The negative ionization mode and selected ion monitoring (SIM) mode were used to examine the compounds of interest thanks to their specificity. In quantitative chromatographic analysis, the peak areas of the following fragment ions $[M\text{-}H]^-$ were measured: 163, 609, 169, 285, 289, 137, 301, 317, 447, 463, 179, and 353.

2.4. Antioxidant Assays

The antioxidant capacity of the extracts was analyzed by three spectrophotometric methods.

2.4.1. DPPH Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed using a previously described method, with minor modifications [55]. The procedure was as follows: 1900 µL of methanol, together with 100 µL of extract and 1000 µL of 0.25 mM DPPH solution, were mixed and kept in the dark for 3 min, after which the absorbance was then read at 517. The following formula was used for the calculations:

$$\text{Radical Scavenging Activity I (\%)} = [\ (\text{Acontrol} - \text{Asample})/\text{Acontrol}] \times 100.$$

IC_{50} value was calculated, representing the concentration of antioxidant required to cause a 50% reduction in the initial DPPH concentration. An IC_{50} value of $\leq$50 µg/mL represents a good antioxidant capacity, whereas an IC_{50} value of $\geq$200 µg/mL reveals an insignificant antioxidant capacity [56].

2.4.2. Determination of Antioxidant Activity by Reducing Power

To determine the antioxidant activity of the extracts by reducing power, the method previously presented by Berker et al. was used [57]. Absorbance was determined at 700 nm, the standard used was ascorbic acid, and calculations were performed using the following formula:

$$\text{Reducing power (\%)} = [(AA - AB)/AA] \times 100$$

where AA = absorbance of sample and AB = absorbance of control.

2.4.3. ABTS Assay

For the ABTS assay, the Rice–Evans method was used with slight modifications; this method is based on the decrease in the ABTS absorbance of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) in the presence of an antioxidant at 731 nm [58]. Thus, to 100 mL of extract, 400 mL of distilled water and 2500 mL of ABTS solution (0.175 mM) were added, mixed, and kept in the dark for 3 min, and then the absorbance at 731 nm was read.

The formula below was used for the calculations, and the data were expressed in TEAC equivalents (Trolox Equivalent Antioxidant Capacity):

$$\text{TEAC}_{\text{sample}} = C_{Trolox} \cdot f \cdot \frac{A_{sample} - A_{blank}}{A_{Trolox} - A_{blank}}$$

where A_{blank} = control absorbance, A_{Trolox} = Trolox absorbance, A_{sample} = sample absorbance, f = dilution factor, and C_{Trolox} = Trolox concentration.

2.5. Enzymes Inhibition Activity

The antidiabetic potential of *S. aucuparia* fruit extracts was assessed by testing their ability to inhibit α-amylase and α-glucosidase. Additionally, the extracts were evaluated for their ability to inhibit lipoxygenase and hyaluronidase, which could indicate anti-inflammatory potential.

2.5.1. Testing the Antidiabetic Potential of the Extracts

The antidiabetic potential of the extracts was evaluated by testing their ability to inhibit the digestive enzymes α-amylase and α-glucosidase, which are involved in carbohydrate metabolism.

- Testing the α-amylase inhibition capacity

The α-amylase inhibition capacity was assayed using the Queiroz method, with minor modifications [59]. The procedure was as follows: 100 µL of extract was mixed with 250 µL of porcine pancreatic α-amylase (EC 3.2.1.1) (1 mg/mL), which was prepared in sodium phosphate buffer (0.02 M, pH 6.9) (Sigma-Aldrich, Steinheim, Germany). The mixture was then incubated at 37 °C for 20 min. Then, 250 µL of a 1% starch solution was added, after which the samples were re-incubated at 37 °C for a further 30 min. Finally, 500 µL of dinitrosalicylic acid (DNS) was added to the samples, which were then boiled for five minutes. Once cooled, 5 mL of distilled water was added to the reaction mixture, after which the absorbance was read at 540 nm using a Jasco V-630 UV-visible spectrophotometer. Acarbose was used as a standard, and the formula was used for calculations.

$$\%\ \text{Amylase inhibition} = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \times 100$$

Linear regression analysis IC_{50} (µg/mL) was used to determine IC_{50} values. Differences in $p < 0.05$ are considered statistically significant.

- α-Glucosidase Inhibition Assay

To test the ability of the extracts to inhibit α-glucosidase, the Ranilla et al. method was used with slight modifications [60]. The following procedure was followed: 120 µL of α-glucosidase (EC 3.2.1.20, Sigma-Aldrich, St. Louis, MO, USA) at a concentration of 0.5 U/mL, 720 µL of 0.1 M sodium phosphate-buffer solution (pH 6.9), and 60 µL of extract were mixed together and incubated at 37 °C for 15 min. Then, 120 µL of a p-nitrophenyl-α-D-glucopyranoside solution (5 mM/L) was added, and the mixture was re-incubated at 37 °C for a further 15 min. Finally, the absorbance was read at 405 nm. Acarbose was used as a standard, and calculations were performed using the following formula:

$$\%\ \text{Glucosidase inhibition} = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \times 100$$

IC_{50} values were calculated, and differences in $p < 0.05$ are considered statistically significant.

2.5.2. Testing the Anti-Inflammatory Potential of the Extracts

The anti-inflammatory potential was tested by analyzing the ability of the extracts to inhibit LOX and HYA, two enzymes involved in the inflammatory process.

- Hyaluronidase inhibition assay

The ability of the extracts to inhibit hyaluronidase was tested using the Morgan–Elson method, with minor modifications [61].

Hyaluronidase acts on hyaluronic acid, producing enzymatic hydrolysis products that react with p-dimethylaminobenzaldehyde to form a pink complex that can be measured using a spectrophotometer at a wavelength of 585 nm.

The procedure is as follows: Add 200 µL of 5% DMSO to 200 µL of extract (500 mg/mL) and 200 µL of enzyme solution (hyaluronidase, EC 3.2.1.35, from bovine testes—1 mg/mL, 4200 U/mL, Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

Incubate at 37 °C for 20 min. Then add 200 µL of 12.5 mM $CaCl_2$ and incubate the samples at 37 °C for a further 10 min. Then, 200 µL of a substrate solution of sodium hyaluronate in bovine vitreous humor (12 mg/mL in 0.1 M acetate buffer at pH 3.5) was added to the reaction mixture. The mixture was then incubated at 37 °C for 40 min. Next, 200 µL of 0.4 M NaOH and 400 µL of 0.2 M sodium borate were added, after which the mixture was incubated for three minutes at 100 °C to stop the reaction. Finally, 200 µL of 1% p-dimethylaminobenzaldehyde was added, after which the mixture was incubated for a further 10 min at 37 °C. The absorbance was then determined at 585 nm. A control sample was obtained under the same conditions, but with buffer solution instead of extract. Ibuprofen was used as the standard, and the following formula was used for calculations:

$$\%\ \text{Hyaluronidase inhibition} = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \times 100$$

- Lipoxygenase inhibition assay

Lipoxygenase inhibition capacity was tested according to a Sigma-Aldrich protocol [62]. The method is based on the formation of 13-hydroperoxyocta-decadienoic acid in the lipoxygenation reaction, which causes an increase in absorbance at 234 nm. Thus, to 100 µL extract, 1600 mL of 0.2 M borate buffer, pH = 9 and 100 mL of LOX solution (2750 U/mL) (EC 1.13.11.12, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) were added, and it is maintained at room temperature for 15 min; finally, 200 µL of linoleic acid substrate (0.017% (v/v)) was added, and after 2 min, the absorbance was read at 234 ± 5 nm, corresponding to the maximum linear rate for the blank and the sample. The standard used was ibuprofen, and the calculations were made according to the formula:

$$\%\ \text{Lipoxygenase inhibition} = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \times 100$$

Linear regression analysis IC_{50} (µg/mL) was used to determine IC_{50} values. Differences in $p < 0.05$ are considered statistically significant.

2.6. In Vitro Cytotoxicity Tests

The cytotoxic potential of the *S. aucuparia* fruit extracts was tested on the mouse fibroblast NCTC clone L929 cell line.

2.6.1. Cell Culture

L929 murine fibroblast cells (NCTC, clone L929) from ECACC (European Collection of Authenticated Cell Cultures, Salisbury, UK) were used to evaluate the in vitro cytotoxicity of *S. aucuparia* samples. These cells were chosen for the evaluation of cytotoxicity, as they

are a standard reference cell line recommended by ISO 10993-5 [63] for the preliminary assessment of cytotoxicity and biocompatibility. This approach provides a reliable initial indication of general cytotoxicity. Specific media were used for cell cultivation, according to the suppliers' recommendations; thus, a minimal essential medium (MEM) was used, to which 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin, neomycin and streptomycin) were added. The cells were kept under standard conditions at 37 °C and in an atmosphere containing 5% CO_2.

In vitro tests were performed by seeding cells at a density of 5×10^4 cells/mL in 96-well cell culture plates, then, after 24 h of incubation, the samples were added. The cells were incubated under standard conditions, after which cell viability was assessed using the quantitative 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at 24 and 48 h [64].

2.6.2. MTT Cell Viability Assay

To assess cell viability, the MTT assay was used as previously described [64]. For this assessment, 0.25 mg/mL MTT solution was added to the treated cells and then incubated at 37 °C for 3 h. Then, the insoluble formazan crystals were dissolved with isopropanol by incubation at room temperature for 15 min under gentle shaking. Absorbance measurement was performed at 570 nm using a SPECTROstar® Nano microplate reader (BMG Labtech, Ortenberg, Germany). There is a direct correlation between the amount of formazan and the number of metabolically active cells. Data were expressed as a percentage of viability compared to the untreated cells (control), which were considered to have 100% viability. Results represent the average of three determinations (mean ± standard deviation).

2.7. Statistical Analysis

Analysis of statistics was accomplished with Excel Office 365 (One Microsoft Way, Redmond, Washington, DC, USA), and the standard deviation (SD) was found to be less than 10%. The statistical analysis employed a Student's *t*-test, with *p*-values less than 0.05 designated as statistically significant. The experiment sought to establish a correlation between the inhibitory activity of antioxidants and enzymes and the content of active principles. Also, the data were analyzed by a one-way repeated measures analysis of variance (RM ANOVA) and Tukey's test, using the SigmaPlote 16.0 software.

3. Results and Discussions

3.1. Phytochemical Analysis and Antioxidant Capacity

The obtained extracts were analyzed for total polyphenols and flavonoids content using both spectrophotometric and HPLC-MS chromatographic methods, as well as for their antioxidant activity. The content of total polyphenols and flavonoids in the extracts determined spectrophotometric, and the extraction yield is presented in Table 1.

Two extraction methods were employed to determine the most effective approach for producing extracts with the highest polyphenol content. An increase in total polyphenols and flavones content, as well as in the extraction yield of the concentrated extracts compared to the initial microfiltered ones, reveals the efficiency of the ultrafiltration process.

ASE was found to be a more efficient method than UAE for extracting the target compounds from *S. aucuparia* (2692.53 ± 37.89 µg/mL CA equivalent for total polyphenols and 433.88 ± 21.12 µg/mL RU equivalent for the content of flavones). This is also evident from the superior extraction yields obtained by ASE, compared to UAE.

The high pressure and temperature used enable the solvent to penetrate the cell wall more effectively, likely enhancing ASE efficiency. Reducing the surface tension

of the solvent releases the phenolic compounds more effectively, thus increasing the extraction efficiency [65].

Table 1. The content of biologically active compounds (total polyphenols, flavonoids) and the extraction yield of extracts from *S. aucuparia* fruits results.

Samples	Extraction Method		Total Polyphenols Concentration ± SD (CA µg/mL)	Flavonoids Concentration ± SD (RU µg/mL)	Yield (%)
S. aucuparia fruit extracts	ASE	Microfiltrate	2265.62 ± 52.63	377.88 ± 17.32	14.09
		Concentrate	2692.53 ± 37.89	433.88 ± 21.12	29.06
	UAE	Microfiltrate	1246.85 ± 28.39	191.12 ± 5.23	13.13
		Concentrate	1576.24 ± 32.78	230.88 ± 10.32	22.12

SD = standard deviation; CA = chlorogenic acid equivalent; RU = rutin equivalent; ASE = accelerated solvent extraction; UAE = ultrasound-assisted extraction. ANOVA results: the differences in the mean values among the treatment groups are not great enough to exclude the possibility that the difference is due to random sampling variability; there was no statistically significant difference ($p = 0.275$).

UAE extraction affects plant cell walls, accelerating heat and mass transfer. This improves the kinetics and release of bioactive compounds, thereby increasing the extraction process yield [47].

The identification of polyphenolic compounds in the extracts was also carried out by chromatographic method, using the HPLC-MS. Good linearity of the method was observed within the studied concentration range (0.1–50 µg/mL). The results obtained regarding the concentrations of the polyphenolic compounds in the tested plant extracts are presented in Table 2 and Figure 1.

Table 2. HPLC-MS analysis of the polyphenolic profile for extracts from the *S. aucuparia* fruit results.

Compound	Extract ASE		Extract UAE	
	Microfiltrate Conc ± SD µg/mL	Concentrate Conc ± SD µg/mL	Microfiltrate Conc ± SD µg/mL	Concentrate Conc ± SD µg/mL
Coumaric acid	0.39 ± 0.01	0.45 ± 0.21	0.22 ± 0.01	0.26 ± 0.01
Gallic acid	-	-	-	-
Caffeic acid	32.67 ± 0.12	34.41 ± 1.21	16.40 ± 0.73	16.27 ± 1.12
Luteolin	0.04 ± 0.01	0.04 ± 0.001	0.02 ± 0.001	0.02 ± 0.001
Kaempferol	0.13 ± 0.01	0.14 ± 0.001	0.09 ± 0.004	0.10 ± 0.001
Ellagic acid	1.59 ± 0.02	3.81 ± 0.02	0.92 ± 0.04	2.20 ± 0.12
Quercetin	1.45 ± 0.01	1.66 ± 0.01	1.33 ± 0.01	1.28 ± 0.01
p-hydroxybenzoic acid	14.71 ± 0.89	15.09 ± 0.89	6.93 ± 0.03	8.81 ± 0.28
Myricetin	-	-	-	-
Chlorogenic acid	473.54 ± 20.12	526.08 ± 23.35	273.98 ± 10.81	296.80 ± 12.35
Quercitrin	2.52 ± 0.13	3.81 ± 0.14	1.34 ± 0.01	1.46 ± 0.01
Quercetin 3-β-D-glucoside	15.82 ± 0.89	17.27 ± 0.56	21.28 ± 1.29	23.37 ± 1.12
Rutin	13.68 ± 0.78	36.07 ± 1.23	4.85 ± 0.14	6.31 ± 0. 23
Epicatechin	4.65 ± 0.23	4.75 ± 0.12	1.15 ± 0.01	1.92 ± 0.02

SD = standard deviation; ASE = accelerated solvent extraction; UAE = ultrasound-assisted extraction, ANOVA results: there is a statistically significant difference ($p < 0.001$).

In the results obtained from ANOVA, it was observed that the differences in the median values among the treatment groups are greater than would be expected by chance; therefore, a Tukey's test was also performed. Between the values obtained for microfiltrate extracts (ASE and UAE), concentrate extracts (ASE and UAE) show a significant difference. Also, between the values obtained for ASE extracts (microfiltrate and concentrate), for

UAE extracts (microfiltrate and concentrate), the *p*-value is greater than the significance level ($p < 0.050$), and there is no statistically significant difference between the means of the two groups.

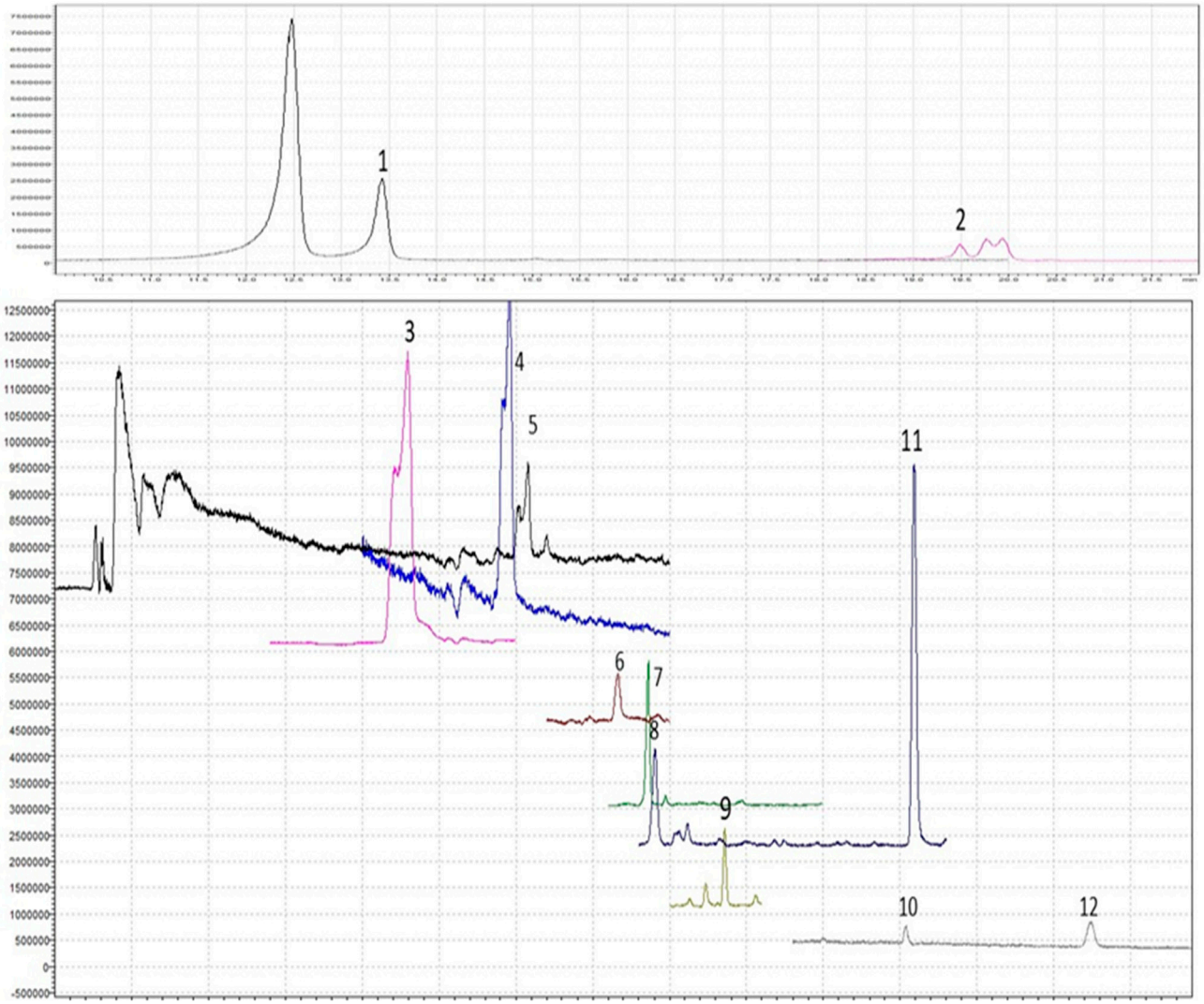


Figure 1. HPLC-MS chromatograms obtained for *S. aucuparia* extract (1-chlorogenic acid, [M-H]− 353; 2-quercetin 3-β-D-glucoside, [M-H]− 463; 3-4-hydroxybenzoic acid, [M-H]− 137; 4-caffeic acid, [M-H]− 179; 5-epicatchin, [M-H]− 289; 6-p-coumaric acid, [M-H]− 163; 7-rutin, [M-H]− 609; 8-ellagic acid, [M-H]− 301; 9-quercitrin, [M-H]− 447; 10-luteolin, [M-H]− 285; 11-quercetin, [M-H]− 301; 12-kaempferol, [M-H]− 285) by HPLC-MS.

HPLC analysis revealed that the predominant polyphenolic compounds were chlorogenic acid (526.08 ± 23.35 μg/mL), rutin (36.07 ± 1.23 μg/mL), caffeic acid (34.41 ± 1.21 μg/mL), quercetin 3-β-D-glucoside (17.27 ± 0.56 μg/mL), p-hydroxybenzoic acid (15.09 ± 0.89 μg/mL), as well as epicatechin, quercitrin, and ellagic acid. The ASE method was more efficient at extracting the targeted compounds than UAE.

The ethanolic rowanberry extract contained high amounts of chlorogenic acid (3970 μg/g), which was higher than in the acetone and aqueous extracts [23]. The same study also found that ethanolic extract had the highest content of rutin, quercetin, and kaempherol-3-O-glucoside, compared to the acetone and aqueous extracts [23]. The main phenolic acids reported in *Sorbus* spp. and in *S. aucuparia* fruits are chlorogenic acids (3-O-caffeoylquinic acid, 3-CQA) and neochlorogenic acids (5-O-caffeoylquinic acid, 5-CQA) [23,66,67]. These acids represent 56–80% of the total phenolic compounds in *Sorbus*

fruits and are present in higher quantities in wild fruits than in cultivated ones [21]. The amount of chlorogenic acids in *Sorbus* fruits is comparable to that in Arabica coffee beans, which are the richest known source of phenolic acids at 0.20 mg/g [68].

Polyphenols act at the cellular level through several mechanisms. One such mechanism is binding to cellular receptors and transcription factors (e.g., Nrf2, NF-κB and AP-1), thereby influencing gene expression [69].

Another mechanism by which polyphenols act is by affecting the expression of microRNAs (miRNAs), which play a role in post-transcriptional regulation as small, non-coding RNAs. This may have implications for cancer, neurodegenerative diseases, and cardiovascular diseases [70].

Polyphenols can also affect the fluidity and permeability of cell membranes, thereby influencing membrane-bound enzymes and receptors, as well as signal transmission and intercellular communication [71].

Caffeoylquinic acids have been reported in *Sorbus* fruits and leaves, although in lower quantities than in inflorescences [72].

The presence of polyphenolic compounds such as chlorogenic acid, hyperin, rutin, catechin, epicatechin, quercetin, and isoquercetin has been reported in rowanberries [12,21].

Another study found that the predominant polyphenolic compounds in rowanberries are isomers of caffeoylquinic acids, with quercetin 3-O-(6-malonyl)-glucoside accounting for over 50% of the flavonols [73].

Other studies have reported the presence of flavonols, such as quercetin, rutin, hyperoside, and isoquercetin glycosides, as well as hydroxycinnamic acids and their derivatives [22,74].

Polyphenolic compounds such as apigenin, baicalin, rutin, kaempferol, scutellariae, isoquercetin, and cinidine-3-O-glucoside, as well as chlorogenic acid, caffeic acid, gallic acid, p-coumaric acid, vanillic acid, and ferulic acid have been determined in 40% ethanolic extracts of *S. acuparia* fruits [75].

Due to the complexity of polyphenolic compounds, several analytical methods are typically employed to assess the antioxidant activity of extracts. In this study, three methods were used to determine antioxidant activity: DPPH, reducing power, and ABTS. These methods are the most common due to their high degree of reproducibility [76]. The results, expressed as IC_{50} for DPPH and reducing power assays and as TEAC equivalents for ABTS, are presented in Table 3.

Table 3. The antioxidant activity of the extracts.

Samples		DPPH Radical Scavenging Activity IC_{50} (μg/mL)		Reducing Power Activity IC_{50} (μg/mL)		$TEAC_{ABTS}$ (μmolTrolox/g)	
		ASE	**UAE**	**ASE**	**UAE**	**ASE**	**UAE**
S. aucuparia fruit extracts	Microfiltrate	43.57 ± 1.62 *	54.98 ± 2.15 *	52.14 ± 2.62 *	75.74 ± 3.25 *	41.90 ± 2.97 *	22.61 ± 0.90
	Concentrate	39.07 ± 0.32 *	50.98 ± 1.1 *	47.36 ± 1.65 *	49.61 ± 1.2 *	50.81 ± 2.87 *	32.33 ± 1.10 *
Vitamin C		46.47 ± 1.38		36.32 ± 1.25		68.61 ± 2.32	

The data represent the average of experiments performed in triplicate; * $p < 0.05$, the reducing power compared with the polyphenol and flavonoid content of the extracts by Student's *t*-test; * $p < 0.05$, the radical scavenging activity compared with the polyphenol and flavonoid content of the extracts by Student's *t*-test.

ASE extracts exhibited superior antioxidant activity compared to UAE. Concentrated extracts demonstrated higher antioxidant activity in comparison to microfiltered extracts, correlated with the content of bioactive compounds (polyphenols and flavones). The existence of a positive correlation between the content of polyphenolic compounds and antioxidant activity has been highlighted by many authors [19,67,68]. In vitro chemical

tests, such as DPPH/ABTS+, have been very popular in this field and have highlighted the existence of this correlation [69]. In the DPPH analysis, the ASE extract exhibited even higher antioxidant activity than the standard (vitamin C). The IC_{50} value of ASEconc extract was 39.07 ± 0.32 µg/mL in comparison to 46.47 ± 1.38 µg/mL for vitamin C. Moreover, the microfiltered ASE extract showed higher antioxidant activity in comparison to the standard. In the reducing power analysis, the concentrated extracts demonstrated comparable antioxidant activity (IC_{50}ASEconc was 47.36 ± 1.65 µg/mL; IC_{50} USconc was 49.61 ± 1.21 µg/mL), which was lower than the standard used—IC_{50} vitamin C was 36.32 ± 1.25 µg/mL. When tested by the ABTS method, the ASE extract also demonstrated the most efficacious antioxidant activity. TEAC_ABTS of ASEconc value was 50.81 ± 2.87 µmol Trolox/g in comparison to the UAE extracts; however, this was still lower than the standard, which had a TEAC_ABTS of vitamin C value of 68.61 ± 2.32 µg/mLTrolox/g.

Studies have reported that rowanberry extracts exhibit antioxidant activity [77], comparable to that of other edible berries such as chokeberries and bilberries [78].

The antioxidant activity of fruits from the genus *Sorbus* has been confirmed in *S. aucuparia*, *S. domestica*, and *S. torminalis* [11].

When the antioxidant activity of the extract was evaluated using the DPPH test, *S. aucuparia* and *S. caucasica* var. *yaltirikii* exhibited SC_{50} values of 0.366 ± 0.009 mg/mL and 0.520 ± 0.023 mg/mL, respectively. These values are comparable to those of the standards used: gallic acid, chlorogenic acid, and quercetin [79].

Methanolic extracts from *S. aucuparia* fruits have been demonstrated to possess antiradical properties, with an inhibition percentage of 16.33 ± 0.96% against hydroxyl radicals, 26.74 ± 1.75% against superoxide anions, and 25.17 ± 1.72% against nitric oxides [80].

The antioxidant properties of the rowanberry are attributable to the presence of polyphenolic compounds, especially chlorogenic acids, flavonols (e.g., quercetin, isoquercetin, rutin, catechin, and epicatechin), as well as anthocyanins and proanthocyanidins [19,21].

Chlorogenic acid (CA) exhibits a variety of important therapeutic properties, including anti-inflammatory, antioxidant, antiviral, and antibacterial effects [81]. CA is a well-known antiradical agent, which was first isolated from coffee beans, where it is found in the highest concentration. CA plays a protective role in pathologies associated with oxidative stress, including neurological, cardiovascular, and cancer [71,82].

Quercetin is a potent antioxidant with protective effects against tissue damage caused by the toxicity of various drugs [83], as well as anti-inflammatory, antidiabetic, and antiviral properties [71].

3.2. Testing Antidiabetic Potential—α-Amylase and α-Glucosidase Inhibition

The antidiabetic potential of the obtained extracts was evaluated by testing their capacity to inhibit α-amylase and α-glucosidase enzymes. The inhibitory capacity of α-amylase and α-glucosidase was compared with the content of polyphenols and flavones. The data were analyzed by Student's test, and the values of $p < 0.05$ were obtained. The results obtained are presented in Table 4.

In the analysis of the α-amylase and α-glucosidase inhibition capacity of *S. aucuparia* fruit extracts, it was observed that the concentrated extracts exhibited higher inhibitory activity in comparison to the initial microfiltrates. The ASE extracts demonstrated higher inhibition than the UAE extracts. The concentrated extracts showed α-amylase inhibition activity that was comparable to that of the standard; specifically, the IC_{50} of the concentrated ASE extract was 23.74 ± 1.32 µg/mL, and the IC_{50} of acarbose was 22.65 ± 1.27 µg/mL. Conversely, the UAE concentrated extract exhibited slightly lower activity than the standard, with an IC_{50} UAEconc of 38.23 ± 2.53 µg/mL.

Table 4. α-amylase and α-glucosidase inhibition activity by analyzed extracts.

Samples		α-Amylase Inhibition IC_{50} (μg/mL)		α-Glucosidase Inhibition IC_{50} (μg/mL)	
		ASE	UAE	ASE	UAE
S. aucuparia extracts	Microfiltrate	32.87 ± 2.15 *	48.38 ± 3.56 *	15.26 ± 0.98 *	25.21 ± 1.36 *
	Concentrate	23.74 ± 1.32 *	38.23 ± 2.53 *	13.50 ± 0.96 *	21.53 ± 1.25 *
Acarbose		22.65 ± 1.27		20.19 ± 1.67	

Values are in form mean ± standard deviation (n = 3); statistical significance (* $p < 0.05$) is obtained for both enzymes' inhibition linked with studied compounds content in extracts.

The extracts obtained by ASE demonstrated inhibitory activity on α-glucosidase that was higher than the standard. This activity was observed in both the microfiltered and concentrated extracts. The most effective inhibition was recorded in the concentrated ASE extract, with the IC_{50} of the ASEconc being 13.50 ± 0.96 μg/mL, lower than that of the standard (20.19 ± 1.67 ug/mL). The inhibitory activity of the extracts from UAE on α-glucosidase was found to be lower than that of the standard that was utilized.

The method employed in this study, ASE, was found to be more efficacious than UAE. This led to an extract containing a higher quantity of bioactive compounds and a higher inhibition yield on the analyzed enzymes. This phenomenon is probably due to the high pressure and temperature, which enhance the extraction process by increasing the diffusion rate of bioactive compounds, as evidenced in other studies [84]. The solvent used for extraction was 50% ethanol, which was also used in previous studies. This solvent led to higher extraction yields in comparison with other solvents, including methanol, acetone, and water [85]. While increasing solvent polarity generally improves extraction, moderately polar solvents like 50% ethanol produce higher yields when compared with absolute ethanol [85].

Among the species of the genus *Sorbus*, the most extensively studied for its antidiabetic effects is *S. decora*, whose bark is traditionally used in folk medicine. Subsequent in vivo and in vitro studies have confirmed the antidiabetic activity of *S. decora* bark [11]. Few studies have been conducted on *Sorbus* fruits (rowanberry) to test antidiabetic activity.

As demonstrated in earlier studies, rowanberry extracts have been shown to possess the capacity to inhibit enzymes involved in the process of carbohydrate digestion, namely, α-amylase, and α-glucosidase, especially extracts rich in chlorogenic acids and proanthocyanidins [86].

Research has demonstrated that chlorogenic acid exerts a regulatory effect on lipid and carbohydrate metabolism, suggesting its potential application in the treatment of hepatic steatosis, cardiovascular diseases, obesity, and even diabetes [81].

Chlorogenic acids have been associated with a reduced risk of type 2 diabetes. They hydrolyze to produce caffeic acid, which decreases glucose absorption, lowers glucose production in the liver, and mitigates oxidative stress in vitro [72].

The aqueous extract of *S. aucuparia* fruits, which is rich in polyphenolic compounds, demonstrated inhibitory activity on α-glucosidase (IC_{50} was 30 μg/mL) comparable to that of acarbose; the extract exhibited a predominant content of chlorogenic acid, over 65% of the total polyphenols [84].

The efficacy of *Sorbus* fruit extracts in reducing blood sugar levels and insulin resistance has also been demonstrated in vivo in mice [87].

As demonstrated in [30], several species within the genus *Sorbus*, including *S. norvegica*, *S. folgneri*, *S. alnifolia*, and *S. aucuparia*, have been shown to possess inhibitory activity on both α-amylase and α-glucosidase. The inhibitory activity was also confirmed in the case of *S. norvegica* fruits by in vivo studies. The antihyperglycemic effect in mice was 36 times

lower than that of acarbose, and both the polyphenolic fraction and the carbohydrate fraction contributed to the inhibitory potential [30].

Rowanberries have been utilized in folk medicine for the treatment of diabetes and in the prophylaxis of cardiovascular diseases [15]. The mechanisms of action underlying these properties, as well as the phytochemical composition of *S. aucuparia* fruits, are not fully known [88].

It has been hypothesized by other studies that polyphenolic compounds may intervene in the modulation of carbohydrate metabolism by inhibiting digestive enzymes such as α-amylase and α-glucosidase, as well as modulating the expression of cytokines involved in the inflammatory process [89,90]. Moreover, polyphenols have been demonstrated to modulate the activity of enzymes such as cyclooxygenase and lipoxygenase, thereby enhancing their anti-inflammatory and chemopreventive properties [91].

3.3. Testing Anti-Inflammatory Potential—Lipoxygenase (LOX) and Hyaluronidase (HYA) Inhibition Activity

The anti-inflammatory potential of the obtained extracts was evaluated by testing their inhibitory capacity on LOX and HYA enzymes. These enzymes form the basis of many synthetic anti-inflammatory drugs. However, plant extracts, particularly those rich in phenolic compounds, can also significantly inhibit their activity [27]. The inhibitory capacity on LOX and HYA was compared with polyphenols and flavones content using Student's test, and values were determined with $p < 0.05$. The results obtained are shown in Table 5.

Table 5. Lipoxygenase and hyaluronidase inhibition activity of the analyzed extracts.

Samples		Lipoxygenase Inhibition IC_{50} (μg/mL)		Hyaluronidase Inhibition IC_{50} (μg/mL)	
		ASE	UAE	ASE	UAE
S. aucuparia extracts	Microfiltrate	40.11 ± 2.21 *	73.63 ± 4.35 *	70.67 ± 2.53 *	65.82 ± 3.16 *
	Concentrate	24.30 ± 1.54 *	31.01 ± 2.36 *	43.04 ± 2.19 *	48.49 ± 3.15 *
Ibuprofen		26.91 ± 1.27		51.54 ± 3.67	

Values are in form mean ± standard deviation ($n = 3$); statistical significance (* $p < 0.05$) is obtained for both enzymes' inhibition linked with studied compounds content in extracts.

The increased efficiency of the ASE method, compared to UAE, as well as the efficiency of the ultrafiltration process, was also evident when testing the inhibitory capacity of the extracts on LOX and HYA. Consequently, ASE extracts demonstrated inhibitory activity on LOX and HYA, which was superior to that of UAE extracts. Furthermore, concentrated extracts exhibited higher efficacy than microfiltrates.

The tested extracts, which were found to be rich in polyphenols, demonstrated the potential to inhibit the activity of the tested enzymes. Consequently, the concentrated extract_ASE demonstrated the most significant inhibitory activity on LOX, as outlined below: the IC_{50}ASEconc was found to be 24.30 ± 1.54 μg/mL, which is higher than the standard used. The IC_{50}ibuprofen was 26.91 ± 1.27 μg/mL, while the UAE extract showed inhibition close to the standard of 31.01 ± 2.36 μg/mL.

With regard to the inhibition of HYA, both concentrated extracts demonstrated inhibitory activities higher than the standard, as follows: the IC_{50}ASEconc was found to be 43.04 ± 2.19 μg/mL, and IC_{50}UAEconc was found to be 48.49 ± 3.15 μg/mL, in comparison to the IC_{50} of ibuprofen, which was 51.54 ± 3.67 μg/mL.

The significant anti-inflammatory activity of *S. aucuparia* extracts may be attributed to their high polyphenol content, particularly chlorogenic acid, a well-known bioactive compound with anti-inflammatory and antioxidant properties. Chlorogenic acid (CA) ex-

erts its anti-inflammatory effects by regulating the production and release of inflammatory mediators such as TNF-α, IL-1β, IL-6, IL-8, NO, and PGE2, which play crucial roles in the initiation and progression of the inflammatory response [92].

Methanol–water extracts of *S. domestica* leaves exhibited LOX inhibitory effect, with an IC_{50} of 115.54 ± 4.99 mg/mL, comparable to the IC_{50} of indomethacin (92.60 ± 3.71 mg/mL). Similarly, extracts demonstrated HYA inhibitory activity, with an IC_{50} of 11.06 ± 0.30 mg/mL, which is also comparable to the IC_{50} of indomethacin (12.77 ± 0.91 mg/mL). The activity of the extracts in the LOX assay was found to be strongly dependent on the total polyphenol content ($r = -0.8969$, $p < 0.05$); the results in the HYA assay were only slightly dependent on the total polyphenol content ($r = -0.3832$, $p > 0.05$), but strongly determined by proanthocyanidins (TPA; $r = -0.8843$, $p < 0.05$) [27].

In another study, methanolic extracts from *S. domestica* leaves demonstrated inhibitory activity against both enzymes, with a stronger effect on LOX—principally due to quercetin and procyanidin—than on HYA, which was mainly influenced by catechins and procyanidins [27]. These effects were comparable to those of the standard reference, indomethacin [93]. Methanolic extracts from *S. aucuparia* flowers exhibited a significant HYA inhibitory effect, surpassing that of indomethacin [94].

The anti-inflammatory activity of *S. commixta* has been confirmed both in vitro and in vivo, with in vivo results comparable to those of ibuprofen [95,96].

Moderate anti-inflammatory activity on lipoxygenase was also revealed by *S. cashmiriana*, which was attributed to two triterpenes, cashmirol A and cashmirol B, with exhibited IC_{50} values of 90.2 μM and 74.9 μM, respectively, (compared to baicalein used as a positive control, with an IC_{50} of 8.0 lM) [97].

3.4. Cytocompatibility of S. aucuparia Samples

The results obtained from the MTT assay, which was used to determine cell viability, are presented in Figure 2. Sample viability was reported relative to the control group (untreated cells), which was considered to have 100% viability. Experiments were conducted to establish the dose–response relationship over a concentration range of 0–1000 μg/mL, with incubation periods of 24 and 48 h.

Cytotoxicity evaluation of the ASE samples (Figure 2A) showed that after 24 h of treatment, the ASE_MF extract was cytocompatible across the entire tested concentration range (0–1000 μg/mL), maintaining cell viability above 80%. In contrast, treatment with the ASE_C extract resulted in a significant decrease in cell viability, with a notable drop to 54% observed at a concentration of 750 μg/mL and 39% at a concentration of 1000 μg/mL.

After 48 h of exposure, a dose-dependent decrease in cell viability was observed for both extracts. At concentrations ranging from 0 to 250 μg/mL, viability remained above 77% for both ASE variants. However, at 500 μg/mL, cell viability decreased to 74% for ASE_MF and further dropped to 38% for ASE_C. The highest concentrations tested (750 and 1000 μg/mL) were cytotoxic, with cell viability being below 38% for both variants.

Regarding samples (Figure 2B), cytotoxicity evaluation showed that after 24 h of treatment, both UAE extracts were cytocompatible across the entire tested concentration range (0–1000 μg/mL), with cell viability values exceeding 77%, except for the UAE_C sample, which resulted in a decline in cell viability to 58%.

After 48 h of treatment, all UAE variants remained cytocompatible within the concentration range of 0-250 μg/mL. Treatment with the UAE_C extract resulted in similar viability rates of 61% and 58% at concentrations of 500 μg/mL and 750 μg/mL, respectively, while the highest concentration reduced viability to only 20%. Conversely, exposure to the UAE_MF extract resulted in elevated cell viability across all concentrations examined, maintaining levels above 67% even at a concentration of 1000 μg/mL.

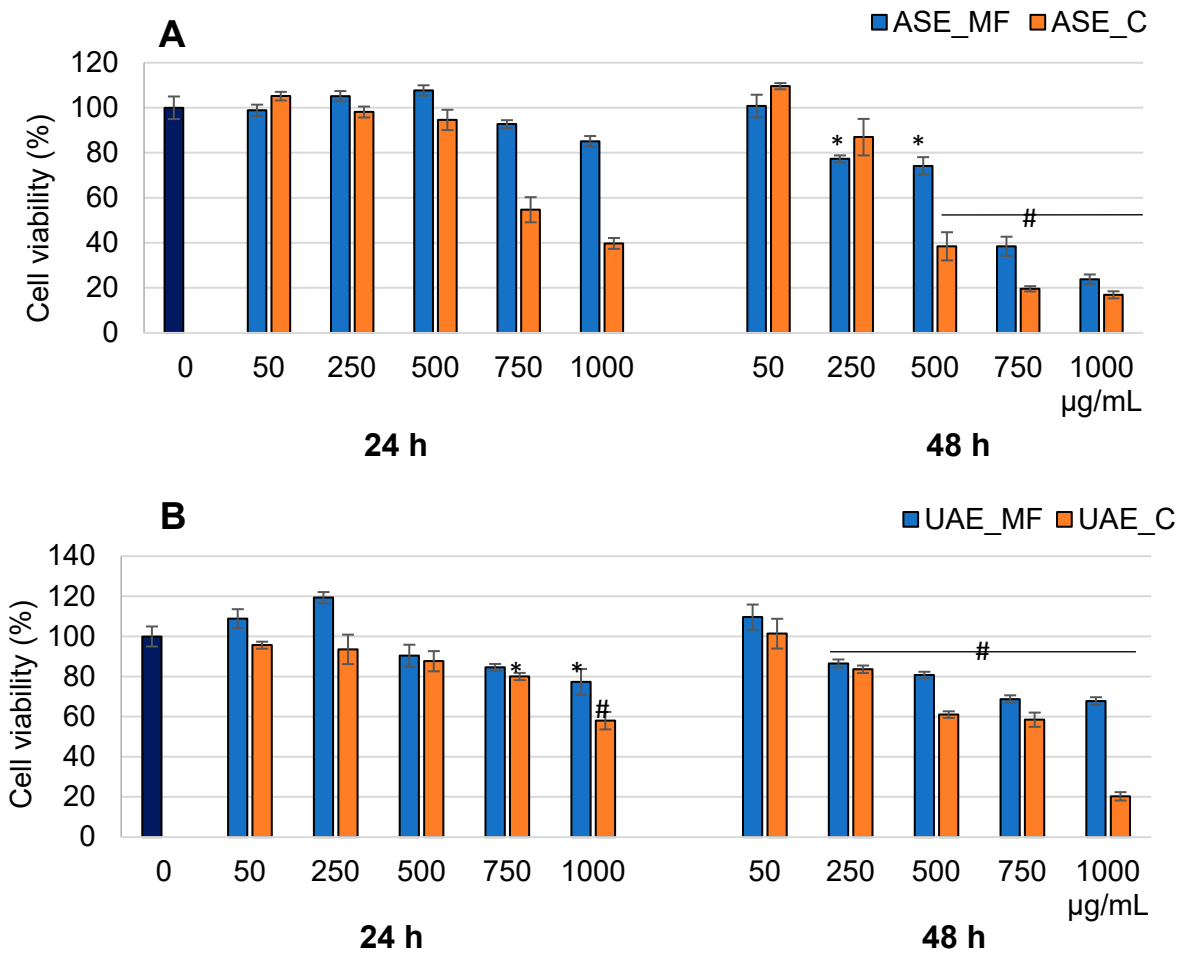


Figure 2. Cell viability of L929 cells after 24 and 48 h of incubation with ASE (**A**) and UAE (**B**) extracts at different concentrations, as determined by the MTT assay. UAE_MF = microfiltrate extract obtained by ultrasound-assisted extraction; UAE_C = concentrate extract obtained by ultrasound-assisted extraction; ASE_MF = microfiltrate extract obtained by accelerated solvent extraction; ASE_C = concentrate extract obtained by accelerated solvent extraction; data were expressed as the average of three replicates (mean ± SD). * $p < 0.05$, # $p < 0.01$.

Literature data indicate that the fruits and seeds of *S. aucuparia* contain parasorbic acid, a toxic compound that can cause digestive and renal problems [98]. This compound is thermolabile and decomposes into harmless sorbic acid when the fruits are harvested after freezing [77]. The compound is almost absent in cultivated species [72]. Another toxic compound present in rowanberry seeds is prunasin, a cyanogenic glycoside that can release HCN (1g of prunasin can release 91.5 mg HCN). A concentration of 2–3 mg/L of HCN has been shown to cause respiratory problems and even death; therefore, it is recommended that seeds be removed during the processing of *S. aucuparia* fruits [72].

One possible explanation for the low viability of the analyzed samples after 48 h could be the presence of toxic compounds such as parasorbic acid and prunasin. These shortcomings could be eliminated by freezing the fruit prior to use; however, we worked with fresh fruit, meaning that parasorbic acid had not yet been transformed into the harmless sorbic acid. Prunasin, on the other hand, is present in the seeds. Using seedless fruit would resolve this problem.

4. Conclusions

The fruit extracts of *S. aucuparia* obtained and analyzed in this study, particularly the ASE concentrates, demonstrated promising antioxidant activity as well as significant enzy-

matic inhibition of α-glucosidase, α-amylase, LOX, and HYA. The concentrated ASE extract of *S. aucuparia* exhibited the highest levels of polyphenols (2692.53 ± 37.89 CA mg/mL) and flavonoids (433.88 ± 21.12 RU mg/mL), along with strong inhibitory effects on the enzymes studied. The IC_{50} values for IC_{50}α-glucosidase, IC_{50}α-amylase, and IC_{50}LOX were determined to be 13.50 ± 0.96 µg/mL, 20.19 ± 1.67 µg/mL, and 23.74 ± 1.32 µg/mL, respectively. The IC_{50} values for IC_{50}HYA and IC_{50}ibuprofen were determined to be 43.04 ± 2.19 µg/mL and 51.54 ± 3.67 µg/mL, respectively.

The data obtained provide scientific support for the use of these fruits in folk medicine for managing diabetes and inflammatory diseases; however, further in vivo studies are needed to confirm these activities. Due to their significant content of polyphenolic compounds, especially chlorogenic acid isomers, which are recognized as powerful antioxidants with beneficial health effects, *S. aucuparia* fruits could be valuable in developing medicinal products, nutraceuticals, or functional foods targeting diabetes and inflammatory diseases.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

UAE	Ultrasound-assisted extraction
ASE	Accelerated solvent extraction
HPLC-MS	High performance liquid chromatography—mass spectrometry
HYA	Hyaluronidase
LOX	Lipoxygenase
TEAC	Trolox Equivalent Antioxidant Capacity
SD	Standard deviation

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