

Phytochemical analysis of the extract from berries of *Schisandra chinensis* Turcz. (Baill.) and its anti-platelet potential in vitro

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Abstract

Schisandra chinensis Turcz. (Baill.) is a dioecious vine, belonging to the Schisandraceae family. Its berries show several beneficial activities, including cardioprotective, antioxidant, and anti-inflammatory. We examined the chemical content of the extract from *S. chinensis* berries, as well as its antiplatelet potential in washed human blood platelets and whole blood *in vitro*. We assessed effect of the extract on several hemostasis parameters, including thrombus formation in full blood, platelet activation and adhesion, and coagulation times. Moreover, we evaluated the cytotoxicity of the extract against blood platelets based on extracellular lactate dehydrogenase (LDH) activity. The most important constituents of the extract were dibenzocyclooctadiene lignans; schisandrin was the dominant compound. The extract inhibited thrombus formation, agonist-stimulated platelet activation and adhesion, and was not cytotoxic. These results suggest that *S. chinensis* berries can be used as a safe, natural supplement with anti-platelet properties. However, more studies are needed to determine their mechanisms of action and *in vivo* efficiency.

1. Introduction

Edible berries are an important component of the human diet, serving as a source of nutrients and bioactive compounds. They contain a variety of phytochemicals that exhibit a wide range of biological properties, including cardioprotective activity (Olas, 2017, 2018, 2023; Sławińska et al., 2023; Sławińska & Olas, 2022).

Schisandra chinensis Turcz. (Baill.) is a dioecious vine, belonging to the Schisandraceae family. It occurs naturally in the forests of south-eastern Siberia (Primorski, Amurski, Khabarovski regions, Sakhalin), north-eastern China, Korea, and Japan. Recently, Valíčková et al. (2023) have shown that *S. chinensis* has the potential to be used for the production of nutrient supplements due to its adaptogenic properties. The primary property of all adaptogens is to eliminate the effects of stress and adapt the body to unfavorable external conditions (Szopa et al., 2016).

S. chinensis fruit, called "fruit of five flavors" (wǔwèizǐ), which results from the fact that the fruit's peel is sweet, the pulp is sour, the seeds are bitter and astringent, and the grain extract is salty, has been used in Chinese medicine to treat different disorders and diseases. Indigenous peoples of the Far-Eastern regions of Russia used it to reduce hunger, thirst, and exhaustion (Panossian & Wikman, 2008; Szopa et al., 2017). Importantly, *S. chinensis* berries also have cardioprotective potential (Chun et al., 2014; Olas, 2023), which is exerted through various molecular pathways. They can also help control oxidative stress, obesity, and inflammation (Lin et al., 2017; Su et al., 2022; Wu et al., 2017). In traditional Chinese medicine, *S. chinensis* is often used in the treatment of palpitation (Su et al., 2022). The 2020-year edition of Chinese Pharmacopoeia contains 100 prescriptions containing *S. chinensis* (K. Yang et al., 2022). Due to medicinal and health promoting properties of this plant, many food supplements made from *S. chinensis* fruit are available on the market.

Different components of *S. chinensis*, including lignans (especially schisandrin, also called schizandrin, schisandrol A, or wǔwèizǐ sù A) play a significant role in the prophylaxis and treatment of cardiovascular diseases (CVDs), for example in myocardial infarction and hypertension. However, there are no experiments that focus on the response of various elements of hemostasis (including blood platelets, plasma, and others) to *S. chinensis* berries. Therefore, the present study examined the anti-platelet activity of the extract from *S. chinensis* berries in washed human blood platelets and human whole blood *in vitro*. Using washed platelets, we studied the activity of this extract on platelet adhesion to two adhesive proteins – collagen and fibrinogen. We also

determined the anti-platelet activity of the tested extract in whole blood using the Total Thrombus-formation Analysis System (T-TAS) and flow cytometry. Moreover, we evaluated the cytotoxicity of the extract against blood platelets based on extracellular lactate dehydrogenase (LDH) activity. We also investigated the effect of *S. chinensis* berry extract on the parameters of hemostasis in plasma (*in vitro*), including coagulation times: thrombin time (TT), prothrombin time (PT), and activated partial thromboplastin time (APTT). The action of the extract from *S. chinensis* berries was compared to the extract from sea buckthorn (*Hippophae rhamnoides* L.) berries, and a commercial product – Aronox (*Aronia melanocarpa* berry extract), which have documented anti-platelet properties (Olas et al., 2008; Skalski et al., 2020, 2021).

2. Materials and methods

2.1. Reagents

Methanol (HPLC grade) was purchased from Fisher Chemical (UK), heptane (for chromatography), and acetonitrile (LC-MS grade) were obtained from Merck (Darmstadt, Germany). Monoclonal antibodies for flow cytometry (CD61-PerCP, CD62P-PE, and PAC-1-FITC) were purchased from Becton Dickinson (New Jersey, USA). Phosphate buffered saline (PBS), fibrinogen, collagen, bovine serum albumin (BSA), tris(hydroxymethyl)aminomethane (Tris), 4-nitrophenyl phosphate, ADP, nicotinamide adenine dinucleotide (NADH), and thrombin were purchased from Merck (Darmstadt, Germany). NaCl, sodium citrate, and citric acid were purchased from POCH (Avantor performance materials, Gliwice, Poland). Reagents needed for coagulation time measurements were purchased from Kselmed (Grudziądz, Poland). All materials and chemicals for T-TAS were purchased from Bionicum Sp. z o.o. (Warsaw, Poland). Other reagents were purchased from commercial distributors and were of the highest available grade.

2.2. Plant material

Crimson-red, ripe *S. chinensis* berries, growing in spike clusters on vines, were collected in mild-September 2022 in the park and palace settlement “Lower Garden” of the Czartoryski family in Pulawy (51°24'47.698" N21° 57' 20.626" E). The plants were planted about 40 years ago and identified by the park's curator, dr. Adam Wołk. After harvesting, the berries were frozen at -18°C, and subsequently lyophilized (Gamma 2–16 LSC, Christ, Osterode am Harz, Germany). The reference sample marked 1/09/2022 is located at the Institute of Soil Science and Plant Cultivation (Pulawy, Poland).

2.3. Preparation of the extract from *S. chinensis* berries

Freeze-dried fruits (100 g) were soaked overnight in 80% methanol (v/v; 1.5 L) at ambient temperature, roughly homogenized using a kitchen blender, and subjected to a 15 min. sonication in an ultrasonic bath. The collected extract was centrifuged (3-16KL, Sigma, Osterode am Harz, Germany), and filtered using a glass filter funnel. The extraction procedure was repeated twice, with new portions of the solvent. The pooled extracts were concentrated in a rotary evaporator and defatted by liquid-liquid extraction with heptane. The defatted extract was rotary evaporated to remove organic solvents and reduce its volume, and freeze-dried. The extract (74.57 g) was subsequently cleaned up by SPE, to remove organic acids, sugars, and other highly polar constituents. It was dissolved in 0.1% formic acid in MilliQ water (v/v) and loaded onto a short C18 column (60 × 50 mm; Cosmosil 140C18-Prep, 140 µm). The column was washed with 0.1% formic acid, and the bound compounds

were eluted with methanol. The eluate was rotary-evaporated, and the residue was freeze-dried to yield 7.61 g of the purified extract.

2.4. Phytochemical analysis of the extract from *S. chinensis* berries

The composition of *S. chinensis* fruits was analyzed by LC-HRMS, using a Thermo Ultimate 3000RS (Thermo Fischer Scientific, Waltham, MS, USA) UHPLC system equipped with a corona-charged aerosol detector (CAD) and coupled with a Bruker Impact II HD quadrupole-time of flight (Q-TOF) mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany). Samples were chromatographed on an ACQUITY UPLC HSS T3 column (2.1 × 150 mm, 1.8 μm), equipped with a pre-column, and maintained at 40°C. The injection volume was 2 μL. The mobile phase A was 0.1% formic acid in MilliQ water, and the mobile phase B was acetonitrile containing 0.1% of formic acid. The flow rate was 0.400 mL min⁻¹. The separation program started from a short isocratic elution with 5% B (0.5 min.), followed by a 31 min. gradient from 5 to 95% of solvent B. The elution was continued at 95% B for 7 min., then the mobile phase composition returned to the initial conditions and the column was equilibrated with 5% B for 5 minutes. MS's analyses were performed in the negative and positive ion mode. MS's settings for negative ion mode: capillary voltage 3 kV; dry gas flow 6 L min⁻¹; dry gas temperature 200°C; nebulizer pressure 2.5 bar; collision RF 750 Vpp; transfer time 100 μs; prepulse storage time 10 μs. Collision energy was set automatically in the range from 2.5 to 80 eV, depending on the *m/z* of a fragmented ion. The scanning values ranged from *m/z* 80 to *m/z* 2000. The settings for positive ion mode: capillary voltage 4 kV; dry gas flow 6 L min⁻¹; dry gas temperature 200°C; nebulizer pressure 2.5 bar; collision RF 750 Vpp; transfer time 100 μs; prepulse storage time 10 μs. Collision energy was set automatically in the range from 2.5 to 50 eV. The scanning range was *m/z* 100–2000.

Quantitative analyses of phenolic compounds were performed using an ACQUITY UPLC® chromatographic system (Waters Inc., Milford, MA, USA), equipped with a PDA detector, and coupled with a triple quadrupole mass detector (TQD; Waters). The *S. chinensis* extract was separated on an ACQUITY UPLC BEH C18 (2.1 × 100 mm, 1.7 μm; Waters) column. The column temperature was 50°C, the injection volume was 2.5 μL. Mobile phase was composed of Mill-Q water with 0.1% formic acid in (solvent A) and acetonitrile with 0.1% formic acid (solvent B); the elution program was as follows: 0.0–0.5 min., 7% B; 0.5–7.0 min., linear gradient 7–25% B; 7.0–16.9 min., linear gradient 25–75% B; 17.0–18.0 min., 95% B; 18.1–20.0 min., 7% B. The TQ mass spectrometer was operated in negative and positive ion mode. The MS settings in negative ion mode were as follows: capillary voltage 2.80 kV, cone voltage 45 V, source temperature 150°C, desolvation temperature 450°C, cone gas flow (N₂) 100 L h⁻¹, desolvation gas (N₂) flow 800 L h⁻¹. In positive ion mode: capillary voltage 3.10 kV, cone voltage 60 V, the remaining parameters were the same as those described above. The scanning range was *m/z* 100–1200. The content of anthocyanins (the peak integration at λ = 475 nm; y = 232.90079x + 82.94488, R² = 0.9989), flavonoids (the peak integration at λ = 350 nm; y = 442.56566x - 46.00717, R² = 0.9999), and lignans (the peak integration at λ = 255 nm; y = 607.19720x + 131.25948, R² = 0.9999) was calculated on the basis of calibration curves, and was expressed as cyanidin, rutin and schisandrin equivalents, respectively.

2.5 Preparation of stock solution of the extract from *S. chinensis* berries, the extract from sea buckthorn berries, and the extract from aronia berries

The extract from *S. chinensis* berries and the extract from sea buckthorn berries were dissolved in 50% DMSO (a universal solvent for many plant substances), however the final concentration of DMSO in the tested human

blood platelets, human plasma, and human blood was below 0.05% (v/v). The addition of a low concentration of DMSO to blood platelets, plasma, and blood has no effect on the hemostatic properties of blood platelets, plasma, and full blood (data not presented).

The extract from sea buckthorn berries contained predominantly various flavonol glycosides, mainly isorhamnetin glycosides and acylated glycosides (Skalski et al., 2021).

A stock solution of commercial product – Aronox (by Agropharm Ltd., Poland; batch No 020/2007k, *A. melanocarpa* berry extract) was prepared in H₂O at a concentration of 5 mg/mL.

2.6. Blood, plasma, and blood platelet samples

Whole human blood was collected at “Diagnostyka” blood collection center (Brzechwy 7A, Lodz, Poland). All donors were non-smoking, healthy volunteers, which did not drink alcohol or take medication and anti-platelet supplementation for two weeks before blood collection. Peripheral blood was drawn into tubes with benzylsulfonyl-D-Arg-Pro-4-amidinobenzylamide (BAPA) (for T-TAS) or with citrate/phosphate/dextrose/adenine (CPDA) anticoagulant (for the remaining assays).

The analysis of blood samples was performed according to the guidelines of the Helsinki Declaration for Human Research. Informed consent form was signed by each participant one day prior to blood collection. Procedures were conducted with the consent of Bioethics Committee at the University of Łódź (2/KBBN-UŁ/III/2014).

Blood for the T-TAS assay was used within 2 hours after collection.

For coagulation times measurements, plasma was separated from whole blood by differential centrifugation (2800 x g, 20 min., at room temperature).

Blood platelets were isolated from fresh blood by differential centrifugation as described previously (Lis et al., 2019). The required number of platelets (2.0×10^8 /mL) was confirmed by a spectrophotometric measurement in a UV-Visible Helios-α at 800 nm.

In every experiment, blood, plasma, or blood platelets were incubated for 30 minutes at 37°C, either with the extract from *S. chinensis* berries at final concentrations of 0.5–50 µg/mL, or the extract from sea buckthorn berries or aronia berries at final concentration 50 µg/mL.

2.7. Blood platelet adhesion

Blood platelet adhesion was measured based on the activation of exoenzyme acid phosphatase in the blood platelets according to the method described by (Bellavite et al., 1994). 96-well plates were coated with 100 µg/mL fibrinogen or 0.04 µg/mL collagen. To achieve this, 100 µL of fibrinogen or collagen was added to the wells, and incubated for 24 h at 4°C, on an orbital shaker. Afterward, the wells were washed three times with TBS (pH 7.5), and 200 µL of 1% BSA was added. The plates were incubated with BSA at 37°C for 2 h. Meanwhile, the extracts were added to washed blood platelets at final concentrations of 0.5–50 µg/mL. A control sample was set up containing only blood platelets with Barber's buffer (a modified Tyrode's buffer); this value was assumed to be 100%. The samples were incubated for 30 min. at 37°C. BSA was removed, and the wells were washed three times with TBS (pH 7.5) with 0.1 mM CaCl₂ and 0.1 mM MgCl₂. The samples were added to the wells in triplicate. Agonists (1 U/mL thrombin or 30 µM ADP) or TBS were added to appropriate wells. Then, the plates

were incubated for 1 h at 37°C. Afterward, the wells were washed three times with PBS. 150 µL of 0.1 M citrate buffer (pH 5.4) with 5 mM *p*-nitrophenyl phosphate and 0.1% Triton X-100 was added; the samples were incubated for 1 h at room temperature. Lastly, 100 µL of 2M NaOH was added to the wells. Absorbance was read at 405 nm with SPECTROstar Nano Microplate Reader (BMG LABTECH, Germany)

2.8. Flow cytometry analysis

Platelet activation was assessed based on platelet-derived microparticles (PMP) formation, and the exposition of P-selectin (CD62P) and the active form of GPIIb/IIIa on the surface of blood platelets. To measure the exposition of P-selectin and the active form of GPIIb/IIIa, full blood was mixed with the extracts at the concentrations of 0.5–50 µg/mL and incubated for 15 min. at 37°C. After 15 minutes, agonists were added to the samples (10 µM ADP, 20 µM ADP, or 10 µg/mL collagen), and the incubation continued for another 15 min. (at 37°C). After the incubation, the samples were diluted 10 times with PBS and incubated with antibodies (CD61-PerCP, CD62P-PE, and PAC-1-FITC, 3 µL of each antibody incubated with 10 µL of diluted samples). The samples were stained for 30 minutes in the dark, at room temperature. Afterward, the samples were fixed with 1% CellFix for 1 h at 37°C. The samples were analyzed with a LSR II Flow Cytometer (Becton Dickinson, San Diego, CA, USA); at least 5000 CD61-PerCP-positive objects were recorded. Blood platelets were gated based on an FSC/SSC (forward scatter/side scatter) plot and positive staining with CD61-PerCP. Then, the percent of CD62-PE and PAC-1-FITC positive platelets was calculated in each of the samples.

To measure the formation of platelet-derived microparticles, the samples were incubated with *S. chinensis* extract for 15 minutes at 37°C. Then, 20 µg/mL collagen was added, and the incubation continued for another 15 minutes. The samples were diluted 10 times with PBS and stained with 2 µL of CD61-PE antibody for 30 minutes in the dark, at room temperature. The samples were fixed with 1% CellFix for 1 hour and analyzed with LSR II Flow Cytometer (Becton Dickinson, San Diego, CA, USA). At least 10000 CD61-PE-positive objects were recorded. PMPs were distinguished from CD61-PE-positive objects based on an FSC/SSC plot on a log/log scale. The analysis was carried out with Floreada software (<https://floreada.io>, accessed on 26.02.2024).

2.9. Total Thrombus-formation Analysis System (T-TAS) (PL-chip)

The T-TAS system can measure the thrombus formation process in semi-physiological conditions. Here, the PL-chip which measures only primary hemostasis was used. The results were recorded as AUC₁₀ (Area Under the Curve) - area under the flow pressure curve recorded for 10 min. after the start of the test. The AUC₁₀ depicts the growth, intensity, and stability of thrombus formation. Data was depicted as % of control. Further information about the assay can be found in (Hosokawa et al., 2011). First, the extracts were incubated with human full blood at final concentrations of 0.5–50 µg/mL (37°C, 30 minutes). A control sample with 0.9% NaCl instead of the extract was set up. Then, 320 µL of each sample was added to the PL-chip, and pressure was recorded for 10 minutes or until it reached 60 kPa (Time of Occlusion). The results were depicted as % of the control sample.

2.10. Measurement of prothrombin time, thrombin time, and activated partial thromboplastin time

Coagulation times were determined coagulometrically, according to the method described by (Malinowska et al., 2012). The extract was incubated with human plasma at 37°C for 30 minutes, at final concentrations of 0.5–50

µg/mL. The measurements were carried out on an Optic Coagulation Analyser, model K-3002 (Kselmed, Grudziadz, Poland). Each sample was measured in duplicate.

2.11. Activity of LDH

The toxic effect of the extract from *S. chinensis* berries on human blood platelets was analyzed by measuring the activity of lactate dehydrogenase (LDH), an enzyme released from damaged blood platelets (Wróblewski & Ladue, 1955). Blood platelets were incubated with the extract at final concentrations of 0.5–50 µg/mL, at 37°C for 30 min. Afterward, the samples were centrifuged (2500 rpm, 15 min., 25°C) and 10 µl of the supernatant was added to the wells of a 96-well plate (in triplicate). Then, 0.1 M phosphate buffer (pH 7.4, 270 µL) and 0.25% nicotinamide adenine dinucleotide (NADH) (10 µl) were added to each well. A blank which contained 280 µL of phosphate buffer and 10 µL of NADH was set up as well. The samples were mixed and incubated for 20 minutes at room temperature. Lastly, 0.25% pyruvate (10 µL) was added to the samples. The samples were immediately mixed, and the absorbance was read at 1 minute intervals for 10 minutes, at 340 nm.

2.12. Statistical analysis

Statistical analysis was performed with Statistica 10 (StatSoft 13.3, TIBCO Software Inc. Palo Alto, CA, USA). The distribution of data was checked by the Shapiro-Wilk test, and the homogeneity of variance by Levene's test. Differences within and between groups were assessed with one-way ANOVA followed by Tukey's test, or Kruskal-Wallis test. Results were presented as means ± SD. The results were considered significant at $p \leq 0.05$. Dixon's Q-test was used to eliminate uncertain data.

3. Results

3.1. Chemical characteristic of the extract from *S. chinensis* berries

The extract contained many lignans, which were the major phenolic constituents (and more generally, main specialized metabolites) of the *S. chinensis* fruit extract (Table 1; Fig. 1). Among them, schisandrin (tentatively identified) was the dominant compound. The red color of the extract can be attributed to the presence of cyanidin- Pen-Hex-dHex. *S. chinensis* fruits also contained lesser amounts of (epi)catechin, dimeric and trimeric B-type proanthocyanidins, quercetin hexoside-deoxyhexoside, and quercetin hexoside. Apart from phenolics, small amounts of highly oxygenated nortriterpenoids (mainly), bisnortriterpenoids, triterpenoids, and homotriterpenoids were also detected (Table 1). Moreover, the extract contained also very high amounts of diverse highly polar constituents (Fig. 1).

Table 1
Specialized metabolites of the *S. chinensis* fruit extract.

No.	tR (min.)	parent ion (<i>m/z</i>)	CID	delta (ppm)	<i>m</i> σ	formula	tentative identification	Ref.
1– 3	0.85– 1.80						mixtures of polar compounds	
4	5.77	203.0823 [–]	186.0552 (5), 159.0921 (7), 142.0641 (7), 116.0595 (17)	1.3	10.2	C ₁₁ H ₁₂ N ₂ O ₂	tryptophan	
5	7.45	577.1352 [–]	407.0773 (90), 289.0720 (100), 245.0821 (23)	-0.2	8.0	C ₃₀ H ₂₆ O ₁₂	(epi)C-(epi)C	
6	7.83	289.0719 [–]	289.0720 (100), 245.0819 (73), 203.03715 (31)	-0.3	1.7	C ₁₅ H ₁₄ O ₆	(epi)catechin	
7	7.90	865.1982 [–]	695.1400 (4), 525.0822 (15), 407.0785 (83), 289.0729 (100), 243.0309 (39)	0.4	15.5	C ₄₅ H ₃₈ O ₁₈	(epi)C-(epi)C- (epi)C	
8	9.30	725.1927 [–]	339.0514 (14), 284.0329 (100)	1.0	7.2	C ₃₂ H ₃₉ O ₁₉	cyanidin-Pen-Hex- dHex	1
9	13.23	441.1769 [–]	441.1769 (78), 397.1854 (28), 330.1319 (100), 217.1227 (30)	-0.6	9.3	C ₂₁ H ₃₀ O ₁₀		
10	13.45	609.1460 [–]	300.0279 (100)	0.2	15.4	C ₂₇ H ₃₀ O ₁₆	rutin	
11	13.87	463.0882 [–]	300.0276 (100)	0.1	13.2	C ₂₁ H ₂₀ O ₁₂	Q-3- <i>O</i> -Hex	
12	16.40	685.2927 ^{–*}	639.2851 (12), 477.2348 (24), 383.1193 (100), 323.0979 (10), 263.0774 (15), 221.0664 (31), 179.0557 (28)	-0.3	8.4	C ₂₈ H ₄₈ O ₁₆	unidentified bisnortriterpenoid	
13	17.09	605.2232 ^{–*}	559.2182 (100), 497.2176 (65), 439.1759 (45),	1.3	12.2	C ₂₉ H ₃₆ O ₁₁	unidentified nortriterpenoid	

No.	tR (min.)	parent ion (<i>m/z</i>)	CID	delta (ppm)	<i>mσ</i>	formula	tentative identification	Ref.
			351.1965 (31), 317.1391 (22)					
14	17.52	575.2131 ⁻	575.2138 (100), 557.2020 (20), 531.2235 (3), 455.1728 (10)	0.5	8.9	C ₂₉ H ₃₆ O ₁₂	unidentified nortriterpenoid	
15	17.74	559.2179 ⁻	559.2209 (100), 541.2087 (65), 523.2031 (50), 465.1557 (47), 447.1440 (55), 439.1785 (70), 421.1656 (81)	1.1	8.0	C ₂₉ H ₃₆ O ₁₁	unidentified nortriterpenoid	
16	18.22	605.2239 ^{-*}	559.2181 (100), 497.2173 (88), 479.2080 (43), 439.1762 (41), 351.1961 (42), 317.1391 (34)	0.1	7.3	C ₂₉ H ₃₆ O ₁₁	unidentified nortriterpenoid	
17	18.45	559.2189 ⁻	559.2196 (100), 541.2082 (19), 465.1531 (5), 445.1495 (6), 439.1760 (7)	-0.7	3.2	C ₂₉ H ₃₆ O ₁₁	unidentified nortriterpenoid	
18	18.85	621.2194 ^{-*}	575.2142 (100), 557.2041 (28), 511.2317 (8), 455.1724 (9), 417.1558 (12)	-0.9	14.6	C ₂₉ H ₃₆ O ₁₂	unidentified nortriterpenoid	
19	19.69	605.2241 ^{-*}	559.2188 (100), 541.2083 (14), 445.1504 (6), 347.1493 (3)	-0.3	9.1	C ₂₉ H ₃₆ O ₁₁	unidentified nortriterpenoid	
20	19.99	589.2296 ^{-*}	543.2244 (47), 525.2134 (60), 507.2025 (100), 481.2219 (60), 463.2128 (89), 419.2235 (94), 383.1863 (43)	-0.9	15.9	C ₂₉ H ₃₆ O ₁₀	unidentified nortriterpenoid	
21	20.24	605.2242 ^{-*}	559.2184 (100), 541.2082 (40), 515.2278 (32), 497.2186 (49)	-0.4	1.6	C ₂₉ H ₃₆ O ₁₁	unidentified nortriterpenoid	

No.	tR (min.)	parent ion (m/z)	CID	delta (ppm)	mσ	formula	tentative identification	Ref.
22	20.62	591.2453 ⁺ *	545.2415 (2), 447.2161 (20), 411.1817 (25), 367.1917 (100)349.1815 (22)	-1.0	11	C ₂₉ H ₃₈ O ₁₀	unidentified nortriterpenoid	
23	20.7	605.2240 ⁺ *	559.2186 (100), 497.1289 (14), 457.1862 (19)	0.0	30.1	C ₂₉ H ₃₆ O ₁₁	unidentified nortriterpenoid	
24	21.38	589.2295 ⁺ *	543.2245 (100), 525.2143 (46), 499.2354 (38), 481.2238 (77), 407.1863 (26)	-0.7	4.3	C ₂₉ H ₃₆ O ₁₀	unidentified nortriterpenoid	
25	21.46	593.2603 ⁺ *	547.2556 (6), 529.2449 (25), 511.2336 (14), 485.2538 (16), 467.2442 (100), 427.2138 (14)	0.1	12.2	C ₂₉ H ₄₀ O ₁₀	unidentified nortriterpenoid	
26	22.83	577.2292 ⁺ *	531.2238 (100), 513.2118 (11), 451.2120 (16), 297.1485 (9)	-0.3	12.8	C ₂₈ H ₃₆ O ₁₀	wuweizidilactone H / isomer	2
27	23.87	603.208 ⁺ *	557.2037 (100)481.1507 (17), 455.1716 (55), 437.1604 (13), 365.1393 (7)	0.5	10.4	C ₂₉ H ₃₄ O ₁₁	unidentified nortriterpenoid	
28	24.17	579.2447 ⁺ *	533.2387 (100), 497.2195 (29), 475.2005 (35), 453.2270 (28), 435.2166 (24)	0.0	13.1	C ₂₈ H ₃₈ O ₁₀	unidentified bisnortriterpenoid	
29	24.6	619.2757 ⁺ *	573.2733 (1), 531.2596 (100), 513.2490 (16), 495.2399 (8), 211.0975 (9)	0.5	10.5	C ₃₁ H ₄₂ O ₁₀	unidentified homotriterpenoid	
30	24.79	633.2554 ⁺ *	587.2515 (1), 509.2182 (29), 491.2076 (13), 465.2284 (19), 447.2180	-0.2	6.6	C ₃₁ H ₄₀ O ₁₁	unidentified homotriterpenoid	

No.	tR (min.)	parent ion (m/z)	CID	delta (ppm)	mσ	formula	tentative identification	Ref.
			(100), 429.2077 (21)					
31	24.79	603.2088 ^{-*}	557.2032 (100), 499.1618 (10), 481.1509 (18), 455.1707 (56), 437.1600 (13)	0.0	17.6	C ₂₉ H ₃₄ O ₁₁	unidentified nortriterpenoid	
32	24.89	635.2710 ^{-*}	589.2654 (4), 571.2546 (21), 509.2544 (100), 491.2440 (14), 467.2435 (54), 449.2329 (34)	-0.1	11.9	C ₃₁ H ₄₂ O ₁₁	unidentified homotriterpenoid	
33	25.36	575.2476 ⁺	557.2372 (11), 497.2162 (27), 479.2057 (69), 461.1951 (33), 437.1953 (51), 419.1846 (100)	1.9	8.9	C ₃₀ H ₃₈ O ₁₁	unidentified triterpenoid	
34	25.47	587.2126 ^{-*}	541.2071 (100), 483.1655 (16), 465.1552 (20), 439.1759 (34), 365.1394 (12), 215.0715 (4)	1.4	3.9	C ₂₉ H ₃₄ O ₁₀	unidentified nortriterpenoid	
35	26.75	577.2638 ⁺	559.2533 (46), 541.2434 (29), 499.2316 (49), 481.2218 (100), 463.2107 (53), 439.2112 (66), 421.2004 (95)	0.9	9.2	C ₃₀ H ₄₀ O ₁₁	unidentified triterpenoid	
36	27.02	619.2764 ^{-*}	573.2706 (71), 531.2591 (100)	-0.6	8.8	C ₃₁ H ₄₂ O ₁₀	unidentified homotriterpenoid	
37	28.12	433.2218 ⁺	415.2112 (100)	0.6	10.7	C ₂₄ H ₃₂ O ₇	schisandrin	3, 4, 5
38	28.93	701.2813 ^{-*}	531.2215 (66), 513.2110 (100), 495.2043 (55), 477.1932 (32), 451.2151 (41), 433.2003 (64), 415.1912 (66)	0.2	10.5	C ₃₅ H ₄₄ O ₁₂	unidentified	

No.	tR (min.)	parent ion (<i>m/z</i>)	CID	delta (ppm)	<i>m</i> σ	formula	tentative identification	Ref.
39	29.13	389.1960 ⁺	389.1963 (100), 357.1699 (7), 319.1180 (3), 287.0920 (3)	-0.4	1.7	C ₂₂ H ₂₈ O ₆	gomisin J / isomer	3, 5
40	29.40	417.1911 ⁺	399.1804 (100), 369.1694 (13)	-0.8	7.3	C ₂₃ H ₂₈ O ₇	schisandrol B / isomer	3, 4, 5
41	30.03	391.2116 ⁺	391.2115 (100), 237.1485 (16)	-0.2	2.1	C ₂₂ H ₃₀ O ₆	pregomisin / isomer	5
42	30.03	501.2482 ⁺	483.2379 (65), 401.1961 (100)	0.1	11.4	C ₂₈ H ₃₆ O ₈	angeloylgomisin H / tigloylgomisin H / isomer	3, 4, 5
43	30.38	501.2483 ⁺	483.2377 (26), 401.1960 (100)	0.1	5.5	C ₂₈ H ₃₆ O ₈	angeloylgomisin H / tigloylgomisin H/ isomer	3, 4, 5
44	30.46	523.2321 ⁺	505.2221 (100), 401.1960 (49)	1.0	2.4	C ₃₀ H ₃₄ O ₈	benzoylgomisin H / isomer	5
45	30.67	548.2850 ⁺⁺	431.2063 (100)	0.7	7.9	C ₂₉ H ₃₈ O ₉	angeloylgomisin Q / tigloylgomisin Q / isomer	4, 5
46	30.70	554.2387 ⁺⁺	415.1754 (100)	-0.5	12.2	C ₃₀ H ₃₂ O ₉	schisantherin A / gomisin G	3, 4, 5
47	30.99	532.2542 ⁺⁺	415.1758 (100)	-0.1	18.1	C ₂₈ H ₃₄ O ₉	schisantherin B / isomer	3, 4, 5
48	31.12	532.2542 ⁺⁺	415.1755 (100), 371.1491 (7)	-0.2	3.0	C ₂₈ H ₃₄ O ₉	gomisin F / angeloylgomisin P / tigloylgomisin P	3, 5
49	31.32	403.2118 ⁺	403.2120 (100), 371.1857 (6)	-0.8	8.1	C ₂₃ H ₃₀ O ₆	schisanhenol / isomer	3, 5
50	32.08	417.2275 ⁺		-0.8	4.7	C ₂₄ H ₃₂ O ₆	schisandrin A (deoxyschisandrin) / isomer	3, 4, 5
51	32.30	401.1962 ⁺	401.1964 (100), 331.1183 (5)	-0.9	2.1	C ₂₃ H ₂₈ O ₆	gomisin N / isomer	3, 5
52	32.40	401.1962 ⁺	401.1965 (100), 331.1183 (5)	-0.9	5.7	C ₂₃ H ₂₈ O ₆	schisandrin B (γ - schisandrin) / isomer	3, 4, 5
53	32.49	385.1649 ⁺	385.1648 (100), 355.1544 (5),	-0.9	5.5	C ₂₂ H ₂₄ O ₆	schisandrin C / isomer	3, 5

No.	tR (min.)	parent ion (<i>m/z</i>)	CID	delta (ppm)	<i>m</i> σ	formula	tentative identification	Ref.
			315.0871 (5), 285.0765 (3)					
54	32.52	485.2536 ⁺	485.2538 (100), 403.2116 (32)	-0.4	7.3	C ₂₈ H ₃₆ O ₇	unidentified	
55	33.31	279.2326 ⁻	279.2326 (100)	1.1	14.3	C ₁₈ H ₃₂ O ₂	octadecadienoic acid	
⁻ - negative ion mode; ⁺ - positive ion mode; * - formic acid adduct; [#] - NH ₄ ⁺ adduct; C – catechin, Q – quercetin, dHex – deoxyhexose; Hex – hexose; Pen – pentose								
1. Ma et al., 2012; 2. Li et al., 2017; 3. He et al., 1997; 4. Yang et al., 2011; 5. Liu et al., 2013								

Results of the quantitative analysis of phenolic compounds are shown in Table 2. As mentioned above, lignans were dominant phenolic compounds of the extract from *S. chinensis* fruit. The total lignan content was 29.61 ± 0.18 mg g⁻¹ of the extract (expressed as schisandrin equivalent). The total flavonoid content was 1.64 ± 0.03 mg g⁻¹ (expressed as rutin equivalent), and the content of anthocyanidins was 17.08 ± 0.35 mg g⁻¹ (expressed as cyanidin equivalent).

Table 2
Content of major phenolic compounds in the extract from the fruit of *S. chinensis*.

tR (min.)	<i>m/z</i> [*]	identification	content (mg g ⁻¹)
2.21	727	cyanidin-Pen-Hex-dHex	17.08 ± 0.35 ^{\$}
4.17	611	rutin	1.12 ± 0.02
4.32	465	quercetin-Hex	0.52 ± 0.01 [#]
11.39	433	schisandrin	23.07 ± 0.31
11.86	389	gomisin J	traces
11.99	417	schisandrol B	1.16 ± 0.01 [^]
12.97	501	angeloylgomisin H / tigloylgomisin H	1.61 ± 0.02 [^]
15.81	401	schisandrin B	2.12 ± 0.11 [^]
16.15	385	schisandrin C	0.84 ± 0.06 [^]
[*] - positive ion mode; ^{\$} - cyanidin equivalent; [#] - rutin equivalent; [^] - schisandrin equivalent; Hex - hexose; dHex - deoxyhexose; Pen - pentose;			

3.2. Effect of the extract from *S. chinensis* berries on the adhesion of washed blood platelets to collagen and fibrinogen

The anti-adhesive activity of the extract was studied using human washed blood platelets. The results were presented as percent of adhesion of the control samples. As shown in Fig. 2A and B the level of adhesion of

resting blood platelets and adhesion of thrombin-activated platelets was significantly reduced in the presence of all used concentrations of the extract from *S. chinensis* berries (0.5–50 µg/mL). For example, at the highest concentration (50 µg/mL), the extract inhibited the adhesion of thrombin-activated blood platelets by about 50% in comparison with control (Fig. 2B). On the other hand, none of the tested concentrations of the extract (0.5–50 µg/mL) demonstrated anti-adhesive properties, when adhesion of thrombin-activated platelets to fibrinogen was measured (Fig. 2C).

For the ADP-activated blood platelets, three tested concentrations of the extract from *S. chinensis* berries (0.5, 1, and 5 µg/mL) did not have anti-adhesive activity (Fig. 2D). Reduced adhesion was only observed for the two highest concentrations (10 and 50 µg/mL) (Fig. 2D).

3.3. Effect of the extract from S. chinensis berries on parameters of blood platelet activation measured with flow cytometry in whole blood

Figures 3 and 4 demonstrate the parameters of activation of platelets stimulated with 10 and 20 µM ADP, and 10 µg/mL collagen (measured with flow cytometry). Changes in platelet activation were noted in whole blood treated with the extract from *S. chinensis* berries at all tested concentrations (0.5–50 µg/mL), but these changes were not always statistically significant (Figs. 3 and 4). For example, the extract from *S. chinensis* berries (at all used concentrations: 0.5–50 µg/mL) inhibited the exposition of the active form of GPIIb/IIIa on the surface of blood platelets stimulated with 10 µM ADP (Fig. 3A). In blood platelets stimulated with 20 µM ADP, statistically significant reduction of the exposition of the active form of GPIIb/IIIa was observed only for the highest concentration of the extract (50 µg/mL) (Fig. 3B). On the other hand, none of the concentrations of the extract (0.5–50 µg/mL) significantly impacted the exposition of GPIIb/IIIa on platelets stimulated by 10 µg/mL collagen, and the exposition of P-selectin on the surface of blood platelets stimulated by 10 and 20 µM ADP (Fig. 3C, 4A and B). However, in platelets activated by collagen, significant inhibition of the exposition of P-selectin was observed for three concentrations of the extract (5, 10, and 50 µg/mL) (Fig. 4C).

Moreover, none of the used concentrations (0.5–50 µg/mL) of the extract from *S. chinensis* berries significantly impacted platelet-derived microparticle formation and the exposition of GPIIb/IIIa and P-selectin on resting platelets (data not presented).

3.4. Effect of the extract from S. chinensis berries on thrombus formation with T-TAS (PL-chip) in whole blood

Only the highest concentration of the extract from *S. chinensis* berries (50 µg/mL) significantly decreased the AUC₁₀ value measured by T-TAS in whole blood (Fig. 5).

3.5. Effect of the extract from S. chinensis berries on coagulation parameters (PT, TT, and APTT) in plasma

The analysis of the effect of the extract from *S. chinensis* berries on the coagulation times of human plasma showed that none of the used concentrations of the extract (0.5–50 µg/mL) changed APTT, PT, and TT (data not demonstrated).

3.6. Effect of the extract from *S. chinensis* berries on a marker of blood platelet damage

To determine the toxic effect of the extract on blood platelets, the level of LDH activity was measured. The results demonstrate no significant difference in platelet viability after exposure to *S. chinensis* berry extract at all used concentrations (0.5–50 µg/mL) (data not presented).

Table 3 compares the effects of the extract from *S. chinensis* berries, the extract from sea buckthorn berries, and the extract from aronia berries (as a positive control) (50 µg/mL) on selected markers of blood platelet activation. The strongest anti-platelet activity was demonstrated by the extract from *S. chinensis* berries and the extract from aronia berries. The anti-platelet potential was observed for six markers.

Table 3

A comparison of the anti-platelet activity of the extract from *S. chinensis* berries (50 µg/mL), the extract from sea buckthorn berries (50 µg/mL), and the extract from aronia berries (50 µg/mL) in washed blood platelets (measured by adhesion to adhesive proteins (collagen and fibrinogen) and in whole blood (measured by T-TAS and flow cytometry).

	<i>S. chinensis</i> berries	Sea buckthorn berries	Aronia berries
Inhibition of adhesion of thrombin-activated platelet to collagen	Anti-platelet activity	No effect	Anti-platelet activity
Inhibition of adhesion of thrombin-activated platelet to fibrinogen	No effect	No effect	No effect
Inhibition of adhesion of ADP-activated platelet to fibrinogen	Anti-platelet activity	Anti-platelet activity	No effect
Inhibition of thrombus formation (measured by T-TAS)	Anti-platelet activity	Anti-platelet activity	No effect
Inhibition of GPIIb/IIIa exposition in 10 µM ADP-activated platelets	Anti-platelet activity	No effect	Anti-platelet activity
Inhibition of GPIIb/IIIa exposition in 20 µM ADP-activated platelets	Anti-platelet activity	No effect	Anti-platelet activity
Inhibition of GPIIb/IIIa exposition in collagen-activated platelets	No effect	No effect	Anti-platelet activity
Inhibition of P-selectin exposition in 10 µM ADP-activated platelets	No effect	Anti-platelet activity	No effect
Inhibition of P-selectin exposition in 20 µM ADP-activated platelets	No effect	Anti-platelet activity	Anti-platelet activity
Inhibition of P-selectin exposition in collagen-activated platelets	Anti-platelet activity	No effect	Anti-platelet activity

4. Discussion

Our LC-HRMS analysis showed that *S. chinensis* berry extract contained diverse lignans, which were its most important phenolic constituents. Most of these tentatively identified compounds were dibenzocyclooctadiene lignans, except for pregomisin, which belongs to the dibenzylbutane type. The presence of dibenzocyclooctadiene lignans is a characteristic feature of Schisandraceae family; they are regarded as the main bioactive constituents of *S. chinensis* (S. Yang & Yuan, 2021). Schisandrin was the dominant compound; putative schisandrol B, angelogomisin H, deoxyschisandrin, and schisandrin B were other major lignans. Our

results are mostly in line with other studies (He et al., 1997; Liu et al., 2013; Sheng et al., 2022; Yang et al., 2011). The red color of *S. chinensis* berries is caused by an anthocyanin, cyanidin- Pen-Hex-dHex. According to the literature data, this compound is most probably cyanidin 3-*O*-xylosylrutinoside (Liao et al., 2016; Ma et al., 2012). Flavonoids, represented rutin and quercetin hexoside, occurred in small quantities. Our results are supported by the work of (Mocan et al., 2014), who found small amounts of rutin, quercetin 3-*O*-glucoside, and quercetin 3-*O*-galactoside in *S. chinensis* berries. Apart from phenolics, the extract also contained numerous terpenoid compounds, mainly highly oxygenated nortriterpenoids, as well as bisnortriterpenoids, triterpenoids and homotriterpenoids, all present in small amounts. Substances with identical or similar molecular formulas were previously isolated from the aerial parts or fruits of *S. chinensis* (Huang et al., 2008; Li et al., 2017; Shi et al., 2014; S. Yang & Yuan, 2021). One of the detected bisnortriterpenoids was tentatively identified (on the basis of its determined formula) as wuweizidilactone H; this compound was earlier purified from the berry of *S. chinensis* by other research groups (Li et al., 2017; Xue et al., 2010).

Hemostasis is a complex process depending on many interconnecting factors, among which blood platelets play a key role (Kannan et al., 2019; Khodadi, 2020). Platelets are small (approximately 2–4 μm), discoid, anucleate blood elements. They can activate due to contact with various agonists, like thrombin, ADP, thromboxane A_2 , or collagen (Gremmel et al., 2016; Rubenstein & Yin, 2018; Tomaiuolo et al., 2017). Upon activation, platelets change their shape and expose various proteins that aid in the coagulation process. One of those proteins is Pselectin, which in resting platelets is located in α granules. After activation, α granules fuse with the cell membrane, which exposes P-selectin on the surface of platelets, allowing for their adhesion to leukocytes and/or endothelial cells. Because P-selectin is exposed only on the surface of activated platelets, it is often used as an activation marker (Kannan et al., 2019; Rubenstein & Yin, 2018). GPIIb/IIIa (integrin $\alpha_{IIb}\beta_3$) is another protein that plays a vital role in hemostasis. In resting platelets, GPIIb/IIIa is exposed on the cell membrane in its low affinity state, and transforms into a high affinity (active) form upon platelet activation. GPIIb/IIIa plays a key role in aggregation, as it binds to fibrinogen, which in turn binds to GPIIb/IIIa on other platelets, facilitating the formation of platelet aggregates (Gremmel et al., 2016; Kannan et al., 2019; Rubenstein & Yin, 2018; Tomaiuolo et al., 2017). Currently, there are two GPIIb/IIIa inhibitors used as anti-platelet drugs - tirofiban and eptifibatide (Tummala & Rai, 2024). Platelet adhesion is mediated mainly by GPIb-IX-V (which binds to von Willebrand factor (vWF), which in turn binds to collagen) and GPIV, which binds to collagen directly (Gremmel et al., 2016; Kannan et al., 2019; Rubenstein & Yin, 2018; Tomaiuolo et al., 2017). Another change that occurs in platelets during activation is increased shedding of platelet-derived microparticles (PMPs), also known as microvesicles. PMPs are 1 μm or less but are bigger than exosomes (30–100 nm). Their plasma membrane exposes negatively charged phospholipids and a number of platelet receptors, including P-selectin, GPIIb/IIIa, and vWF (Nignpense et al., 2019; Kannan et al., 2019).

Cardiovascular diseases are a leading cause of death worldwide. An imbalance in hemostasis which leads to excessive clotting is a major issue in many CVDs and can lead to severe conditions, like myocardial infarction or stroke (Kannan et al., 2019; Khodadi, 2020). Increased platelet activation plays a major role in the pathology of many CVDs and is associated with adverse prognosis. To prevent this, many cardiovascular patients must take anti-platelet medication, among which acetylsalicylic acid (aspirin) is used most often. Aspirin inhibits cyclooxygenase (COX) activity, decreasing the production of thromboxane A_2 (Kannan et al., 2019). Unfortunately, aspirin and many other anti-platelet drugs can cause adverse effects, including bleeding from the

gastrointestinal tract. For this reason, researchers are searching for new anti-platelet compounds with a lower risk of side effects (Gremmel et al., 2016; Olas, 2020).

The most important aspect of our findings is the confirmation of the anti-platelet activity of the extract from *S. chinensis* berries in various *in vitro* models. We used flow cytometry and TTAS to study platelet activation in whole blood, which is a more natural environment than media in which blood platelets are suspended after isolation. For the first time, we noted that *S. chinensis* extract (at the highest tested concentration – 50 µg/mL) significantly prolonged the time of occlusion, showing anti-platelet activity *in vitro*. We also observed that all the tested

concentrations (0.5–50 µg/mL) of the extract inhibit the exposition of the active form of GPIIb/IIIa in 10 µM ADP-stimulated platelets. In addition, we observed the anti-adhesive potential of the extract from *S. chinensis* berries using an *in vitro* model based on human washed blood platelets. Results of (Chang et al., 2005) also demonstrated that the extract from *S. chinensis* inhibits arachidonic acid-induced blood platelet aggregation. Moreover, they suggested that the inhibition of cyclooxygenase is its primary mechanism of action. On the other hand, our results demonstrate that *S. chinensis* berry extract did not influence plasma coagulation times.

Various studies showed that lignans (including schisandrin, schisandrin A, B, and C) isolated from *S. chinensis* are its main active constituents, and possess a variety of nutritional properties and biological activities (Jia et al., 2023; Wang et al., 2024; Zhou et al., 2021). We propose that the most active component of *S. chinensis* berry extract may be schisandrin, which could be the major determinant of its anti-platelet activity *in vitro*. Other *in vitro* and *in vivo* models have also demonstrated that schisandrin has cardioprotective properties (Gong et al., 2021; Zhang et al., 2019). For example, (Gong et al., 2021) observed its cardioprotective activity in a myocardial ischemia/reperfusion injury murine model (*in vivo*) and H9c2 cardiomyocyte cell line subjected to hypoxia/reoxygenation injury (*in vitro*). (Zhang et al., 2019) also found that schisandrin promotes the recovery of myocardial tissues by enhancing cell viability and migration. Recently, more information about the protective effect of schisandrin on the cardiovascular system has been described in a review paper by (Wang et al., 2024). However, it did not cover the effect of this compound on blood platelets.

The bioavailability and toxicity of various plant preparations (including extracts and chemical compounds) that could be used as drugs or supplements are a crucial element in the evaluation of their biological properties. In our study, none of the used concentrations of the extract from *S. chinensis* berries caused damage to human blood platelets (which was determined by a measurement of LDH that leaked out from the damaged cells into the extracellular medium). These results indicate, that this extract should be safe for use as a natural supplement with anti-platelet activity, but it still lacks validation in clinical settings. However, it has been found that extract from *S. chinensis* berries has little to no toxicity toward various animals, including mice, rats, pigs, and rabbits (Panossian & Wikman, 2008; K. Yang et al., 2022). Moreover, the results of (Chen et al., 2018) demonstrated that it did not have toxic effects in an atherosclerosis rat model.

Researchers have incorporated schisandrin as a key ingredient in various formulations, including tablets, capsules, liquids, and injections, to investigate its efficacy. Interestingly, schisandrin exhibited a good level of absorption. Hydroxylation and demethylation pathways are its main metabolic modifications (Wang et al., 2024).

Another active components that we identified in *S. chinensis* berries are triterpenoids, which display a wide range of biological activities, including antitumor, antiviral, hepatoprotective, neuroprotective, and anti-

inflammatory (Jia et al., 2023). Moreover, other studies indicate that triterpenoids and their derivatives present in fractions and extracts from various organs of sea buckthorn have anti-platelet activity (Skalski et al., 2021, 2023).

Other compounds that can significantly affect the circulatory system, including blood platelets, are phenolic compounds. They occur in large quantities in many plant species, including sea buckthorn and aronia berries (Olas, 2017, 2018, 2023; Olas et al., 2008; Skalski et al., 2020, 2021). Some mechanisms of their anti-platelet activity are the inhibition of cyclooxygenase and blocking the binding of surface receptors to adhesion proteins. An additional advantage of supplementation with phenolic compounds is the lack of side effects (Luo et al., 2017; Olas, 2017).

To conclude, our results indicate that *S. chinensis* berries could be used to produce natural nutrient supplements with cardioprotective potential, including anti-platelet activity. Future investigations into this extract and its chemical components, including schisandrin, should prioritize the following areas: (1) further understanding of the molecular mechanisms underlying its anti-platelet action, and the identification of specific targets; (2) bridging the gaps in knowledge about its *in vivo* efficiency by using animal models and performing clinical studies on healthy people and patients with different CVDs.

Abbreviations

ADI - Acceptable Daily Intake; APTT - activated partial thromboplastin time; BSA - bovine serum albumin; CPDA - citrate/phosphate/dextrose/adenine; CVDs – cardiovascular diseases; FDA - Food and Drug Administration; LDH - lactate dehydrogenase; PBS - Phosphate buffered saline; PT - prothrombin time; T-TAS - Total Thrombus-formation Analysis System; TT - thrombin time; WHO - The World Health Organization

Declarations

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The data availability: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Author Contribution

N.S. and J.Z. - methodology, formal analysis, investigation, writing-original draft, prepared figures; B.L., J.B., and K. Z. - methodology, formal analysis, investigation; B.M.Sz. , P.B., and A.S. - methodology; B.O. - conceptualization, writing-review and editing. All authors reviewed the manuscript.

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Figures

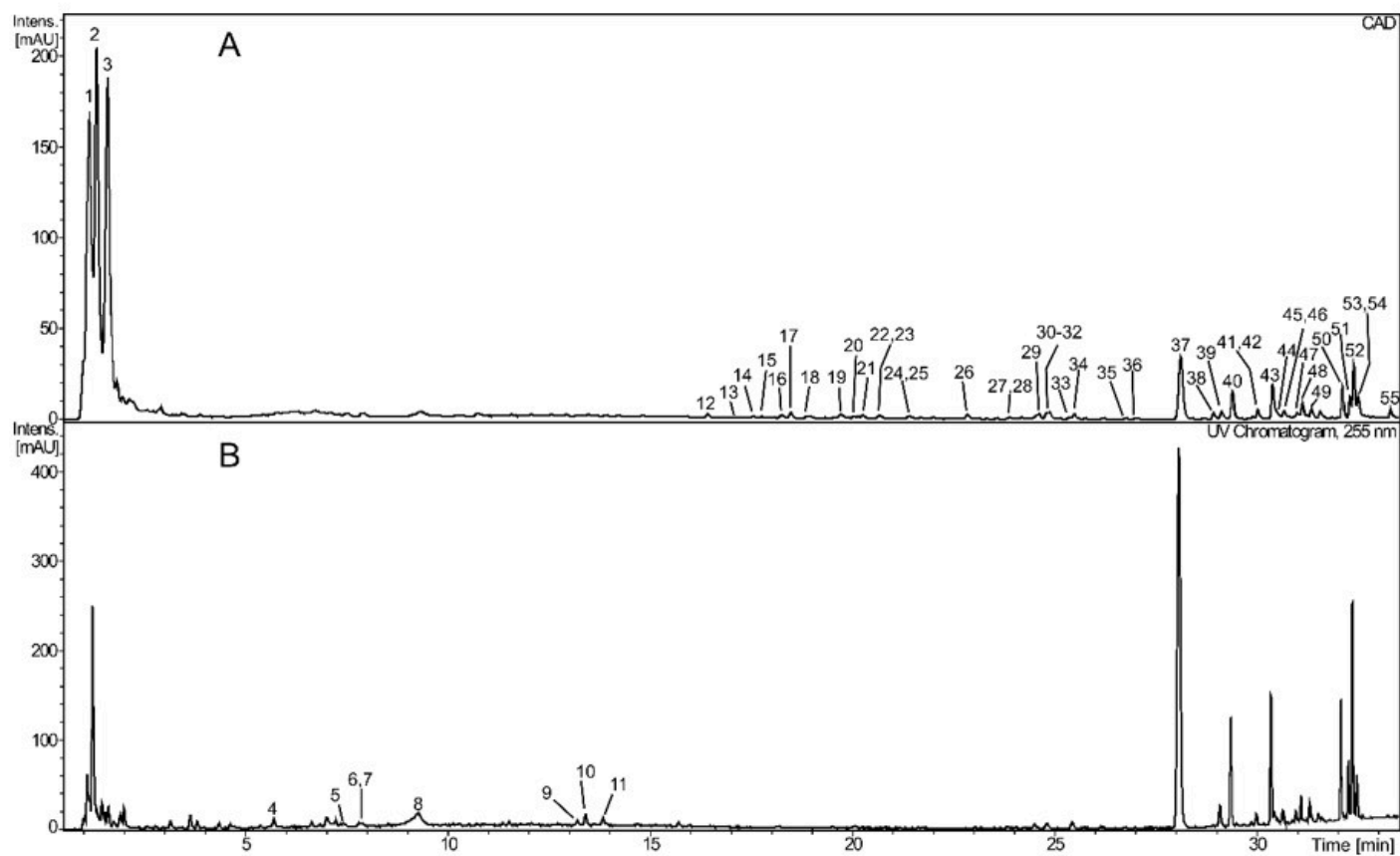


Figure 1

UHPLC-CAD (A), and UV ($\lambda = 255$ nm (B)) chromatograms of the extract from the fruit of *S. chinensis*. Numbers above the chromatographic peaks correspond to those from Table 1.

Fig. 2

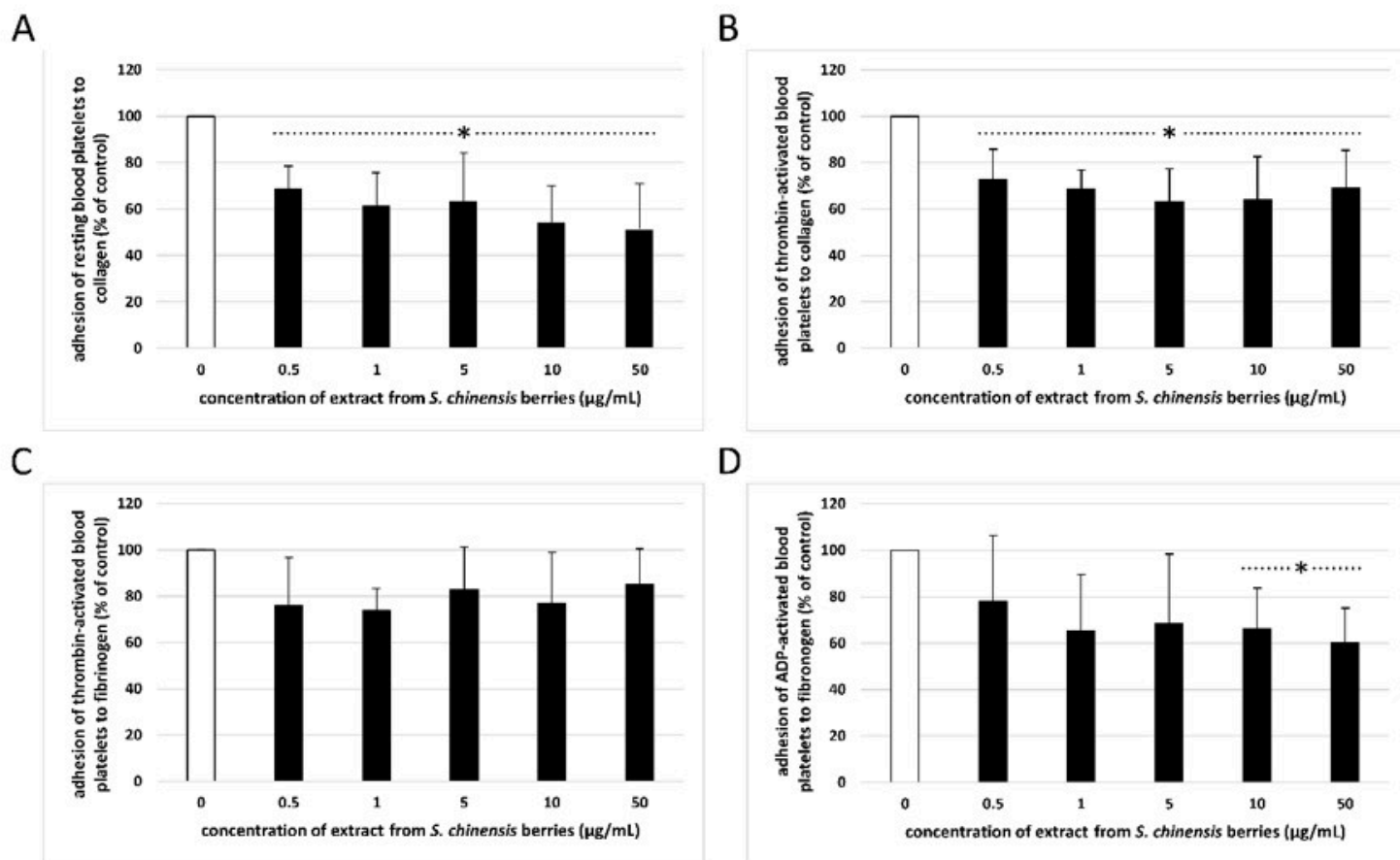


Figure 2

Effect of the extract from *S. chinensis* berries (at concentrations 0.5, 1, 5, 10, and 50 µg/mL) on the adhesion of resting platelets to collagen (A), thrombin-activated platelets to collagen (B), thrombin-activated platelets to fibrinogen (C), and ADP-activated platelets to fibrinogen (D) (n=9). In the graphs, platelet adhesion is expressed as a percentage of the control sample (blood platelets without the tested extract). The data are expressed as means $\pm$ SD. The results were considered significant at $p < 0.05$ (* $p < 0.05$).

Fig. 3

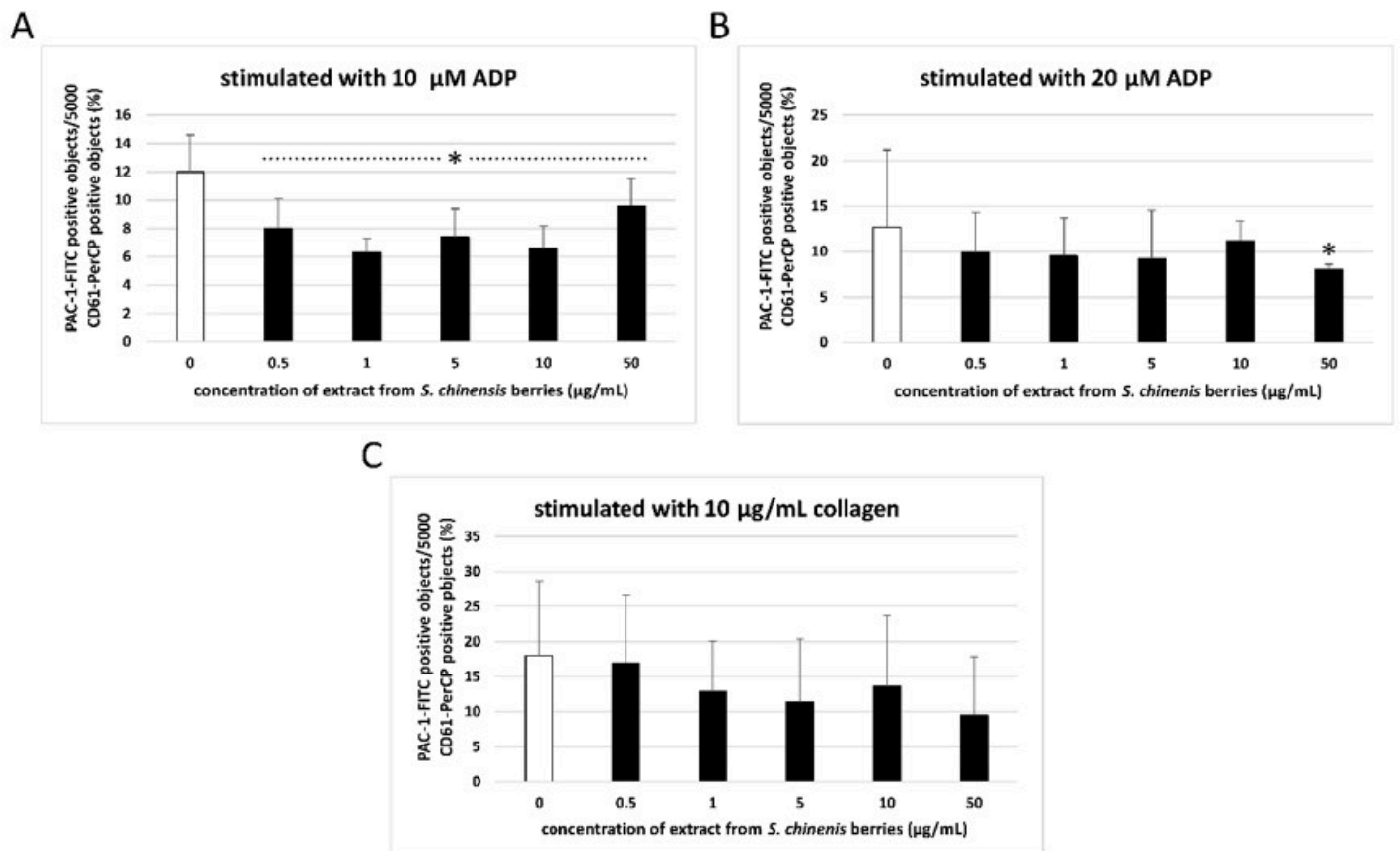


Figure 3

Effect of the extract from *S. chinensis* berries (at concentrations 0.5, 1, 5, 10, and 50 μ g/mL) on the exposition of the active form of GPIIb/IIIa on 10 μ M ADP-stimulated blood platelets (A), 20 μ M ADP-stimulated blood platelets (B), and 10 μ g/mL collagen-stimulated blood platelets (C) in whole blood samples. Blood platelets were distinguished based on the exposition of CD61. For each sample, 5000 CD61-positive objects were acquired. For the assessment of GPIIb/IIIa exposition, samples were labeled with fluorescently conjugated monoclonal antibody PAC-1/FITC. Results are shown as the percentage of platelets binding PAC-1/FITC. Data represent the means $\pm$ SD. The blood samples were drawn from 6 healthy volunteers. The activity of the tested extract was compared to the control sample. The results were considered as significant at $p < 0.05$ (* $p < 0.05$).

Fig. 4

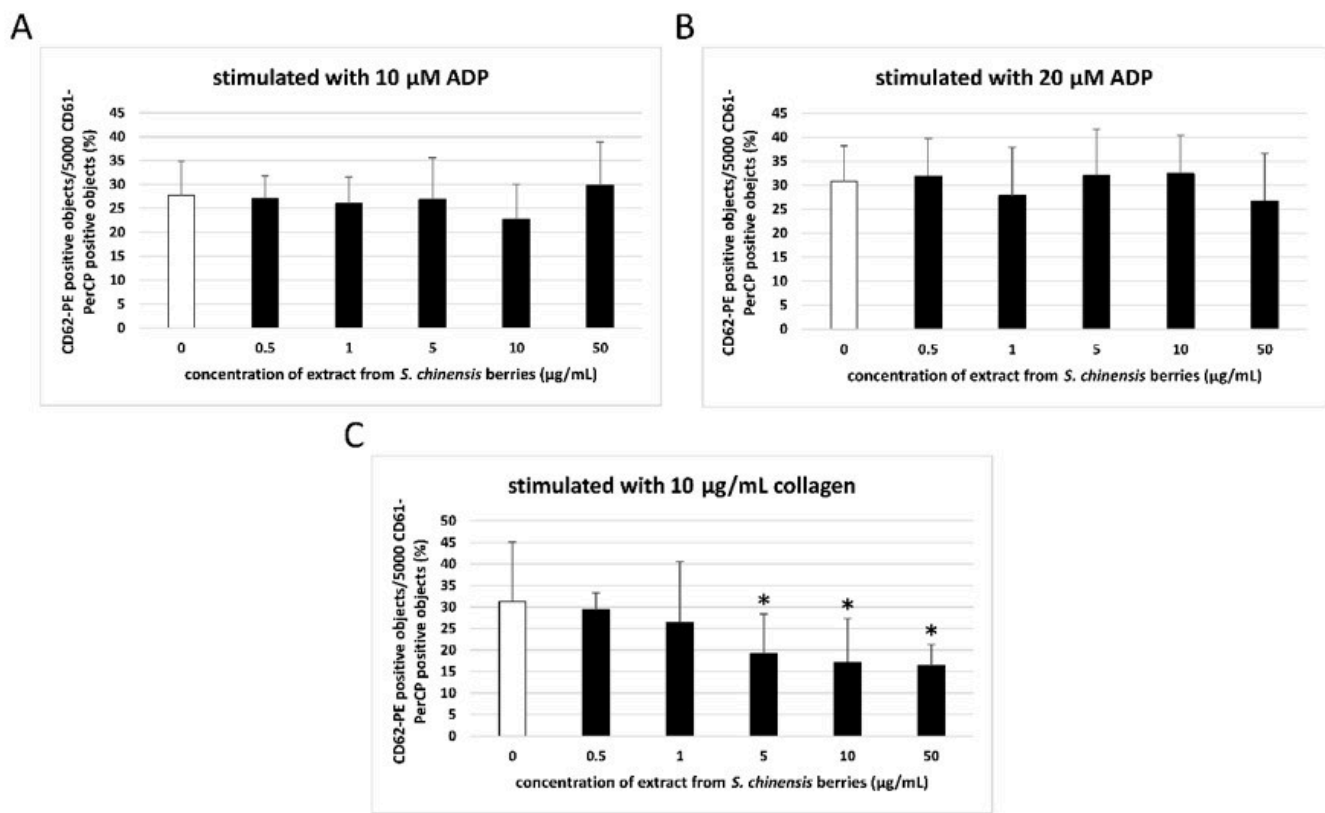


Figure 4

Effect of the extract from *S. chinensis* berries (at concentrations 0.5, 1, 5, 10, and 50 μg/mL) on the exposition of P-selectin on 10 μM ADP-stimulated blood platelets (A), 20 μM ADP-stimulated blood platelets (B), and 10 μg/mL collagen-stimulated blood platelets (C) in whole blood samples. Blood platelets were distinguished based on the exposition of CD61. For each sample, 5000 CD61-positive objects were acquired. For the assessment of P-selectin exposition, samples were labeled with fluorescently conjugated monoclonal antibody CD62P/PE. Results are shown as the percentage of platelets expressing CD62P. Data represent the means ± SD. The blood samples were drawn from 6 healthy volunteers. The activity of the tested extract was compared to the control sample. The results were considered as significant at $p > 0.05$ (* $p < 0.05$).

Fig. 5

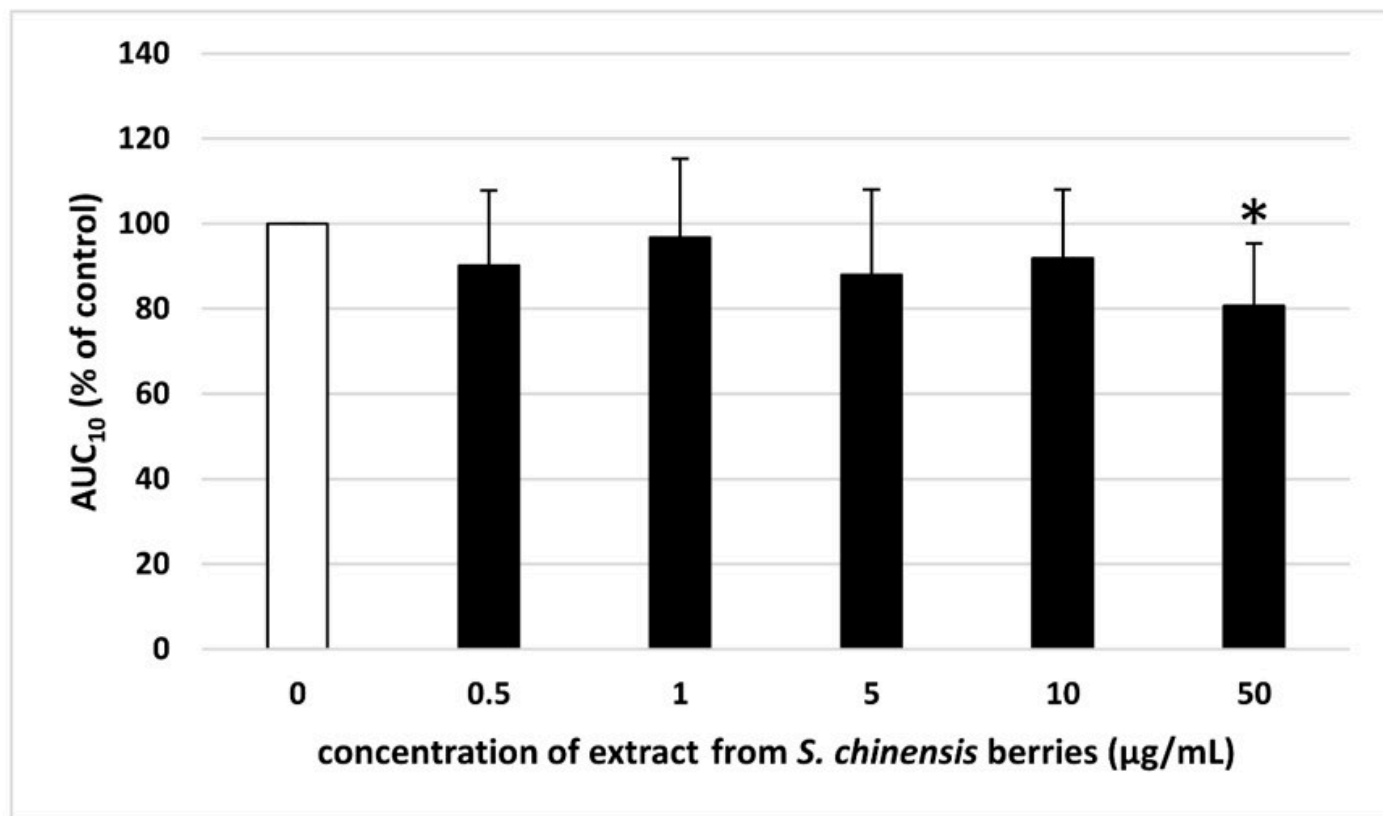


Figure 5

Effect of the extract from *S. chinensis* berries (at concentrations 0.5, 1, 5, 10, and 50 $\mu\text{g/mL}$) on thrombus formation in whole blood ($n=7$). The samples were analyzed with T-TAS PL-chip, at the shear stress rates of 1500/s. The results are calculated as AUC_{10} (area under the curve). In the graphs, AUC_{10} is expressed as a percentage of the control sample (blood without the tested extract). The data are expressed as means $\pm$ SD. The results were considered significant at $p < 0.05$ (* $p < 0.05$).