The Anti-platelet and Anticoagulant Potential of Isorhamnetin and Its New Derivatives Isolated From Sea Buckthorn Fruits in Whole Blood

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Abstract

Blood platelets play a crucial role in hemostasis, the process responsible for keeping blood flowing in the circulatory system. However, unnecessary platelet activation can lead to aggregation at the site of atherosclerotic plaque rapture and the formation of a thrombus, which promotes atherothrombotic diseases. Various dietary components, such as phenolic compounds, are known to demonstrate antiplatelet and anticoagulant properties, and it is possible that these could form an important element in the prophylaxis and therapy of cardiovascular diseases. Our present study examines the biological activity of isorhamnetin (compound 1) and two isorhamnetin derivatives, compound 2 (3-O-beta-glucoside-7-O-alpha-rhamnoside) and compound 3 (3-O-beta-glucoside-7-O-alpha-(3°-isovaleryl)-rhamnoside), isolated from the phenolic fraction of sea buckthorn fruit, against human washed blood platelets and human whole blood in vitro. The anti-platelet and anticoagulant potential was determined using (A) flow cytometry, (B) the thrombus-formation analysis system (T-TAS) and (C) colorimetry. The tested flavonoids demonstrated anticoagulant and anti-platelet potential, including anti-adhesive activity, with these effects being more intense in compound 2 than isorhamnetin. Compound 2 inhibited GPIIb/IIIa and P-selectin expression on blood platelets from whole blood, and demonstrated anti-adhesion properties in washed blood platelets and anti-coagulant potential in whole blood, measured by T-TAS.

1. Introduction

Blood platelets play a crucial role in hemostasis, a process responsible for keeping the blood flowing in the circulatory system. Platelets are generated from megakaryocytes in bone marrow, with the daily production providing 150–400×10⁹ platelets per liter of blood. The cells display a range of surface receptors and adhesion molecules, and contain numerous granules; these are used to initiate coagulation cascades in primary hemostasis, where blood platelets adhere to the extracellular matrix [1]. Platelet activation may be stimulated by platelet secretion products and local prothrombotic factors, including tissue factors. There are two main pathways leading to platelet activation, but both result in the rapid binding of platelets to damaged blood vessels. This aggregation prevents excessive bleeding by the formation of thrombin.

Stable platelet adhesion requires an interaction between glycoprotein GPVI, integrin-α₂β₁, and collagen, and between integrin-α₅β₁ and fibronectin. The binding of collagen to GPVI results in platelet activation and the release of soluble agonists, including ADP and thromboxane A₂, leading to activation of GPIIb/IIIa, a major receptor for fibrinogen. As a result of this process, the platelets aggregate and form a thrombus [2]. However, unnecessary platelet activation can lead to the development of atherothrombotic diseases caused by aggregation and thrombus formation at the site of an atherosclerotic plaque rupture. The rupture of an atherosclerotic plaque or acute erosion of the endothelial layer can result in the formation of a pathological arterial thrombus, leading to exposure of highly-reactive subendothelial matrix protein, such as collagen and von Willebrand factors (vWF) [1].

Arterial thrombosis is responsible for myocardial infarction and ischemic stroke, two of the leading causes of death globally. They are often treated pharmacologically with antiplatelet drugs, due to the fundamental role of blood platelets in the development of atherothrombosis. A significant role in clotting is played by GPIIb/IIIa signaling, with inside-out GPIIb/IIIa signaling being activated during platelet activation. However, ligand-bound GPIIb/IIIa can result in outside-in signaling events that mediate the cytoskeletal reorganization. The initial arterial
thrombus is reinforced by thrombin generation, which further increases platelet activation and activates coagulation, leading to greater stabilization of the fibrin mesh [2].

Four main classes of antiplatelet drugs are currently used in therapy. The first type are cyclooxygenases 1 (COX1) inhibitors such as aspirin, which blocks thromboxane A$_2$ production [2]. Other drugs are used to inhibit the P$_2$Y$_{12}$ receptors for ADP; these are divided into two groups: thienopyridines, such as clopidogrel, and nucleoside-nucleotide derivates, such as cangrelor. A third group comprises proteinase-activated receptor 1 (PAR1) antagonists such as vorapaxar, which was developed to target thrombin; the platelet reaction to thrombin is mainly mediated by two G protein-coupled PARs: PAR1 and PAR4. Finally, GPIIb/IIIa inhibitors can be used, such as abciximab, a human antigen-binding fragment of mouse monoclonal antibody, tirofiban, a nonpeptidic small molecule mimicking the fibrinogen binding site, and eptifibatide, a cyclic heptapeptide with lysine-glycine-aspartic acid and a motif mimicking the fibrinogen binding sequence within GPIIb/IIIa. Unfortunately, in numerous cases, the use of these drugs is connected with an elevated risk of bleeding [2].

Various *in vitro* and *in vivo* analyses indicate that certain dietary components with antiplatelet and anticoagulant properties, such as phenolic compounds, may play a valuable role in the prophylaxis and therapy of cardiovascular diseases (CVDs) [3]. For example, isorhamnetin and two of its derivatives isolated from the phenolic fraction of sea buckthorn fruit, viz. 3-O-beta-glucoside-7-O-alpha-rhamnoside and 3-O-beta-glucoside-7-O-alpha-(3‴-isovaleryl)-rhamnoside, have been found to have antioxidant and anti-aggregation potential against washed blood platelets, and anticoagulant potential against plasma [4]; however, their mechanisms of action in whole blood remain poorly understood. Therefore, the aim of the present study is to determine the biological activity of isorhamnetin (compound 1), 3-O-beta-glucoside-7-O-alpha-rhamnoside (compound 2) and 3-O-beta-glucoside-7-O-alpha-(3‴-isovaleryl)-rhamnoside (compound 3), (Fig. 1) using human washed blood platelets and human whole blood *in vitro*. The anti-platelet and anticoagulant potential was evaluated using three key analytical approaches: (A) flow cytometry, measuring the cell-surface expression of P-selectin (CD62P) and the existence of the active form of GPIIb/IIIa (PAC-1 binding); this was performed in resting or agonist (ADP or collagen)-stimulated blood platelets after incubation with tested compounds and plant fraction; (B) the thrombus-formation analysis system (T-TAS), used to determine the influence of the tested compounds and plant fraction on thrombus formation in whole blood; (C) colorimetry, used to assess the changes of platelet adhesion to collagen after incubation with the tested compounds and plant fraction.

## 2. Material And Methods

### 2.1. Chemicals

All flow cytometry reagents were acquired from Becton Dickinson (USA). The PL-chip, reservoir kit for PL-chip and BAPA tubes (3 mL) and other equipment needed for the T-TAS were purchased from Bionicum (Poland). Collagen and ADP were obtained from Chrono-Log Corporation (Havertown, USA). Dimethylsulfoxide (DMSO), isorhamnetin, Triton X-100 and p-nitrophenylphosphate were purchased from Sigma-Aldrich (USA). The remaining reagents, including NaCl, Tris, NaOH and glucose were obtained from POCh (Poland).

### 2.2. Plant material
Sea buckthorn fruits (*E. rhamnoides* (L.) A. Nelson) were acquired from a horticultural farm in Sokółka (Podlaskie Voivodeship, Poland (53 ° 24'N, 23 ° 30'E). The frozen fruits were ground, freeze-dried (Gamma 2-16 LSC, Christ, Osterode am Harz, Germany) and stored in a refrigerator. All plant studies involved in the research were carried out in accordance with relevant institutional, national or international guideline.

2.3. Preparation and quantification of the phenolic fraction from sea buckthorn fruits

The tested phenolic fraction from sea buckthorn fruits was prepared as previously described by Olas et al. [4]. Its main components are isorhamnetin glycosides, acylated isorhamnetin glycosides, triterpenoids and acylated triterpenoids.

2.4. Stock solutions of tested compounds and plant fraction

Stock solutions of sea buckthorn phenolic fraction, and of isorhamnetin (compound 1) and its two derivatives were made in 50% DMSO: the derivatives were isorhamnetin 3-O-beta-glucoside-7-O-alpha-rhamnoside (compound 2) and isorhamnetin 3-O-beta-glucoside-7-O-alpha-(3”-isovaleryl)-rhamnoside (compound 3) [5]. The DMSO concentration in the final samples did not exceed 0.05% and its effects were checked in every experiment.

2.5. The samples of blood

Fresh human blood was collected in the L. Rydygier hospital in Lodz, Poland. All donors were healthy volunteers, none were smokers or reported taking drugs. Blood was collected in tubes with CPDA anticoagulant (citrate/phosphate/dextrose/adenine; 8.5:1; v/v; blood/CPDA).

The blood used in the flow cytometry and T-TAS assays was incubated (30 min, at 37°C) with *E. rhamnoides* (L.) fraction, isorhamnetin and its derivatives at final concentrations of 5 and 50 μg/mL.

Confirmation by human participants

All experiments were approved by the University of Lodz Committee for Research on Human Subjects and carried out under permission number 3/KBBN-UL/II/2016.

We confirm that all experiments were performed in accordance with relevant guidelines and regulations. All donors were informed about the purpose of the study and gave their informed consent to participate.

2.6. Isolation of blood platelets

Fresh human blood (1200 rpm, 15 min, at 25°C) was centrifuged. Platelet-rich plasma (supernatant) was collected in Falcon tubes and centrifuged again at 1200 rpm for 15 min at 25°C. The obtained platelets were suspended in Berber's buffer (0.14 M NaCl, 0.014 M Tris, 5 mM glucose, pH 7.4). The platelet concentrations in the suspensions used in the experiments ranged from 2 to 2.5×10⁸/mL, as indicated spectrophotometrically [6]. Blood platelet suspensions were incubated (30 min, at 37°C) with *E. rhamnoides* (L.) fraction, isorhamnetin and its derivatives at final concentrations of 5 and 50 μg/mL.

2.7. Markers of hemostasis
2.7.1 Platelet adhesion to collagen

The test was performed to measure the activity of the platelet exoenzyme acid phosphatase. The platelets were first dissolved in Triton X-100. The phosphatase substrate p-nitrophenylphosphate was then added. The resulting formation of p-nitrophenol was determined spectrophotometrically at a wavelength $\lambda = 405$ nm. Finally, a color was obtained by adding 2M NaOH. The absorbance of the control (which included only blood platelets with Barber’s buffer) was expressed as 100 %. The method is fully described in Bellavita et al. [7].

2.7.2 Flow cytometry analysis

Changes in platelet activation were determined using an LSR II flow cytometer (Becton Dickinson, San Diego, CA, USA). Whole blood was incubated with the test compounds or phenolic fraction and platelet activators (ADP and collagen). The samples were then diluted with PBS with Mg$^{2+}$ ions. Antibodies (CD61/PerCP; CD62/PE and PAC-1/FITC) were added to the cytometry tubes. The platelets were fixed in CellFix and incubated for one hour at $37^\circ$C. The blood platelets were distinguished from other blood cells based on a forward light scatter (FCS) vs. side light scatter (SSC) plot on a log/log scale (first gate) and by positive staining with monoclonal anti-CD61/PerCP antibodies (second gate). In each sample, the percentages of CD62P-positive and PAC-1-positive platelets were measured. FlowJo software (Becton Dickinson, San Diego, CA, USA) was used to analyze the obtained results. The precise details of the method are described by Rywaniak et al. [8].

2.7.3 Total Thrombus-formation Analysis System (T-TAS)

The plate plug formation process was determined using a real-time hydrodynamic model. Whole blood (400 µl) was incubated with the tested fraction, isorhamnetin or its two derivatives (30 min, 37°C). Next, 350 µl of blood was drawn for analysis. The results were obtained as AUC$_{10}$ (Area Under the Curve) using a PL chip. A detailed description of the method can be found in Hosokawa et al. [9].

2.8. Data analysis

The Q-Dixon test was used to discard uncertain data. All values are presented as means ± SD. N – number of donors. Statistical analysis was carry out using the one-way ANOVA for repeated measurement test using Statistica 13.1.

3. Results

The results of the T-TAS teste indicate that the AUC$_{10}$ of the tested phenolic compounds (compound 1, 2 and 3; 50 µg/mL) was markedly reduced compared to control values (Fig. 2 and 3).

While changes in blood platelet activation were noted in whole blood treated with all tested phenolic compounds (1-3) and the phenolic fraction from sea buckthorn fruits, these changes were not always statistically significant (Fig. 3-6). However, at a concentration of 50 µg/mL, compound 2 significantly reduced the expression of GPIIb/IIIa on platelets activated by 20 µM ADP, reduced the expression of CD62P in collagen-activated platelets, and inhibited PAC-1 binding in platelets activated with 10 µM ADP. Similar effects were observed for compound 3 (50 µg/mL) (Fig. 3-6).
The non-activated blood platelets and thrombin-activated platelets also demonstrated significantly lower collagen adhesion following incubation with compounds 1, 2, and 3 (5 and 50 µg/mL), as well as with the phenolic sea buckthorn fruit fraction (Fig. 7).

Table 1 compares the effects of isorhamnetin and its derivatives (50 µg/mL) on hemostasis in whole blood and washed blood platelets. The strongest anti-platelet and anti-coagulant potential was demonstrated by compound 2, which inhibited the expression of GPIIb/IIIa and P-selectin on blood platelets in whole blood; it also demonstrated anti-adhesion properties in washed blood platelets and anti-coagulant potential in whole blood, as measured by T-TAS.

Table 1. Comparative effects of isorhamnetin and its derivatives (at 50 µg/mL) on parameters of hemostasis (using whole blood and washed blood platelets).

| Tested phenolic compound | Inhibition of adhesion of non-activated platelets to collagen (%) | Inhibition of adhesion of thrombin-activated platelets to collagen (%) | Inhibition of thrombus formation (%) | Inhibition of expression of GPIIb/IIIa on platelets activated by 20 µM ADP (%) | Inhibition of expression of GPIIb/IIIa on platelets activated by collagen (%) | Inhibition of expression of P selectin on platelets activated by 10 µM ADP (%) |
|-------------------------|---------------------------------------------------------------|---------------------------------------------------------------|-----------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Compound 1              | 18.4 ± 10.4                                                   | 26.7 ± 7.8                                                   | 42.4 ± 10.4                      | No effect vs. control                                                             | 26.7 ± 4.5                                                                     | No effect vs. control                                                             |
|                         | Positive effect (antiadhesive action) vs. control             | Positive effect (antiadhesive action) vs. control             | Positive effect (anticoagulant action) vs. control |                                                                                 | Positive effect (antiplatelet action) vs. control                               |                                                                                 |
| Compound 2              | 46.1 ± 12.8                                                   | 30.4 ± 9.9                                                   | 61.4 ± 12.3                      | 28.4 ± 8.2                                                                       | 32.4 ± 5.1                                                                     | 21.4 ± 3.8                                                                       |
|                         | Positive effect (antiadhesive action) vs. control             | Positive effect (antiadhesive action) vs. control             | Positive effect (anticoagulant action) vs. control |                                                                                 | Positive effect (antiplatelet action) vs. control                               | Positive effect (antiplatelet action) vs. control                               |
| Compound 3              | 40.0 ± 13.5                                                   | 48.4 ± 10.1                                                   | 65.2 ± 11.0                      | No effect vs. control                                                             | 26.1 ± 4.2                                                                     | 25.5 ± 4.1                                                                       |
|                         | Positive effect (antiadhesive action) vs. control             | Positive effect (antiadhesive action) vs. control             | Positive effect (anticoagulant action) vs. control |                                                                                 | Positive effect (antiplatelet action) vs. control                               | Positive effect (antiplatelet action) vs. control                               |

4. Discussion

The flavonoids are an important group of polyphenolic compounds produced widely by plants. Their high number of hydroxyl groups impart a reducing character, thus bestowing them with strong antioxidant activity, and making them valuable biological agents for scavenging and inactivating reactive oxygen species. This antioxidant potential can be used to prevent lipid peroxidation and oxidative damage in membrane lipids and to
inhibit various prooxidant enzymes. They also have a protective effect against damage induced by UV radiation. Indeed, they can also demonstrate greater antioxidant potential than vitamin C and E [12].

The compounds are known to demonstrate a range of pro-health effects on the human body, including various anti-inflammatory, antilipidemic, antiviral and antibacterial, hepatoprotective and cardioprotective properties [12]. These properties allow them to be used as active ingredients in various nutraceutical, cosmetic and even pharmaceutical preparations, and can be found in red wine, tea, coffee and medical herbs, as well as in fruits and vegetables [10,12].

Isorhamnetin is a flavonoid isolated from the leaves, flowers and fruits of *E. hamnoides, Ginkgo biloba* and other plants. It shows a broad range of pharmacological activity against cardiovascular disease, tumors and some neurovegetative diseases, like Alzheimer’s disease. Its therapeutic effect against cardiovascular and cerebrovascular diseases is based on its ability to protect endothelial cells, and its anti-atherosclerosis, anti-hypotension and anti-thrombosis activity [10, 13]. It can also improve nerve function and enhance memory. It has also demonstrated a bacteriostatic effect and has been proposed as a candidate for antibacterial drug research [12]. Isorhamnetin has also demonstrated some anti-tumor effects, manifested in the inhibition of human cervical cancer cell, lung cancer cells, breast cancer cell and liver cancer cells. It inhibits the proliferation of tumor cell by inducing apoptosis and regulation of tumor suppressor genes and signal pathways [10-13].

The anti-inflammatory effects of isorhamnetin derive from its ability to regulate the production of inflammatory mediators such as cytokines, and to inhibit the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway, which regulates a range of inflammatory molecules; indeed, its influence on NF-κB signaling is known to protect endothelial cells against inflammation and oxidative damage [13]. Isorhamnetin has also been found to reduce the risk of thrombosis by inhibiting collagen-stimulated platelet aggregation and various aspects of signal transduction; in addition, like all flavonoids, it has been found to demonstrate strong antioxidant activity by scavenging radicals and inhibiting lipid peroxidation [13]. Moreover, isorhamnetin can inhibit cardiac hypertrophy and fibrosis by blocking the activation of phosphatidylinositol 3-kinase-AKT (protein kinase B) signaling pathway. In *in vitro* studies, isorhamnetin attenuates cardiomyocyte hypertrophy in neonatal rat cardiomyocytes induced by angiotensin II [13]

It has known that phenolic compounds isolated from various parts of plants have anti-platelet activity. For example, they may inhibit P-selectin expression, which has a crucial role in connection of inflammation and hemostasis. P-selectin is a integrin belonging to heterodimeric transmembrane receptors, formed by noncovalent association of α and β chains; its main role is to mediate interactions with adhesion molecules on other cells and extracellular matrix molecules [1]. Flavonoids also show some cardiovascular activity; this is mostly connected with their antiplatelet activity, i.e. preventing primary clot formation and inhibiting platelet aggregation. Structure-activity analysis has attributed this antiaggregatory activity to the C-ring structure of flavonoids: non-methylated flavonoids with a double bond between C2 and C3 have the strongest antiaggregatory activity [14]. In addition, the antiplatelet activity of plants is often related to their flavonoid and phenolic content. The leaves from *Melissa officinalis*, whose main compounds are flavonoids, reduced ADP-induced platelet aggregation by up to 18% [14, 15].

Sea buckthorn fruits have been found to be a safe and valuable source of phenolic compounds such as flavonoids. Isorhamnetin and two isorhamnetin derivatives, 3-O-beta-glucoside-7-O-alpha-rhamnoside
(compound 2) and 3-O-beta-glucoside-7-O-alpha-(3''-isovaleryl)-rhamnoside (compound 3), have been reported to have antioxidant, anti-platelet and anticoagulant properties against plasma and washed platelets in vitro [4]. The present study examines the effects of these three flavonoids (compounds 1-3) and a phenolic fraction from sea buckthorn fruits on selected aspects on hemostasis in whole blood. It is the first study to compare the effects of these three phenolic compounds on inhibiting thrombus formation and their anticoagulant properties in whole blood measured by T-TAS; this approach imitates in vivo conditions to assess whole blood thrombogenicity.

In the present study, compound 2 (50 µg/mL) was found to reduce GPIIb/IIIa expression in platelets activated by 20 µM ADP. The GPIIb/IIIa complex is recognized by PAC-1, which promotes platelet aggregation. These findings suggest that the compound has an inhibitory effect on platelet aggregation. In addition, all the tested flavonoids (i.e. compounds 1-3) also inhibited activation of platelets stimulated by collagen.

Compounds 2 and 3 also appeared to inhibit P-selectin expression in stimulated platelets, again indicating anti-platelet activity. The tested flavonoids also inhibited adhesion of washed platelets to collagen, as indicated colorimetrically. Compound 3 (50 µg/mL) showed the greatest anti-adhesive properties against thrombin-stimulated platelets.

Previous studies have also found isorhamnetin to have strong antiplatelet potential: isorhamnetin administered at 1-100 µM significantly inhibited platelet aggregation induced by collagen and thrombin receptor activator peptide [16]. It also reduced the level of ATP (adenosine triphosphate) in collagen-stimulated platelets [16].

Compound 2 was found to demonstrate stronger anti-platelet and anti-coagulant potential than compound 3 and isorhamnetin in whole blood; these differences may be due to their chemical structure. The most significant innovative finding of our experiment is that the tested flavonoids often demonstrate similar, or stronger, anti-platelet and anti-coagulant properties than the whole phenolic fraction. In addition, it important to note that the flavonoids and phenolic fraction were administered in two concentrations, 5 and 50 µg/mL, the lower of which may correspond to the physiological concentration of plant-derived phenolic compounds available after oral supplementation.

An important novel finding of the present study is that the flavonoids isolated from sea buckthorn fruits possess anticoagulant and anti-platelet (including anti-adhesive) potential against both washed blood platelets and whole blood. Of the tested compounds, compound 2 exhibited stronger properties than isorhamnetin, probably due to the differences in its structure.

**Declarations**

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**Declaration of Interest statement**

None to declare.
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Figure 1

Chemical structure of isorhamnetin (compound 1) and its derivatives: compound 2 (3-O-beta-glucoside-7-O-alpha-rhamnoside) and compound 3 (3-O-beta-glucoside-7-O-alpha-(3''-isovaleryl)-rhamnoside) isolated from the phenolic fraction of *E. rhamnoides* (L.) A. Nelson fruits.
Figure 2

Effects of isorhamnetin, its derivatives and the phenolic fraction of *E. rhamnoides* (L.) A. Nelson fruits (5 and 50 µg/mL; 30 min) on the T-TAS using the PL-chip in whole blood samples. Whole blood samples were analyzed by the T-TAS at the shear rates of 1000 s⁻¹ on the PL-chips. The area under the curve (AUC₉) in PL are shown as closed circles. Data represent the means ± SD of five healthy volunteers. * p<0.05 vs. control
Figure 3

Flow pressure analysis reflect the platelet thrombus formation process using the PL-chip in whole blood (control – blood treated without derivatives of isorhamnetin; blood treated with 50 µg/mL derivatives of isorhamnetin: compound 2 and 3) within 10 minutes.

Figure 4

Effects of isorhamnetin, its derivatives and the phenolic fraction of *E. rhamnoides* (L.) A. Nelson fruits (5 and 50 µg/mL; 30 min) on the expression of P-selectin on resting (a) or agonist-stimulated blood platelets: 10 µM ADP (b), 20 µM ADP (c) and 10 µg/mL collagen (d) in whole blood samples. The blood platelets were distinguished based on the expression of CD61/PerCP. For each sample, 10000 CD61-positive objects (blood platelets) were acquired. For the assessment of P-selectin expression, samples were labeled with fluorescently conjugated
monoclonal antibody CD62P. Results are shown as the percentage of platelets expressing CD62P. Data represent the means ± SD of 5 healthy volunteers. * p<0.05 vs. control

Figure 5

Effects of isorhamnetin, its derivatives and the phenolic fraction of *E. rhamnoides* (L.) A. Nelson fruits (5 and 50 µg/mL; 30 min) on the expression of the active form of GPIIb/IIIa on resting (a) or agonist-stimulated blood platelets: 10 µM ADP (b), 20 µM ADP (c) and 10 µg/mL collagen (d) in whole blood samples. The blood platelets were distinguished based on the expression of CD61. For each sample, 10000 CD61-positive objects (blood platelets) were acquired. For the assessment of GPIIb/IIIa expression, 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 ± SD of 5 healthy volunteers. *p<0.05 vs. control
Figure 6

Effects of compound 2 (50 µg/mL; 30 min) on the expression of P-selectin (a) and the active form of GPIIb/IIIa (b) in platelets stimulated by 10 µg/mL collagen in whole blood samples. Figure demonstrates selected diagrams.
Effects of isorhamnetin, its derivatives and the phenolic fraction of *E. rhamnoides* (L.) A. Nelson fruits (5 and 50 µg/mL; 30 min) on the collagen adhesion of resting (A) and thrombin-activated platelets (B). Blood platelets not treated with phenolic compounds/plant fraction were used as control samples (positive control). Adhesion is expressed as a percentage of the control samples (100%). Data represent means ± SD of 6 healthy volunteers (experiments performed in triplicate). Action of phenolic compounds and phenolic fraction was compared to control: *p<0.05, **p<0.01