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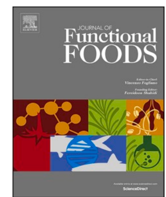

Anti-platelet activity of phytocompounds in various dandelion organs in human whole blood model *in vitro*

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ABSTRACT

Numerous studies indicate that the usage of natural compounds, such as those found in dandelion, might modulate blood platelets' function. Our previous investigations indicated that fractions obtained from different organs of dandelion possessed anti-platelet properties and inhibited activation of washed platelets. However, the impact of those fractions on platelets in whole blood is still unknown. Therefore, the aim of the presented study was to evaluate the anti-platelet potential of four fractions obtained from various parts of dandelion (fractions A and B - roots; fraction C - leaves; fraction D - petals) on platelets activation and thrombus formation in whole blood and an impact of tested fractions on platelets' proteome. The level of activation of resting or agonist-stimulated (ADP or collagen) whole blood platelets after incubation with different dandelion fractions was determined based on their cell-surface expression of P-selectin (CD62P) and presence of an active form of GPIIb/IIIa (PAC-1 binding) by flow cytometry technique. The influence of the tested fractions on whole blood thrombus formation was estimated by the thrombus-formation analysis system (T-TAS). The changes in platelets' proteome after incubation with dandelion fractions were analyzed using gel electrophoresis (both, native and SDS-PAGE). We found that fraction C from dandelion leaves reduced: 1) the thrombus formation and 2) platelets' activation upon stimulation with collagen (statistically significant decrease in P-selectin expression and presence of an active form of GPIIb/IIIa) in whole blood. None of the tested fractions caused changes in the platelet proteome. This may represent a novel avenue of research on antiplatelet supplementation in the prevention and treatment of cardiovascular diseases caused by hyperactivation of platelets.

1. Introduction

Hemostasis is the whole of various defensive mechanisms developed by the organism, which serve to maintain a constant flow of circulating blood and at the same time protect against blood loss in case of discontinuity of blood vessels. The correct hemostasis is the result of achieving a balance between the factors that activate and inhibit clotting processes. This condition in the body requires the interaction of many elements, including blood vessels, clotting, and fibrinolytic proteins, inhibitors, and activators of both systems, phagocytic system, monocytes, and neutral granulocytes, as well as blood platelets (Ziolkowska, Ziolkowski, Madra-Gackowska, Zekanowska, & Kedziora-Kornatowska, 2018).

Platelets are one of the smallest morphotic elements of the blood that do not have a cell nucleus and are formed in the bone marrow during the thrombopoiesis process. Platelets occur as discoidal fragments of blood cells whose cytoskeleton is very well organized and contain specific secretory granules and a unique membrane receptor system which

Abbreviations: ADP, adenosine diphosphate; AUC, area under the curve; cAMP, cyclic adenosine monophosphate; CVDs, cardiovascular diseases; CPDA, citrate/phosphate/dextrose/adenine; FDA, Food and Drug Administration's; GRAS, generally recognized as safe; PGE_1, prostaglandin E_1; PRI, Platelet Reactivity Index; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TXA_2, thromboxane A_2; T-TAS, Total Thrombus formation Analysis System; VASP, vasodilator-stimulated phosphoprotein.

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makes them very reactive (Korzonek-Szlacheta, Hudzik, Zubelewicz-Szkodzińska, & Gąsior, 2018). Through their receptors, platelets can adhere to many components of subendothelial connective tissue, including collagen, fibronectin, laminin, and vitronectin. The platelets are activated by their adhesion to the subendothelial connective tissue and by various substances called agonists. Activation of the platelets *in vivo* model is mainly initiated by: thrombin (the strongest physiological platelet agonist), collagen, adrenaline, platelet activator (PAF), and ADP. Substances released from the platelets themselves: ADP, thromboxane A_2, and serotonin contribute to further activation, including aggregation. The activation process of blood platelets may lead to a change of their shape and in the result, can provide to intensive release of various compounds in platelet granules. Among the most numerous granules in the platelets, are alpha-granules. However, their content undergoes rapid exocytosis during platelet activation, and under the influence of released mediators, haemostasis and inflammatory processes intensify (Periayah, Halim, & Mat Saad, 2017). In addition, some mediators such as P-selectin (otherwise known as CD62P) can significantly facilitate interaction between blood platelets, white blood cells, plasma proteins, and the wall of the vascular (Korzonek-Szlacheta et al., 2018). P-selectin is a protein present in α-granules of platelets. Activation of the platelets exposes P-selectin on their surface. Another possibility to measure platelet activation in the diagnosis of platelet anomalies, as well as to control the effectiveness of pharmacological treatment, may be P-selectin determination located on the surface of activated platelets (Fox et al., 2019). Additionally, platelet glycoprotein IIb/IIIa ($\alpha_{IIb}\beta_3$-integrin) participates indirectly in cell adhesion and plays an essential role in haemostasis and clot formation (Ma, Qin, & Plow, 2007). Phosphoprotein is another example of protein that occurs in large quantities in platelets stimulated by the vasodilator (VASP), whose presence has an important function in the negative regulation of secretion and adhesion in these cells. VASP is controlled by the cyclic adenosine monophosphate (cAMP) cascade. PGE_1 (prostaglandin E_1) activates this cascade, whereas it is inhibited by adenosine diphosphate (ADP) through $P2Y_{12}$ receptors (which is a predominant receptor involved in the ADP-stimulated activation of the glycoprotein IIb/IIIa receptor). Activation of the glycoprotein IIb/IIIa receptor contributes to stabilization of the platelet net formation and facilitation of thrombus formation. The adhesion, activation and aggregation of blood platelets are considered to play an important role in the development of cardiovascular diseases (CVDs) (Damman, Woudstra, Kuijt, de Winter, & James, 2012). Reducing platelet aggregation through antiplatelet therapy has an effect on the disturbance of formation and progression of thrombotic processes. Platelet inhibitors include various forms of cyclooxynase inhibitors (aspirin); thienopyridines (clopidogrel) or non-thienopyridine (elinogrel). Nevertheless, the effects of various synthetically produced antiplatelet drugs can be associated with a number of side effects, for example: risk of excessive or recurrent bleeding, haematological malignancy, idiopathic thrombocytopenic purpura or heart failure (Levy & McKee, 2007; Fox et al., 2019). Thus, an important aspect is the constant search for alternatives, such as pharmaceuticals based on constituents of natural origin. One example of a plant that has a significant impact, not only on the plasma system, but also on haemostasis, is dandelion *(Taraxacum officinale)*. Our previous papers demonstrated that dandelion organs including leaves, petals and roots are a safe and valuable source of various natural components with biological effects such as: antioxidant, anticoagulant and anti-platelet activities using washed blood platelets and plasma in an *in vitro* model (Lis, Jedrejek, Stochmal, & Olas, 2018A and B; Jedrejek, Lis, Rolnik, Stochmal, & Olas, 2019; Lis, Jedrejek, Moldoch, Stochmal, & OlasA, 2019 and B). However, their mechanisms of action in whole blood are still unknown. Hence, the present study aims to evaluate the effects of four chemically different fractions from dandelion organs including roots (fractions A and B), leaves (fraction C) and petals (fraction D) on platelets activation and thrombus formation in whole blood. Furthermore, the effects of these fractions on platelets' proteome were studied.

Table 1
Simplified phytochemical composition of the A-B fractions of dandelion roots (relative content (%Area UV) and Total Phenolic Content (mg GAE/g of dry weight (DW))), fraction C of leaves (mg/g DW) and fraction D of petals (mg/g DW). The information provided is a simplified results of the characteristics of the tested A-D fractions included in our previous publications (Jedrejek et al., 2017; 2019).

Chemical component	Fraction A (%Area UV)	Fraction B (%Area UV)	Fraction C (mg/g DW)	Fraction D (mg/g DW)
Hydroxycinnamic acids (HCA):	16.74	100.00	418.64 ± 5.67	214.33 ± 5.18
- L-chicoric acid	-	63.04	351.58 ± 4.72	117.17 ± 3.18
Hydroxyphenylacetate inositol esters (PIEs)	80.61	–	–	–
Flavonoids	–	–	19.17 ± 0.64	38.73 ± 2.55
Total Phenolic Content (mg GAE/g DW)	483.69 ± 2.81	771.91 ± 1.76	ND	ND

ND – not determined.

The anti-platelet potential was determined based on the various parameters (using different techniques), such as: (A) flow cytometry technique (the level of activation of resting or agonist (ADP or collagen)-stimulated blood platelets after incubation with tested dandelion fractions was measured through their cell-surface expression of P-selectin (CD62P) and presence of an active form of GPIIb/IIIa (PAC-1 binding); (B) thrombus formation (the influence of tested fractions on whole blood); (C) the platelets' proteome changes after incubation with dandelion fractions (using gel electrophoresis).

2. Material and methods

2.1. Plant material

The tested organs of the dandelion (*Taraxacum officinale* L.), i.e. roots, leaves and petals, were acquired from a small farm in Rzeszow, Poland. The extraction, fractionation and chemical analyses were developed in collaboration with the Institute of Soil Science and Plant Cultivation, State Research Institute in Pulawy, Poland. Extensive information on the plant material, preparation and phytochemical characteristics of the fractions used in the current research can be found in our previous publications: A-B fractions obtained from roots (Jedrejek et al., 2019), C-D fractions obtained from leaves and petals, respectively, (Jedrejek et al., 2017). Briefly, the finely ground plant material (leaves, petals, and roots) was extracted with 80% methanol. The obtained extracts were then purified and fractionated using the solid-phase extraction technique and/or column chromatography in the reverse-phase or Sephadex-LH20 system. The obtained fractions were freeze-dried and phytochemically characterized using an ACQUITY UPLC system (Waters), equipped with a photodiode array detector (PDA) and a tandem quadrupole mass spectrometer (TQD). Total phenolic content (TPC) in the A-B fractions was determined with the Folin-Ciocalteu assay, as described by Lis et al. (2019A). Quantitative determinations of phenolic acids and flavonoids in the C-D fractions were carried out using the external standard method and LC-UV analysis at 325 nm (phenolic acids, 5-caffeoylquinic acid as group standard) and 255 nm (flavonoids, rutin as group standard). The detailed information on the extraction and fractionation procedures, and UPLC-PDA-MS characteristics of the A-D fractions are described in our previous papers (Jedrejek et al., 2017; 2019).

The simplified characteristics of dandelion A-D fractions is presented in Table 1.

2.2. Blood collection and isolation human blood platelets from whole blood

Fresh human blood was obtained from regular, medication-free and non-smoking donors (male and female, age 23–30 years old) from a Medical Center in Lodz, Poland. The blood was collected into CPDA-1 solution (citrate/phosphate/ dextrose/adenine; 8.5:1; v/v; blood/ CPDA) or BAPA (benzylsulfonyl-D-argininyl-prolyl-4-amidinobenzylamide) – thrombin and factor Xa inhibitor, enables blood anticoagulation without interfering with physiological calcium levels. None of the volunteers had taken any medication or addictive substances such as tobacco, alcohol, antioxidant supplementation or aspirin, or any other antiplatelet drugs before blood collection. The protocol was accepted by the Committee for Research on Human Subjects of the University of Lodz (the number of permission is 2/KBBN-UŁ/II/ 2016).

Human blood platelets were separated using the method of differential centrifugation of blood, according to description as described by Wachowicz and Kustron (1992). The platelet pellet was twice time washed with modified Tyrode's buffer (pH 7.4), and then the platelets were suspended in the same buffer. The concentration of platelets in suspensions (used in the experiments), determined spectrophotometrically at wavelength 800 nm (Walkowiak, Michalak, Koziołkiewicz, & Cierniewski, 1989), amounted to 2–2.5 × 10^8 /mL.

Samples for gel electrophoresis: suspensions of blood platelets were incubated (30 min, at 37 °C) with fractions A-D of dandelion at the final concentrations of 10 μg/mL.

Samples for thrombus formation assay using: whole blood collected on BAPA was incubated (30 min, at 37 °C) with fractions A-D of dandelion at the final concentrations of 10 μg/mL.

Samples for flow cytometry: whole blood collected on CPDA-1 was incubated (30 min, at 25 °C) with fractions A-D of dandelion at the final concentrations of 1, 10 and 50 μg/mL.

Samples for VASP test: whole blood collected on CPDA-1 was incubated (30 min, at 37 °C) with fractions A-D of dandelion at the final concentrations of 10 μg/mL.

2.3. Flow cytometry

The changes in the activation and reactivity of resting or agonist-stimulated blood platelets were observed using a LSR II Flow Cytometer (Becton Dickinson, San Diego, CA, USA). At first, whole blood samples (150 μL) were incubated with the tested dandelion fractions (30 min., 25 °C) and then stimulated with ADP (10 and 20 μM, 15 min., 37 °C) or collagen (10 μg/mL, 15 min., 37 °C). After incubation, all the samples (control or agonist-stimulated) were diluted (1:9) in sterile PBS with Mg^{2+}, and immediately the antibodies mixture (CD61/PerCP; CD62/PE; PAC-1/FITC) were added to the test tubes. Parallel to the tested samples, the compensation samples or isotope control (CD61/ PerCP, FITC isotype, PE isotype) were prepared. All the samples were stained in the dark (30 min., 25 °C), and then fixed with 1%CellFix (60 min., 37 °C) followed by cytometric measurement (Rywaniak, Luzak, & Watała, 2012). All data analyses were performed in FACSDiva program.

2.4. Vasp

The VASP (Vasodilator Stimulated Phosphoprotein) assay was used to the monitoring of specific platelet ADP receptor ($P2Y_{12}$) antagonists by flow cytometry. The PLT VASP/ $P2Y_{12}$ test (Biocytex, France) was

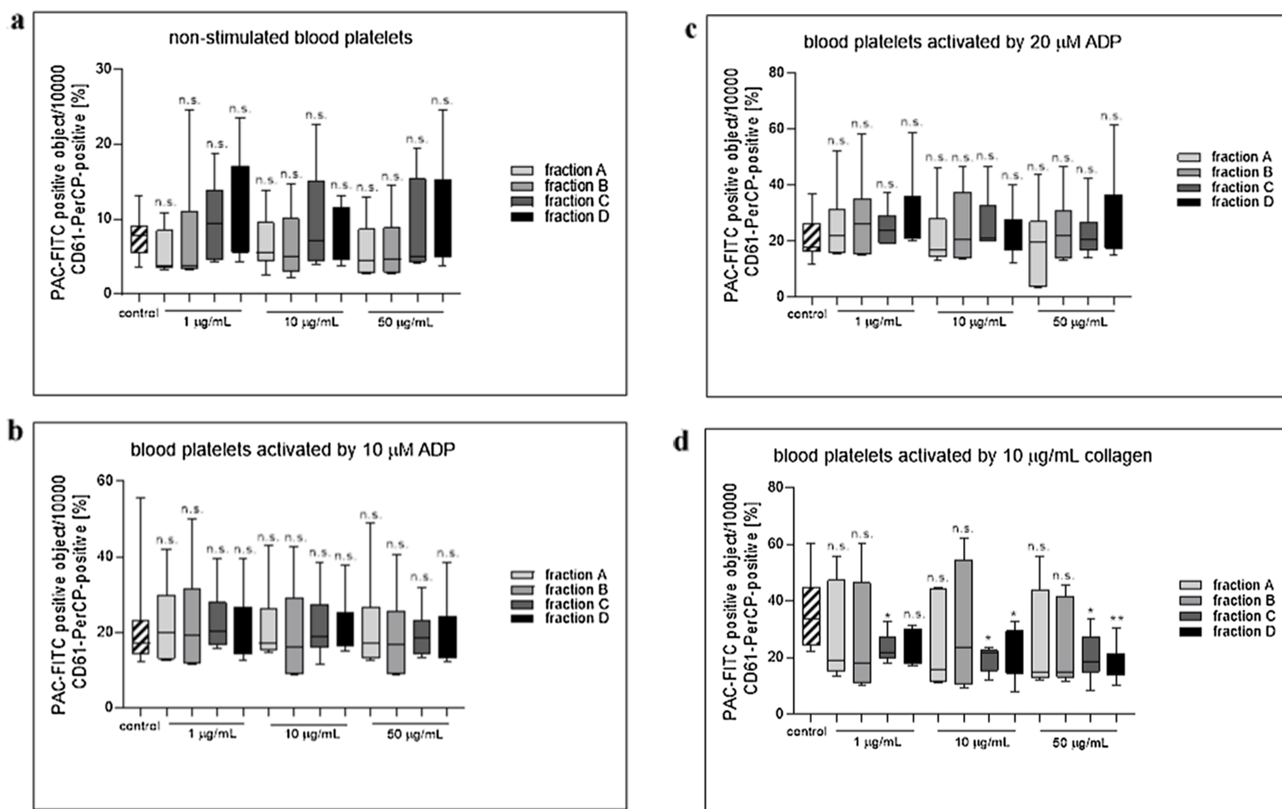

Fig. 1. Effects of fractions (A and B) of dandelion roots, fraction C of dandelion leaves, and fraction D of dandelion petals (at concentrations: 1, 10 and 50 μg/mL, incubation time – 30 min) on the expression of the active form of GPIIb/IIIa on resting (a) or agonist-stimulated blood platelets: 10 μM 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, 10,000 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 medians ± SD of 6 healthy volunteers. *$p < 0.05$ (vs. control platelets).

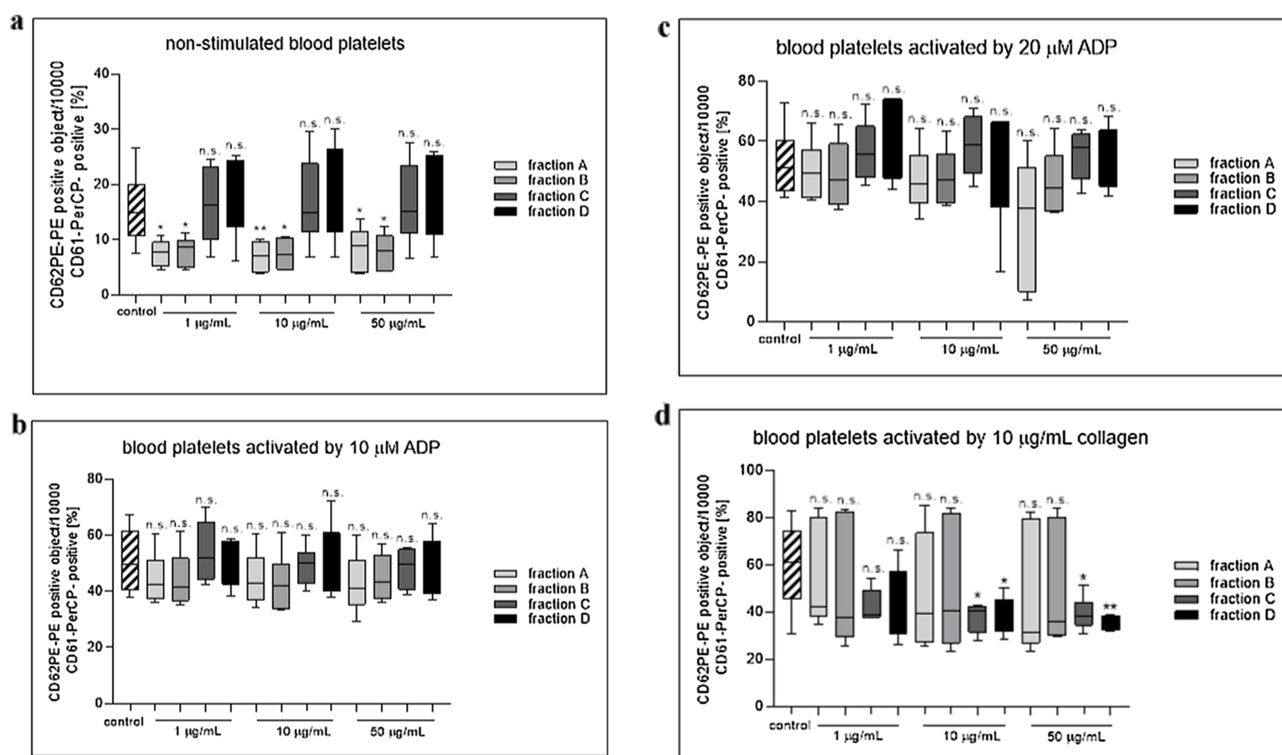

Fig. 2. Effects of fractions (A and B) of dandelion roots, fraction C of dandelion leaves, and fraction D of dandelion petals (at concentrations: 1, 10 and 50 μg/mL, incubation time – 30 min) on the expression of P-selectin on resting (a) or agonist-stimulated blood platelets: 10 μM ADP (b), 20 μM ADP (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, 10,000 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 medians ± SD of 6 healthy volunteers.

done according to the manufacturer's protocol. All the samples were measured within an hour after fluorescent staining and analyzed according to the instructions for Becton–Dickinson flow cytometers. The reported values were expressed as PRI (Platelet Reactivity Index).

2.5. Gel electrophoresis

For electrophoretic measurements, the isolated platelets in modified Tyrod's buffer were incubated (30 min., 37 °C) with different fractions (A-D) of dandelion at a final concentration of 10 μg/mL. Then, platelet lysates were prepared by adding lysis buffer and sonication of the samples. Next, the samples were centrifugated (5000 × g, 5 min., 4 °C) and total protein concertation in all the samples was determined according to Bradford (Bradford, 1976).

Native electrophoresis: The protein preparations were dissolved in sample buffer (0.0625 M Tris/HCl, 10% glycerol, pH 6.8) where the final protein concentration was 1 mg/ml.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE): The protein preparations were carried out in complexes with SDS, after dissolution in sample buffer (0.0625 M Tris/HCl, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, pH 6.8) where the final protein concentration was 1 mg/mL.

The electrophoresis was performed according to Laemmli's method (Laemmli, 1970) using Mini-PROTEAN® Tetra Vertical Electrophoresis Cell (Bio-Rad, Poland). Protein samples were loaded in a 4–20% precast polyacrylamide gel and electrophoresed. After the end of electrophoresis, the gel was stained in a dye solution (0.125% Coomassie brilliant blue R-250, 50% methanol, 10% acetic acid) for 30 min. Then, the excess dye was removed with the decoloring solution (25% methanol, 10% acetic acid) and the protein bands were located on the obtained electropherogram using a protein molecular weight markers - Precision Plus Protein™ Standards (Bio-Rad, Poland).

2.6. Thrombus formation assay

The thrombus formation in whole blood under flow conditions was determined with thrombus-formation analysis system (T-TAS) on PL-chips (coated with collagen). PL-chip is designed for quantitative analysis of primary hemostasis, i.e. the whole process of platelet clot formation (adhesion, aggregation, granule secretion and thrombus formation). Whole blood (400 μL) anticoagulated by BAPA (benzylsulfonyl-D-argininyl-prolyl-4-amidinobenzylamide) was incubated with the tested fractions by 30 min. at the 37 °C. Subsequently, the 340 μL of samples were transferred to the PL-chip (26 collagen-coated microcapillaries) and measurement was done on T-TAS®01 apparatus (Fujimori Kogyo Co., Ltd., Japan). The AUC_{10} parameter that is an area under the pressure curve from the start of the test to a time of 10 min was studied. The above parameter determines the growth, intensity, and stability of the formation of platelet or clot (Hosokawa et al., 2011).

2.7. Data analysis

Statistical analysis was realized with GraphPad Prism 8. Due to elimination of unreliable data, Q-Dixon test was done. Normal distribution of data was verified through normal probability plots and homogeneity of variance was confirmed by Levene's test. Differences within and between groups were studied using the Kruskal-Wallis test. For the sake of clarity, only changes between the tested fractions and control/control positive were marked. The significance was assessed as $p < 0.05$.

3. Results

As can be seen in Table 1, all four (A-D) dandelion fractions are enriched with phenolic compounds, however, the A fraction of

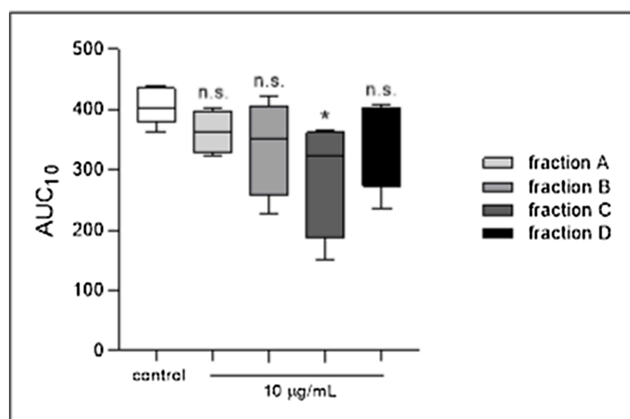

Fig. 3. Effects of fractions (A and B) of dandelion roots, fraction C of dandelion leaves, and fraction D of dandelion petals (at concentration: 10 μg/mL, incubation time – 30 min) on the T-TAS using the PL-chip in whole blood samples. Whole blood samples were analyzed by the T-TAS at the shear rates of 1000 s^{-1} on the PL-chips. The area under the curve (AUC_{10}) in PL are shown as closed circles. Data represent the medians ± SD of 6 healthy volunteers.

dandelion roots is dominated by the hydroxyphenylacetate inositol esters, while in the B-D fractions the main components are hydroxycinnamic acids, in particular L-chicoric acid.

The impact of dandelion fractions (A-D) on resting or agonist-stimulated (ADP or collagen) whole blood platelets was determined based on platelets' surface expression of P-selectin and presence of an active form of GPIIb/IIIa (PAC-1 binding) by flow cytometry. The obtained results demonstrated that all the tested fractions (A-D) did not affect the P-selectin expression on resting or ADP-stimulated platelets (Fig. 1a, b, and c). On the other hand, fraction C (at all tested concentrations: 1, 10, and 50 μg/mL) from dandelion leaves statistically significantly reduced P-selectin expression on platelets after activation with 10 μg/mL collagen by 31, 31 and 42%, respectively (Fig. 1d). Fraction D from dandelion petals (at 10 and 50 μg/mL) also decreased cellular expression of P-selectin (Fig. 1d) by 37 and 47%, respectively. The determination of the level of the active form of GPIIb/IIIa on cell-surface of platelets in whole blood showed that only fractions A from dandelion roots (at all tested concentrations: 1, 10, and 50 μg/mL) statistically significantly decreased active GPIIb/IIIa expression level on resting platelets by 47, 52 and 40%, respectively; as well as fraction B from dandelion roots (at all tested concentrations: 1, 10, and 50 μg/mL) caused a significant decrease of about 42, 51 and 46%, respectively. However, fractions C (from leaves) used at concentrations of 10 and 50 μg/mL - reduced it after platelet stimulation with collagen of about 33 and 37%, respectively; and D (from petals) at 10 and 50 μg/mL caused a significant decrease of about 36 and 43%, respectively (Fig. 2d). All four tested fractions (A, B, C, and D) did not change the expression active GPIIb/IIIa in ADP (10 and 20 μM) – activated blood platelets (Fig. 2b and c).

During blood flow through the PL-chip analysis pathway, platelets adhere and aggregate on the surface of the capillary canals, which are covered with collagen. The resulting platelet aggregates gradually increase, leading to the closure of the vessel, while at the same time increasing the change in blood flow pressure over time. In this way, changes in blood pressure reflect the process of platelet clot formation. The analysis of the effect of the tested fractions (A-D) on total thrombus-formation in whole blood showed that only fraction C (dandelion leaves fraction, 10 μg/mL) statistically significantly decreased AUC_{10} about 20% (Figs. 3 and 4). Other studied plant fractions (A, B, and D) did not change this parameter (Fig. 3).

The electrophoretic data showed, that all of the dandelion fractions (A-D) did not affect platelets' proteome in comparison to control platelets (not treated with fractions) (Fig. 5). Our result clearly showed that none of the examined fractions affected PRI, a parameter which is corresponding with VASP phosphorylation level (Fig. 6).

Table 2 summarizes the effects of all tested fractions (A-D) from different parts of dandelion on the level of platelet activation (P-selectin and active form of GPIIb/IIIa expression), VASP phosphorylation level (PRI), thrombus formation and platelet proteomics. Based on obtained results, we found that fraction C from dandelion leaves had the greatest anti-platelet potential: fraction C reduced the P-selectin expression and presence of an active form of GPIIb/IIIa on collagen-stimulated platelets and inhibited thrombus formation.

4. Discussion

Dandelion may be consumed in various forms, because it is listed on the U.S. Food and Drug Administration's (FDA) "generally recognized as safe" (GRAS) as a food and supplement (Martinez et al., 2015; Lis & Olas, 2019). Our earlier findings have also shown that dandelion roots are a safe and highly valuable source of natural ingredients with several biological properties, especially anti-platelet activity, based on an *in vitro* model of washed blood platelets (Jedrejek et al., 2019; Lis et al.B,

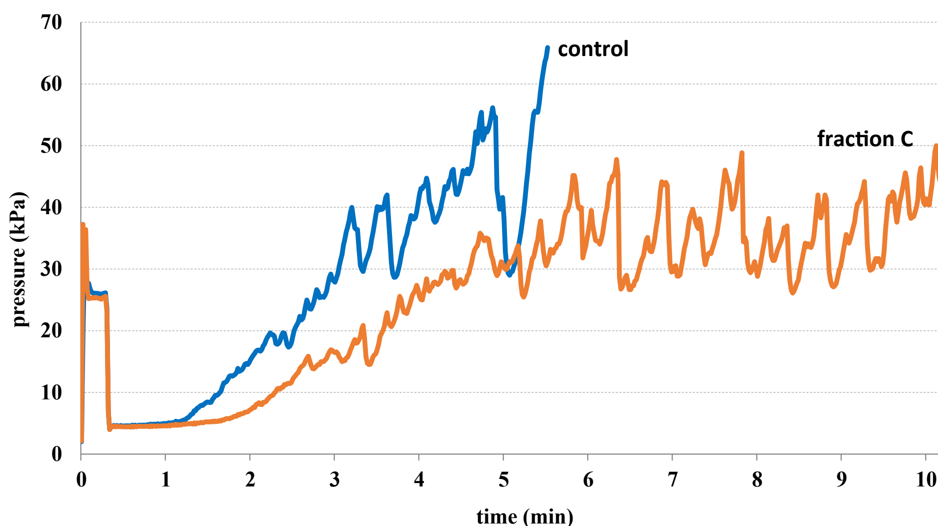

Fig. 4. Flow pressure analysis reflect the platelet thrombus formation proces using the PL-chip in whole blood (control – blood treated without fraction C of dandelion leaves; fraction C – blood treated with 10 μg/mL fraction C of dandelion leaves) within 10 min.

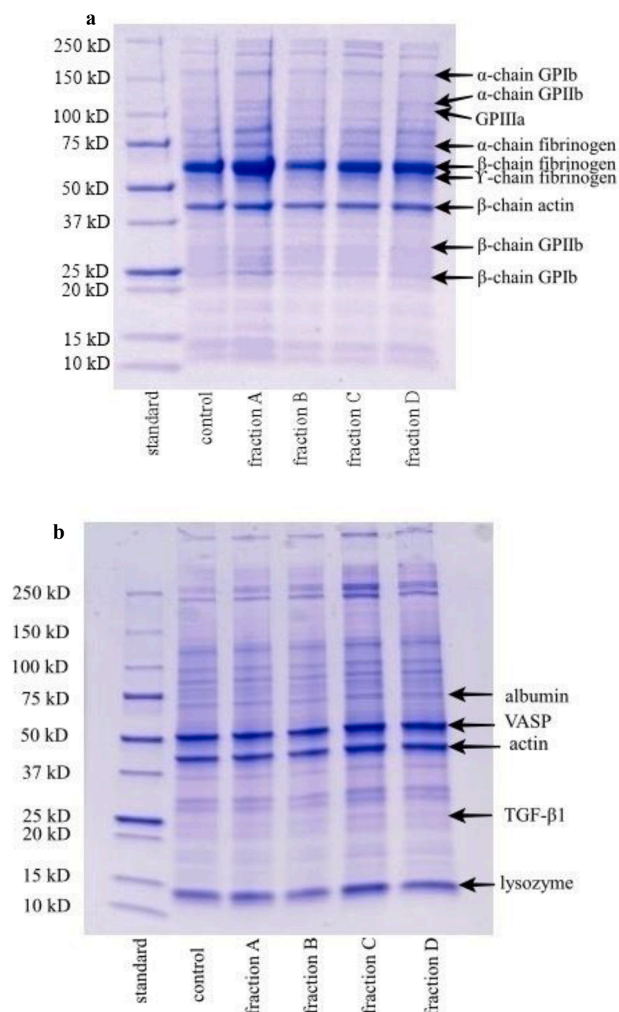

Fig. 5. Electrophoretic separation of platelet proteins treated with different dandelion fractions (A-D) at the final concentration of 10 μg/mL. Dandelion preparations were incubated with blood platelets (37 °C, 30 min.). Protein samples in SDS buffer were subjected to electrophoresis under non-reducing conditions through a 4–20% TGX (Tris-Glycine eXtended) gel. The gel was stained with Coomassie blue R-250. The molecular mass standard is indicated in kilodaltons. Electrophoretic patterns of blood platelet proteome in the presence of different plant fractions (A-D; 10 μg/mL; 30 min): reducing conditions (a), non-reducing conditions (b).

2019). Further, our previous *in vitro* tests (also with the usage of washed blood platelets) indicated that the preparations (rich in phenolic acids, including chicoric acid) extracted from dandelion leaves and petals have been shown to have antiplatelet effect (Lis et al., 2019A). Results of Majewski et al. (2020) demonstrated that these fractions are the modulators of the antioxidant status and lipid profile in an *in vivo* study. In this experiment, Wistar rats were supplemented for four weeks with these fractions. Other authors studied the effect of water extract from dandelion leaves on haematological profile *in vivo*. Wistar Albino rats treated with carbon tetrachloride (CCl_4) had elevated levels of white blood cells (WBC) and bilirubin; as well as a decrease in blood cell volume (PCV) and haemoglobin (Hb) was observed in compared to control. After applying 100 mL and 200 mL of dandelion water extract, within 3 weeks there was a decrease in WBC and bilirubin levels, and at the same time an increase in PCV and Hb (Berezi, Monago, & Adelagun, 2013). However, the anti-platelet potential of preparations isolated from different dandelion organs in whole blood remained unknown. For the first time, we used T-TAS and flow cytometry technique to analyze the anti-platelet activity of four fractions (A-D) obtained from different

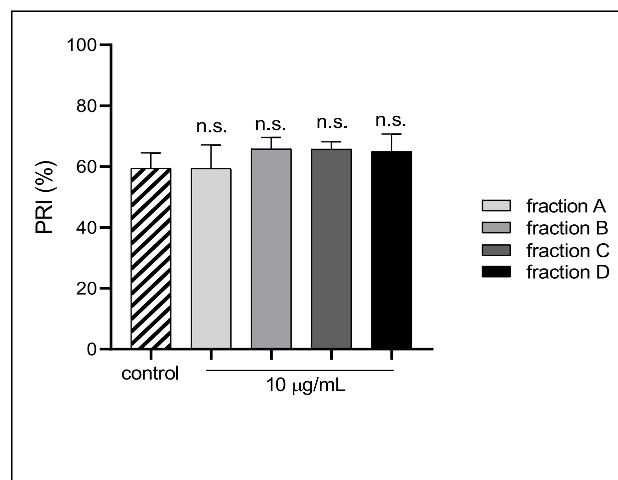

Fig. 6. Effects of different plant fractions (A-D) at the final concentration 10 μg/mL on VASP phosphorylation in ADP – activated blood platelets. Data represent the mean ± SD of 2 healthy volunteers.

Table 2
Comparative anti-platelet potential of four fractions (A-D; tested concentration – 10 μg/mL) isolated from different dandelion organs.

Experiment	Fraction A isolated from roots	Fraction B isolated from roots	Fraction C isolated from leaves	Fraction D isolated from petals
GPIIb/IIa expression				
Non-stimulated platelets	Anti-platelet potential	Anti-platelet potential	No effect	No effect
10 μM ADP-activated platelets	No effect	No effect	No effect	No effect
20 μM ADP-activated platelets	No effect	No effect	No effect	No effect
10 μg/mL collagen-activated platelets	No effect	No effect	Anti-platelet potential	Anti-platelet potential
P-selectin expression				
Non-stimulated platelets	No effect	No effect	No effect	No effect
10 μM ADP-activated platelets	No effect	No effect	No effect	No effect
20 μM ADP-activated platelets	No effect	No effect	No effect	No effect
10 μg/mL collagen-activated platelets	No effect	No effect	Anti-platelet potential	Anti-platelet potential
VASP phosphorylation	No effect	No effect	No effect	No effect
T-TAS	No effect	No effect	Anti-platelet potential	No effect

dandelion organs: roots, leaves, and petals in whole blood. T-TAS (a microchip-based flow chamber system) imitates *in vivo* conditions to assess whole blood thrombogenicity. The T-TAS platelet chip is under the flow pressure curve (AUC) and evaluates primary hemostasis, also is sensitive to the therapeutic effects of different anti-platelet drugs, supplements or other preparations (Kaikita, Hosokawa, Dahlen, & Tsujita, 2019). In our present experiment, we used PL-chip surfaces coated with collagen, and we observed that only one fraction – fraction C (isolated from dandelion leaves, and riches in phenolic acids) has anti-coagulant properties. Moreover, a novel findings of the present study is that two

fractions (C and D, riches in phenolic acids) isolated from dandelion leaves and petals decreased surface expression of P-selection and the active form of GPIIb/IIIa on blood platelets stimulated by collagen in whole blood. On the other hand, no changes in platelets' activation state were observed for two fractions isolated from dandelion roots (fraction A and B) in platelets activated by collagen or ADP. These fractions only reduced the expression active form of GPIIb/IIIa on resting platelets. The obtained results indicate that especially fraction C has the greatest anti-platelet properties, which were observed using T-TAS and flow cytometry techniques. It seems that the action of fraction C is due to bioactive components in the form of hydroxycinnamic acids, and above all, a significant content of chicoric acid compared to the other fractions used. Taking these facts into account, enriching the diet with products containing dandelion raw materials rich in polyphenols, can prevent cardiovascular diseases.

VASP phosphorylation experiment is often used to examine the interaction of anti-platelet drugs with $P2Y_{12}$ receptors (Fedor et al., 2013.; Marginean, Bănescu, Scridon, & Dobreanu, 2016). For the first time, our results reveal that all used dandelion fractions (A-D) did not change the blood platelet reactivity index (PRI; VASP phosphorylation) in whole blood. Therefore, we suppose that the mechanisms of anti-platelet action of these dandelion fractions (A-D) are not associated with interactions between chemical compounds of dandelion fractions and blood platelet surface membrane $P2Y_{12}$ receptor. Moreover, tested fractions did not affect the blood platelet proteome.

The differences in the anti-platelet potential of tested dandelion fractions (A-D, observed in P-selectin expression, GPIIb/IIa expression and thrombus formation) might be correlated with their different chemical profile. Our current observations are in agreement with the others. For example, results of various authors (Ivanov, 2014; Treml & Smejkal, 2016; Lis et al.A, 2019) demonstrate that the phenolic compounds (especially phenolic acids and flavonoids) present in dandelion organs possess the greatest beneficial impact on different elements of hemostasis. It is observed that flavonoids have a mitigating effect on the process induced by various molecules involved in the aggregation pathway, such as collagen, ADP, thrombin, PAF, cyclooxygenase 1 (COX-1), and arachidonic acid (AA). In particular, collagen and ADP are involved in platelet adhesion and activation (Khan et al., 2018). Thus, the presence of both phenolic acids and flavonoids may act synergistically and prove the best antiplatelet properties of the C and D fractions from dandelion.

The bioavailability of phenolic compounds is an important element in the evaluation of their biological activity, including anti-platelet potential. The concentrations tested in this study demonstrated a non-cytotoxic effect in our earlier experiments (Lis et al., 2019A). Moreover, the concentration of dandelion fractions (C and D; $\leq$ 50 μg/mL) in whole blood, which were used in our study, corresponds with the physiological concentrations of phenolics after oral administration (Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004; 2005). Interestingly, metabolites of phenolic acids ($\leq$100 μg/mL, *in vitro*) have been demonstrated to have a stronger antiplatelet potential than their phenolic precursors (Baeza et al., 2017).

To conclude, for the first time, thanks to the analysis conducted in whole blood, we could successfully observe the anti-platelet potential of two tested fractions (C and D, riches in phenolic acids) isolated from dandelion leaves and petals. Thus, the different dandelion organs including roots, leaves or flowers are a valuable source of biologically active substances with anti-platelet potential, whose consumption in the diet in the form of various products (leaf salad, coffee from roasted roots, syrup/wine from flowers) can have a beneficial effect in the prevention and treatment of cardiovascular diseases caused by hyperactivation of platelets. However, these results encourage further investigations of the bioactive potential of dandelion, as well as its mechanisms of action in blood platelets not only in *in vitro* model, but also in *in vivo* model.

5. Ethics statement

The protocol was approved by the Committee for Research on Human Subjects of the University of Lodz (number 2/KBBN-U Ł/II/2016).

CRediT authorship contribution statement

Bernadetta Lis: Methodology, Funding acquisition, Investigation, Writing - original draft. **Joanna Rywaniak:** Methodology. **Dariusz Jedrejek:** Methodology, Writing - original draft. **Aleksandra Szustka:** Methodology. **Anna Stochmal:** Methodology. **Beata Olas:** Conceptualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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