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Extracts of tamarillo, horned melon, and raspberry, but not extract of pear, inhibit human blood platelet aggregation: Investigating the underlying factors for their differential mechanisms

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ABSTRACT

In addition to their roles in thrombosis, hyperactive platelets contribute to atherosclerosis. Since anti-platelet drugs are not recommended for the vulnerable population with hyperactive platelets to reduce the risk of cardiovascular disease, a search is on to find alternative dietary antiplatelets that can be safely used. We here describe that in a dose-dependent manner, sugar-free extracts of tamarillo, horned melon (kiwano), and raspberry inhibited ADP-induced platelet aggregation in platelet-rich plasma, whereas pear extract had no effects. Furthermore, analysis of untargeted metabolites revealed the presence of platelet inhibitory components such as benzoic acid, caffeic acid, and gallic acid in the sugar-free extracts of tamarillo, raspberry, and kiwano but absent in pear extract. All these three fruit extracts inhibited the platelet production of TxB_2 and the release of platelet factor 4. Our work suggests that these fruits inhibit platelet aggregation partly due to anti-platelet compounds such as benzoic, caffeic, and gallic acids.

1. Introduction

Human blood platelets play a crucial role in hemostasis and thrombosis and are also involved in other biological processes, including inflammation, immunity, wound healing, cancer, and angiogenesis (O'Kennedy et al., 2021; Jackson, 2007; Lusis, 2000; Berenson et al., 1998). Platelets contribute to homeostasis via their surface receptor-ligand interactions, granule release, mitochondrial secretion, and RNA transfer, subsequently regulating hemostatic events and immune processes. Activated platelets release mediators, such as serotonin, ADP, ATP, and lysophosphatidic acid, and their hyperactivation is the causal factor for the initiation and progression of atherosclerosis and cardiovascular disease (CVD) (O'Kennedy et al., 2006; O'Kennedy et al., 2021). Atherosclerosis, the primary contributor to CVD, is a chronic inflammatory state in the arterial blood vessel walls, leading to thrombus formation (Jackson, 2007; Lusis, 2000). Factors contributing to the development of atherosclerotic include adverse environments and personal lifestyle choices such as smoking, a high-fat diet, lack of exercise, and genetic factors, all of which ultimately lead to plaque formation (Lusis, 2000; Berenson et al., 1998; Graham et al., 2012). Regular blood platelet activity maintains hemostasis and adequate blood flow (Jackson, 2007). However, hyperactive platelets interact with vessel walls by shedding membrane macro-particles, secreting adhesive growth factors, 5-hydroxytryptamine (serotonin), ADP, ATP, and lysophosphatidic acid and inflammatory cytokines, interrupting blood flow and promoting a pro-thrombotic state (Duhamel et al., 2007). This is particularly evident in individuals with obesity, diabetes, a sedentary lifestyle, hypertension, and smokers (Natarajan et al., 2008; Massberg et al., 2002; Metharom et al., 2015; Kaplan and Jackson, 2011). Therefore, platelet activation triggers and maintains the pro-inflammatory and pro-thrombotic state in obesity. Thus, the hyperactive platelets create a feedback loop involving adipose tissue and

Abbreviations: CVD, Cardiovascular disease; PF4, platelet factor 4; PRP, Platelet-rich plasma; NCE, normalized collision energy; PCA, Principal component analysis; Tx, Thromboxane.

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vascular endothelium that shifts towards the atherothrombotic vascular events (Fuentes et al., 2016; Ferroni et al., 2008). Hence, hyperactive platelets contribute to plaque formation and have thus been proposed as a CVD risk factor (Badimon et al., 2012; Davi and Patrono, 2007).

Aspirin (acetylsalicylic acid) remains a cornerstone in anti-platelet treatment to prevent vascular thrombosis. However, resistance to aspirin treatment occurs in 25–30 % of patients, and aspirin can also lead to several serious side effects, particularly bleeding, rendering it often unsuitable for primary CVD prevention (Cai et al., 2016; Hennekens and Dalen, 2014; Das et al., 2022). In addition, other platelet inhibitors such as dipyridamole, ADP-receptor-, and glycoprotein antagonists also confer side effects, making it challenging to use them to prevent CVD. In line with this, aspirin and other anti-platelet drugs are not recommended for primary CVD prevention or in subjects with low CVD risk. Therefore, finding alternative safe anti-platelet inhibitors for those with hyperactive platelets is essential to reduce CVD risk.

There is growing interest in naturally occurring compounds with potential anti-platelet effects that lack the side effects of current anti-platelet inhibitors (Das et al., 2022). In line with this, nutraceuticals with anti-platelet compounds are suggested to prevent CVD (Tsoupras et al., 2020). Various fruits are being investigated for their cardioprotective effects due to their high polyphenol content and bioactive component profile (Duttaroy, 2018). To this end, emerging data indicate that fruit extracts may be cardioprotective via favorable modulation of the platelet-blood vessel interaction (O'Kennedy et al., 2017).

Therefore, we now systematically investigate bioactive compounds' impact in high polyphenol-content fruits on human blood platelet aggregation. In addition, we aim to utilize the findings to characterize the mechanisms involved in this process to identify potential dietary anti-platelet components.

Dietary polyphenols have been shown to reduce platelet activation and aggregation via multiple pathways (Das et al., 2022; Ludovici et al., 2018; O'Kennedy et al., 2006). Plant polyphenols, such as flavonols, phenolic acids, anthocyanins, and procyanidins, are found in exceptionally high concentrations in various berries, e.g., tamarillo and kiwano (horned melon) (Skrovankova et al., 2015; Williamson, 2017; Diep et al., 2020; Busuioc et al., 2020). Tamarillo (*Solanum betaceum Cav*.) is a sub-tropical fruit known for its high nutritional value and beneficial effect on CVD, attributed to numerous phytochemicals (Diep et al., 2022). Kiwano (*Cucumis metuliferus*), a fruit from the *Cucurbitaceae* family, also contains several polyphenols (Sovljanski et al., 2022). Raspberry (*Rubus idaeus*) is another fruit that is an excellent source of polyphenols (Balawejder et al., 2022). Although these fruits contain extensive high polyphenols, their effects on platelet aggregation have not been studied. Thus, our first objective was to investigate the effects of extracts prepared from these fruits on ADP-induced platelet aggregation. Secondly, we aimed to unravel their mechanism of action on platelet activation. In this paper, we report that these fruits exhibited potent anti-platelet activity, and their anti-platelet effects can be due to benzoic acid, caffeic acid, and gallic acid. These fruits can be exploited to develop cardioprotective functional food.

2. Materials and methods

Tamarillo (*Solanum betaceum),* Kiwano or horned melon (*Cucumis metulifer*), Raspberry (*Rubus idaeus),* and pears (*Pyrus communis)* were obtained from local grocery shops in Oslo. Hydro-benzoic acid, caffeic acid, and gallic acid were obtained from Sigma (Oslo, Norway). TxB_2 ELISA kit was obtained from Cayman Chemical (Ann Arbor, Michigan, USA), whereas a human platelet factor 4 (PF4) ELISA kit was obtained from Thermo Fisher (Waltham, USA). Fruitflow® (sugar-free tomato aqueous extract) was kindly provided by DSM Nutritional Products (Basel, Switzerland). ADP was obtained from Helena (New York, USA). All other reagents used were of analytical-grade quality. All volunteers abstained from platelet-interfering medications for at least 14 days before the study. Exclusion criteria were (a) the presence of overt vascular, hematological, or respiratory disease, hypertension, or infection, (b) frequent consumption of drugs that affect platelet function (e.g., aspirin, paracetamol, steroids, chronic consumption of omega-3 fatty acid supplements, and tomato), (c) previous or current cardiovascular, neoplastic, infectious, or immune disease, those on specific dietary restrictions (e.g., vegetarians and vegans), and (d) failure to comply with oral/written instructions. Ethical approval for this project was obtained from the Regional Committee for Medical and Health Research Ethics (no. 2015/396/REK Sør-Øst C), Norway.

2.1. Preparation of fruit extracts and the removal of sugars

To prepare 100 % fruit juice, peeled fruits were homogenized with a Brown Turbo Mixer for 90–200 s at the highest speed, and the homogenate was centrifuged at 2500*g* for 15 min at 22 °C. The first centrifugation removed most seeds and fruit pulp and was excluded from the experimental workflow. Then, the supernatant was centrifuged at 3000*g* for 15 min at 22 °C to obtain a more clarified fruit extract to be stored at −20 °C. The supernatant was then concentrated, dried, and reconstituted in PBS (pH 7.4). As prepared above, the fruit extracts usually contain soluble sugars and polyphenols. Therefore, 1 ml of the fruit extracts was freeze-dried for 72 h after being stored at −80° overnight. When prepared as above, the fruit extracts had 50–60 % water-soluble sugars. Solid phase extraction column chromatography was used to remove soluble sugars using a J.T.Baker® Bakerbond SPE 1 ml disposable extraction columns. Typically, 1 ml fruit extract (as prepared above) was loaded onto the cartridge after conditioning the column with 1 ml methanol and 1 ml distilled water. The cartridge was thoroughly dried before eluting the non-sugar components with 100 % methanol. The eluted fractions were dried under N_2 at 45 °C (O'Kennedy et al., 2006; O'Kennedy et al., 2006; Dutta-Roy et al., 2001).

The freeze-dried and sugar-free materials from each extraction step were tested for anti-platelet activity. The platelet-rich plasma (PRP) was incubated with fruit extracts to measure its effects on ADP-induced platelet aggregation (Dizdarevic et al., 2014). As previously described, inhibition of platelet aggregation by the fruit extracts was expressed as a decrease in the area under the curve compared with the control (Uddin et al., 2018).

2.2. Platelet-rich plasma preparation and platelet aggregation study

Venous blood (20–30 ml) was collected from volunteers who had not taken any medications for at least 14 days before donation. Blood was collected in a Vacutainer containing sodium citrate buffer (135 mM) (ratio 9:1) to prevent blood coagulation (Dizdarevic et al., 2014). The PRP was prepared as described earlier (Biswas et al., 2014; Hunter et al., 2000). The anti-platelet activity of the fruit extracts prepared at different steps was investigated for anti-platelet factors. Fruit extracts at various concentrations (0–1.50 mg/ml) were incubated with 0.45 ml of PRP at 37 °C for 15 min, after which the effect of the extract on agonist-induced platelet aggregation was monitored with the addition of aggregating agent, ADP (10 μM). Controls were run parallel using 10 μl of PBS (pH 7.4) instead of the fruit extract. The platelet aggregation in PRP was monitored using Aggram, Aggregometer (Helena, USA) at a constant stirring speed of 1000 rpm at 37 °C (Dizdarevic et al., 2014). The extent of ADP-induced platelet aggregation in PRP was measured at each time point. The maximal aggregation (100 %) was defined as the maximum change in light transmission observed over 15 min without fruit extract. Inhibition of platelet aggregation was expressed as the decrease in the area under the curve compared with the control. Platelet inhibition was calculated as: inhibition of platelet aggregation (%) = 100 − ((platelet aggregation of in the presence of fruit extract/platelet aggregation of negative control) × 100) (Sepulveda et al., 2019).

IC_{50} determination

Typically, the PRP (250 μl) was incubated with different

concentrations of fruit extracts for 15 min at 37 °C. After the incubation, the ADP-induced platelet aggregation was measured to determine the inhibition of platelet aggregation by the presence of different concentrations of fruit extracts. The IC_{50} values (the concentration necessary to reduce the induced platelet aggregation by 50 % with respect to control) were obtained from concentration-effect curves, as described before (Dizdarevic et al., 2014).

2.3. Untargeted sample analysis

Chromatographic analyses were conducted to identify the unknown compounds in the sugar-free fruit extracts derived from tamarillo, kiwano, raspberry, and pear. The analysis was carried out by preparing the samples consistently and reproducibly by selecting appropriate sample matrices and extraction methods to ensure the extraction of a broad range of polar and non-polar metabolites from the biological samples.

The prepared samples were then subjected to liquid chromatography-mass spectrometry (LC-MS) analysis. We employed state-of-the-art chromatographic techniques to achieve high-resolution separation of metabolites. The LC system had a column facilitating efficient separation based on compound hydrophobicity and other physicochemical properties. The LC system was coupled to a high-resolution mass spectrometer, enabling accurate mass measurements of the separated compounds. We used positive and negative ionization modes to capture a comprehensive range of metabolites. The acquired mass spectra provided information about the elemental composition of the compounds.

Chromatographic separation was performed with a Dionex Ultimate 3000 UHPLC system, coupled with a Q-Exactive hybrid quadrupole-orbitrap mass spectrometer, in turn, equipped with a heat electrospray ionization (HESI) source (Bremen, Germany). The UPLC column used was HSS T3 C18 (Waters, Milford). The flow rate was 600 l $min^{-1,}$ and the injection volume was 3 µl. Separations were performed using a binary gradient. The mobile phase was 0.1 % formic acid in reagent water (A) and 0.1 % formic acid in 70 % acetonitrile and 30 % methanol (B). A gradient of both mobile phases (running time 11 min) was used: 1–98 % A at 0–8 min, 1 % A for 9–10 min, 1 % A for 10–11 min. The mass spectrometer was operated both in positive and negative ion mode, with the ion source parameters optimized, and the operating conditions were as follows: 3.0 kV (positive mode) and 2.5 kV (negative mode); sheath gas flow-rate 50 arbitrary unit; auxiliary gas flow-rate 13 arbitrary unit; heater temperature 425 °C; capillary temperature 263 °C; capillary voltage 45 V and lens voltage 60 V. Both ionization in positive (ESI+) and negative (ESI−) mode was used. The system operated in full-scan mode (70–1050 *m/z*) at a resolving power of 70,000 (200 ms). The resolution of the entire M.S. scan was 70,000, and the range was 700–1050 *m/z*. In MS2 mode at 35,500, samples were analyzed at 10, 30, and 60 normalized collision energy (NCE).

The raw data obtained from the mass spectrometer were processed using advanced data analysis software. We employed peak detection, alignment, and deconvolution algorithms to convert the raw mass spectra into a list of detected features (ions) with associated retention times and *m/z* values. This step allowed us to generate a data matrix for all samples. The data matrix was subjected to multivariate statistical analysis, such as principal component analysis (PCA) or partial least squares-discriminant analysis (PLS-DA), to identify metabolites showing significant differences between sample groups. Subsequently, we performed tandem mass spectrometry (MS/MS) experiments to elucidate the fragmentation patterns of the selected metabolites, aiding in their identification.

2.4. Inhibition of platelet factor 4 (PF4) release by sugar-free fruit extracts

To measure the inhibitory effect of different fruit extracts on PF4 release, PRP (450 µl) was incubated in the presence of these extracts at different concentrations and followed by 10 µM ADP treatment, as described earlier (Hua et al., 2004). After 5 min of incubation, PRP was centrifuged to prepare platelet-poor plasma, and PF4 was measured using the PF4 assay kit using a HuPF-4 ELISA kit (ThermoFisher), as described earlier (Dizdarevic et al., 2014).

2.5. Thromboxane B_2 assay

Thromboxane (Tx) A_2 is produced from arachidonic acid, 20:4n-6, released cell membrane phospholipids after activation/aggregation, and causes platelet aggregation. Thromboxane (Tx)B_2, the breakdown product of TxA_2, was estimated using a TxB_2 assay kit (Cayman Chemical, Ann Arbor, USA), as described before (Dizdarevic et al., 2014). PRP (450 µl) was incubated with 10 µl of sugar-free fruit extracts. At the end of the platelet aggregation experiment, plasma was centrifuged at 3000*g* for 5 min at 22C. The supernatants were collected and stored at −80 °C and subsequently used for TxB_2 assay using the ELISA Kit according to the manufacturer's instructions.

2.6. Data processing

For data acquisition, Thermo Xcalibur 3.0 (Thermo Scientific, San Jose, CA, USA) and Compound Discover (CD) software 3.1 (Thermo Scientific) were used. Natural product analysis and untargeted metabolomics workflow were selected, and the databases included the MZcloud database. Principal component analysis (PCA), a method for reducing the dimensionality of such datasets and increasing interpretability while minimizing information loss, was used to detect variation between data groups. Partial least squares discriminant analysis (PLS-DA), a method that reduces the variables to predict to a smaller set of predictors, was used to distinguish the compounds between two data groups. Differential peak area analysis was performed to normalize and compare the relative intensity of chromatographic peaks. This analysis allowed for the estimation of p-values through data transformation, specifically using log-10 areas. Peaks with larger differences in intensity and smaller p-values are considered more significant.

2.7. Statistics

The Results are presented as the mean ± S.D. Results were analyzed by the Student's *t*-test. Other statistical analyses were performed using ANOVA where appropriate; values were considered significantly different when $p < 0.05$. A two-tailed student *t*-test calculated statistical test, and the p-value was adjusted using Benjamin-Hochberg correction for a false discovery rate.

3. Results

3.1. Isolation of water-soluble extracts of fruits

PRP was prepared from the blood drawn from the overnight fasted, healthy volunteers (n = 18) of both genders, aged 20–39 years. Samples of different fruit extracts were evaluated regarding the anti-aggregation effect on platelet-rich plasma. 100 % juice (w/v) was initially used for the platelet aggregation studies. All the fruit extracts had a dose-

Table 1
The inhibitory effect of different fruit extracts on platelet aggregation in PRP.

Fruit extract	Inhibition of platelet aggregation (n = 9)
Raspberry	77 ± 8 %
Tamarillo	81 ± 11 %
Kiwano	75 ± 12 %
Pear	3 ± 2 %

Values are means ± SD.

dependent inhibition of platelet aggregation; the maximum inhibition was around 70 %−80 % (Table 1), whereas pear extract had little or no inhibitory effect (2–5 %).

Soluble sugars were removed by using SPE column chromatography. Under the experimental conditions, polar compounds were eluted earlier than non-polar compounds. The de-sugared extracts contained less than 0.2 mg/ml of glucose, fructose, and no detectable sucrose. The soluble sugars showed no activity toward platelet aggregation (data not shown). One hundred grams of tamarillo, kiwano, or raspberry produced 6.57 mg, 1.59 mg, and 9.6 mg of sugar-free extract containing anti-platelet factors. Fig. 1 shows the effect of different sugar-free fruit extracts on ADP-induced platelet aggregation in PRP. The dose-dependent inhibition of ADP-induced aggregation was also demonstrated. The IC_{50} (minimum concentration required for 50 % inhibition of platelet aggregation induced by ADP in 450 µl of PRP of various fruit extracts was around 5–15 µl (100 % juice). Because the juice prepared from these three fruits (kiwano, raspberry, tamarillo) had the maximum anti-platelet activity, we compared the yield of anti-platelet factors and their potency against platelet aggregation. The sugar-free extracts containing cardioprotective compounds yielded 6.57 mg, 1.59 mg, and 9.6 mg per 100 g of tamarillo, kiwano, and raspberry, respectively. The IC_{50} was 2.2 ± 0.2 mg/ml, 2.2 ± 0.2 mg/ml, and 2.6 ± 0.11 mg/ml for tamarillo, kiwano, and raspberry, respectively ($p < 0.05$) (Fig. 2.). Because, among all fruits, tamarillo, kiwano, and raspberry have a considerable amount of anti-platelet factors, we isolated and compared their yields and potencies. Pear extract had no detectable effect. As expected, aspirin and Fruitflow® (Das et al., 2021) markedly inhibited platelet aggregation (95 %). Kiwano, raspberry, and tamarillo showed their anti-platelet effect. However, Tamarillo extract was the most potent platelet aggregation inhibitor.

3.2. Effect of fruit extracts on platelet TxA_2 Synthesis

To determine whether the inhibitory effect of different fruit extracts (sugar-free) on platelet aggregation was due to the reduced synthesis of TxA_2, the levels of TxB_2, the stable breakdown product of TxA_2, were measured in PRP in the presence and absence of these fruit extracts (500 µg) and ADP. Incubation of PRP with fruit extracts inhibited TxB_2 production. The inhibition of platelet aggregation by sugar-free fruit extracts was concomitantly associated with the inhibition of TxB_2 synthesis. All three sugar-free extracts inhibited ADP-induced TxB_2 synthesis by 80–90 % (996 ± 79 pg/ml) compared with their respective controls (6574 ± 256 pg/ml)($p < 0.05$).

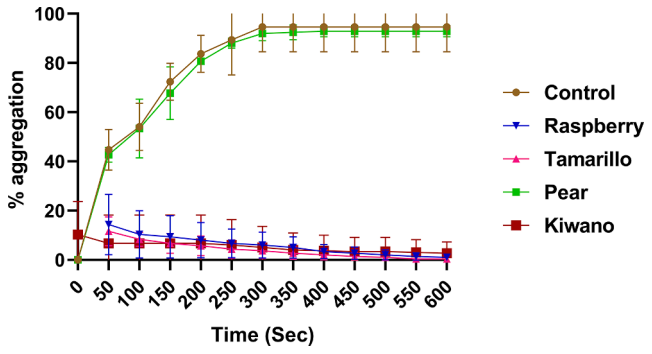

Fig. 1. Inhibition of platelet aggregation by different fruit extracts and Fruitflow. PRP was prepared as described in the "Methods" section. PRP (final volume, 0.225 ml) was then incubated with sugar-free fruit extracts (3 mg/ml) for 15 min at 37 °C before ADP-induced aggregation was initiated. Aggregation was followed at 37 °C with stirring. A representative inhibition profile of different fruit extracts on ADP-induced aggregation of platelets is shown. For details, see the section "Methods.".

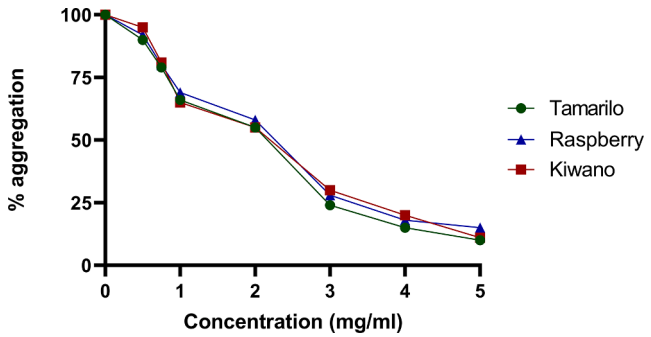

Fig. 2. Dose-dependent inhibition of platelet aggregation by different fruit extracts PRP was prepared as described in the "Methods" section. PRP (final volume, 0.225 ml) was then incubated with different amounts of sugar-free fruit extracts (0–5 mg/ml) for 15 min at 37 °C before ADP-induced aggregation was initiated. Aggregation was followed at 37 °C with stirring. A representative inhibition profile of different fruit extracts on ADP-induced aggregation of platelets is shown. For details, see the section "Methods.".

3.3. Effects of fruit extracts on platelet factor 4 (PF4) release

The extracellular release of granule contents of PF4 (a constituent of platelet-derived α granules) in the supernatants from ADP-stimulated platelets was determined in the presence and absence of different fruit extracts. The level of PF4 was determined in PRP in duplicates. The concentration of PF4 in control was around a maximum of ≈3100 pg/ml. All three sugar-free extracts lowered the concentration of PF-4 by around 45–50 % (1678 ± 98 ng/ml) compared with the control (3415 ± 125 ng/ml) ($p < 0.05$).

3.4. Chemical analysis of sugar-free fruit extracts

Upon thorough analysis of the processed data, we observed significant differences in the abundance levels of benzoic acid, caffeic acid, and gallic acid among the sample groups. These differences were confirmed through statistical analysis, and MS/MS fragmentation patterns were consistent with reference standards for these compounds. The differential abundance of these metabolites could be attributed to various factors, including biological variation and treatment effects. Significant Differences in Benzoic Acid, Caffeic Acid, and Gallic Acid: Chemical analysis showed that benzoic acid, caffeic acid, and gallic acid were present significantly in all the fruit extracts (tamarillo, raspberry, and kiwano) that had anti-platelet activity (Fig. 3). In contrast, the pear extracts did not contain detectable amounts of these compounds. Benzoic acid, caffeic acid, and gallic acid were then further evaluated on their platelet anti-aggregatory effect to assess whether they had a role in the inhibitory activity of these fruits. Benzoic, caffeic, and gallic acids showed potent anti-platelet activity (Fig. 4).

4. Discussion

The potent anti-platelet factors were identified in water-soluble extracts of tamarillo, kiwano, and strawberry, which significantly inhibited platelet aggregation. In contrast, the extract of pear had no such effect. Untargeted metabolite analysis suggested that benzoic acid, caffeic acid, and gallic acid were significant in all the fruit extracts (tamarillo, raspberry, and kiwano) but not in pear extracts. We also reported earlier that pear extract had no or little anti-platelet activity (Dutta-Roy et al., 2001).

We also found a decrease in TxB_2 and PF4 levels in response to all three fruits, indicating that these molecular mechanisms may contribute to their anti-platelet effects. These effects are already impressive in their pure form but could be improved with synthetic or recombinant alternatives.

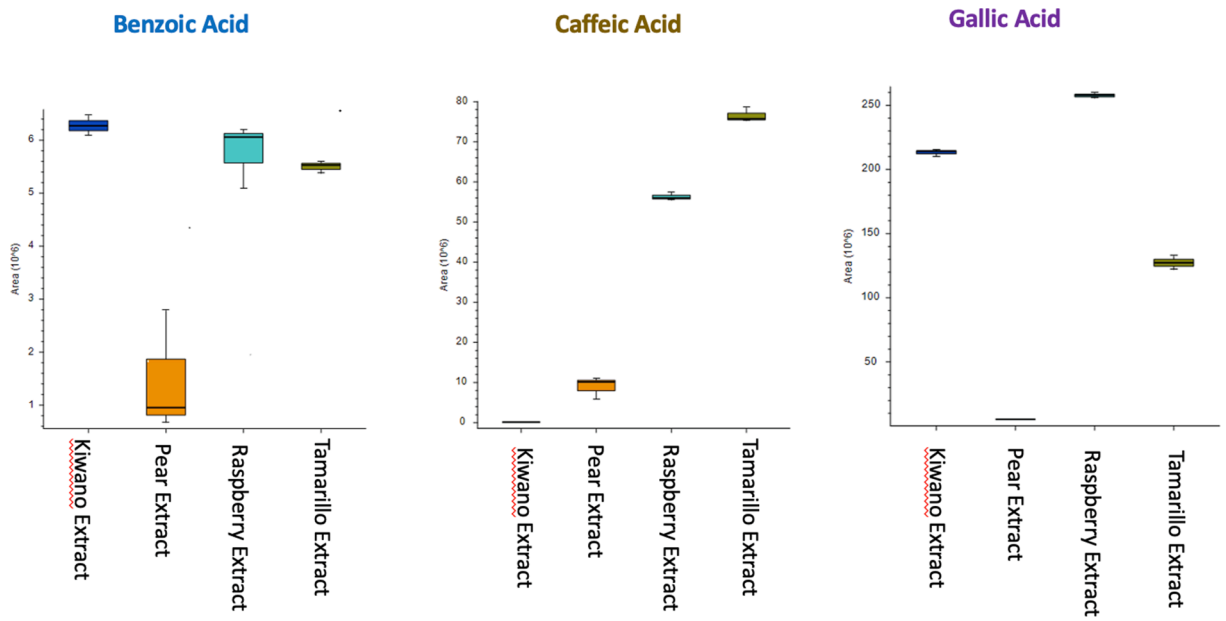

Fig. 3. The presence of benzoic acid, caffeic acid, and gallic acid in the sugar-free raspberry, kiwano, and tamarillo extract was determined by the untargeted analysis. Further chromatographic analyses were conducted to identify the unknown compounds in the sugar-free fruit extracts derived from tamarillo, kiwano, raspberry, and pear. Pear was used again as a negative control to underline differences in components in other fruit extracts.

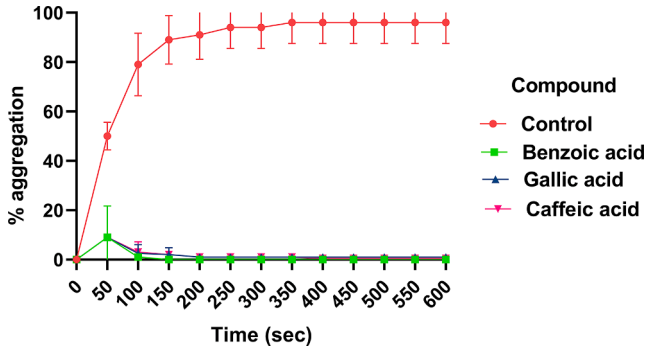

Fig. 4. Inhibition of ADP-induced platelet aggregation in PRP by gallic, benzoic, and caffeic acids PRP was incubated with 10 µl of gallic acid, benzoic acid, and caffeic acid (2 %) for 15 min before adding the ADP (10 µM). The aggregation was measured as described in Fig. 1.

Nutraceuticals are bioactive substances found in everyday food or botanical-based sources. They can be delivered as dietary supplements or functional food, providing beneficial effects and essential nutritional components. Several studies have demonstrated the significant beneficial effects of nutraceuticals on immune system functions, such as boosting immunomodulatory activity and reducing the impacts of autoimmune disorders and hypersensitivity (Basak and Gokhale, 2022; Ooi and Pak, 2021).

Maintaining regular platelet activity is critical to overall hemostasis. However, due to several side effects, anti-platelet drugs (aspirin, etc.) cannot be used as primary prevention. Therefore, alternative safe anti-platelet inhibitors for the population with hyperactive platelets are being sought to reduce the risk of developing CVD. This paper demonstrates that extracts of three fruits, tamarillo, raspberry, and kiwano (horned melon), inhibited ADP-induced platelet aggregation.

In conclusion, the study's findings indicate the promising anti-aggregation potential of these fruit extracts, but further understanding of their mechanisms of action and evaluation for possible clinical use is needed. Human trials should also be conducted to assess the in vitro effects of these fruits. Nevertheless, our data suggest that these fruits have the potential therapeutical and/or preventive application against CVDs and potentially a broader range of platelet-related acquired disorders.

CRediT authorship contribution statement

Agnese Barin: Methodology, Investigation, Formal analysis, Data curation. **Ranjit K. Das:** Methodology, Investigation, Data curation. **Nasser E. Bastani:** Validation, Software, Methodology, Formal analysis, Data curation. **Per Ole Iversen:** . **Asim K. Duttaroy:** .

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.

Data availability

Data will be made available on request.

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Declarations:

Ethical Approval and Consent to Participate: Ethical approval for this project was granted by the Regional Committee for Medical and Health Research Ethics (no. 2015/396/REK Sør-Øst C), Norway.

Consent for publication: All authors have approved the manuscript and agree with its submission for publication in this journal.

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