Heliyon 10 (2024) e25013

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Effects of quercetin and extracts from *Phyllanthus emblica*, *Morus alba*, and *Ginkgo biloba* on platelet recovery in a rat model of chemotherapy-induced thrombocytopenia

Kriangkrai Chawansuntati [a], Sayamon Hongjaisee [a], Kittichai Sirita [a], Kornkamon Kingkaew [a], Kritsadee Rattanathammethee [a], Benjawan Kumrapich [a], Sakaewan Ounjaijean [a], Aphisek Kongkaew [b], Nongkran Lumjuan [a,*]

[a] *Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, 50200, Thailand*
[b] *Research Administration Section, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand*

ARTICLE INFO

Keywords:
Ginkgo biloba
Morus alba
Phyllanthus emblica
Platelet
Quercetin
Thrombocytopenia

ABSTRACT

Background: Chemotherapy-induced thrombocytopenia (CIT) is a major reason for chemotherapy delays, dose reduction, or even treatment discontinuation, which may impact oncologic outcomes. We investigated the effects of quercetin and extracts of *Phyllanthus emblica* fruit (PEE), *Morus alba* leaf (MAE), and *Ginkgo biloba* leaf (GBE) on platelet recovery in a rat model of chemotherapy-induced thrombocytopenia.

Methods: The total phenolic content (TPC), total flavonoid content (TFC), quercetin content, and antioxidant activities of all the extracts were determined. Sixty male Sprague Dawley rats were categorized into healthy controls and CIT groups. The CIT groups was administered a cyclophosphamide solution, while the control group received a saline solution. Each group was then subdivided into five subgroups of six animals which were administered with PEE, MAE, GBE, quercetin, or a vehicle for 15 days.

Results: The highest quercetin content was found in PEE, followed by MAE and GBE, which correlated with their antioxidant properties. Administration of these extracts and quercetin did not significantly change the platelet counts in healthy rats. Thrombocytopenic rats treated with PEE, MAE, and GBE also were not associated with significant changes in platelet counts. However, more rapid platelet count recovery was observed in all groups receiving extracts. On day 11, platelet counts in the PEE, MAE, and GBE groups returned to near baseline levels with a mean of 4.29 %, −40.77 %, and −14.24 %, respectively, compared to −71 % in the CIT group. In thrombocytopenic rats treated with quercetin, there was a significant increase in platelet counts on days 9 and 11, with a mean decrease of 5.41 % from baseline on day 11.

Conclusion: Quercetin improved platelet recovery in the animal model of CIT. This finding merits for further investigation to better elucidate the health benefits of quercetin and quercetin-rich plants and potential pharmacokinetics underpinning their activity in thrombocytopenia.

* Corresponding author. Research Institute for Health Sciences, Chiang Mai University, 110 Intavaroros Road, Sriphum, Muang, Chiang Mai, 50200, Thailand.
E-mail address: nongkran.l@cmu.ac.th (N. Lumjuan).

https://doi.org/10.1016/j.heliyon.2024.e25013
Received 27 October 2023; Received in revised form 17 January 2024; Accepted 18 January 2024
Available online 19 January 2024

1. Introduction

Thrombocytopenia is a condition in which the platelet count falls below 100×10^9/L and may be a result of decreased platelet production from bone marrow stem cells, increased platelet destruction, or sequestration of platelets in the spleen [1]. Thrombocytopenia may be due to viral infection, cancer, immune thrombocytopenic purpura (ITP), toxic chemicals, radiation therapy, and drugs, including chemotherapy. Chemotherapy drugs treat cancer by targeting rapidly dividing cells, including bone marrow cells responsible for generating blood cells and platelets. Chemotherapy-induced thrombocytopenia (CIT) is a potential complication in many cancer patients. Chemotherapy drugs affect platelet production through several pathways including inhibiting megakaryocyte production in the bone marrow, enhancing platelet clearance, and increasing the rate of platelet destruction [2]. CIT is a major cause of treatment delay or chemotherapy dose reduction, which directly affects the treatment of cancer. It has been reported that the relative dose intensity (RDI) of over 80 % of each chemotherapy agent is associated with an improved survival rate [3,4]. Therefore, treatment of thrombocytopenia is an important part of optimizing chemotherapy.

Platelet transfusions are recommended for patients with severe thrombocytopenia to reduce the incidence of bleeding. However, platelet transfusions increase the risk of adverse reactions such as transfer of pathogens, intolerance to blood components, or platelet refractoriness [5]. Moreover, platelet transfusion is not practical to maintain platelet counts through a course of chemotherapy treatment. At present, recombinant human thrombopoietin (rhTPO) and thrombopoietin receptor agonists (TPO-RA) are not approved by the Food and Drug Administration (FDA) for CIT treatment. This is because of their adverse effects and the lack of evidence showing their effectiveness in reducing bleeding or improving survival rates [2]. Recombinant human IL-11 is the only approved drug for the treatment of CIT. However, this drug was discontinued due to its adverse effects such as cardiac and renal fibrosis [6]. Many patients with CIT have limited treatment options due to existing limitations of thrombocytopenia treatments. Therefore, efficacious alternatives to improve platelet recovery after chemotherapy-induced thrombocytopenia, including in medicinal plants is needed.

Medicinal plants have long been used in traditional medicine for the treatment of ailments, including for bleeding diatheses. Papaya juice and leaf extract have been demonstrated to improve platelet count in human and animal models [7]. Guava leaf extract has also been found to increase platelet counts significantly in thrombocytopenic rats treated with cyclophosphamide [8]. Co-administration of Indian gooseberry (*Phyllanthus emblica*) extract in mice treated with arsenic significantly increased platelet count [9]. Rats exposed to gamma irradiation had a significant decrease in hematological indices including platelet counts, while rats treated with ethanolic extract of mulberry (*Morus alba*) leaves before irradiation showed significant improvement in these parameters [10]. Supplementation of *Ginkgo biloba* leaf extract increased platelet count in a dose-dependent manner in fluoride-associated thrombocytopenia in rats [11]. The active ingredient in these extracts that is specifically responsible for these changes currently unknown. However, all are rich in flavonoids. Flavonoids are natural plant compounds belonging to the polyphenol family. They exhibit antioxidant effects and anti-inflammatory activities that contribute to various health benefits for disease prevention [12]. Papaya leaf extract contains seven types of flavonoids such as quercetin, quercetin glycoside, kaempferol, kaempferol glycoside, and myricetin glycoside [13]. Meanwhile, various flavonoid compounds especially quercetin, have been reported in guava [14], Indian gooseberry [15], mulberry [16], and ginkgo [17].

Quercetin is a flavonol that belongs to the flavonoid subclass and can be found in fruits and vegetables such as green leafy vegetables, onions, lettuce, apples, blueberries, and cranberries [18]. Quercetin is a flavonoid compound that contains various pharmacological activities. It is considered to be the most potent flavonoid compound. It is widely known that quercetin possesses strong antioxidant properties. Quercetin has anti-cancer properties in several cancer models by inhibiting tumor proliferation, invasion, and tumor metastasis [19]. Quercetin may also have protective effects against cardiovascular diseases, diabetes, and liver disease [20–22]. Currently, extensive research is being conducted on the health benefits of quercetin in several diseases. Therefore, the mechanism of action against thrombocytopenia may be due to the quercetin compounds in these plants.

In this study, we investigated the effects of quercetin and the extracts of *Phyllanthus emblica* fruits, *Morus alba* leaves, and *Ginkgo biloba* leaves on platelet recovery in animal model of chemotherapy-induced thrombocytopenia.

2. Materials and methods

2.1. Reagents and chemicals

All the reagents and chemicals used in the experiments were of analytical grade. The chemicals, Folin-Ciocalteu reagent, sodium carbonate, aluminum chloride, sodium nitrate, ABTS (2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)), potassium persulfate, and DPPH (2,2-diphenyl-1-picrylhydrazyl) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Cyclophosphamide (Baxter Oncology GmbH, Nordrhein-Westfalen, Germany) was purchased from the pharmacy units of Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand. Carboxymethylcellulose (CMC) was purchased from Loba Chemie (Mumbai, India). Gallic acid, quercetin, and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ginkgo biloba leaf extract powder (20:1) was purchased from Shaanxi Hongda Phytochemistry Co., Ltd., China. The HPLC grade solvent was purchased from J.T. Baker, USA.

2.2. Plant material and extraction

Dried *Phyllanthus emblica* fruits and *Morus alba* (mulberry) leaves were purchased from the local market in Chiang Mai Province, Thailand. These were ground with an electronic mill and passed through a sieve (80 mesh) to obtain a fine powder. The powder was kept dry at −20 °C and protected from humidity and light exposure until extraction.

The extraction process was optimized by varying the ethanol concentration in distilled water to obtain the polyphenol-rich fractions. Briefly, 30 mg sample powder was extracted at room temperature for 3 h with 1.5 mL of 100 %, 75 %, 50 %, 25 %, and 0 % ethanol solutions prepared with distilled water and distilled water without ethyl alcohol. The extracted solutions were filtered and then centrifuged at 13,000×*g* for 20 min at 25 °C. The supernatants were collected and stored at 4 °C until determination of total phenolic and flavonoid contents and antioxidant capacities. After obtaining the appropriate extraction solvent, the extraction process was performed as follows: 40 g of sample powder was mixed with 800 mL of extraction solvent (solid/liquid ratio: 1:20) at room temperature in a shaking incubator for 24 h. The extraction mixture was then centrifuged at 13,000×*g* for 10 min, and then filtered using Whatman grade No. 4 filter paper. The supernatant was then evaporated to remove ethanol until no droplets of the liquid residue were present in the receiving flask using a rotary evaporator at 40 °C, pressure 70 mbar, then lyophilized. All extracts were stored at −20 °C for further analysis.

2.3. Determination of total phenolic content (TPC)

The TPC of the plant extracts was measured using the Folin-Ciocalteu Assay [23] with slight modifications. Briefly, gallic acid was used as the reference standard (50, 100, 150, 250, and 500 μg/mL). The Folin-Ciocalteu reagent was freshly prepared by dilution to 1:10 (v/v) using distilled water. The reaction mixture consisted of 20 μL of extract, 100 μL of Follin-Cicocalteu reagent, and 80 μL of saturated Na_2CO_3 solution. The absorbance of the reaction mixture was measured immediately at 700 nm using the CLARIOstar® Plus Multi-mode Microplate Reader (BMG LABTECH). The TPC values were estimated by plotting on the gallic acid calibration curve and were expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

2.4. Determination of total flavonoid content (TFC)

The total flavonoid content of the crude extracts was determined using the aluminum chloride colorimetric method [24]. Quercetin was used as reference standard (50, 100, 150, 250, and 500 μg/mL). The reaction mixture consisted of 10 μL of extract, 200 μL of distilled water, and 30 μL of $NaNO_2$ solution (5 % w/v). The mixture was mixed and incubated at 37 °C for 5 min, and then 30 μL of aluminum chloride solution (10 %; w/v) was added. After incubation at 37 °C for 6 min, the reaction was mixed with 20 μL of 10 mM NaOH solution. The absorbance of the reaction mixture was measured at 490 nm using the CLARIOstar® Plus Multi-mode Microplate Reader (BMG LABTECH). The TFC values were estimated by plotting on the calibration curve and were expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE/g).

2.5. Quantitative determination of quercetin content

A high-performance liquid chromatographic (HPLC) method was developed to quantify quercetin in the extracts. Briefly, 0.25 g of dried extracts were dispersed in 5 mL of 6 N HCl in 50 % (v/v) of methanol/water. The mixture was sonicated at 60 °C for 30 min. The solution was filtered through 2.5 μm filter paper followed by a 0.2 μm nylon membrane filter. The solution was collected and subjected to HPLC analysis using a Shimadzu-LC20A system (Shimadzu, Japan) equipped with a CBM-20A controller, LC-20AT pump, DGU-20A5 prominence degasser, SIL-20A autosampler SPD-20AV detector, and CTO-10ASvp column oven. Chromatographic separations were achieved using an Inertsil ODS-C18 column (150 mm × 4.6 mm inner diameter, 3.5 μm particle size). A reverse-phase HPLC assay was carried out using isocratic elution with a flow rate of 1.0 mL/min, column temperature of 30 °C, a mobile phase of 100 % methanol, and a detection wavelength of 360 nm. The injection volume was 20 μL of each solution. The total run time was 10 min for each injection. Data was acquired and processed using LC-Solution Software.

2.6. ABTS radical scavenging capacity measurement

The ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) free radical assay was performed using a previously described method [25] with some modifications. Briefly, the ABTS radical solution was generated by mixing equal volumes of ABTS solution (7 mM) and potassium persulfate solution (2.45 mM), and the mixture was kept in the dark at room temperature for 12–16 h. Before use, the ABTS solution was diluted with ethanol to obtain an absorbance of 0.7 (±0.02) at 734 nm. Ascorbic acid and quercetin were used as standard antioxidants. The ABTS free radical assay was performed by mixing 10 μL of extract or various concentrations of standard antioxidant with 195 μL of ABTS solution. After 30 min of incubation in the dark at room temperature, the decreased absorbance was measured at 734 nm. The percentage radical scavenging activity (% RSA) was calculated using the following formula:

$$\%\ \mathrm{RSA} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100$$

Abs control was the absorbance of the blank (without samples), and Abs sample was the absorbance of tested sample. Dose-response curves were plotted with a five-parameter regression model using the GraphPad Prism 5.01 software. 50 % effective concentration (EC50) values were calculated.

2.7. DPPH radical scavenging capacity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay was performed [26] with minor modifications. In brief, the reaction mixture consisted of 100 μL of the extract or standard antioxidant and 100 μL of DPPH radical solution prepared in absolute ethanol (0.2 mM). The absorbance of the reaction mixture was measured at 517 nm after 30 min incubation in the dark at room temperature. The percentage radical scavenging activity (% RSA) was calculated using the following formula:

$$\%\ \mathrm{RSA} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100$$

Abs control was the absorbance of the blank (without samples), and Abs sample was the absorbance of the tested sample. Dose-response curves were plotted with a five-parameter regression model using the GraphPad Prism 5.01 software. EC50 values were calculated.

2.8. Animals

Male Sprague Dawley (SD) rats aged 7–8 weeks (weighing 200–250 g) were purchased from Nomura-Siam International Co. Ltd., Thailand. The rats used in this study were housed in 2 rats per individual ventilated cages (IVC) and maintained at room temperature (25 ± 2 °C) with a controlled cycle of 12 h light and dark and access to food and water ad libitum. Animals were acclimatized to the experimental conditions for one week before the initiation of the experiment. The study was approved by the Institutional Animal Ethics Committee of The Faculty of Medicine, Chiang Mai University, Thailand (code no. 12/2565).

2.9. Animal experimental design

Sixty rats were divided into two main groups, the healthy control groups and the thrombocytopenic groups. The healthy control groups received normal saline solution at 5 mL/kg body weight injected subcutaneously once a day on days 0, 1, and 2 for a total of 3 doses. The healthy control groups were further subdivided into five subgroups of six animals in each group. Group I served as the control group that received saline solution. Groups II, III, and IV were control groups that were given 400 mg/kg body weight of the plant extracts, *Phyllanthus emblica* fruit extract (PEE), *Morus alba* leaf extract (MAE), and *Ginkgo biloba* leaf extract (GBE), respectively. Group V was a control group that received a 50 mg/kg body weight dose of quercetin in 0.5 % (w/v) sodium carboxymethylcellulose aqueous solution. In the thrombocytopenic groups, thrombocytopenia was induced in the rats by administering 50 mg/kg of cyclophosphamide solution subcutaneously once a day on days 0, 1, and 2. These were similarly subdivided into five subgroups of six animals. Group VI served as the thrombocytopenic group that received saline solution only (CIT). Groups VII, VIII, and IX were the thrombocytopenic groups that received the plant extracts, PEE, MAE, and GBE, respectively. Group X served as the thrombocytopenic group that received a quercetin solution. All groups were given saline solution or plant extracts or quercetin on day 0 of the study by intragastric administration for 15 days. On days 0, 3, 7, 9, 11, 13 15, 17, and 22, blood was collected from the leg veins in EDTA-containing tubes for measurement of hematologic parameters.

2.10. Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM). Data was calculated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using GraphPad Prism (Version 9.0 - GraphPad Software, San Diego, CA, United States). The significance of the difference between the mean values of the control and treated animals was determined by an independent samples *t*-test. $p < 0.05$ was considered to be statistically significant.

Table 1
Total phenolic, and flavonoid content of extracts from *Phyllanthus emblica* fruits and *Morus alba* leaves.

Extraction solvent	*Phyllanthus emblica* fruit extract		*Morus alba* Leaf extract	
	TPC	TFC	TPC	TFC
aqueous	103.93 ± 2.93[a]	87.72 ± 3.20[a]	8.73 ± 0.24[a]	11.89 ± 0.70 [b]
25% Ethanol	117.59 ± 2.40[a]	110.09 ± 4.64[a]	8.55 ± 0.08[a]	12.28 ± 0.67 [b]
50 % Ethanol	132.67 ± 1.75[a]	105.43 ± 2.63[a]	7.37 ± 0.27[a]	9.95 ± 0.36 [b]
75% Ethanol	133.47 ± 2.55[a]	109.03 ± 0.90[a]	4.38 ± 0.17[a]	14.26 ± 0.97 [b]
100% Ethanol	47.31 ± 0.76 [b]	47.54 ± 1.48 [b]	1.81 ± 0.47 [b]	25.93 ± 1.23[a]

The values represent the mean ± SEM (n = 3). The different letters a and b within the same column showed a statistically significant difference at a confidence level of 95 % ($p < 0.05$) analyzed using one-way ANOVA followed by Tukey's Honest Significant Difference test. TPC, total phenolic content (mg GAE/g dry weight); TFC, total flavonoid content (mg QE/g dry weight); GAE, gallic acid equivalents; QE, quercetin equivalents.

3. Results

3.1. Optimization of the extraction solvent

This study aimed to determine the optimal ethanol concentration for extracting flavonoids. The results suggest that the extraction efficiency of flavonoid compounds is influenced by the ratio of ethanol to water used (Table 1). *Morus alba* leaf extracted with ethanol-water solvent at concentrations of 0, 25, 50, 75, and 100 % showed high total phenolics content in the ethanol-water solvent at concentrations of 0 %, and 25 %, while the total flavonoids contents were high in the ethanol-water mixture at the concentrations of 100 %, 75 %, and 25 %, respectively (Table 1). The phenolics and flavonoids were high in *Phyllanthus emblica* fruits extracted with the ethanol-water mixture at concentrations of 25 %, 50 %, and 75 %. The antioxidant capacity of *Morus alba* leaf extract and *Phyllanthus emblica* fruit extract were high in the extraction solvents containing ethanol at concentrations of 25–75 % (Table 2). This study evaluated the antioxidant capacity, phenolic and flavonoid content, and determined the most effective extraction solvent while considering the use of ethanol. The results showed that 25 % ethanol was the best solvent for extraction, as it was able to extract essential substances with high biological activity.

3.2. Extraction yield, total phenolic, flavonoid, and quercetin contents

In this study, 25 % ethanol was used to extract flavonoids from plant materials for animal studies. The extraction yield, total phenolic content, and total flavonoid content of the extracts are shown in Table 3. *Phyllanthus emblica* fruit extract and *Morus alba* leaf extract had an extraction yield of 41.53 and 24.07 g per 100 g dried weight, respectively. In the determination of total phenolic content by the Folin-Ciocalteu method, the extracts of *Phyllanthus emblica* fruits had the highest phenolic content at 274.61 ± 4.26 (mg GAE/g dry extract), followed by *Morus alba* leaves (30.04 ± 0.77 mg GAE/g dry extract) and *Ginkgo biloba* leaves (17.23 ± 0.28 mg GAE/g dry extract). *Phyllanthus emblica* fruit extract contained the highest amount of flavonoid compounds at 101.99 ± 1.79 mg QE/g dry extract, followed by *Morus alba* leaf extract and *Ginkgo biloba* leaf extract (34.56 ± 0.20 and 14.50 ± 0.58 mg QE/g dry extract, respectively). Quercetin content in the extracts was determined by HPLC after acid hydrolysis. The analysis revealed that *Phyllanthus emblica* fruits had the highest concentration of quercetin, followed by *Morus alba* leaves and then *Ginkgo biloba* leaves. These results are consistent with the total flavonoid content in the extracts.

3.3. Antioxidant activity

The antioxidant activity of the extracts were evaluated using the ABTS and DPPH assays, and the results are shown in Table 4. Antioxidant capacities were highest in *Phyllanthus emblica* fruit extract followed by *Morus alba* leaf extract and then *Ginkgo biloba* leaf extract. *Phyllanthus emblica* fruit extract showed the most potent antioxidant activity against free radical ABTS (EC50 value = 4.43 ± 0.07 μg/mL) and DPPH (EC50 value = 9.57 ± 0.74 μg/mL) compared to *Morus alba* leaf extract and *Ginkgo biloba* leaf extract. The extract from *Phyllanthus emblica* fruits exhibited anti-oxidative bioactivity comparable to ascorbic acid and quercetin standardized compounds. The antioxidant capacities of the extracts were positively correlated with the total phenolic, total flavonoid, and quercetin contents (Table 3).

3.4. Changes in hematological parameters after cyclophosphamide treatment in rat model

Administration of cyclophosphamide (50 mg/kg subcutaneously) resulted in statistically significant reductions in the number of white blood cells (WBCs), red blood cells (RBCs), platelet (PLTs), and hemoglobin content (HGB) compared with baseline (Day-0) (Table 5). There were no significant changes in hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). Changes in the WBC, RBC, and platelet count, along with HGB in rats receiving saline solution and cyclophosphamide are shown in Fig. 1. After the administration of cyclophosphamide, the WBC counts immediately decreased from day 1 and reached a nadir

Table 2
EC50 values of antioxidants in ABTS and DPPH radical scavenging assays.

	EC50 of *Phyllanthus emblica* Extract		EC_{50} of *Morus alba* Extract	
	ABTS (μg/mL)	DPPH (μg/mL)	ABTS (μg/mL)	DPPH (μg/mL)
aqueous	7.21 ± 0.66	5.39 ± 0.59[a]	249.97 ± 20.76[a]	837.75 ± 107.25[b]
25%Ethanol	7.17 ± 0.39	4.82 ± 0.19[a]	210.80 ± 15.72[a]	222.33 ± 8.42[a]
50 % Ethanol	5.84 ± 0.57	4.38 ± 0.43[a]	202.57 ± 9.28[a]	198.23 ± 19.05[a]
75%Ethanol	7.24 ± 0.35	4.75 ± 0.14[a]	300.00 ± 0.00[a]	354.75 ± 19.75[a]
100%Ethanol	10.75 ± 2.42	8.54 ± 0.49[b]	788.90 ± 50.20[b]	–

Each value represents the mean ± SEM (n = 3). The different letters a and b within the same column showed a statistically significant difference in the antioxidant capacity of the compounds at a confidence level of 95 % ($p < 0.05$) analyzed using one-way ANOVA followed by Tukey's Honest Significant Difference test. nd indicates not detectable. EC50 value, defined as the effective concentration of the extracts that are required to scavenge ABTS and DPPH radicals by 50 %. Dash = not determined. ABTS, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl.

Table 3
Total phenolic, flavonoid, and quercetin content of extracts from *Phyllanthus emblica* fruits, *Morus alba* leaves, and *Ginkgo biloba* leaves.

Extracts	Extraction yield (%)	TPC (mg GAE/g dry extract)	TFC (mg QE/g dry extract)	Quercetin content (mg/g dry extract)
PEE	41.53	274.61 ± 4.26[a]	101.99 ± 1.79[a]	10.20 ± 1.04[a]
MAE	24.07	30.04 ± 0.77[b]	34.56 ± 0.20[b]	6.29 ± 1.20[b]
GBE	nd	17.23 ± 0.28[c]	14.50 ± 0.58[c]	3.40 ± 0.11[c]

Each value represents the mean ± SEM (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Different letters within the same column indicate statistically significant differences ($p < 0.05$). nd indicates not detectable. Quercetin content was determined by HPLC after acid hydrolysis. TPC, total phenolic content; TFC, total flavonoid content; GAE, gallic acid equivalents; QE, quercetin equivalents; PEE, *Phyllanthus emblica* fruit extract; MAE, *Morus alba* leaf extract; GBE, *Ginkgo biloba* leaf extract.

Table 4
Antioxidant activities of different plant extracts on ABTS and DPPH assays.

Plant Extracts/standards	Antioxidant activity (EC50 (μg/mL))	
	ABTS	DPPH
PEE	4.43 ± 0.07[a]	9.57 ± 0.74[a]
MAE	82.80 ± 6.00[b]	180.70 ± 0.38[b]
GBE	175.40 ± 13.00[c]	287.30 ± 54.25[c]
Ascorbic acid	3.83 ± 0.03[a]	3.80 ± 0.06[a]
Quercetin	1.60 ± 0.00[a]	2.67 ± 0.03[a]

Each value represents the mean ± SEM (n = 3). Different letters a and b within the same column showed a statistically significant difference in the antioxidant capacity of the compounds at a confidence level of 95 % ($p < 0.05$) analyzed using one-way ANOVA followed by Tukey's Honest Significant Difference test. nd indicates not detectable. PEE, *Phyllanthus emblica* fruit extract; MAE, *Morus alba* leaf extract; GBE, *Ginkgo biloba* leaf extract; ABTS, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl.

Table 5
Hematological parameters in Sprague Dawley rats after cyclophosphamide treatment.

Hematological parameters	Days after cyclophosphamide injection								
	Day-0	Day-3	Day-7	Day-9	Day-11	Day-13	Day-15	Day-17	Day-22
WBC (x10^3/μL)	8.32 ± 0.82	1.01 ± 0.06[c]	0.32 ± 0.03[c]	1.15 ± 0.35	3.04 ± 0.74	10.38 ± 2.62	13.22 ± 4.07	10.54 ± 2.46	5.63 ± 0.98
RBC (x10^6/μL)	7.89 ± 0.20	7.68 ± 0.11	7.27 ± 0.11	6.03 ± 0.66[c]	5.91 ± 0.59 [b]	5.52 ± 0.51[a]	5.58 ± 0.42[a]	6.03 ± 0.33 [b]	7.73 ± 0.29
HGB (g/dl)	16.30 ± 0.26	15.75 ± 0.34	14.66 ± 0.33[a]	11.93 ± 1.10[a]	11.62 ± 0.89[a]	11.14 ± 0.87[a]	11.48 ± 0.83[a]	12.32 ± 0.62[a]	15.22 ± 0.46
HCT (%)	47.80 ± 0.65	45.72 ± 0.94	41.94 ± 1.02	34.00 ± 3.28	33.78 ± 2.79	33.44 ± 3.11	35.62 ± 2.42	38.42 ± 1.68	37.28 ± 1.37
MCV (fL)	60.72 ± 1.33	59.57 ± 1.23	57.70 ± 1.62	56.73 ± 1.18	57.56 ± 1.62	60.74 ± 1.99	64.28 ± 2.43	64.04 ± 2.29	61.38 ± 1.68
MCH (pg)	20.72 ± 0.49	20.52 ± 0.46	20.16 ± 0.55	19.93 ± 0.52	19.88 ± 0.62	20.34 ± 0.56	20.66 ± 0.63	20.50 ± 0.69	19.78 ± 0.68
MCHC (g/dL)	34.07 ± 0.13	34.45 ± 0.14	34.98 ± 0.16	35.13 ± 0.20	34.52 ± 0.36	33.54 ± 0.63	32.18 ± 0.37[a]	32.04 ± 0.30[a]	32.18 ± 0.35[a]
RDW-CV (%)	12.38 ± 0.30	12.53 ± 0.30	12.12 ± 0.28	12.03 ± 0.32	12.02 ± 0.34	15.52 ± 1.51	24.40 ± 1.92[a]	22.48 ± 0.61[a]	18.74 ± 0.52[a]
RDW-SD (fL)	31.58 ± 0.80	31.37 ± 1.06	29.38 ± 1.13	28.60 ± 0.29	28.48 ± 0.96	37.60 ± 3.82	64.42 ± 7.01[a]	59.94 ± 2.83[a]	47.84 ± 1.31[a]
PLT (x10^5/μL)	10.47 ± 0.31	6.82 ± 0.22	0.59 ± 0.20 [b]	0.77 ± 0.15 [b]	2.89 ± 0.67[c]	10.67 ± 4.71	11.99 ± 2.37	15.68 ± 1.31	10.18 ± 1.06
MPV (fL)	6.43 ± 0.12	6.17 ± 0.11	7.38 ± 0.32 [b]	7.13 ± 0.27[c]	7.46 ± 0.05[a]	7.32 ± 0.09 [b]	7.16 ± 0.19[c]	6.70 ± 0.17	6.52 ± 0.07
PDW	15.15 ± 0.02	14.98 ± 0.04	15.34 ± 0.21	15.40 ± 0.14	15.70 ± 0.09 [b]	15.92 ± 0.12[a]	15.72 ± 0.12 [b]	15.44 ± 0.10	15.32 ± 008
PCT (%)	0.67 ± 0.03	0.42 ± 0.01[c]	0.04 ± 0.01[a]	0.05 ± 0.01[a]	0.21 ± 0.05[a]	0.45 ± 0.11	0.65 ± 0.15	0.85 ± 0.01	0.67 ± 0.08

Results are expressed as mean ± SEM, (n = 4–6/group). One-way ANOVA, followed by Tukey's post-hoc test was used. Different letters within the same row indicate statistically significant differences; [a] $p < 0.001$, [b] $p < 0.01$, and [c] $p < 0.05$ when compared to baseline (Day-0). WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red blood cell distribution width coefficient of variation; RDW-SD, red blood cell distribution width standard deviation; PLT, platelet; MPV, mean platelet volume; PDW, platelet distribution width; PCT, platelet crit.

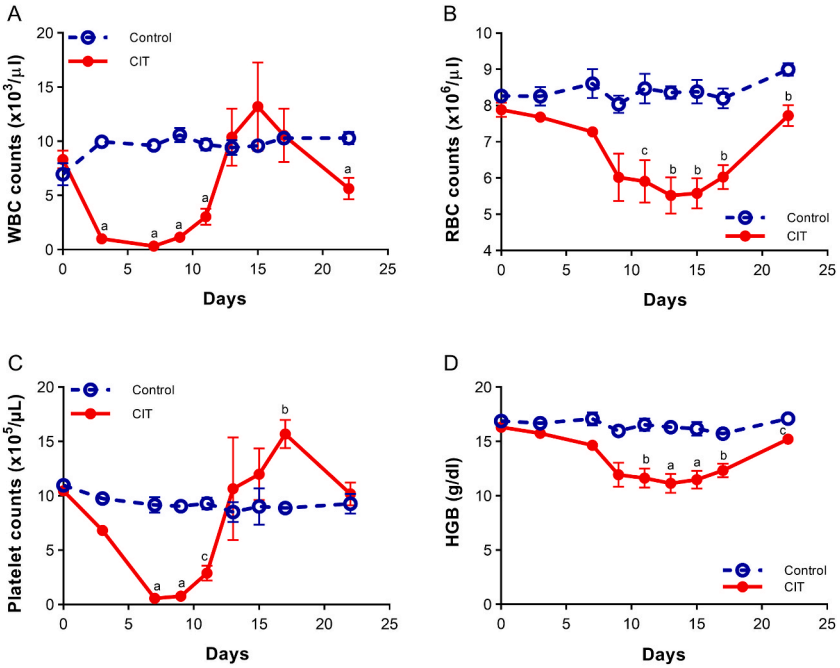

Fig. 1. The effect of saline solution (open circle) and cyclophosphamide (filled circle) on white blood cell count (A), red blood cell count (B), platelet count (C), and hemoglobin level (D) of Sprague Dawley rats. Rats were given 50 mg of cyclophosphamide per kg of body weight subcutaneously for 3 days on days 0, 1, and 2. Blood was drawn from the saphenous vein for hematologic examination. The numbers of white cells, red cells, platelets, and hemoglobin were determined by a blood analyzer on days 0, 3, 7, 9, 11, 13, 15, 17, and 22 after cyclophosphamide treatment. Results are expressed as mean ± SEM, (n = 4–6/group). One-way ANOVA, followed by Tukey's post-hoc test was used. Different letters indicate statistically significant differences; $^{a}p < 0.001$, $^{b}p < 0.01$, and $^{c}p < 0.05$ when compared to the control group at the respective time. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

on days 3–7 before returning to normal range by day 17 (Fig. 1A). The RBC count and hemoglobin content of cyclophosphamide-treated animals exhibited a similar pattern of decline, reaching the lowest point on days 7–15 before progressing to near-baseline by day 22 (Fig. 1B and D). Cyclophosphamide administration resulted in biphasic changes in platelet counts in the

Table 6
The effects of plant extracts or quercetin on platelet counts in cyclophosphamide-induced rats.

Groups	Platelet count (x10^5/µL)								
	Day-0	Day-3	Day-7	Day-9	Day-11	Day-13	Day-15	Day-17	Day-22
Control	10.98 ± 0.44	9.77 ± 0.47	9.17 ± 0.70	9.06 ± 0.12	9.30 ± 0.56	8.53 ± 0.91	9.03 ± 1.66	8.89 ± 0.43	9.29 ± 0.90
PEE	10.31 ± 0.99	10.35 ± 2.60	8.97 ± 1.03	9.47 ± 0.52	9.54 ± 0.53	8.58 ± 0.75	8.80 ± 0.85	8.75 ± 0.63	8.87 ± 0.59
MAE	10.09 ± 1.03	8.00 ± 1.02	10.05 ± 0.58	10.33 ± 0.71	9.45 ± 0.52	8.68 ± 0.97	10.34 ± 0.92	8.23 ± 0.96	8.80 ± 0.44
GBE	10.45 ± 0.55	8.59 ± 0.57	9.77 ± 0.54	9.31 ± 0.50	9.73 ± 0.49	10.19 ± 1.43	9.37 ± 0.43	9.14 ± 0.59	9.04 ± 0.48
Q	9.79 ± 0.67	8.64 ± 0.33	9.97 ± 0.53	8.55 ± 0.66	9.34 ± 0.70	9.70 ± 0.71	9.04 ± 0.99	9.83 ± 0.30	8.47 ± 0.41
CIT	10.47 ± 0.31	6.82 ± 0.22^{c}	0.59 ± 0.21^{c}	0.77 ± 0.15^{c}	2.89 ± 0.67^{c}	10.67 ± 4.71	11.99 ± 2.37	15.68 ± 1.31	10.18 ± 1.06
CIT + PEE	8.72 ± 1.36	6.89 ± 0.42	0.50 ± 0.11^{c}	1.78 ± 0.59^{c}	6.88 ± 1.98	12.88 ± 2.76	16.53 ± 1.26	15.31 ± 1.72	8.95 ± 0.33
CIT + MAE	10.15 ± 0.39	7.01 ± 0.37^{c}	0.73 ± 0.22^{c}	1.99 ± 0.63^{c}	5.83 ± 1.53	11.99 ± 2.12	16.76 ± 0.83	14.33 ± 1.20	8.25 ± 0.36
CIT + GBE	9.86 ± 0.68	6.30 ± 0.59^{c}	1.09 ± 0.22^{c}	2.71 ± 0.73^{c}	8.16 ± 2.21	13.59 ± 2.26	15.31 ± 0.65	15.83 ± 2.37	9.27 ± 0.94
CIT + Q	10.06 ± 0.61	6.85 ± 0.40^{c}	1.00 ± 0.18^{c}	3.23 ± 0.68 ^{bc}	8.98 ± 1.65 ^{b}	12.54 ± 1.44	15.41 ± 0.83	13.44 ± 0.88	8.38 ± 0.40

Results are expressed as mean ± SEM, (n = 4–6/group). An independent samples *t*-test was used; $^{a}p < 0.05$ in PEE, MAE, GBE, and quercetin groups when compared to the control group at the respective time, $^{b}p < 0.05$ in thrombocytopenic groups received PEE, MAE, GBE, and quercetin when compared to the CIT group at the respective time, and $^{c}p < 0.05$ when compared to the baseline (Day-0). CIT, chemotherapy (cyclophosphamide)-induced thrombocytopenia; PEE, *Phyllanthus emblica* fruit extract; MAE, *Morus alba* leaf extract; GBE, *Ginkgo biloba* leaf extract.

thrombocytopenic group. The platelet count decreased by about 30 % of baseline on day 3, reaching a nadir of approximately 89–90 % of the baseline on days 7–9 before recovery, reaching a peak of 50 % above the baseline on day 17. The values then returned to baseline by day 22 (Fig. 1C).

3.5. The effects of plant extracts or quercetin on platelet counts in cyclophosphamide-induced rats

The effects of cyclophosphamide treatment and co-administration of plant extracts or quercetin on platelet counts are presented in Table 6. Administration with PEE, MAE, GBE, and quercetin did not significantly alter platelet counts compared to saline administration in the healthy control group rats. Platelet counts decreased statistically significantly in all groups receiving cyclophosphamide, reaching a nadir on days 7 and 9. On days 9 and 11, rats treated with cyclophosphamide and quercetin had significantly higher platelet counts ($p < 0.05$) compared to the CIT group. Thrombocytopenic rats that received PEE, MAE, and GBE did not show any changes in platelet values compared to the CIT group. Responses of rats in each group, presented by the percentage change in platelet count from baseline, are shown in Fig. 2. No statistically significant increase in platelet counts was observed in thrombocytopenic rats receiving PEE, MAE, and GBE (Table 6). Thrombocytopenic rats treated with PEE, MAE, and GBE showed more rapid platelet count recovery compared to the CIT group. By day 11, platelet counts in the PEE, MAE, and GBE groups returned to near baseline levels with a mean of 4.29 %, −40.77 %, and −14.24 %, respectively, compared to −71 % in the CIT group (Fig. 2A–C). On days 9 and 11, thrombocytopenic rats receiving quercetin showed a statistically significant increase in platelet count when compared to the CIT group, with a mean decrease of 5.41 % from baseline (Table 6 and Fig. 2D).

4. Discussion

We evaluated the effect of quercetin and the extracts of *Phyllanthus emblica* fruit (PEE), *Morus alba* leaf (MAE), and *Ginkgo biloba* leaf (GBE) on an animal model of chemotherapy-induced thrombocytopenia. The PEE contained the highest quercetin content followed by MAE and GBE which were related to their anti-oxidative bioactivity. Quercetin and all three extracts were not associated with a statistically significant increase in platelet counts in normal healthy rats. In contrast, quercetin supplementation was linked to more rapid recovery of platelet counts in cyclophosphamide-induced thrombocytopenia in rats. However, there was no statistically significant increase in platelet counts in thrombocytopenic rats receiving PEE, MAE, and GBE.

We observed that quercetin was associated with a faster recovery of platelets in rats with cyclophosphamide-induced thrombocytopenia. The role of quercetin in modulating platelet count is less studied in animal models of CIT. However, the link between quercetin administration and the recovery of platelet counts in CIT in our study is consistent with previous studies on thrombocytopenia in other experimental animals models. In rats treated with cadmium and polychlorinated biphenyl, administration of quercetin was linked to significantly increased platelet counts compared to treated groups [27,28]. These studies demonstrated that quercetin effectively enhances platelet recovery and regulates platelet count in thrombocytopenia caused by various chemical agents. Administration of quercetin can reduce abnormally high platelet counts to normal levels in rats treated with difenoconazole [29]. Furthermore, our experiment showed that quercetin did not affect platelet count in normal animals. These findings suggest that quercetin may be the compound of choice because it modulates abnormal platelet counts.

We found that PEE has the highest concentration of quercetin, followed by MAE and GBE. However, PEE and GBE increased platelet counts to near baseline faster than MAE but not significantly. The results of this study are not in line with the hypothesis. This may be due to the low quercetin content in these extracts, or the different types of quercetin glycosides found in these plants. Due to limited resources and a lack of equipment, phytochemical profiles were not identified in this study. However, these plants have been found to contain quercetin derivatives, as reported by multiple studies. Quercetin, kaempferol, and their derivatives, including quercetin 3-β-D-glucopyranoside and kaempferol 3-β-D-glucopyranoside, have been identified in *Phyllanthus emblica* fruits [30]. The flavonol glycosides quercetin-3-(6-malonyl glucoside) and quercetin-3-*O*-rutinoside are found in mulberry leaves extracted with 60 % ethanol, while the water extract of mulberry leaves contains flavonols in the following order: quercetin-3-β-D-glucose, quercetin-3-*O*-glucose-6-acetate, rutin, and quercetin, respectively [31,32]. The *Ginkgo biloba* leaves consist of more than 30 flavonoids, mainly flavonol glycosides such as quercetin, kaempferol, and isorhamnetin [33]. It has been noted that consuming quercetin-rich foods does not guarantee the highest bioavailability of quercetin. This is because the bioavailability of quercetin is influenced by various factors, including the forms of the sugar moiety [18]. Therefore, the role of quercetin in platelet recovery might differ between various quercetin forms.

One of the limitations of this study is that the mechanisms, including histopathological data on the bone marrow of quercetin activity and platelet recovery, have not been entirely elucidated in animal models. However, quercetin-3-glucuronide, a quercetin metabolite, can increase platelet count by inhibiting platelet apoptosis in rats with immunological bone marrow failure [34]. Quercetin has also been found to increase the number of bone marrow cells, including platelets, in animal models of cisplatin-induced myelosuppression by elevating levels of hematopoietic growth factors [35]. Quercetin and its metabolites have demonstrated therapeutic activities against various diseases as it has antioxidant, antimicrobial, antidiabetic, anti-inflammatory, anti-Alzheimer's, antiarthritic, anticancer, anti-atherogenic, and wound-healing effects [36]. Its role in platelet recovery in CIT has been little studied. These findings highlight the potential medicinal properties of quercetin in specific health conditions related to thrombocytopenia.

In conclusion, *Phyllanthus emblica* fruit extract (PEE) exhibited higher antioxidant activities than *Morus alba* leaves extract (MAE) and *Ginkgo biloba* leaves extract (GBE) which may be related to the high content of total phenolic, flavonoid, and quercetin contents. Quercetin, PEE, MAE, and GBE did not affect platelet counts in normal healthy rats. We have found that platelet count recovers more quickly for PEE, MAE, and GBE in thrombocytopenic rats. Although, there were no significant changes in platelet counts. However, only quercetin improved platelet recovery in a rat model of chemotherapy-induced thrombocytopenia. This data may serve as the basis

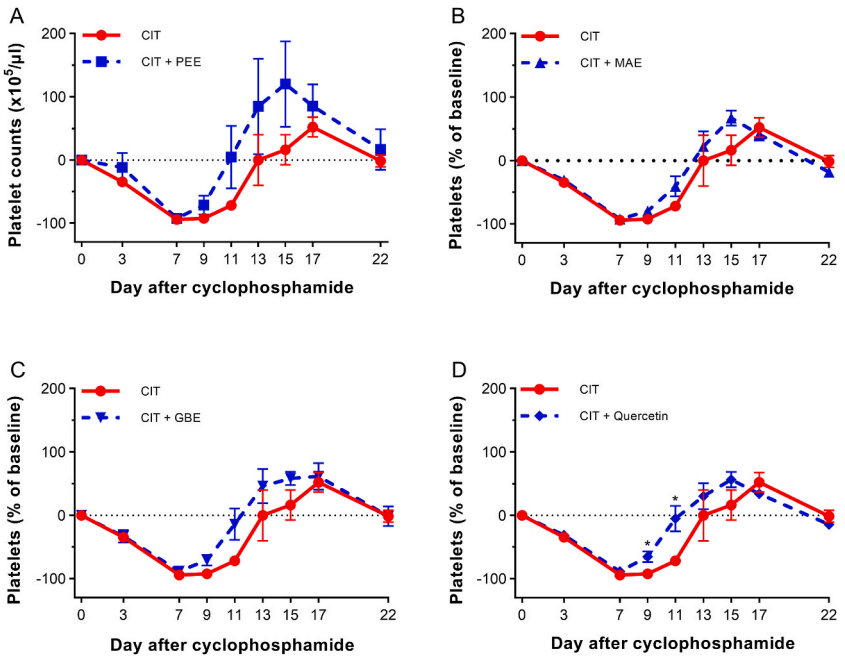

Fig. 2. Effect of PEE (A), MAE (B), and GBE (C) and quercetin (D) on the mean percentage change in platelet counts from baseline (Day 0) in male Sprague-Dawley rats after cyclophosphamide treatment.
Rats were given 50 mg of cyclophosphamide per kg of body weight subcutaneously for 3 days on days 0, 1, and 2. Rats began receiving saline solution or the test substances: PEE, MAE, and GBE extract at a dose of 400 mg/kg body weight, or quercetin at a dose of 50 mg/kg body weight on day 0 of the study by intragastric administration. Blood collection from the veins of the legs was performed on days 0, 3, 7, 9, 11, 13 15, 17, and 22. Data are presented as the mean percentage change of baseline in platelet count (Day 0) of each rat and expressed as mean ± S.E.M. (n = 4–6/group). An independent samples *t*-test was used. A significant difference is indicated by *($p < 0.05$) when compared to the CIT group at the respective time. CIT, chemotherapy (cyclophosphamide)-induced thrombocytopenia; PEE, *Phyllanthus emblica* fruit extract; MAE, *Morus alba* leaf extract; GBE, *Ginkgo biloba* leaf extract.

for investigating the health benefits of quercetin and quercetin-rich plants in thrombocytopenia.

Funding

This research project was supported by Fundamental Fund 2022, Chiang Mai University, Thailand.

Ethical statement

The animal experimental protocol was approved by the Institutional Animal Ethics Committee of the Faculty of Medicine, Chiang Mai University, Thailand (code no. 12/2565).

Data availability statement

The data for this study has not been deposited in any public repository. However, it is available from the corresponding author upon request.

CRediT authorship contribution statement

Kriangkrai Chawansuntati: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Sayamon Hongjaisee:** Writing – original draft, Formal analysis. **Kittichai Sirita:** Investigation. **Kornkamon Kingkaew:** Investigation. **Kritsadee Rattanathammethee:** Investigation. **Benjawan Kumrapich:** Investigation. **Sakaewan Ounjaijean:** Investigation, Formal analysis. **Aphisek Kongkaew:** Supervision. **Nongkran Lumjuan:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization.

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.

References

[1] K.J. Smock, S.L. Perkins, Thrombocytopenia: an Update, vol. 36, 2014, pp. 269–278, https://doi.org/10.1111/ijlh.12214, 3.
[2] D.J. Kuter, Treatment of chemotherapy-induced thrombocytopenia in patients with non-hematologic malignancies, Haematologica 107 (6) (2022) 1243–1263, https://doi.org/10.3324/haematol.2021.279512.
[3] S. Loibl, T. Skacel, V. Nekljudova, H.J. Lück, M. Schwenkglenks, T. Brodowicz, et al., Evaluating the impact of Relative Total Dose Intensity (RTDI) on patients' short and long-term outcome in taxane- and anthracycline-based chemotherapy of metastatic breast cancer- a pooled analysis, BMC Cancer 11 (2011) 131, https://doi.org/10.1186/1471-2407-11-131.
[4] A. Luciani, C. Bertuzzi, G. Ascione, E. Di Gennaro, S. Bozzoni, S. Zonato, et al., Dose intensity correlate with survival in elderly patients treated with chemotherapy for advanced non-small cell lung cancer, Lung Cancer 66 (1) (2009) 94–96, https://doi.org/10.1016/j.lungcan.2008.12.019.
[5] V. Kiefel, Reactions induced by platelet transfusions, Transfus. Med. Hemotherapy 35 (5) (2008) 354–358, https://doi.org/10.1159/000151350.
[6] I. Tepler, L. Elias, J.W. Smith 2nd, M. Hussein, G. Rosen, A.Y. Chang, et al., A randomized placebo-controlled trial of recombinant human interleukin-11 in cancer patients with severe thrombocytopenia due to chemotherapy, Blood 87 (9) (1996) 3607–3614.
[7] S. Munir, Z.W. Liu, T. Tariq, R. Rabail, P.L. Kowalczewski, J. Lewandowicz, et al., Delving into the therapeutic potential of Carica papaya leaf against thrombocytopenia, Molecules 27 (9) (2022), https://doi.org/10.3390/molecules27092760.
[8] D.P. Bordoloi, D.V. Devi, D.S. Dasgupta (Eds.), A Comparative Study of the Platelet Augmentation Potential of Leaf Extracts of Psidium Guajava with Carica Papaya in Thrombocytopenic Rats, 2016.
[9] M.K. Singh, P.K. Singh, S.S. Yadav, U.S. Singh, P. Dwivedi, R.S. Yadav, Attenuation of Arsenic-Induced Dyslipidemia by Fruit Extract of Emblica Officinalis in Mice, vol. 8, 2018, pp. 3–9, https://doi.org/10.4103/ijnpnd.ijnpnd_69_17, 1.
[10] N.E. Mohamed, S.E. Ashour, Role of ethanolic extract of Morus alba leaves on some biochemical and hematological alterations in irradiated male rats, Int. J. Radiat. Biol. 94 (4) (2018) 374–384, https://doi.org/10.1080/09553002.2018.1433888.
[11] R. Sugavasi, S. Sivanesan, K. Gudemalla, R. Mundugaru, M. Swaminathan, Effect of ginkgo biloba extract on hematological and biochemical alterations in fluoride intoxicated Wistar rats, Res. J. Pharm. Technol. 12 (2019) 3839, https://doi.org/10.5958/0974-360X.2019.00659.0.
[12] M. Li, M. Qian, Q. Jiang, B. Tan, Y. Yin, X. Han, Evidence of flavonoids on disease prevention, Antioxidants 12 (2) (2023), https://doi.org/10.3390/antiox12020527.
[13] A. Nugroho, H. Heryani, J. SueChoi, H.J. Park, Identification and quantification of flavonoids in *Carica papaya* leaf and peroxynitrite-scavenging activity, Asian Pac. J. Trop. Biomed. 7 (3) (2017) 208–213.
[14] W. Zhang, J. Wang, Y. Chen, H. Zheng, B. Xie, Z. Sun, Flavonoid compounds and antibacterial mechanisms of different parts of white guava (Psidium guajava L. cv. Pearl), Nat. Prod. Res. 34 (11) (2020) 1621–1625, https://doi.org/10.1080/14786419.2018.1522313.
[15] S. Kumar, A. Singh, B. Kumar, Identification and characterization of phenolics and terpenoids from ethanolic extracts of Phyllanthus species by HPLC-ESI-QTOF-MS/MS, J Pharm Anal 7 (4) (2017) 214–222, https://doi.org/10.1016/j.jpha.2017.01.005.
[16] W.T. Ju, O.C. Kwon, H.B. Kim, G.B. Sung, H.W. Kim, Y.S. Kim, Qualitative and quantitative analysis of flavonoids from 12 species of Korean mulberry leaves, J. Food Sci. Technol. 55 (5) (2018) 1789–1796, https://doi.org/10.1007/s13197-018-3093-2.
[17] P.C. Chan, Q. Xia, P.P. Fu, Ginkgo biloba leave extract: biological, medicinal, and toxicological effects, J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 25 (3) (2007) 211–244, https://doi.org/10.1080/10590500701569414.
[18] W.M. Dabeek, M.V. Marra, Dietary quercetin and kaempferol: bioavailability and potential cardiovascular-related bioactivity in humans, Nutrients 11 (10) (2019), https://doi.org/10.3390/nu11102288.
[19] P. Asgharian, A.P. Tazekand, K. Hosseini, H. Forouhandeh, T. Ghasemnejad, M. Ranjbar, et al., Potential mechanisms of quercetin in cancer prevention: focus on cellular and molecular targets, Cancer Cell Int. 22 (1) (2022) 257, https://doi.org/10.1186/s12935-022-02677-w.
[20] P. Papakyriakopoulou, N. Velidakis, E. Khattab, G. Valsami, I. Korakianitis, N.P. Kadoglou, Potential Pharmaceutical Applications of quercetin in cardiovascular diseases, Pharmaceuticals 15 (8) (2022), https://doi.org/10.3390/ph15081019.
[21] P. Ansari, S.T. Choudhury, V. Seidel, A.B. Rahman, M.A. Aziz, A.E. Richi, et al., Therapeutic potential of quercetin in the management of type-2 diabetes mellitus, Life 12 (8) (2022), https://doi.org/10.3390/life12081146.
[22] X. Zhao, J. Wang, Y. Deng, L. Liao, M. Zhou, C. Peng, Y. Li, Quercetin as a protective agent for liver diseases: a comprehensive descriptive review of the molecular mechanism, Phytother Res. 35 (9) (2021) 4727–4747, https://doi.org/10.1002/ptr.7104.
[23] V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.
[24] J. Zhishen, T. Mengcheng, W. Jianming, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, Food Chem. 64 (4) (1999) 555–559.
[25] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (9–10) (1999) 1231–1237, https://doi.org/10.1016/s0891-5849(98)00315-3.
[26] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 28 (1) (1995) 25–30, https://doi.org/10.1016/S0023-6438(95)80008-5.
[27] H.H. Donmez, N. Donmez, I. Kısadere, I. Undag, Protective effect of quercetin on some hematological parameters in rats exposed to cadmium, Biotech. Histochem. 94 (5) (2019) 381–386, https://doi.org/10.1080/10520295.2019.1574027.
[28] K. Selvakumar, S. Bavithra, S. Suganya, F. Ahmad Bhat, G. Krishnamoorthy, J. Arunakaran, Effect of quercetin on haematobiochemical and histological changes in the liver of polychlorined biphenyls-induced adult male wistar rats, J Biomark 2013 (2013) 960125, https://doi.org/10.1155/2013/960125.
[29] S. Kasmi, I. Bkhairia, B. Harrabi, H. Mnif, R. Marrakchi, H. Ghozzi, et al., Modulatory effects of quercetin on liver histopathological, biochemical, hematological, oxidative stress and DNA alterations in rats exposed to graded doses of score 250, Toxicol. Mech. Methods 28 (1) (2018) 12–22, https://doi.org/10.1080/15376516.2017.1351507.
[30] X. Liu, C. Cui, M. Zhao, J. Wang, W. Luo, B. Yang, Y. Jiang, Identification of phenolics in the fruit of emblica (Phyllanthus emblica L.) and their antioxidant activities, Food Chem. 109 (4) (2008) 909–915, https://doi.org/10.1016/j.foodchem.2008.01.071.
[31] T. Katsube, N. Imawaka, Y. Kawano, Y. Yamazaki, K. Shiwaku, Y. Yamane, Antioxidant flavonol glycosides in mulberry (Morus alba L.) leaves isolated based on LDL antioxidant activity, Food Chem. 97 (1) (2006) 25–31, https://doi.org/10.1016/j.foodchem.2005.03.019.
[32] G.-N. Kim, H.-D. Jang, Flavonol content in the water extract of the mulberry (Morus alba L.) leaf and their antioxidant capacities, J. Food Sci. 76 (6) (2011) C869–C873, https://doi.org/10.1111/j.1750-3841.2011.02262.x.
[33] X.-G. Liu, S.-Q. Wu, P. Li, H. Yang, Advancement in the chemical analysis and quality control of flavonoid in Ginkgo biloba, J. Pharmaceut. Biomed. Anal. 113 (2015) 212–225, https://doi.org/10.1016/j.jpba.2015.03.006.
[34] L.-M. Xia, A.-P. Zhang, Q. Zheng, J. Ding, Z. Jin, H. Yu, et al., Quercetin-3-O-β-D-glucuronide inhibits mitochondria pathway-mediated platelet apoptosis via the phosphatidylinositol-3-kinase/AKT pathway in immunological bone marrow failure, World J Tradit Chin Med 8 (1) (2022) 115–122, https://doi.org/10.4103/wjtcm.wjtcm_44_21.
[35] C.-H. Chuang, Y.-C. Lin, J. Yang, S.-T. Chan, S.-L. Yeh, Quercetin supplementation attenuates cisplatin induced myelosuppression in mice through regulation of hematopoietic growth factors and hematopoietic inhibitory factors, The Journal of nutritional biochemistry 110 (2022) 109149, https://doi.org/10.1016/j.jnutbio.2022.109149.
[36] B. Salehi, L. Machin, L. Monzote, J. Sharifi-Rad, S.M. Ezzat, M.A. Salem, et al., Therapeutic potential of quercetin: new insights and perspectives for human health, ACS Omega 5 (20) (2020) 11849–11872, https://doi.org/10.1021/acsomega.0c01818.