RESEARCH ARTICLE

Anti-thrombocytopenic Activities of 15 Selected Indonesian Medicinal Plants: *Psidium guajava* Leaves, *Phyllanthus niruri* Aerial Parts, and *Andrographis paniculata* Leaves as Potential Sources in Enhancing Platelet Levels in Rats

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Abstract

BACKGROUND: Thrombocytopenia is a medical condition characterized by a low platelet count in the blood, which can cause excessive bleeding and serious complications. Current conventional treatments for thrombocytopenia often present significant side effects and limitations, necessitating the exploration of alternative therapeutic options. Therefore, this study was conducted to evaluate the potential of Indonesian ethnomedicinal plants as alternative therapies.

METHODS: The research was conducted by ethanol maceration of 15 medicinal plants selected based on their use in traditional Indonesian medicine to treat thrombocytopenia. Male white rats, aged three month and weighing between 150 and 250 g, were divided into four groups, which each group consists of five rats: the negative control group, group receiving 250 mg/kg BW extract, group receiving 500 mg/kg BW extract, and the normal control group. The effects of the test preparations on platelet count, erythrocyte count, and hematocrit were observed after 7 days of treatment.

RESULTS: Among of 15 medicinal plants extract tested at a dose of 500 mg/kg BW, *Psidium guajava* L. leaves extract showed to increase blood platelets counts in thrombocytopenic mice by 60.82% (p<0.01) followed by *Phyllanthus niruri* L. aerial parts extract at (48.22%; p<0.01), and *Andrographis paniculata* (Burm.f.) Wall. ex Nees leaves extract (47.14%; p<0.01).

CONCLUSION: The extracts of *P. guajava* leaves, aerial parts of *P. niruri*, and *A. paniculata* leaves exhibited significant potential in enhancing platelet levels. These findings suggest that some Indonesian medicinal plants can be used as alternative therapy for thrombocytopenia.

KEYWORDS: dengue hemorrhagic fever, *Phyllanthus niruri, Psidium guajava*, *Andrographis paniculata*, thrombocytopenia

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Introduction

Dengue Hemorrhagic Fever (DHF) has become an endemic disease that occurs almost all over the world. The most infected areas are tropical regions, such as Southeast Asia and the Western Pacific. The disease is caused by the rapid transmission of the *Aedes aegypti* mosquito, and the cases in Indonesia have ranked second among endemic countries.(1) According to data from the Ministry of Health of the Republic of Indonesia, dengue cases in March 2024 had reached 53,131 and the death rate was 404 people. The prevalence of these cases tripled from 2023 with 17,434 cases and 118 deaths.(2) Transmission of the infection starts with a mosquito bite, which then the virus enters the blood vessels and causes thrombocytopenia. Interpretation of the severity of DHF disease experienced can be seen from the thrombocytopenia.

Thrombocytopenia, a hematological condition characterized by a platelet count lower than the normal physiological range, is a dangerous condition which leads to the formation of thrombosis. It can trigger various problems in the cardiovascular system, such as acute coronary syndrome. Platelets, also known as thrombocytes, play a crucial role in the hemostatic process by facilitating blood clot formation. A reduced platelet count compromises the body's ability to effectively form clots, leading to challenges in halting bleeding and increasing the risk of hemorrhagic complications.(3)

Treatment of thrombocytopenia is commonly referred to as anti-thrombocytopenic, usually uses glucocorticoids, immunoglobulins, thrombopoietin receptor agonists (TPO-RAs), splenectomy, rituximab, and immunosuppressants. These treatments have proven to have good effectiveness in increasing platelet production and inhibiting autoantibody production. However, there are some adverse side effects, such as TPO-RAs that can increase the risk of thrombosis. This is due to its mechanism of increasing platelet count and stimulating platelet production. In addition, short-term side effects of glucocorticoid use can cause insomnia, dyspepsia, and hyperglycemia.(4,5) Vaccines can be used to prevent DHF. However, vaccines have several constraints such as age, side effects, long-term effects, and a history of infection.(6) Therefore, alternative therapies using natural products are needed.

Indonesian people often use natural ingredients, specifically plants that are known from generation to generation to increase blood platelet levels. The natural ingredients that are commonly used by Indonesians for this purpose include papaya leaves (*Carica papaya* L.) and guava fruit (*Psidium guajava* L.).(7) In addition, plants such as aerial parts of alang-alang (*Imperata cylindrica* (L.) Beauv.), sambiloto leaves (*Andrographis paniculata* (Burm.f.) Wall. ex Nees), aerial parts of pegagan (*Centella asiatica* (L.) Urban), dewa leaves (*Gynura divaricata* (L.) D.C), sweet potato leaves (*Ipomoea batatas* L. Poir.), temu ireng rhizomes (*Curcuma aeruginosa* Roxb.), kencur rhizomes (*Kaempferia galanga* L.), tapak liman leaves (*Elephantopus scaber* L.), red betel leaves (*Piper crocatum* Ruiz & Pav), green beans (*Vigna radiata* (L.) R. Wilczek), rosella (*Hibiscus sabdariffa* L.), and aerial parts of meniran (*Phyllantus niruri* L.) are also often used by the community to raise platelets.(8-13) Results of a survey of herbal medicine use in the community in West Java, Indonesia also showed that these plants are often used especially in the treatment of DHF, especially in increasing platelet counts because they contain compounds such as flavonoids especially quercetin. However, information on the efficacy of these plants is still unclear and there is no scientific evidence so that their use is without clear rules and doses.(14) Therefore, further research is needed as scientific evidence regarding the effectiveness of therapy with the use of these plants. Alternative anti-thrombocytopenia derived from plants is needed and expected to have low side effects and low toxicity to the body.(15)

The high number of dengue cases in Indonesia and other tropical areas and the lack of drugs that effectively increase platelet levels make research on the anti-thrombocytopenic activity of plants essential. In addition, research on the effects of anti-thrombocytopenia has not been widely studied. It is hoped that these plants can be developed as an alternative therapy in increasing the number of platelets in the blood. So, this research aims to prove the effects of ethnomedicine plants commonly used to treat thrombocytopenia using test animals, specifically white rats.

Methods

Sampling and Determinations

The plants used in this study were selected based on the results of literature studies regarding plants that were traditionally used for the treatment of DHF cases. The plants were collected from The Manoko Experiment Garden (Lembang, Indonesia). The plant materials was produced by drying the cleaned parts of the plant in indirect sunlight. The determination was carried out at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Bandung (No. 476/HB/01/2021). Determination results were shown in Table 1.

Extraction Process

Extraction was performed to each medicinal plant using a maceration method with 70% ethanol solvent for 3×24 hours with solvent replacement every 24 hours. The extract was collected in an Erlenmeyer flask and then concentrated using a rotary evaporator at a temperature higher than 40°C. To determine the amount of condensed extract, the yield calculation was carried out. The results of the extract yield calculation from the 15 plants were also listed in Table 1.

Table 1. Taxonomy and yield result of 15 medicinal plants used.

Spesies	Family	Local Name	Parts Used	Quantity (g)	Yield (%)
Imperata cylindrica (L.) Beauv.	Poaceae	Alang-alang	Aerial parts	329	11.09
Andrographis paniculata (Burm.f.) Wall. ex Nees	Acanthaceae	Sambiloto	Leaves	493.98	8.33
Psidium guajava L.	Myrtaceae	Jambu Biji	Fruits	934.6	7.27
Centella asiatica (L.) Urban	Apiaceae	Herba Pegagan	Aerial parts	467.5	8.40
Gynura pseudochina (L.) D.C	Asteraceae	Dewa	Leaves	942.41	6.79
Ipomoea batatas (L.) Poir.	Convolvulaceae	Ubi Jalar	Leaves	527	7.04
Curcuma aeruginosa Roxb.	Zingiberaceae	Temu Ireng	Rhizome	918.84	15.90
Kaempferia galanga L.	Zingiberaceae	Kencur	Rhizome	950	22.88
Elephantopus scaber L.	Asteraceae	Tapak Liman	Leaves	389.07	22.96
Piper crocatum Ruiz & Pav.	Piperaceae	Sirih Merah	Leaves	335.69	11.36
Vigna radiata (L.) R. Wilczek	Fabaceae	Kacang Hijau	Seed	1250	3.46
Hibiscus sabdariffa L.	Malvaceae	Rosella	Flower	307	9.48
Psidium guajava L.	Myrtaceae	Jambu Biji Merah	Leaves	500.47	28.8
Phyllanthus niruri L.	Euphorbiaceae	Herba Meniran	Aerial parts	285.03	23.66
Carica papaya L.	Caricaceae	Pepaya	Leaves	400	17.85

Animal Model

The animals used were male white rats (*Rattus norvegicus*) Wistar strain aged 3 months weighing 150 to 250 grams. The test animals were obtained from D'Wistar, Bandung (No. RL/D'WISTAR/20.11.2021). Rat cages were lined with wood shavings because they were easy to replace, can absorb liquids, have no pungent odor, and are free of chemicals. Each cage was equipped with food and drink containers. Before use, the test animals were acclimatized for 7 days. The body weight of the test animals was weighed, and their behavior was observed periodically. Animals are declared fit and can be used if they do not lose more than 10% of their weight.(16) The protocol experiment had been approved by the Research Ethics Commission of Universitas Padjadjaran (No. 620/UN6.KEP/EC/2021).

Anti-thrombocytopenic Activity Test

All 15 test extracts were tested against Wistar male white rats for to analyze its anto-thrombocytopenic activity. Rats were divided into 4 test groups, each group consisted of 5 rats, including: the negative control group, test group 1 which received 250 mg/kg BW of each extract, test group 2 which received 500 mg/kg BW of each extract, and the normal control group. Animals in the negative control group were only administered with 150 IU Heparin (Na-Heparin, Inviclot; Fahrenheit, Jakarta, Indonesia) solution inducer intraperitoneal. Animals in test group 1 and 2 were induced with the same dosage of heparin, then given the extracts orally at a dose of 250 mg/kg BW and 500 mg/kg BW, respectively. Animals in the normal control group did not receive any treatment and were observed at their normal condition. For 7 days, the rats in test group 1 and 2 were given the test extract. The blood of the rats was taken on the 7th day after treatment, so that the number of platelet, erythrocyte, and hematocrit levels can be calculated (Figure 1).(17)

Rat blood was collected by putting the rats into an isolation cage, after which the rat's tail was cleaned with 70% alcohol using a cotton swab. The rat's tail was irradiated with a lamp with a distance of 30 cm for 15 minutes to dilate the blood vessels. The rat tail was cut approximately 0.5 cm from the tip and the blood was collected in an anticoagulant-coated blood tube. In calculating the number of platelet, erythrocyte, and hematocrit counts, the Sysmex XE-5000 hematology analyzer (Sysmex America, Lincolnshire, IL, USA) was used.

Statistical Analysis

Statistical analyses were performed using Statistical Package for Social Science (SPSS) version 29 (IBM Corporation, Armonk, NY, USA). The normality of the data was tested with the Shapiro-Wilk test. The data analysis was done with the Independent T-Test for the parametric tests. The confidence interval in this study was 95% so that if the p<0.05 then it could be stated meaningful in statistics.

Results

Platelet, Erythrocyte and Hematocrit Counts in Negative and Normal Control

The number of platelet, erythrocyte and hematocrit in each blood was observed. The data for each test extract was then compared with the negative control to get the percentage

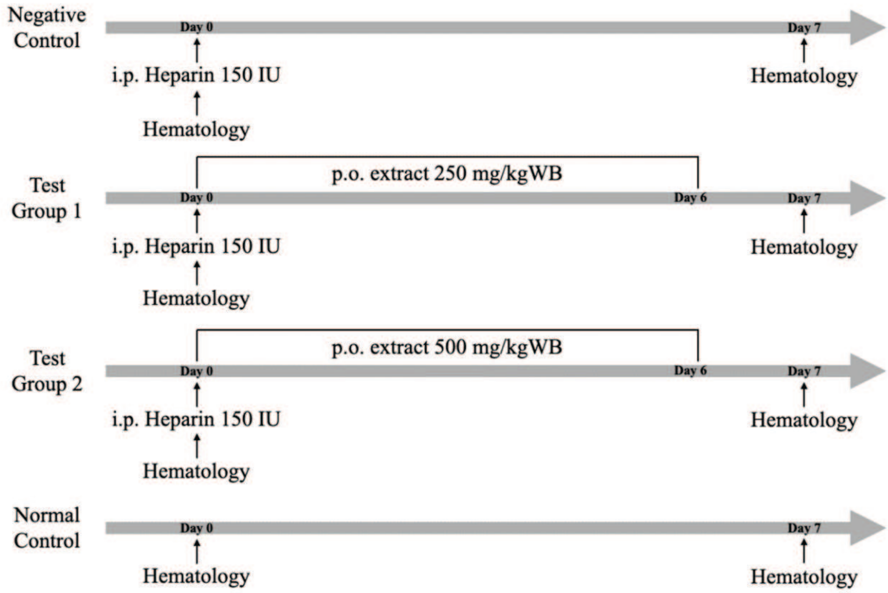

Figure 1. Study scheme and timeline of the *in vivo* anti-thrombocytopenic activity test.

value of the increase in platelet, erythrocyte and hematocrit, so that it can make it easier to see which test extract has the best anti-thrombocytopenic activity.

The average value of platelet, erythrocyte and hematocrit counts in normal control group before treatment were $3.75 \pm 0.16 \times 10^5$, $10.58 \pm 0.27 \times 10^5$, and 51.25 ± 0.95, respectively. Whereas the platelet, erythrocyte, and hematocrit counts on the 7th day of the negative control and normal control could be seen in Table 2.

Platelet, Erythrocyte and Hematocrit Counts Increased After the Administration of Extracts

The average increase in platelet, erythrocyte and hematocrit counts after treatment of the 15 extracts at dose of 250 mg/kg BW and 500 mg/kg BW were presented in Table 3. It was found that almost all test extracts gave anti-thrombocytopenic activity.

This was shown by the increase in the value of platelet counts in experimental animals after administration of plant extracts at the dose of 250 mg/kg BW and 500 mg/kg BW by 1.93-45.62% and 6.70-60.82%, respectively. *P. guajava* leaves, aerial parts of *P. niruri*, and *A. paniculata* leaves provide the best platelet-raising (anti-thrombocytopenia) activity at a dose of 500 mg/kg BW. These results were supported by statistical tests showing a significant difference ($p<0.01$) in the increase in platelet count after treatment with extracts of *P. guajava*, *P. niruri*, and *A. paniculata*. The largest average increase in the number of erythrocytes was given by *C. aeruginosa* extract at a dose of 500 mg/kg BW, and 250 mg/kg BW and *P. guajava* leaves at a dose of 500 mg/kg BW. This shows that besides having the highest anti-thrombocytopenic activity, *P. guajava* leaves can also significantly increase the number of erythrocytes or red blood cells. Meanwhile the average percentage of blood hematocrit rats on the 7th day showed the greatest increased after given 500 mg/kg BW *G. pseudochina*.

From the observations of the increase in platelet, erythrocyte and hematocrit, the potential extracts for making natural herbal medicine preparations for thrombocytopenia which also increase erythrocyte levels and do not trigger plasma leakage were the aerial parts of *P. niruri* at a dose of 500 mg/kg BW and *P. guajava* leaves both at doses of 250 and 500 mg/kg BW).

Discussion

From the 15 extracts, there are 3 extracts that shown the greatest effects in increasing blood platelets, namely *P. guajava* leaves, *P. niruri* aerial parts, and *A. paniculata* leaves. The increase in blood platelet levels in the three plants was significantly different based on statistical ($p<0.01$). Results of the experiment showed that the administration of 250 and 500 mg/kg BW of the test preparation increases the platelet, erythrocyte, and hematocrit counts in the rats. The three parameters were measured because they are interconnected with each other. Low platelets can trigger plasma leakage which is indicated by an increase in hematocrit. While hematocrit will affect erythrocytes.(18)

In this study, heparin was used to induced thrombocytopenia. Heparin induction resulted in a 53.33% decrease in platelet levels in the test animals (Table 2).

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Table 2. Average results of the platelet, erythrocyte and hematocrit counts in rat blood on the 7th day observation (n=5).

Group	Platelet Count	Erythrocyte Count	Hematocrit Count
Negative	1.75±0.43 x 10^5	7.59±0.55 x 10^5	45.5±0.57
Normal	3.69±0.14 x 10^5	10.29±0.33 x 10^5	51.27±0.52

Additionally, there was a decrease of 28.26% in erythrocyte levels and 11.21% in hematocrit levels. This is in accordance with another previous study, where after heparin exposure, platelet numbers decline rapidly, sometimes by 50% or more from baseline in 90% respondent.(19) From Table 3, the administration of the extract at the test dose did not cause an increase in hematocrit above 20%, so that the administration of the test extract will not trigger plasma permeation in dengue fever subjects.(20)

P. guajava from the *Myrtaceae* family contain quercetin, avicularin, apigenin, guaijaverin, kaempferol, hyperin, myricetin, gallic acid, catechin, epicatechin, chlorogenic acid, epigallocatechin gallate, and caffeic acid.(21) *P. guajava* leaves extract have several pharmacological activities such as anticancer (22), antioxidant, antidiabetic (23), antimicrobial (24), and hepatoprotective (25). Aqueous extracts of *P. guajava* leaves were found to significantly increase platelet count after cyclophosphamide-induced thrombocytopenia. The leaf extract has benefits such as reducing the severity of dengue bleeding.(8) From the results of the phytochemical screening, polyphenols, tannins, saponins, flavonoids, monoterpenoids, sesquiterpenoids, triterpenoids, and quinones were found in the extract.

From the experimental results, it was found that ethanol extract of *P. guajava* leaves increases the number of platelet, erythrocyte, and hematocrit counts. This is also proportional to the increase in dose, the higher the dose given, the greater the increase in the number of parameters studied. The flavonoid content of quercetin can increase platelet count because it is assisted by amino acids which have a function as a building substance that can affect thrombopoietin, which is a megakaryocyte stimulating factor.(26,27) This finding is also supported by previous study that has proven the megakaryocyte increasing activity of *P. guajava* leaves extract in white Wistar rats. Megakaryocytes are the precursor of platelets. Therefore, the increase of megakaryocytes could help in increasing the platelet counts.(27) Not only the leaves, *P. guajava* fruit extract is also shown to have anti-thrombocytopenic activity through the enhancement of stem cell factor expression.(28,29)

P. niruri is a plant that belongs to the *Euphorbiaceae* family. This plant has several activities such as antiviral activities because it can inhibit DNA polymerase, can increase the body's immune system, antioxidant, antibacterial, antifungal, and can treat dengue fever. This

Table 3. Average results of the increase in platelet, erythrocyte and hematocrit counts in rat after receiving plant extracts (n=5).

Plant	Increase in Platelet Count (%)		Increase in Erythrocyte Count (%)		Increase in Hematocrit Count (%)	
	250 mg/kg BW	500 mg/kg BW	250 mg/kg BW	500 mg/kg BW	250 mg/kg BW	500 mg/kg BW
Imperata cylindrica (L.) Beauv.	15.55±1.56	26.68±3.84	7.34±0.66	8.05±0.83	1.37±0.06	7.14±0.27
Andrographis paniculata (Burm.f.) Wall. ex Nees	30.39±0.58	47.17±0.97	8.52±1.51	9.44±2.01	2.96±1.46	6.15±1.63
Psidium guajava L. (Fruit)	9.35±3.47	28.27±6.17	8.57±1.61	9.12±1.18	2.85±0.13	4.22±1.36
Centella asiatica (L.) Urban	6.19±0.96	34.00±1.69	5.11±1.94	8.05±0.93	1.54±0.16	4.19±0.74
Gynura pseudochina (L.) D.C	5.12±0.65	16.03±0.51	3.44±3.03	19.44±4.32	4.94±1.99	19.97±0.05
Ipomoea batatas (L.) Poir.	2.74±0.83	11.13±1.69	6.62±1.29	10.95±0.17	4.68±1.55	9.08±1.69
Curcuma aeruginosa Roxb.	24.84±1.21	26.27±6.32	24.83±1.26	26.98±1.28	2.28±0.34	8.2±0.77
Kaempferia galanga L.	3.28±0.37	6.47±0.29	4.64±0.15	11.48±1.73	2.12±0.07	6.28±0.29
Elephantopus scaber L.	5.51±0.16	8.37±0.35	4.21±1.07	7.99±0.67	5.13±0.16	8.55±0.35
Piper crocatum Ruiz & Pav.	5.33±0.81	6.66±1.47	5.52±0.88	7.99±1.84	5.34±1.46	5.72±1.47
Vigna radiata (L.) R. Wilczek	17.21±4.83	29.47±3.47	4.61±1.77	12.56±1.65	1.32±0.02	11.19±1.51
Hibiscus sabdariffa L.	34.81±7.16	40.28±9.4	7.62±0.92	9.45±1.89	6.12±1.53	8.77±1.53
Psidium guajava L. *(Leaves)*	45.62±4.67	60.82±8.46	18.1±1.74	20.41±1.48	12.65±1.75	13±2.02
Phyllanthus niruri L.	33.27±1.71	48.22±1.95	17.1±0.59	17.43±1.94	8.03±1.75	8.12±0.08
Carica papaya L.	26±3.83	35.62±3.67	12.28±3.63	13.18±1.42	11.09±0.83	11.39±0.57

is because *P. niruri* contains several compounds, including tannins, quinones, triterpenoids, flavonoids, essential oils, coumarins, and polyphenols.(9) Bioactive compounds from *P. niruri* are niruriside which has antiviral activity (30) and corilagin which has anticancer activity (31). Therefore, this plant recommended can be used as an alternative medicine in treating a disease such as DHF.

Based on the data obtained, the average examination results of platelet, erythrocyte and hematocrit counts increased with increasing doses. Hematocrit itself is related to blood viscosity. The higher the hematocrit value, the higher the blood viscosity. High or low hematocrit values can cause an increase or can slow blood flow in capillaries.(32) According to these results, *P. niruri* at the test dose will not trigger plasma permeation in dengue fever patients. In thrombocytopenia, the value of erythrocytes decreases, so according to the results obtained, *P. niruri* also has activity in increasing the number of blood erythrocytes.

P. niruri is also proven to have an activity in increasing the platelet counts. The increase in platelet count can occur due to the presence of bioactive flavonoid and tannin compounds as anti-aggregation of platelets to inhibit the formation of blood clots.(9) As well as from the results of the phytochemical screening, alkaloids, polyphenols, tannins, saponins, flavonoids, triterpenoids, and quinones were found in the extract. In addition, flavonoids can also inhibit the enzyme hyaluronidase which functions in the degradation of hyaluronate. Hyaluronic acid in the bone marrow can secrete mast cell interleukin (IL)-6 to induce the liver to secrete thrombopoietin and stimulate megakaryocytes so that platelet cells increase.(33) A study done previously also supports this finding. Instead of ethanol extract, this study used aqueous extract of *P. niruri* that has proven to be active in increasing the platelet counts in white rats that have phenytoin-induced thrombocytopenia.(34)

A. paniculata contains several compounds, such as phenols, alkaloids, saponins, flavonoids, farnesol, and tannins.(35) A. paniculata is often used as herbal medicine for various condition, from sepsis to anemia.(35,36) Based on previous research, *A. paniculata* also has activity, especially in inhibiting Dengue Virus type 1 (DENV1) replication. Dengue virus infection can cause platelet deficiency or known as thrombocytopenia.(10) Based on the data obtained, *A. paniculata* has an effect in increasing platelet count with an average percentage increase at a dose of 500 mg/kg BW is 47.17%. *A. paniculata* is the third highest order of plants that can increase the platelet count of white rats.

A. paniculata has several bioactive compounds, such as andrographolide, neoandrographolide, didehydroandrographolide.(38) From the results of the phytochemical screening, alkaloids, polyphenols, saponins, flavonoids, monoterpenoids, sesquiterpenoids, and quinones were found in the extract. Andrographolide , other than a well known , is an effective anti-inflammatory agent (36), is also terpenoids that is an inhibitor of platelet activation by reducing the formation of hydroxy radicals (OH) initiated by collagen involving the activation of endothelial nitric oxide synthase-nitric oxide (eNOS-NO)/cyclic guanosine 3',5'-cyclic monophosphate (GMP) pathway and inhibiting PI3 kinase/Akt-p38 mitogen-activated protein kinase (MAPK) and phospholipase C gamma 2-protein kinase C (PLCγ2-PKC) cascade (38). Therefore, andrographolide is the expected compound in *A. paniculata* that are responsible for the anti-thrombocytopenic activity. A previous study proves this hypothesis whereas both *A. paniculata* extract and andrographolide are shown to have activity in increasing platelet counts. However, the crude water extract of *A. paniculata* shows slightly better platelet increasing activity than pure andrographolide.(39) Because of that, we assumed that while still be the most active andrographolide is not the only compound in *A. paniculata* that has anti-thrombocytopenic activity.

From the experimental results of current study, almost all extracts gave an increase in platelets when the dose increased. However, in *C. aeruginosa* rhizome extract, the platelet counts at a dose of 250 mg/kg BW is greater than at a dose of 500 mg/kg BW. This can happen because *C. aeruginosa* rhizome extract does not depend on the dose increase. If the dose is increased, the pharmacological activity is not optimal.(40)

This research proves the empirical study of ethnomedicine in Indonesia, whereas plants that are often used by the community for DHF treatment do have anti-thrombocytopenic activity. All plants, especially *P. guajava, P. ninuri*, and *A. paniculata* are potential to be further studied as novel anti-thrombocytopenic medications. However, the limitation of this study is that the effective dose and active compounds of anti-thrombocytopenia from these three plants are unknown yet. Therefore, further study will focus on obtaining effective doses and isolating active anti-thrombocytopenic compounds from *P. guajava*, *P. ninuri*, and *A. paniculata*. Nonetheless, *P. guajava*, *P. ninuri*, and *A. paniculata* can be developed into pharmaceutical preparations and they may be further investigated as new anti-thrombocytopenic drugs.

Conclusion

From the results of testing the anti-thrombocytopenic activity of 15 plants, it is known that the three most potential extracts in increasing blood platelets are *P. guajava* leaves (60.82%), *P. niruri* (48.22%), and *A. paniculata* (47.14%) at a dose of 500 mg/kg BW. The three plants are recommended for handling dengue fever, as well as ethnomedicine studies, so it can be developed into herbal medicines with anti-thrombocytopenic activity

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Authors Contribution

YS, EH and AT were involved in concepting and planning the research, FFS and AT performed the data acquisition/collection, YS and EH calculated the experimental data and performed the analysis, AA and HPD drafted the manuscript and designed the figures, YS and EH aided in interpreting the results. All authors took part in giving critical revision of the manuscript.

References

1. Kementerian Kesehatan Republik Indonesia. Strategi Nasional Penanggulangan Dengue 2021-2025. Jakarta: Kementerian Kesehatan RI; 2021.
2. Yuningsih R. Kebijakan Penggerakan Partisipasi Masyarakat dalam Penanggulangan DBD. Jakarta: Pusat Analisis Keparlemenan Badan Keahlian DPR RI; 2024.
3. Jinna S, Khandhar PB. Thrombocytopenia. Treasure Island: StatPearls Publishing; 2023.
4. Tjepkema M, Amini S, Schipperus M. Risk of thrombosis with thrombopoietin receptor agonists for ITP patients: A systematic review and meta-analysis. Crit Rev Oncol Hematol. 2022; 171:103581. doi: 10.1016/j.critrevonc.2022.103581.
5. Yamasaki S. Bisphosphonate Use for glucocorticoid-induced osteoporosis in older patients with immune thrombocytopenia: A clinical perspective. Ann Hematol. 2023; 102(7): 1645-56.
6. See KC. Dengue Vaccination: A Practical Guide for Clinicians. Vaccines. 2025; 13(2): 145. doi: 10.3390/vaccines13020145.
7. Sumartini R. Pengaruh tanaman herbal terhadap peningkatan kadar trombosit pada anak demam berdarah dengue (DBD). Profesional Health Journal. 2023; 5(1): 311-9.
8. Bordoloi P, Devi D, Dasgupta. A comparative study of the platelet augmentation potential of leaf extracts of Psidium guajava with Carica papaya in thrombocytopenic rats. Sch J App Med Sci. 2016; 4(8A): 2774-2782.
9. Lee NY, Khoo WK, Adnan MA, Mahalingam TP, Fernandez AR, Jeevaratnam K. The pharmacological potential of Phyllanthus niruri. J Pharm Pharmacol. 2016; 68(8):953-69.
10. Juan-Pablo BP, David PE, Mónica SR, Ashutosh S, Daniel NA, Dealmy DG, *et al.* Medicinal plants with anti-dengue and immunomodulatory activity. Curr Pharm Biotechnol. 2023; 24(4): 486-94.
11. Prasetyaningsih Y, Sari N, Prasetya HR, Wulandari R, Naer VG. Potensi etnomedicine daun ubi jalar ungu (Ipomoea batatas L. Poir) dan daun ubi jalar putih (Ipomoea batatas L.) sebagai obat demam berdarah di Sleman DIY. Journal of Health. 2019; 6(1): 6-11.
12. Salehi B, Zakaria ZA, Gyawali R, Ibrahim SA, Rajkovic J, Shinwari ZK, *et al.* Piper species: A comprehensive review on their phytochemistry, biological activities and applications. Molecules. 2019; 24(7): 1364. doi: 10.3390/molecules24071364.
13. Djati MS, Christina YI, Rifa'i M. The combination of Elephantopus scaber and Sauropus androgynus promotes erythroid lineages and modulates follicle-stimulating hormone and luteinizing hormone levels in pregnant mice infected with Escherichia coli. Vet World. 2021; 14(5): 1398-404.
14. Mohan V, Doss A, Tresina P. Ethnomedicinal Plants with Therapeutic Properties. CRC Press: Boca Raton. 2019.
15. Munir S, Liu ZW, Tariq T, Rabail R, Kowalczewski PL, Lewandowicz J, *et al*. Delving into the therapeutic potential of Carica papaya Leaf against thrombocytopenia. Molecules. 2022; 27(9):2760. doi: 10.3390/molecules27092760.
16. Beaudry AG, Law ML. Variable body and tissue weight reporting in preclinical cachexia literature may alter study outcomes and interpretation. Dis Model Mech. 2023; 16(7): dmm050148. doi: 10.1242/dmm.050148.
17. Fithria RF, Wulandari R, Hidayati DN, Annisa P, Putri NE. Antithrombocytopenia activity of peanut shell (Arachis hypogea L.) extract and infusa on heparin induced balb/C mice. Jurnal Tumbuhan Obat Indonesia. 2019; 12(1): 1-9.
18. Jundi M, Tadasa E, Adissu W. Correlation of red blood cell parameters and platelet count among adult anemic patients attending Madda Walabu University Goba Referral Hospital, Goba, Southeast Ethiopia: A comparative cross-sectional study. Medicine. 2025; 104(2): e41156. doi: 10.1097/MD.0000000000041156.
19. Warkentin TE, Kelton JG. Temporal aspects of heparin-induced thrombocytopenia. N Engl J Med. 2001; 344(17): 1286-92.
20. Hossain MJ, Das M, Shahjahan M, Islam MW, Towhid ST. Clinical and hematological manifestation of dengue patients in 2022 outbreak: A tertiary care hospital-based cross-sectional study. Health Sci Rep. 2025; 8(1): e70356. doi: 10.1002/hsr2.70356.
21. Kumar M, Tomar M, Amarowicz R, Saurabh V, Nair MS, Maheshwari C, *et al.* Guava (Psidium guajava L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. Foods. 2021; 10(4): 752. doi: 10.3390/foods10040752.
22. Jiang L, Lu J, Qin Y, Jiang W, Wang Y. Antitumor effect of guava leaves on lung cancer: A network pharmacology study. Arab. J. Chem. 2020; 13(11): 7773-97.
23. Simamora A, Paramita L, Azreen N, Santoso AW, Timotius KH. In vitro antidiabetic and antioxidant activities of aqueous extract from

the leaf and fruit of Psidium guajava L. Indones Biomed J. 2018; 10(2): 156-64.

24. Pereira GA, Chaves DSA, Silva TME, Motta REA, Silva ABRD, Patricio TCDC, *et al.* Antimicrobial activity of Psidium guajava aqueous extract against sensitive and resistant bacterial strains. Microorganisms. 2023; 11(7): 1784. doi: 10.3390/microorganisms11071784.
25. Laily N, Kusumaningtyas RW, Sukarti I, Rini MRDK. The potency of Guava Psidium guajava (L.) leaves as a functional immunostimulatory ingredient. Procedia Chem. 2015; 14: 301-7.
26. Handayani V, Nurfadillah. Kajian farmakognostik herba meniran hijau (Phyllanthus niruri L.) dan herba meniran merah (Phyllanthus urinaria L.). Jurnal Fitofarmaka Indonesia. 2018; 1(1): 18-25.
27. Widhawati R, Solehah M. Pengaruh jambu biji terhadap kenaikan trombosit pasien demam berdarah dengue (DBD) di Ruang Ayana Rumah Sakit Permata Ibu Kunciran Tangerang. Jurnal Kesehatan. 2018; 7(1): 1-9.
28. Wiyasihati SI, Wigati KW, Wardani T. Comparing the effect of red yeast rice, date palm, and guava leaf extract on thrombocyte and megakaryocyte count in thrombocytopenic white rats. Folia Medica Indonesiana. 2013; 49(2): 82-7.
29. Atik N, Tarawifa S, Avriyanti E, Rahmadi AR, Hilmanto D. Psidium guajava L. extract increases platelet count through enhancement of stem cell factor expression in thrombocytopenic mice model. Int J Pharm Pharm Sci.2018; 10(1): 23-6.
30. Manisha P, Alekhya KP, Tejaswini A, Swathi K, Venkat Sai K. Screening of Phyllanthus niruri leaves phytoconstituents for antiviral and antibacterial activity by molecular docking studies. Adv Exp Med Biol. 2023; 1423: 115-22.
31. Zheng ZZ, Chen LH, Liu SS, Deng Y, Zheng GH, Gu Y, *et al.* Bioguided fraction and isolation of the antitumor components from Phyllanthus niruri L. Biomed Res Int. 2016; 2016: 9729275. doi: 10.1155/2016/9729275.
32. Zimmerman R, Tsai AG, Salazar Vázquez BY, Cabrales P, Hofmann A, Meier J, *et al.* Posttransfusion increase of hematocrit per se does not improve circulatory oxygen delivery due to increased blood viscosity. Anesth Analg. 2017; 124(5): 1547-54.
33. Kartika RW, Alwi I, Suyatna FD, Yunir E, Waspadji S, Immanuel S, *et al.* Advance-platelet rich fibrin and hyaluronic acid combination improves interleukin-6 and granulation index in diabetic foot ulcer patients. Indones Biomed J. 2021; 13(2): 170-7.
34. Sumiati RL, Baehaki F, Nurdin N, Pakpahan SE. Meniran leaf extract (Phyllanthus niruri L.): How does it affect the number of platelets in the blood?. Stannum Jurnal Sains dan Terapan Kimia. 2024; 6(1): 48-56.
35. Patin EW, Zaini MA, Sulastri Y. Pengaruh variasi suhu pengeringan terhadap sifat fisiko kimia teh daun sambiloto (Andrographis paniculata). Pro Food. 2018; 4(1): 251-8.
36. Ardika RG, Budiono BP, Widiastiti NS, Maharani N, Susilaningsih N, Sandra F. Andrographis paniculata leaves extract inhibit TNF-α and caspase-3 expression of septic rats' intestinal tissues. Indones Biomed J. 2024; 16(1): 66-71.
37. Eziefule OM, Arozal W, Wanandi SI, Louisa M, Wuyung PE, Dewi S, *et al*. Andrographis paniculata ethanolic extract improved doxorubicin-induced cardiac inflammation, alterations in liver function parameters and anemia. Mol Cell Biomed Sci. 2024; 8(2): 117-26.
38. Chang CC, Duann YF, Yen TL, Chen YY, Jayakumar T, Onge ET, *et al.* Andrographolide, a novel NF-kB inhibitor, inhibits vascular smooth muscle cell proliferation and cerebral endothelial cell Inflammation. Acta Cardiol Sin. 2014; 30: 308-15.
39. Venkataraman N, Pamukuntla S, Banoth J, Boini P, Sampathi S, Swami V, *et al.* Platelet augmentation activity of Andrographis paniculata extract and andrographolide against cyclophosphamide induced thrombocytopenia in rats. Pharm Pharmacol Int J. 2015; 2(4): 126-31.
40. Korn EL, Moscow JA, Freidlin B. Dose optimization during drug development: Whether and when to optimize. J Natl Cancer Inst. 2023; 115(5): 492-7.