Journal of Vocational Health Studies

https://e-journal.unair.ac.id/JVHS

THE EFFECTIVENESS OF PAPAYA LEAF EXTRACT (CARICA PAPAYA L.) AS AN ANTIAGGREGATION AGENT ON PLATELET COUNT AND PLATELET AGGREGATION: IN VITRO STUDY

EFEKTIVITAS EKSTRAK DAUN PEPAYA (CARICA PAPAYA L.) SEBAGAI ANTIAGREGRASI TERHADAP JUMLAH TROMBOSIT DAN AGREGRASI TROMBOSIT

Original Research Article
Penelitian

Ganjar Noviar, Eem Hayati, Riski Nur Ramadhani

Medical Laboratory Technology Faculty, Bandung Ministry of Health Polytechnic, Bandung, Indonesia

A B S T R A C T

Background: *In hypertensive patients, platelet hyperaggregation may lead to acute ischemic stroke, which can increase mortality or neurological deficits. Papaya (Carica papaya L.) has potential effect to mitigate complications arising from hyperaggregation, because its effectiveness as antiplatelet aggregants.* ***Purpose:*** *The aim of this study was to determine the effectiveness of papaya leaf extract as an antiaggregation on platelet count and platelet aggregation.* ***Method:*** *A quasi-experimental design was adopted observing platelet counts utilizing a hematology analyzer medonic M32 and assessing platelet aggregation values through the velaskar method. These assessments were conducted on citrated blood samples, including a control group, aspirin at 5 ppm, and a group with citrated blood augmented with papaya leaf extract at concentrations of 5 ppm, 10 ppm, and 15 ppm.* ***Result:*** *The platelet count in citrated blood was 186.000 cells/µL and platelet aggregation was 75%. When with aspirin at 5 ppm, the platelet count was 153.000 cells/µL and platelet aggregation was 71%. In ethanol extract from papaya leaves at 5 ppm, a platelet count of 160.750 cells/µL and platelet aggregation of 70% were exhibited. At 10 ppm, the platelet count was 149.125 cells/µL and platelet aggregation was 65%. At 15 ppm, the platelet count was 105.675 cells/µL and platelet aggregation was 56%. The results of the General Linear Model (GLM) test indicated that p (Sig.) < 0.05.* ***Conclusion:*** *The ethanol extract of papaya leaves can be used effectively as an antiplatelet agent, reducing both platelet count and platelet aggregation at minimum concentrations of 5 ppm and 10 ppm of papaya leaf extract.*

A R T I C L E I N F O

Received 03 January 2024
Revised 08 January 2024
Accepted 22 June 2024
Available Online 31 July 2025

Correspondence:
Riski Nur Ramadhani

E-mail :
riskinr13@gmail.com

Keywords:
Platelet aggregation, Platelet count, Papaya leaf extract, Velaskar

A B S T R A K

Latar belakang: Pada pasien hipertensi terjadi hiperagregasi trombosit. Hiperagregasi trombosit akan memberikan keluaran yang buruk pada stroke infark akut, berupa kematian atau defisit *neurologic*. Salah satu spesies tanaman dapat berperan mencegah terjadinya komplikasi hiperagregrasi adalah pepaya (*Carica papaya L.*) karena berkhasiat sebagai antiagregrasi. **Tujuan:** Penelitian ini bertujuan untuk mengetahui efektivitas ekstrak daun pepaya sebagai antiagregasi trombosit terhadap jumlah trombosit dan agregasi trombosit. **Metode:** Bersifat kuasi eksperimen, dilakukan pengamatan jumlah trombosit menggunakan *hematology analyzer medonic* M32 dan nilai agregrasi trombosit metode velaskar. Pemeriksan ini dilakukan pada sampel darah sitrat, termasuk kelompok kontrol, aspirin 5 ppm, dan kelompok darah sitrat dengan penambahan ekstrak daun pepaya pada konsentrasi 5 ppm, 10 ppm, dan 15 ppm. **Hasil:** Jumlah trombosit dalam darah sitrat 186.000 sel/µL dan agregrasi trombosit 75%. Ketika diberikan aspirin pada 5 ppm, jumlah trombosit 153.000 sel/µL dan agregrasi trombosit 71%. Dalam ekstrak etanol dari daun papaya pada 5 ppm, jumlah trombosit 160.750 sel/µL dan agregrasi trombosit 70%. Pada 10 ppm, jumlah trombosit 149.125 sel/µL dan agregrasi trombosit 65%. Pada 15 ppm, jumlah trombosit 105.675 sel/µL dan agregrasi trombosit 56%. Hasil uji General *Linear* Model (GLM) menunjukkan p (Sig.) < 0.05. **Kesimpulan:** Ekstrak etanol daun papaya dapat digunakan secara efektif sebagai antiagregrasi terhadap jumlah trombosit dan agregrasi trombosit dengan konsentrasi minimal ekstrak daun papaya 5 ppm dan 10 ppm.

Kata kunci:
Agregasi trombosit, Jumlah trombosit, Ekstrak daun papaya, Velaskar

Journal of Vocational Health Studies p-ISSN: 2580–7161; e-ISSN: 2580–717x
DOI: 10.20473/jvhs.V9.I1.2025.38-46

INTRODUCTION

Platelets are tiny fragments derived from the cytoplasm of megakaryocytes (Apriliani, 2016). Their primary role is to create a physical obstruction as part of the typical hemostatic response to vascular injuries (Sherwood *et al*., 2018; Hall and Hall, 2020). Platelet count serves as a crucial diagnostic tool to enhance diagnostic accuracy, track therapeutic outcomes, gauge the progression of an illness, predict prognosis, and assess the severity of a condition (Sujud *et al*., 2015). Platelet count measurements can be expressed in units such as cells/mm^3, cells/µL, x 10^3 cells/mL, or x 10^6 cells/L. Among these units, cells/mm^3 or cells/µL are the more commonly employed ones in platelet count assessments (Kuman, 2019). Higher platelet count was observed to have a significant causal effect on the elevated risk of hypertension through this bi-directional MR study (Chiu *et al*., 2021). Hypertension is commonly referred to as "The Silent Killer" because patients typically do not exhibit noticeable symptoms. This lack of symptoms can often lead to complications, including stroke, heart and blood vessel diseases, kidney disorders, and other health issues, if left untreated and unmanaged. Ultimately, these complications can lead to disability or even death (Rilantono, 2012). Hypertensive individuals commonly experience platelet hyperaggregation. The process of platelet hyperaggregation plays a significant role in the development of cardiovascular and cerebrovascular diseases, as it leads to platelet accumulation and the formation of thrombi. Platelet aggregation is observed to play a role in influencing the development of occlusions within cerebral blood vessels. Occlusions resulting from platelet hyperaggregation may serve as an outcome marker in acute infarction stroke. Platelet hyperaggregation is associated with unfavorable outcomes in acute infarction stroke, such as death or neurological deficits (Khan *et al*., 2009; WHO, 2013).

One of the plant species with potential in preventing complications associated with hyperaggregation is the papaya leaf (*Carica papaya L*.), commonly found in numerous tropical and subtropical countries, including Indonesia (Lopes et al., 2019). Papaya (*Carica papaya L*.) is extensively utilized by the community for medicinal purposes, encompassing the use of its leaves, fruits, seeds, and sap. These components are known to be employed in the treatment of various ailments such as typhoid fever, wound infections, asthma, diarrhea, hypertension, and more (Maniyar and Bhixavatimath, 2012). Flavonoids, steroids, and tannin compounds in free form and tannin-protein complexes are efficacious as antiaggregants (Yogiraj *et al*., 2014; A'yun and Laily, 2015; Pamungkas *et al*., 2020). Furthermore, papaya is also valuable for its antimicrobial and anti-inflammatory properties (Gupta *et al*., 2017) and analgesic (Danborno *et al*., 2018) and antioxidant (Mandal *et al*., 2015). Given the numerous advantages associated with papaya leaf (*Carica papaya L*.), a research was undertaken to assess the efficacy of papaya leaf extract (*Carica papaya L*.) in reducing platelet aggregation and impacting platelet count (Sathyapalan et al., 2020). The high ratio of potassium to sodium is very beneficial for preventing the occurrence of hypertension as too much sodium in the body is a signal to increase blood pressure. So, with the presence of high potassium in *Carica papaya L*. can compensate for the amount of sodium (Wahdi *et al*., 2020). Several studies have demonstrated a noteworthy correlation between the administration of papaya leaf extract and an increase in platelet levels (Dharmarathna *et al*., 2013; Kumar *et al*., 2018).

The innovative aspect of this research lies in examining the effects of ethanol extract from papaya leaves (*Carica Papaya L*.) on platelet count and platelet aggregation. Currently, the cost of platelet aggregation examination using automated methods or turbidimetry remains high. Therefore, this research conducts platelet aggregation testing using the velaskar method in vitro or manually, with a more economical examination cost, serving as a screening for platelet aggregation. Thus, the outcomes of this research can provide information on the effects of ethanol extract from papaya leaves (*Carica Papaya L*.) on platelet count and platelet aggregation using an alternative platelet aggregation examination method employing the velaskar method.

MATERIAL AND METHOD

This research employed a quasi-experimental design, involving the assessment of platelet count using the hematology analyzer medonic M32 and the measurement of platelet aggregation through the velaskar method. The research used citrated blood samples taken from those who were considered normal by random sampling with normal platelet count from the Department of Medical Laboratory Technology at the Polytechnic of the Ministry of Health in Bandung, including a control group, a group with citrated blood supplemented with aspirin at 5 ppm (aspirin control), and a group with citrated blood enhanced with papaya leaf extract (*Carica papaya L*.) at concentrations of 5 ppm, 10 ppm, and 15 ppm. This research has been approved by the ethics committee with number 10/KEPK/EC/X/202 3 issued on 29th October 2023 by the Health Research Ethics Commission of the Bandung Ministry of Health Polytechnic.

The research involved four samples, two male and two female students, who were considered normal, from the Department of Medical Laboratory Technology at the Polytechnic of the Ministry of Health in Bandung. Each student provided a blood sample of ± 12 mL, which was then used to conduct platelet aggregation assessments utilizing the velaskar method and platelet count measurements using the hematology analyzer

medonic M32. The research results were handled anonymously to protect the confidentiality of the samples. Additionally, the results were exported for data analysis using IBM SPSS Statistics 26.

Blood samples from each respondent were collected using a syringe, approximately 12 mL of blood each, then divided into four tubes, each containing 2.7 mL of blood and 0.3 mL of 3.2% sodium citrate anticoagulant in a ratio of (9 : 1). Subsequently, aspirin at a concentration of 5 ppm and papaya leaf extract at concentrations of 5 ppm, 10 ppm, and 15 ppm were added to the citrate blood samples as seen in Table 1. Additionally, sample stability was maintained by storing them in a refrigerator at a temperature of 2 – 6 ^{0}C to prevent damage and decrease in platelet count.

Table 1. Aspirin and papaya leaf extract concentration for blood samples

Concentration	Stock 100 ppm of aspirin (µL)	Stock 100 ppm of papaya leaf extract (µL)	Citrated blood (µL)
5 ppm aspirin	50	-	950
5 ppm ethanol extract from papaya leaves	-	50	950
10 ppm ethanol extract from papaya leaves	-	100	900
15 ppm ethanol extract from papaya leaves	-	150	850

RESULT

The examination of platelet aggregation and platelet count involved the use of papaya leaf extract, with aspirin control serving as the standard reference. Table 2 the results of examination of platelet counts using the hematology analyzer medonic M32 and platelet aggregation using the velaskar method. The citrated blood (negative control) shows an average platelet count of 186.000 cells/µL and an average platelet aggregation of 75%. Conversely, the citrated blood supplemented with aspirin at 5 ppm (positive control) displayed an average platelet count of 153.500 cells/µL and an average platelet aggregation of 71%. These outcomes indicate the antiaggregation impact of aspirin on both platelet count and platelet aggregation.

While in citrated blood with the addition of ethanol extract from papaya leaves (*Carica papaya L.*), 5 ppm showed an average platelet count of 160.750 cells/µL and an average platelet aggregation of 70%, 10 ppm showed an average platelet count of 149.125 cells/µL and average platelet aggregation of 65% and 15 ppm showed an average platelet count of 105.675 cells/µL and average platelet aggregation of 56%. The above results showed a decrease in platelet count and platelet aggregation in citrated blood added with ethanol extract of papaya leaves (*Carica papaya L.*).

Decrease in platelets compared to control before addition of aspirin and papaya leaf ethanol extract

Frequencies of abnormal platelet counts in a population-based setting, including 41.5% of the entire age-specific population of the Principality of Liechtenstein, were compared by using age and sex independent RIs and the RLs obtained in the present study. The males (n = 542), 95% RIs for platelet counts were defined as follows: 150 – 300 × 109/L (60 – 69 years); 130 – 300 × 109/L (70 – 79 years); and 120 – 300 × 109/L (80 years and above). The females (n = 661), the consolidated age independent 95% RI was 165 – 355 × 109/L (Hermann *et al*., 2020). The results of platelet reduction are presented in Table 3.

As presented in Table 3, the citrated blood supplemented with aspirin at 5 ppm (positive control) exhibited an average reduction in platelet count of 32.500 cells/µL (17%). In contrast, citrated blood enhanced with ethanol extract from papaya leaves (*Carica papaya L.*) at 5 ppm displayed an average decrease in platelet count of 25.250 cells/µL (13%), while at 10 ppm it showed an average decrease of 36.875 cells/µL (19%). Moreover, at 15 ppm, there was a notable average decrease in platelet count amounting to 80.325 cells/µL (43%).

Decrease in platelet aggregation compared to control before the addition of aspirin and papaya leaf ethanol extract

As indicated in Table 4, the citrated blood supplemented with aspirin at 5 ppm (positive control) exhibited an average reduction in platelet aggregation of 4%. On the other hand, citrated blood enriched with ethanol extract from papaya leaves (*Carica papaya L.*) at 5 ppm displayed an average decrease in platelet aggregation of 5%, while at 10 ppm it demonstrated an average decrease of 10%. Furthermore, at 15 ppm, there was a significant average reduction in platelet aggregation amounting to 19%.

Data processing results using SPSS

Normality test results

The normality test results cab be seen in Table 5, shown indicate that the p (Sig.) value for platelet count and platelet aggregation in the data groups, including aspirin control and papaya leaf extract at 5 ppm, 10 ppm, and 15 ppm, is greater than 0.05. Consequently, it can be inferred that the data distribution in the examination is normal. Therefore, the statistical test employed is the General Linear Model (GLM) repeated measure.

Table 2. Platelet count and platelet aggregation of blood samples

No. sample	Control		Aspirin control		Papaya leaf extract					
	Blood citrate		5 PPM		5 PPM		10 PPM		15 PPM	
	Platelet count (SEL/ µL)	Platalet aggregation (%)	Platelet count (SEL/ µL)	Platalet aggregation (%)	Platelet count (SEL/ µL)	Platalet aggregation (%)	Platelet count (SEL/ µL)	Platalet aggregation (%)	Platelet count (SEL/ µL)	Platalet aggregation (%)
1	206.000	80	162.000	77	171.000	77	163.000	75	113.200	63
2	171.000	74	152.000	70	162.500	69	148.000	63	103.000	51
3	152.500	74	137.000	69	137.000	71	131.000	62	92.000	57
4	214.500	72	163.000	68	172.500	62	154.500	61	114.500	52
Average	186.000	75	153.500	71	160.750	70	149.125	65	105.675	56

Table 3. Reduction of platelet count of blood samples

No. sample	Control	Aspirin control		Papaya leaf extract					
	Blood citrate	5 PPM		5 PPM		10 PPM		15 PPM	
	Platelet count (SEL/ µL)	Decreased platelet count (SEL/ µL)	Decreased (%)	Decreased platelet count (SEL/ µL)	Decreased (%)	Decreased platelet count (SEL/ µL)	Decreased (%)	Decreased platelet count (SEL/ µL)	Decreased (%)
1	206.000	44.000	21	35.000	17	43.000	21	92.800	45
2	171.000	19.000	11	8.500	5	23.000	13	68.000	40
3	152.500	15.500	10	15.500	10	21.500	14	60.500	40
4	214.500	51.500	24	42.000	20	60.000	28	100.000	47
Average		32.500	17	25.250	13	36.875	19	80.325	43

Table 4. Reduction of platelet aggregation of blood samples

No. sample	Control	Aspirin control		Papaya leaf extract					
	Blood citrate	5 PPM		5 PPM		10 PPM		15 PPM	
	Platelet count (SEL/ µL)	Decreased platelet count (%)	Decreased (%)	Decreased platelet count (%)	Decreased (%)	Decreased platelet count (%)	Decreased (%)	Decreased platelet count (%)	Decreased (%)
1	80	3	4	4	4	6	7	17	21
2	74	4	5	5	6	11	14	23	31
3	74	6	7	4	5	12	16	17	23
4	72	4	6	10	14	12	16	20	28
Average		4	5	5	7	10	13	19	26

Table 5. Normality test results of platelet count and platelet aggregation

Data group	Storage time	Sig. value	Results	Conclusion
Platelet count	Aspirin control	0.310	p-value > 0.05	Normal distribution
	Blood citrate control	0.517		
	Papaya leaf extract 5 ppm	0.149		
	Papaya leaf extract 10 ppm	0.816		
	Papaya leaf extract 15 ppm	0.411		
Platelet aggregation	Aspirin control	0.115		
	Blood citrate control	0.195		
	Papaya leaf extract 5 ppm	0.941		
	Papaya leaf extract 10 ppm	0.054		
	Papaya leaf extract 15 ppm	0.473		

Table 6. Homogeneity test result of platelet count and platelet aggregation

Data group	Levene statistic	df1	df2	Sig.
Platelet count (cells/uL)	3.296	4	15	.060
Platelet aggregation (%)	.487	4	15	.745

Table 7. General Linear Model (GLM) test results of platelet count and platelet aggregation

Data group	Sig. value		Conclusion
Platelet count Platelet aggregation	0.000	p-value < 0.05	There is an influence

Table 8. Pairwise comparisons test results of platelet count

Platelet count	Concentration of papaya leaf extract (ppm)	Sig. value	Results	Conclusion
Aspirin 5 ppm control	5	0.588	p-value > 0.05	There is no difference
	10	1.000	p-value > 0.05	
	15	0.000	p-value < 0.05	There is a difference

Table 9. Pairwise comparisons test results of platelet aggregation

Platelet aggregation	Concentration of papaya leaf extract (ppm)	Sig. value	Results	Conclusion
Aspirin 5 ppm control	5	1.000	p-value > 0.05	There is no difference
	10	0.193	p-value > 0.05	
	15	0.020	p-value < 0.05	There is a difference

Homogeneity test results

The homogeneity test results presented Table 6 indicate that the p (Sig.) value for platelet count and platelet aggregation in the data groups, including aspirin control and papaya leaf extract at 5 ppm, 10 ppm, and 15 ppm, is greater than 0.05. As a result, it can be concluded that the data distribution in the examination is homogeneous. Consequently, the statistical test employed is the GLM repeated measure.

General Linear Model (GLM) test results

According to the Table 7, the p (Sig.) value for platelet counts and platelet aggregation in the data groups, including aspirin control and papaya leaf extract at 5 ppm, 10 ppm, and 15 ppm, is less than 0.05. Therefore, it can be deduced that the ethanol extract of papaya leaves (*Carica papaya L.*) is effective as an antiplatelet agent, exhibiting an impact on both platelet count and platelet aggregation.

Pairwise comparisons test results

Based on Table 8, the Sig. value of the output is obtained as, when comparing the data group of platelet counts in blood citrate with aspirin at 5 ppm to the data groups of blood citrate with papaya leaf ethanol extract at 5 ppm and 10 ppm, the p (Sig.) values were found to be 0.588 and 1.000, respectively, both of which are greater than α (0.05). Therefore, it can be concluded that there is no significant difference. This means that papaya leaf ethanol extract at 5 ppm and 10 ppm exhibits antiplatelet aggregation effectiveness that is comparable to aspirin at 5 ppm regarding platelet count. In the dataset comparing platelet counts in blood samples treated with aspirin at a concentration of 5 ppm to the dataset of blood samples treated with papaya leaf ethanol extract at a concentration of 15 ppm, the significance level (p-value) obtained was 0.000, which is less than the predetermined alpha level of 0.05.

Therefore, we can conclude that there is a statistically significant difference between the two groups. This suggests that papaya leaf ethanol extract at 15 ppm does not exhibit anti-aggregation effectiveness similar to aspirin at 5 ppm on platelet counts. The results of the comparisons test on platelet aggregation are presented in Table 9.

Based on Table 9, the Sig. value of the output is obtained as in the dataset comparing platelet aggregation in citrated blood with aspirin at 5 ppm to the dataset comparing citrated blood aggregation with papaya leaf ethanol extract at 5 ppm and 10 ppm, the p-values are 1.000 and 0.193, respectively, both of which are greater than the significance level α (0.05). Therefore, we can conclude that there is no significant difference. This suggests that papaya leaf ethanol extract at 5 ppm and 10 ppm exhibits antiaggregation effectiveness against platelet aggregation similar to aspirin at 5 ppm. In the dataset comparing platelet aggregation in citrated blood with aspirin at 5 ppm to the dataset comparing citrated blood with papaya leaf ethanol extract at 15 ppm, the p-value is 0.000, which is less than the significance level α (0.05). Therefore, we can conclude that there is a significant difference. This suggests that papaya leaf ethanol extract at 15 ppm does not exhibit antiaggregation effectiveness against platelet aggregation similar to aspirin at 5 ppm.

In this research, experimentation was conducted in vitro, thus the intervention was not directly administered to the respondents. This research serves as preliminary investigation to ascertain the reduction in platelet count and aggregation resulting from the addition of ethanol extract from papaya leaves. It is anticipated to provide descriptive information for further research, with the hope that, in the future, it could be applied in the development of an herbal remedy derived from papaya leaves for use in cardiovascular patients with cases of platelet hyperaggregation.

DISCUSSION

Based on the results of the generalized linear model (GLM) test, the significance value (Sig.) of the output for both platelet count and platelet aggregation is found to be p-value (Sig.) < 0.05 in the data groups comprising aspirin control and papaya leaf extract at concentrations of 5 ppm, 10 ppm, and 15 ppm. Therefore, we can conclude that the ethanol extract of papaya leaves (*Carica papaya L*.) is effective as an antiplatelet agent, as it significantly affects both platelet count and platelet aggregation.

Based on the results, it shows that the effectiveness of ethanol extract of papaya leaf (*Carica papaya L*.) as antiaggregation on platelet count and platelet aggregation. In this research, ethanol extract of papaya leaf (*Carica papaya L*.) at concentrations of 5 and 10 ppm showed the same effectiveness as citrated blood control with the addition of 5 ppm aspirin against a decrease in platelet count and platelet aggregation.

Platelet hyperaggregation occurs in individuals with hypertension. The accumulation of platelets and the formation of blood clots as a result of hyperaggregation play a significant role in the development of cardiovascular and cerebrovascular diseases. It is believed that platelet aggregation plays a crucial role in the formation of blockages in cerebral blood vessels. The blockages resulting from platelet hyperaggregation may serve as an indicator of outcomes in cases of acute ischemic stroke. Platelet hyperaggregation will give poor outcome in acute infarction stroke, in the form of death or neurologic deficit (Khan *et al*., 2009; WHO, 2013). One of the plant species that can play a role in preventing hyperaggregation complications is papaya (*Carica papaya L*.) which is found in most tropical and subtropical countries such as Indonesia. Papaya fruit is utilized by the community and can act as an anti-aggregate (Yogiraj *et al*., 2014; A'yun and Laily, 2015; Pamungkas *et al*., 2020). The platelet count has an impact on the incidence of cardiovascular disease triggered by platelet hyperaggregation. Platelets play a critical role in situations involving wounds or the leakage of blood vessels (Sa'adah, 2018).

The phytochemical testing results of the ethanol extract obtained from papaya leaves (*Carica papaya L*.) indicated positive outcomes, confirming the presence of phenolic, alkaloid, steroid, tannin, and saponin compounds. It is noteworthy that flavonoids, steroids, and tannin compounds, both in their free form and as tannin-protein complexes, exhibit effectiveness as antiaggregants (Parle and Bhardwaj, 2011; A'yun and Laily, 2015; Pamungkas *et al*., 2020). Proteinolytic enzymes like papain and chymopapain have the potential to raise platelet counts, playing a crucial role in exerting anti-thrombocytopenic effects. Furthermore, flavonols and flavonoids exhibit a stimulating effect

on the production of blood cells (Sundarmurthy *et al*., 2017). Studies conducted at Temple University School of Medicine in Philadelphia have demonstrated that Arachidonate 12-lipoxygenase (ALOX 12), also referred to as platelet-type Lipoxygenase, plays a crucial role in platelet production and activation. The increased activity of the ALOX 12 gene is necessary for these processes. This gene is expressed in megakaryocytes and is responsible for the generation of 12-Hydroxyeicosatetraenoic acid (12-HETE) from platelets. Furthermore, ALOX12 is a direct target of the transcription factor RUNX1 in both megakaryocytes and platelets. RUNX1 is a transcription factor responsible for controlling the expression of genes specific to hematopoiesis. In cases where there is a deficiency of RUNX1, it has a notable impact on hematopoiesis as a whole, resulting in reduced expression of ALOX 12 within platelets. Additionally, this reduction in ALOX 12 expression leads to a decrease in the production of 12-Hydroxyeicosatetraenoic acid (12-HETE) when platelets are stimulated by agonists. This observation serves as further confirmation of the association between platelet production and the expression of ALOX 12 (Subenthiran *et al*., 2013).

The results of this research, contrary to research conducted by Lobang *et al*. (2020), state that papaya leaves can increase platelet count and function as anti-inflammatory (Lobang *et al*., 2020), whereby the results of this research showed a decrease in platelet count and platelet aggregation in line with the addition of the concentration of ethanol extract of papaya leaves (*Carica papaya L*.).

Papaya leaves (*Carica papaya L*.) contain several alkaloid compounds (carpain, pseudocarpain, dehydrokarpain I, dehydrokarpain II, and emetine). The total alkaloids of papaya leaves (*Carica papaya L*.) have been proven to increase the percentage of inhibition of platelet aggregation (antiplatelet) and reduce the value of platelet aggregation (Rohmah and Fickri, 2020).

According to Moss *et al*. (2010), in hypertensive patients without any treatment platelet hyperaggregation will generally occur. Aspirin administration is expected to provide normoaggregation results so as to prevent the incidence of cardiovascular and cerebrovascular diseases (Moss *et al*., 2010). This can be seen in Table 1 of the research results, where platelet aggregation using aspirin control, citrate blood control, and papaya leaf extract 5 ppm, 10 ppm, and 15 ppm obtained low results. This aligns with the outcomes of a research conducted by Ick *et al*. (2014), which reported a distinction in platelet aggregation levels among hypertensive patients who received aspirin compared to those who did not receive aspirin.

In this research, experimentation was conducted on citrated blood from normal patients in vitro, with platelet counts within the normal range (150.000 – 400.000 cells/µL). Meanwhile, patients with platelet hyperaggregation had platelet counts below 120.000 cells/µL, a known tendency to bleed, and chronic use of immunosuppressants, antiplatelet therapy, or other drugs affecting hemostasis (such as NSAIDs, SSRIs, and anticoagulants) (Zijverden *et al*., 2023). The reduction in platelet count and platelet aggregation following the addition of ethanol extract from papaya leaves at concentrations of 5 ppm and 10 ppm has been shown to decrease platelet levels to below normal values in the body. Therefore, further research is needed to investigate the effects of papaya leaf extract on samples with platelet counts and platelet aggregation above normal or on patients with platelet hyperaggregation

CONCLUSION

The conclusions drawn from the results of this research are as follows, (1) The phytochemical test results of ethanol extract from papaya leaves (*Carica papaya L*.) showed positive indications for containing phenolics, alkaloids, steroids, tannins, and saponins, which are known as antiplatelet aggregation agents, (2) Citrated blood with the addition of aspirin at 5 ppm (positive control) showed an average decrease in platelet count of 17%. Meanwhile, citrated blood with the addition of ethanol extract from papaya leaves (*Carica papaya L*.) at 5 ppm demonstrated an average decrease in platelet count of 13%, at 10 ppm of 19%, and at 15 ppm of 43%, and (3) Citrated blood with the addition of aspirin at 5 ppm (positive control) showed an average decrease in platelet aggregation of 4%. Meanwhile, citrated blood with the addition of ethanol extract from papaya leaves (*Carica papaya L*.) at 5 ppm demonstrated an average decrease in platelet aggregation of 5%, at 10 ppm of 10%, and at 15 ppm of 19%, (4) The ethanol extract from papaya leaves (*Carica papaya L*.) at concentrations of 5 ppm and 10 ppm is effective as an antiplatelet agent against both platelet count and platelet aggregation.

ACKNOWLEDGMENTS

The authors express their gratitude to Bandung Ministry of Health Polytechnic, PT MRK Diagnostic, and all individuals and organizations who contributed to the facilitation of this research. Furthermore, the authors declare that they have no conflicts of interest with any of the parties involved in this research.

REFERENCE

Apriliani, T., 2016. Gambaran Hitung Jumlah Trombosit dengan Antikoagulan K3EDTA 10%.

A'yun, Q., Laily, A.N., 2015. Analisis Fitokimia Daun Pepaya (Carica Papaya L.) di Balai Penelitian Tanaman Aneka Kacang dan Umbi, Kendalpayak, Malang. In: Seminar Nasional Konservasi dan Pemanfaatan Sumber Daya Alam 2015. Presented at The Seminar Nasional Konservasi dan Pemanfaatan Sumber Daya Alam 2015, Sebelas Maret University, Pp. 134-137.

Chiu, P.-C., Chattopadhyay, A., Wu, M.-C., Hsiao, T.-H., Lin, C.-H., Lu, T.-P., 2021. Elucidation of a Causal Relationship Between Platelet Count and Hypertension: A Bi-Directional Mendelian Randomization Study. Frontiers in Cardiovascular Medicine Vol. 8, Pp. 743075.

Danborno, A.M., Ibrahim, S.H., Mallo, M.J., 2018. The Anti-Inflammatory and Analgesic Effects of The Aqueous Leaves Extract of Carica Papaya. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) Vol. 13(3), Pp. 60-63.

Dharmarathna, S.L.C.A., Wickramasinghe, S., Waduge, R.N., Rajapakse, R.P.V.J., Kularatne, S.A.M., 2013. Does Carica Papaya Leaf-Extract Increase The Platelet Count? An Experimental Study in A Murine Model. Asian Pacific Journal of Tropical Biomedicine Vol. 3(9), Pp. 720-724.

Gupta, A., Patil, S., Pendharkar, N., 2017. Antimicrobial and Anti-Inflammatory Activity of Aqueous Extract of Carica Papaya. Journal of HerbMed Pharmacology Vol. 6(4), Pp. 148-152.

Hall, J.E., Hall, M.E., 2020. Guyton and Hall Textbook of Medical Physiology E-Book: Guyton and Hall Textbook of Medical Physiology E-Book, 14th edn. Elsevier Health Sciences, Philadelphia.

Hermann, W., Risch, L., Grebhardt, C., Nydegger, U.E., Sakem, B., Imperiali, M., Renz, H., Risch, M., 2020. Reference Intervals for Platelet Counts in the Elderly: Results from the Prospective SENIORLAB Study. Journal of Clinical Medicine Vol. 9(9), Pp. 2856.

Ick, B.L., Mongan, A.E., Memah, M., 2014. Perbandingan Nilai Agregasi Trombosit pada Pasien Hipertensi yang Diberi Aspirin dan Tidak Diberi Aspirin di RSUP. Prof. DR. R. D. Kandou Manado. eBiomedik Vol. 2(2), Pp. 523-531.

Khan, N.A., Hemmelgarn, B., Herman, R.J., Bell, C.M., Mahon, J.L., Leiter, L.A., Rabkin, S.W., Hill, M.D., Padwal, R., Touyz, R.M., Larochelle, P., Feldman, R.D., Schiffrin, E.L., Campbell, N.R.C., Moe, G., Prasad, R., Arnold, M.O., Campbell, T.S., Milot, A., Stone, J.A., Jones, C., Ogilvie, R.I., Hamet, P., Fodor, G., Carruthers, G., Burns, K.D., Ruzicka, M., DeChamplain, J., Pylypchuk, G., Petrella, R., Boulanger, J.-M., Trudeau, L., Hegele, R.A., Woo, V., McFarlane, P., Vallée, M., Howlett, J., Bacon, S.L., Lindsay, P., Gilbert, R.E., Lewanczuk, R.Z., Tobe, S., Canadian Hypertension Education Program, 2009. The Canadian Hypertension Education Program Recommendations for The Management of Hypertension: Part 2--Therapy. The Canadian Journal of Cardiology Vol. 29(5), Pp. 287-298.

Kuman, M.Y., 2019. Perbedaan Jumlah Eritrosit, Leukosit dan Trombosit pada Pemberian Antikoagulan Konvensional an EDTA Vacutainer (Diploma). Poltekkes Kemenkes Kupang, Program Studi Analisis Kesehatan.

Kumar, M.S., M, G., Shah, M.J., Goyal, M., D, S.L., 2018. Evaluation of Efficacy of Carica Papaya Leaf Extracts to Increase Platelet Count in Hydroxyurea Induced Thrombocytopenia in Albino Rats. International Journal of Basic & Clinical Pharmacology Vol. 7(1), Pp. 173-178.

Lobang, E.W.N., Putri, I.M., Hanafi, Z., Widhiyastuti, E., 2020. Pengaruh Kombinasi Ekstrak Carica Papaya dan Propolis terhadap Peningkatan Trombosit. Jurnal Farmasi Vol. 9(2), Pp. 26-32.

Lopes, A.C.A., Eda, S.H., Andrade, R.P., Amorim, J.C., Duarte, W.F., 2019. New Alcoholic Fermented Beverages—Potentials and Challenges. In: Grumezescu, A., Holban, A.M. (eds.), Fermented Beverages. Woodhead Publishing, Pp. 577-603.

Mandal, S. de, RR, L., Vabeiryureilai, M., Senthil Kumar, N., Lalnunmawii, E., 2015. An Investigation of the Antioxidant Property of Carica Papaya Leaf Extracts from Mizoram, Northeast India. Research & Reviews: Journal of Botanical Sciences Vol. 4(2), Pp. 43-46.

Maniyar, Y., Bhixavatimath, P., 2012. Antihyperglycemic and Hypolipidemic Activities of Aqueous Extract of Carica Papaya Linn. Leaves in Alloxan-Induced Diabetic Rats. Journal of Ayurveda and Integrative Medicine Vol. 3(2), Pp. 70-74.

Moss, M.B., Siqueira, M.A., Mann, G.E., Brunini, T.M., Mendes-Ribeiro, A.C., 2010. Platelet Aggregation in Arterial Hypertension: Is There A Nitric Oxide-Urea Connection? Clinical and Experimental Pharmacology & Physiology Vol. 37(2), Pp. 167-172.

Pamungkas, K., Dewi, P., Tandiono, E., 2020. Potensi Quercetin dalam Ekstrak Daun Psidium guajava dan Papain dalam Ekstrak Daun Carica Papaya Linn sebagai Terapi Demam Berdarah Dengue. ESSENTIAL:Essence of Scientific Medical Journal Vol. 17(2), Pp. 22-28.

Parle, M., Bhardwaj, G., 2011. Basketful Benefits of Papaya. International Research Journal of Pharmacy Vol. 2(7), Pp. 6-12.

Rilantono, L.I., 2012. Penyakit Kardiovaskular (PKV) : 5 Rahasia. Yayasan Bina Pustaka.

Rohmah, M., Fickri, D., 2020. Uji Aktivitas Antiplatelet, Antikoagulan, dan Trombolitik Alkaloid Total Daun Pepaya (Carica papaya L.) secara in Vitro. Jurnal Sains Farmasi & Klinis Vol. 7(2), Pp. 115.

Sa'adah, S., 2018. Sistem Peredaran Darah Manusia. Universitas Kristen Artha Wacana, Program studi Pendidikan Biologi Fakultas Tarbiyah dan Keguruan UIN Sunan Gunung Djati Bandung.

Sathyapalan, D.T., Padmanabhan, A., Moni, M., P-Prabhu, B., Prasanna, P., Balachandran, S., Trikkur, S.P., Jose, S., Edathadathil, F., Anilkumar, J.O., Jayaprasad, R., Koramparambil, G., Kamath, R.C., Menon, Veena, Menon, Vidya, 2020. Efficacy & Safety of Carica Papaya Leaf Extract (CPLE) in Severe Thrombocytopenia (≤30,000/ Ml) in Adult Dengue – Results of A Pilot Study. PLoS One Vol. 15(2), Pp. e0228699.

Sherwood, L., Mahendera, L.I., Hartanto, H., 2018. Fisiologi Manusia: Dari Sel ke Sistem, 9th edn. EGC, Jakarta.

Subenthiran, S., Choon, T.C., Cheong, K.C., Thayan, R., Teck, M.B., Muniandy, P.K., Afzan, A., Abdullah, N.R., Ismail, Z., 2013. Carica Papaya Leaves Juice Significantly Accelerates The Rate of Increase in Platelet Count among Patients with Dengue Fever and Dengue Haemorrhagic Fever. Evidence-Based Complementary and Alternative Medicine: eCAM, Pp. 616737.

Sujud, S., Hardiasari, R., Nuryati, A., 2015. Perbedaan Jumlah Trombosit pada Darah EDTA yang Segera Diperiksa dan Penundaan Selama 1 Jam di Laboratorium RSJ Grhasia Yogyakarta. Medical Laboratory Technology Journal Vol. 1(2), Pp. 91-95.

Sundarmurthy, D., R, J., Kuntegowdanahalli, L., 2017. Effect of Carica Papaya Leaf Extract on Platelet Count in Chemotherapy-Induced Thrombocytopenic Patients : A Preliminary Study. National Journal of Physiology, Pharmacy and Pharmacology Vol. 7(6), Pp. 685-692.

Wahdi, A., Astuti, P., Puspitosari, D., Maisaroh, S., Pratiwi, T., 2020. The Effectiveness of Giving Papaya Fruit (Carica Papaya) Toward Blood Pressure on Elderly Hypertension Patients. IOP Conference Series: Earth and Environmental Science Vol. 519(1), Pp. 012007.

WHO, 2013. Measure Your Blood Pressure, Reduce Your Risk.

Yogiraj, V., Goyal, P.K., Chauhan, C.S., Goyal, A., Vyas, B., 2014. Carica Papaya Linn: An Overview. International Journal of Herbal Medicine Vol. 2(5), Pp. 1-8.

Zijverden, L.M. van, Schutte, M.H., Madsen, M.C., Bonten, T.N., Smulders, Y.M., Wiepjes, C.M., van Diemen, J.J.K., Thijs, A., 2023. The Eficacy of Aspirin to Inhibit Platelet Aggregation in Patients Hospitalised with A Severe Infection: A Multicentre, Open Label, Randomised Controlled Trial. Clinical and Experimental Medicine. Clinical and Experimental Medicine Vol. 23(7), Pp. 3501-3508.