The Effectiveness Of Additional Tomato (*SOLANUM LYCOPERSICUM*) Water Extract As Antiagregation Of Thrombocytes In Whole Blood On The Number Of Thrombocytes And Thrombocytes Antiagregation

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ABSTRACT

In hypertensive patients, there is hyperaggregation of platelets. Platelet accumulation and thrombus formation due to hyperaggregation are important in the pathogenesis of cardiovascular and cerebrovascular diseases. Tomato (Solanum lycopersicum) is a horticultural product that contains adenine which is useful for preventing platelet aggregation. The purpose of this study was to determine the effectiveness of minimal concentrations of aqueous extract of tomato (Solanum lycopersicum) as anti-platelet aggregation in whole blood on platelet count and platelet aggregation. This study was a quasi-experimental study by observing platelet counts using a hematology analyzer. In addition, platelet aggregation was measured using the velaskar method with aspirin (control) 5 ppm and with the addition of aqueous extract of Tomato (Solanum lycopersicum) with concentrations of 5, 10 and 15 ppm. The research data obtained is presented in tabular form and then processed using the Normality Test and One Way ANOVA Test. The results showed that the water extract of tomato (Solanum lycopersicum) was effective as an anti-platelet aggregation in whole blood on platelet count and platelet aggregation with a minimum concentration of 5 ppm.

Keywords: *Tomato, Platelet, Velaskar Platelet Aggregation*

1. Introduction

Platelet hyperaggregation occurs which is important in the pathogenesis of cardiovascular and cerebrovascular diseases. Platelet aggregation is considered to influence the formation of occlusion in the blood vessels of the brain. Occlusion resulting from platelet hyperaggregation can provide an outcome marker in acute stroke infarction and result in a poor outcome in the form of death or neurological deficits [1][2].

Platelet aggregation is the formation of platelet cross-links through active GPIIb/IIIa receptors with fibrinogen bridges. Platelets in an inactivated state possess approximately 50–80,000 GPIIb/IIIa receptors, which do not bind fibrinogen, von Willebrand factor (VWF) or other ligands. Stimulation of platelets by ADP will cause an increase in GPIIb/IIIa molecules, allowing cross-linking of platelets to fibrinogen bridges (reversible) [3].

As a positive feedback, cytosolic phospholipase A2 (PLA2) is activated which then converts platelet phospholipids into arachidonic acid. Arachidonic acid is then converted by the cyclooxygenase enzyme (Cox-1) into prostaglandin G2 (PGG2) which will then be

converted into prostaglandin H2 (PGH2) by the peroxidase enzyme. PGH2 will be converted by the enzyme thromboxane synthase to thromboxane A2 (TXA2). TXA2 is a potent platelet aggregator that will strengthen platelet aggregation to form more stable (irreversible) aggregates. TXA2 acts on surface receptors and activates phospholipase C which causes the formation of inositol triphosphate which causes an increase in intracellular calcium. Calcium converts inactive GPIIb/IIIa receptors on the platelet membrane to a conformation with high affinity for fibrinogen that forms cross-links between platelets and causes platelet aggregation. So, it is highly recommended in patients with hyperaggregation to use aspirin to prevent this platelet aggregation [4][5].

Platelet count affects the occurrence of cardiovascular disease which results in platelet hyperaggregation, where the normal value of platelets is 150,000-400,000 cells/µL. But this value can vary because it can be influenced by age, gender, and race [6]. Horticultural crop products in Indonesia are still very limited. Tomato (Solanum lycopersicum) is a horticultural product that has the potential to be healthy and has quite promising market prospects. Tomatoes, both fresh and processed, have a complete and good nutritional composition. Tomato fruit consists of 5-10% dry weight without water and 1% skin and seeds. If the tomatoes are dried, glucose and fructose, the remaining organic acids, minerals, pigments, vitamins, and lipids [7].

The health benefits of tomatoes include reducing fat levels that cause obesity, building muscle, preventing cancer, scavenging free radicals, keeping teeth and bones strong and healthy, controlling cholesterol that causes hypertension (high blood pressure), preventing platelet aggregation. The content of compounds in tomatoes include solanine (0.007%), saponins, folic acid, malic acid, citric acid, bioflavonoids (including lycopene, α and ß-carotene), protein, fat, vitamins, minerals and histamine[8]. The first paragraph after a heading is not indented (Bodytext style).

According to Ruman's research in 2010, regarding the anti-aggregation effect of tomato water extract, it was proven that tomatoes can inhibit platelet aggregation by up to 75%. Adenine is one of the chemical compounds contained in tomatoes that may function as an inhibitor of platelet aggregation. The mechanism of adenine has not been fully elucidated, but adenine is more potent in inhibiting platelet aggregation induced by collagen than by ADP [9].

Compounds contained in tomatoes are more polar compounds, one of which is adenine which is well soluble in water. Adenine is polar and dissolves well in water with a solubility level in water of 0.103 g / 100 mL [10] [11]. Examination of peripheral blood smears to assess platelet aggregation function was introduced by Velaskar DS and Chitre in 1982. The material used for this examination was whole blood with 3.8% sodium citrate anticoagulant.

In examining the function of platelet aggregation by the peripheral blood smear method using epinephrine 1 mg / mL and ADP 1 mg / mL. This assay is performed to evaluate differences in platelet aggregation with and without taking into account initial platelet aggregation or circulating platelet aggregation. Velaskar read the entire zone from the edge of the smear to the next edge, and calculated the percentage of aggregated platelets compared to the total platelets [12].Other paragraphs are indented (BodytextIndented style).

2. Methods

The research was conducted at the Basic Chemistry Laboratory and Hematology Laboratory, Department of Technology, Medical Laboratory, Poltekkes, Ministry of Health, Bandung, from March 2021 to January 2022. Approval of research ethics by the Health Research Ethics Commission Team, Poltekkes, Ministry of Health, Bandung, number 25 / KEPK / EC / XII / 2021 which applies until 20 December 2022.

The research design was quasi-experimental, with the research population being tomatoes (Solanum lycopersicum) from tomato plantations in Padaasih Village, Cisarua District, West Bandung Regency. The sample for this research was aqueous extract of tomato (Solanum lycopersicum) with concentrations of 5 ppm, 10 ppm and 15 ppm added to whole blood. Then an examination of the platelet count was carried out using the Medonic M32 hematology analyzer and examination of the value of platelet aggregation using the Velaskar method on citrate blood (control), citrate blood with the addition of 5 ppm aspirin (aspirin control) and citrate blood with the addition of Tomato Fruit (Solanum lycopersicum) aqueous extract with a concentration 5, 10 and 15 ppm with 4 repetitions.

3. Simplicia and tomatoes water extract from tomatoes (Solanum lycopersium)

A total of 4 kg of tomatoes were cleaned of organic matter and impurities by soaking them in water for a while, then washing them thoroughly, cutting them into small pieces, grinding them with a blender and filtering them. The simplicia that was filtered was then subjected to a lyophilization process to obtain aqueous extract of tomatoes in solid or powder form. Identification of adenine levels in tomato aqueous extract using the HPLC method at the integrated Lab of the Poltekkes Kemenkes Bandung. Tomato fruit water extract was weighed 1 gram dissolved in 10 mL of 3.8% citric blood so that it had a concentration of 100 ppm (mother liquor) which was diluted to a concentration of 5 ppm, 10 ppm and 15 ppm using 3.8% citrate blood solvent.

3.1.1. Preparation of control and working solution of tomato water extract. The concentration of 5-ppm extract, pipette the tomato aqueous extract 250 µL of mother liquor, put it into a 5 mL volumetric flask and dilute with 3.8% citrate blood to the volume mark. For the 10-ppm extract, pipette the tomato aqueous extract 500 µL of mother liquor, put it into a 5 mL volumetric flask and dilute with 3.8% citrate blood to the volume mark. For the 15-ppm extract, pipette the aqueous extract of tomato with 750 µL of mother liquor, put it into a 5 mL volumetric flask and dilute with 3.8% citrate blood to the volume mark, homogenize. For the control of 5 ppm aspirin, 5 mg aspirin was weighed, mashed, and put into a 5 mL volumetric flask and dissolved with 3.8% citrate blood to the volume mark, so that a concentration of 1000 ppm was obtained. Then pipetted 25 µL of aspirin mother liquor (1000 ppm), put into a 5 mL volumetric flask, diluted with 3.8% citrate blood to the volume mark, homogenized.

4. Determination of Platelet Count

Firstly, venous blood collection was carried out at the cubital fossa vein as much as 45 mL using a 23G Terumo winged needle and a 10 ml syringe. The blood taken was then added with 5 mL of 3.8% sodium citrate (ratio of 9:1). Venous blood collection was carried out at the cubital fossa vein as much as 45 mL using a 23G Terumo winged needle

and a 10 ml syringe. The blood taken was then added with 5 mL of 3.8% sodium citrate (ratio of 9:1). Secondly, this blood is then examined for platelet counts in each treatment carried out with 4 repetitions using a Hematology Analyzer Medonic 32 from MRK diagnostic volumetric impedance measurement method using an electrolyte solution (diluent) mixed with blood cells sucked through the aperture. Then it is measured by two electrodes consisting of internal and external electrodes based on cell size (Manual Book Medonic, 2016) [13].

5. Preparation of Epinephrine Inductors and Preparation of Peripheral Blood Smear

epinephrine inductor concentration of 1 mg/mL was prepared at room temperature. there are five tubes which all contain blood which is given anticoagulant sodium citrate 3.8% which will be treated. The first tube as a blank was not given any treatment. the second tube contained 5-ppm aspirin control and 1mg/mL epinephrine inductor, 1000 uL and 100 uL, respectively. while the third to fifth tubes were treated with the same amount of inductor and tomato water extract with a concentration of 5, 10 and 15 ppm. Citrate blood that had been treated was allowed to stand for 3 minutes, then 10 µL of each tube was taken for peripheral blood smear preparation. The smear preparation was waited for to dry and fixed with methanol for 5 minutes and then stained with Giemsa color which was diluted 10x. Examination of the peripheral blood smear was carried out at the border of the lateral, medial and mediolateral zones, and blood tails. The percentage of aggregated platelets is calculated based on the number of aggregated platelets compared to the total platelet count. This method is quoted from Sotianingsih (2011) [14].

$$\%\ Aggregation = \frac{Aggregation\ Platelet}{Total\ Platelet}\ x\ 100\%$$

$$Aggregation\ with\ correction = \frac{(\%\ total\ aggregation - \%\ initial\ Aggregation)}{(100 - \%\ initial\ Aggregation)}$$

Interpretation of the results of the Velaskar method of platelet aggregation examination, for Hypoaggregation shows a value of < 54%, Normoaggregation shows a value of 54-70% and Hyperaggregation shows a value of > 70%.

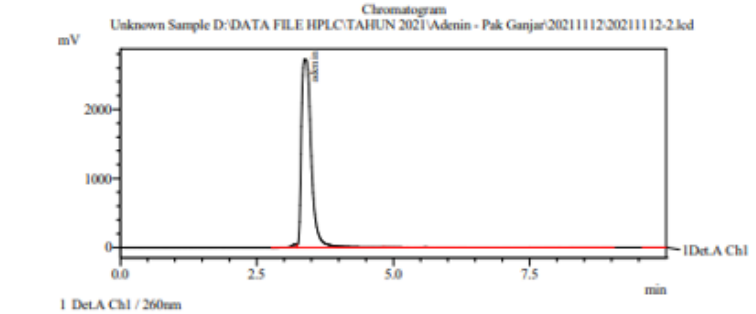
6. Results and discussion

The plant determination results from the Life Sciences Research Organization Bogor Biology Research Center Office with letter number B.947/V/DI.05.07/12/2021 stated that the subject of this study was a tomato (Solanum lycopersicum) and the results of testing the phytochemical content of the fruit water extract tomato (Solanum lycopersicum) conducted at the Basic Chemistry Laboratory, Department of Medical Laboratory Technology, Health Polytechnic, Ministry of Health, Bandung, showed that the water extract of tomato (Solanum lycopersicum) contains flavonoids, saponins and phenols (table 1).

Table 1. Phytochemical test results of tomato water extract

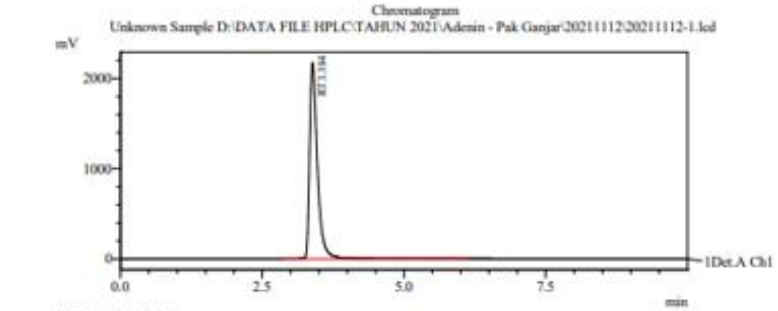
No	nato water extract	Testing						
		Alkaloids	Flavonoids	Steroids	terpenoids	Saponins	Phenol Tannins	
1.	5-ppm concentration	-	+	-	+	+	+ -	
2.	10-ppm concentration	-	+	-	+	+	+	-
3.	15-ppm concentration	-	+	-	+	+	+	-

The chemical content test was continued with the prediction of the adenine content in the tomato extract samples. This supporting analysis used High Performance Liquid Chromatography (HPLC) with reversed phase and gradient elution. HPLC results indicated the presence of 100-ppm adenine (Figure 1).



Detector A

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
adenin	3.383	34675466	98.935	0.736	1.713	2167.223	0.061



Detector A

Name	Ret. Time	Area	Area %	Resolution	Tailing Factor	Theoretical Plate#	k'
RT3.384	3.384	20957911	100.000	0.000	1.735	3093.169	0.000

Figure 1. Chromatogram of Adenine in Tomato Extract

The results of measuring the number of platelets using a hematology analyzer can be seen in Figure 2. The number of platelets in citrate blood alone used as a blank was 279,250 cells/µL. each treatment of epinephrine inductor and aspirin control and 5 -15 ppm extract. The results of the study obtained data that there was a decrease of 244,000 cells/µL each; 232,750 cells/µL; 227,250 cells/µL; 208,000 cells/µL. Treatment of tomato extract in this study proved to reduce the number of platelets.

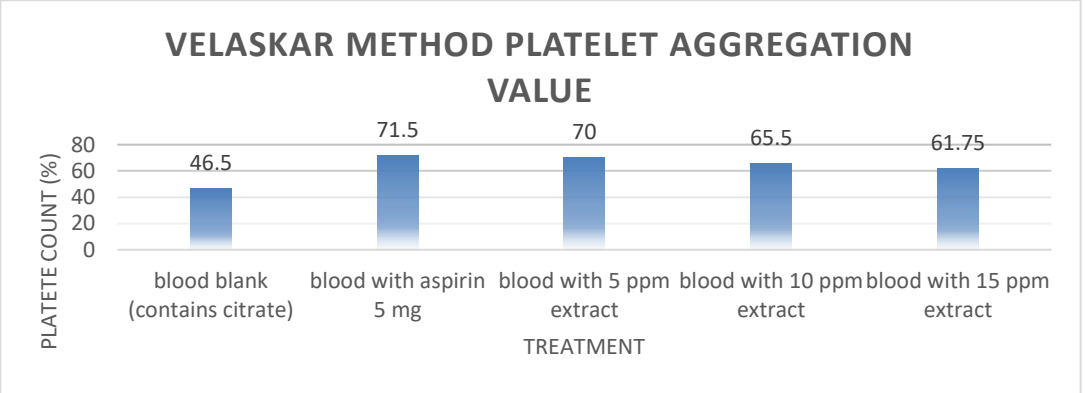


Figure 2. Average of Platelet counts

Table 2. shows the significant value of all research treatment data showing sig > α (0.005), so it can be concluded that all data are normally distributed. The homogeneity test showed a significant value of 0.646 > α (0.005), so that it can be concluded that the platelet aggregation data is homogeneous and the parametric One-Way ANOVA test can be performed.

Table 2 Tests of Normality Shapiro-Wilk

Dependent Variables	**Research Treatment**	**Sig.**
Platelet count (cell/µL)	Blood citric 3.8%	.166
	Citrate blood 3.8% + Aspirin 5 ppm	094
	Citrate blood 3.8% + Tomato Extract 5 ppm	.455
	Citrate blood 3.8% + Tomato extract 10 ppm	.500
	Citrate blood 3.8% + Tomato extract 15 ppm	.948

Table 3 shows a significant value of 0.000 < α (0.005), so that there is a significant difference in the value of platelet aggregation in each blood sample treatment. From the above results it can be concluded that the water extract of tomato (Solanum lycopersicum) is effective as an anti-platelet aggregation in whole blood against platelet aggregation.

Table 3 One Way Anova

		Sig.
Platelet aggregation (%)	Between Groups	.000
	Within Groups	
	Total	

Based on the results of the One-Way Anova test obtained from the SPSS output in table 4.12 Multiple Comparisons above, the results obtained. The difference in average platelet aggregation of Citrate blood 3.8% and Aspirin 5-ppm and citrate blood as a blank obtained Sig. 0.000 (Sig. < 0.05). It can be concluded that there is a difference in platelet aggregation in 5-ppm Aspirin treatment and Citrate blood 3.8%. The difference in average platelet aggregation of citrate blood 3.8% and 5-ppm Aspirin and citrate blood 3.8% and 5-ppm Tomato Extract obtained Sig. 0.993 (Sig. > 0.05). It can be concluded that there is no difference in platelet aggregation between Citrate blood 3.8% and Aspirin 5-ppm and Citrate blood 3.8% and Tomato Extract 5 ppm.

The difference in average platelet aggregation of Citrate blood 3.8% and Aspirin 5 ppm and Citrate blood 3.8% and Tomato Extract 10 ppm obtained the Sig. 0.485 (Sig. > 0.05). It can be concluded that there is no difference in platelet aggregation between Citrate blood 3.8% and Aspirin 5-ppm and Citrate blood 3.8% and Tomato Extract 10 ppm.

The difference in average platelet aggregation of Citrate blood 3.8% and 5-ppm Aspirin and Citrate blood 3.8% and 15-ppm Tomato Extract obtained Sig. 0.101 (Sig. > 0.05). It can be concluded that there is no difference in platelet aggregation between Citrate blood 3.8% and Aspirin 5-ppm and Citrate blood 3.8% and Tomato Extract 15-ppm. From the data it can be concluded that the minimum concentration of tomato water extract which is effective as an anti-aggregation agent in whole blood against platelet aggregation is 5 ppm.

Table 4. Multi Comparison Turkey HSD

Dependent Variables	(i) Treatment Study	(J) Treatment Study	Sig.
Aggregation thrombocyte (%)	Citrate blood 3.8% + Aspirin 5 ppm	Blood citric 3.8%	.000
		Blood citric 3.8% + Tomato Extract 5 ppm	.993
		Citrate blood 3.8% + Tomato extract 10 ppm	.485
		Citrate blood 3.8% + Tomato extract 15 ppm	.101

6.1. Discussion

In this research, tomato aqueous extract was made because the compounds contained in tomatoes are more polar compounds, one of which is adenine which dissolves well in water solvents. Adenine is polar and dissolves well in water with a solubility level in water of 0.103 g / 100 mL.

Examination of the platelet count using a medonic M32 hematology analyzer which has been carried out through a quality control process using normal control material with lot 22109-12 M-Series and the expiry date is February 4, 2022. The results of the control examination showed a value of 228,000 cells / µL and was included in the value range of 228,000 cells/µL ± 30,000. So, it can be concluded that the Medonic M32 hematology analyzer functions optimally for platelet examination.

For examination of platelet aggregation using the peripheral blood smear method introduced by Velaskar and Chitre. This assay is performed to evaluate differences in platelet aggregation regardless of initial platelet aggregation (or so-called circulating platelet aggregation). Velaskar read the entire zone from the edge of the smear to the next edge, and calculated the percentage of aggregated platelets compared
with total platelets. Examination of peripheral blood smears to assess platelet aggregation using an inductor as a trigger for platelet aggregation. Inductors that are often used in platelet aggregation tests are ADP, epinephrine and collagen. The inductor used in this study was 1mg/mL Epinephrine. The ability of epinephrine itself to induce platelet aggregation in vitro is highly dependent on the metabolism of arachidonic acid by the cyclooxygenase pathway. The results showed that the platelet aggregation value was still in the normal range, namely 54 -70%, but it was seen that there was a decrease in the platelet aggregation value according to the addition of aqueous extract of tomatoes to whole blood, this indicates the activity of the aqueous extract for tomatoes which can inhibit Platelet aggregation after administration of epinephrine inductors.

Platelet aggregation depends on the presence of free calcium, with the presence of agonists/inductors that will activate platelets causing an increase in intracytoplasmic calcium ions of platelets. An increase in platelet free calcium concentration results in a number of structural and functional changes in platelets. Morphologically, the platelets change from a disc shape to a spiny ball. The granules in the platelets are concentrated and their contents are discharged into the lumen of the open canaliculi and then out. Platelet shape changes are mediated by the platelet cytoskeleton, which is composed of a network of microtubule actin filaments and a number of related proteins associated with various platelet signaling molecules

The results of the One ANOVA test concluded that there was no significant difference between Citrate Blood 3.8% + Aspirin 5 ppm and all additional treatments aqueous extract of tomatoes, sig > α = 0.05 so that it can be concluded that aqueous extracts of tomatoes are effective as an anti-aggregation agent in whole blood on platelet count and platelet aggregation and the minimum concentration of tomato aqueous extract is 5 ppm.

This is in line with Ruman's research in 2010, regarding the anti-aggregation effect of tomato water extract, proving that tomatoes can inhibit platelet aggregation by up to 75%. Adenine is one of the chemical compounds contained in tomatoes that may function as an inhibitor of platelet aggregation. Some other literature mentions inhibition of platelet aggregation by inhibiting glycoprotein IIb/IIIa in platelets. The mechanism is that glycoprotein IIb/IIIa binds to adenine so that platelets become inactive and can prevent platelet aggregation by blocking fibrinogen binding to its receptor on the platelets. Adenine more strongly inhibits platelet aggregation induced by collagen than by ADP [14]

Other research results according to the Rambang, Fatmaria, Martani literature study in 2021 show several other plants besides tomatoes that have a function as anti-platelet aggregation including strawberry extract, grape seed, Allium sp., bay leaves, mango skin, Hawthorn leaves, C.aromatica Salisb ., red ginger rhizome, G.verrucosa, S.polycystum, olive leaf, M.alba, P.baumii, R.vernicflua, A.shikokiana, katuk leaves, tempuyung leaves, red cabbage, M.obovata, lempeni leaves, leaves kajahoi, starfruit leaves, U.macrocarpa, M.citrifolia, C.limon, L.japonica, E.bicyclis, S.desert, V.labrusca. Active compound [15] [16]

The most commonly found to have antiplatelet activity is the flavonoid, the fraction of which is quercetin. Mechanism of action on COX-1, AA, TXA2, P13K pathways, cAMP increase, VASP stimulation. Methods for extracting active compounds by maceration, reflux, soxhlet, sonication, juice, distillation [17] [18]

7. Conclusion

Water extract of tomato (Solanum lycopersicum) is effective as an anti-platelet aggregation in whole blood on platelet count and platelet aggregation and the minimum concentration of aqueous extract of tomato (Solanum lycopersicum) as anti-platelet aggregation in whole blood on platelet count and platelet aggregation is 5 ppm

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