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Research Article

Preliminary Identification and Quantification of Four Secondary Metabolites, Total Tannin and Total Flavonoid Contents in Guava Fruit Ethanol Extract

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ABSTRACT

Introduction: The study on guava fruit ethanol extract from Dukuhwaluh Village, Purwokerto, Central Java, Indonesia showed increased megakaryocytes and platelet numbers in thrombocytopenic mice model. The study of acute oral toxicity of the extract did not show toxic effects in the kidney and liver at doses of 2000 and 5000 mg/kg b.w. The aim of the study was to determine the profile and quantity of four metabolite compounds and total tannin and flavonoid in the extract. **Materials and Method:** gallic acid, ellagic acid, rutin and kaemferol in the extract were identified and quantified by using high-performance liquid chromatography (HPLC) method with column LiChroCART 250-4,6 RP 18E, isocratic mobile phases with the composition of mixture:0.1% acetic acid, acetonitrile and methanol (40:50:10) respectively, and at temperature 30^{0}C. The total tannin and flavonoid were determined using the by spectrophotometry method, Fe(III) chloride and 1.10-phenanthroline at wavelength 510 nm for tannin and aluminium chloride and rutin at wavelength 422 nm for flavonoid, respectively. **Results**: Percentage of gallic acid, ellagic acid, rutin and kaempferol were 0.77%, 1.37 %, 0.41 % and 0.35 %, respectively. Total tannin and flavonoid contents were 1.20% (TAE) and, 1.18% (RE) respectively. **Conclusion**: The guava fruit ethanol extract contained gallic acid, ellagic acid, rutin, kaempferol, tannin and flavonoid.
Key words: *Psidium guajava*, Guava fruit, Tannin, Flavonoid.

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INTRODUCTION

Infection by the dengue virus will cause a dangerous condition marked by the presence of varying degrees of thrombocytopenia.

Thrombocytopenia is a condition in which the platelet count is below the lower limit of normal.

Platelets are blood cells that are important to help blood clot.

Guava fruit extract from Dukuh Waluh Village, Purwokerto, Central Java, Indonesia has been tested for its activity and acute toxicity. A study by Atik *et al.* proved that guava fruit extract was shown to have a statistically significant activity to increase the number of megakaryocytes in thrombocytopenic mice, but not in normal mice. The acute toxicity was examined in the previous studies with the Up-and-Down Procedure showed safety and was not harmful to the liver and kidneys of mice. Also the phytochemical screening red guava fruit extract contained flavonoids, tannins, quinone, saponins, and triterpenoid/steroid.[1,2]

Guava plant is used as a traditional medicine to manage many diseases, among others some parts of the plant which are frequently used as follows: leaves for antidiarrheal, fruits for antidiabetic and anti-hypercholesterolemia, stem for leukorrhea, roots for dysentery, etc.[3,4]

A previous study reported some results, such as phytochemical screening red guava fruit extract containing flavonoids, tannins, quinone, saponins, and triterpenoid/steroid.[2]

The secondary metabolite compounds in guava fruits are ellagic acid, gallic acid, catequin, chlorogenic acid, and rutin.[5] Between ripe and green fruits, the catequin, rutin, syringic, elagic, transcinamic, cafeic and p-coumaric acids are the most contributing compounds as bioactive phenolics compounds.[5] Flesh of fruits contained flavonoid which is higher than fruit peel. Kaempferol is the mayor flavonoid in pink guava fruit.[6] Schottenol, kaempferol, quercetin, among many others.[7] Cyanidin-3-O-sophoroside is one of the major pigments in pink and yellow fruits, and five other pigments: myricitrin, myricetin, quercetin, quercitrin, and chlorogenic acid may be contributing to the formation of the red color,[8] lycopene, carotene, lectin.[9] Rich of carotenoids (beta-carotene, beta-cryptoxanthin, and lycopene), vitamin C and polyphenols, nine triterpenoids: ursolic acid; 1beta, 3beta-dihydroxyurs-12-en-28-oic acid; 3beta,19alpha-dihydroxyurs-12en-28-oic acid; 2alpha,3beta-dihydroxyurs-12-en-28-oic acid; 3beta, 23-dihydroxy urs-12-en-28-oic acid; 19a-hydroxylurs-12-en-28-oic acid-3-O-alpha-L-arabinopyranoside; 3beta, 19alpha, 23beta-tri-hydroxylurs-12-en-28-oic acid; 3alpha,19alpha,23,24-tetrahydroxyurs-12-en-28-oic acid; and 2alpha, 3beta,19alpha, 23betatetra-hydroxyurs-12-en-28-oic acid were found in a previous study.[10] There were also three benzophenone glycosides: 2, 6-dihydroxy-3-methyl-4-O-(6”-Ogalloyl-beta-D-glucopyranosyl)-benzophenone; 2, 6-dihydroxy-3, 5-dimethyl-4-O-beta-Dglucopyranosyl-benzophenone, and 2, 6-dihydroxy-3, 5-dimethyl-4-O-(6”-O-galloyl-beta-D-glucopyranosyl) benzophenone.[10]

The activities of guava fruits were reported to have antioxidant properties,[5,7] regulate blood

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pressure, maintain sodium and potassium levels in the body, lower triglycerides and low-density lipoprotein (LDL), and increase high-density lipoprotein (HDL). The guava fruits also showed antibacterial activity in *Shigella*, *E.coli*, *Staphylococcus*, *Pseudomonas*, *Bacillus* and *Clostridium*.[9] Prevention of cardiovascular damage is associated with lycopene content, while anti-cancer is associated with ursolic acid and other triterpenoids. Reduce the triglycerides accumulation were associated with Benzophenone glycosides.[10]

Its antioxidant properties protect against oxidative damage of an in vitro model of human skin fibroblasts which is suppressed using the stressor 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH).[11] Gallic acid, ellagic acid and its derivates, rutin, kaempferol, and also tannin were reported to increase platelet number and against dengue virus, and to accelerate blood clotting.[12-16]

Based on the prior studies described above some phytoconstituents in guava fruits had activity to increase the number of megakaryocytes in thrombocytopenia, among others gallic and ellagic acids and their derivatives, tannins, etc. Therefore, a study on the guava fruits ethanol extract from Dukuhwaluh that had activity to increase the number of megakaryocytes was continued to examine its content which was active to increase the number of megakaryocytes, namely ellagic acid, gallic acid, rutin, kaempferol, total flavonoid and tannin contents.

MATERIALS AND METHODS

Materials

Ethanol extract of guava fruits was obtained from Dukuhwaluh Village, Purwokerto, Central Java, Indonesia. Ellagic acid, and kaempferol reference standards were from ChemFaces; gallic acid, rutin, tannic acid from Sigma Aldrich; acetic acid; methanol; acetonitrile; and Milli Q-water (Merck) (all reagents were HPLC grade), ethylene diamine tetraacetic acid (EDTA), iron (III) chloride hexahydrate, sodium chloride, hydrochloric acid, gelatine, sulfuric acid, acetic acid, sodium acetate, potassium acetate, and aluminium chloride hexahydrate were from Merck (all reagents were analytical grade).

Equipment

The equipment used to examine the extract were *Waters Alliance e2695*HPLC; detector UV/Vis 2489, Column: LiChroCART 250-4,6RP 18E wavelength: UV 254 nm. Eppendorf Biospectrometer Basic AG 22331 Hamburg series: 6135 BJ.

Quantification of gallic acid, ellagic acid,rutin, and kaempferol by High-performance liquid chromatography (HPLC)[17]

Preparation of sample and reference standards for HPLC analysis[17]

Precisely 25 mg guava fruit extract was dissolved in 25 mL methanol, and filtered through a Millipore 0.45 µm filter. Then, 10 µL of the guava fruits solution was taken and injected into the HPLC instrument. In the same way for the reference standards procedure, each reference standard compound gallic acid, ellagic acid, rutin and kaempferol was weighed with the following concentrations: 10, 20, 30, 40 and 50 mg, respectively. Then, each reference standard compound was dissolved into 10 ml of methanol and filtered through a Millipore 0.45 µm filter. Then, 10 µL was injected into the HPLC instrument.

The condition of samples analysis was as follows, a column LiChroCART 250-4,6 RP 18E with heating at 30 ^{0}C was used as stationary phase and eluted with isocratic mobile phases consisting of 0.1% acetic acid in water - acetonitrile - methanol (40:50:10). The flow rate was 1 ml/min, separation time was 30 min, and detection was at 254 nm. Chromatogram data result was performed with the aid of sma4w148e.

Then, in similar conditions with the samples, reference standard curves of gallic acid, ellagic acid, rutin, and kaempferol were quantified by various concentrations: 10, 20, 30, 40, and 50 ppm, respectively. Standard calibration curves were made by plotting the retention time against the peak area.

Measurement of total tannin in the extract[17]

Preparation of reference standards calibration curve

Prepared reference standard tannic acid in concentrations were as follows: 1.0, 2.0, 3.0, 4.0 and 5.0 µg/mL in 25.00 mL the volumetric flasks, respectively. Precisely 2.50 mL of iron (III) chloride hexahydrate solution (0.01 mol/L) was added to each flask and the mixture was incubated in a water bath for 20 minutes at 80°C. Then, 2.50 mL of acetic buffer (pH 4.4), 5.0 mL of 1,10-phenanthroline solution (0.015 mol/L) and 0.50 mL of EDTA (0.05 mol/L) were added to each flask to let the solution cool. Afterwards, each flask was added up with distilled water to the limit mark. The absorbance of the solution was measured at a wavelength of 540 nm relative to the blank. The reference standard tannic acid calibration curve was made by plotting the concentration against absorbance.

Preparation sample of guava fruits extract

The guava fruits extract was prepared by dissolving 0.025 grams extract to 100 ml solution with distilled water. The next step followed the same steps in the preparation of the calibration curve.

Preparation of blank for guava fruits extract

Aliquots of 10 ml of guava fruits extract solution above was put to 100.00 mL volumetric flask containing 10 mL of acidic NaCl solution, and 0.50 mL of gelatine solution (0.3% m/V), and added distilled water to the limit mark. Then, the mixture was shaken and filtered (Filtrate No.1).Aliquots of 10 ml of Filtrate No. 1 was pipette and then the same way to Fitrat No.1 procedure was performed at which the result was called Filtrate No. 2. Aliquots of 10 ml of Filtrate No. 2 was put into a 25 mL flask and the next step followed the same steps in the calibration curve preparation.

Preparing the gelatin blank

The gelatin blank samples were created with the same procedure as the blank guava fruits samples. The sample of guava fruits was substituted with distilled water. All works were performed in triplicate, and the absorbance of the solutions above were measured at a wavelength of 540 nm.

Then the tannin content was counted with the equation of calibration curve of standard reference of tannic acid and the absorbance of the tannins in the sample.

The absorbance of the tannins in the sample was counted with the equation below:

Anbs = Abbs – Agbs

At =As - Anbs

where:

Anbs= the net blank samples absorbance,

Abbs= the blank *P. guava* fruit samples absorbance

Agbs= the gelatine blank sample absorbance for the corresponding sample.

At= the absorbance of the tannins in the sample

As= the *P. guava* fruit sample absorbance.

Measurement of total flavonoids in the extract[18,19]

Preparation of rutin reference standard calibration curve

Rutin reference standard (100 mg/25 mL) was pipetted to 10.00 mL the volumetric flasks as follows: 0.125; 0.250; 0.375; 0.500, and 0.675 mL, respectively. The final concentration of each rutin reference standard was 50, 100, 150, 200, 250 ppm.

Aliquots of 0.5 mL of each rutin reference standard solution was pipetted and was added with 1.5 mL methanol 80%, 0.1 mL potassium acetate 1 M, and 0.1 mL aluminium chloride 10% and was mixed. Moreover, distilled water 2,8 mL was added and shaken. Then, the mixture was incubated at room temperature for 30 minutes. The solution absorbance was measured at 422nm relative to the blank. All rutin reference standard solutions were measured at 422 nm to make the rutin reference standards curve. In addition, all measurements were performed in triplicate. The reference standard calibration curve was made by plotting the concentration against absorbance.

Preparation of guava fruit extract

The first step was to make a guava fruit extract stock solution (250 mg/25 mL).

Aliquots of 0.5 mL of guava fruit extract stock solution was pipetted, and the next step followed the procedure in the preparation of the calibration curve.

Measurement was performed in triplicate.

The flavonoid content was counted with the equation of the standard curve of rutin reference, and $F1 = \frac{C \times V \times F \times 100\%}{m}$

Where:

F=Total flavonoids content

C= rutin equivalence

V=volume of extract (mL),

F= dilution factor

m= extract weight(g)

Time and place

This study was carried out in the period of April 2021 - July 2021 in the Central Laboratory Universitas Padjadjaran and Biochemistry and Molecular Biology Laboratory, Faculty of Medicine Universitas Padjadjaran, Jatinangor, Indonesia.

RESULT

Identification and quantification of gallic acid, ellagic acid, rutin and kaempferol from guava fruits extract

Measurement of gallic acid, ellagic acid, rutin and kaempferol in the extract by HPLC were shown in Table 1 and Figure 1, respectively.

Chromatogram between references standard and guava fruits extract was shown in Figure 2.

Table 1: Retention time and area of gallic acid, rutin, ellagic acid, and kaempferol from P.guajava fruits extract .

Name of compound	Retention time (min)	Area
Gallic acid	3.658	114200
Rutin	14.017	16519
Ellagic acid	15.353	211663
Kaempferol	25.643	21295

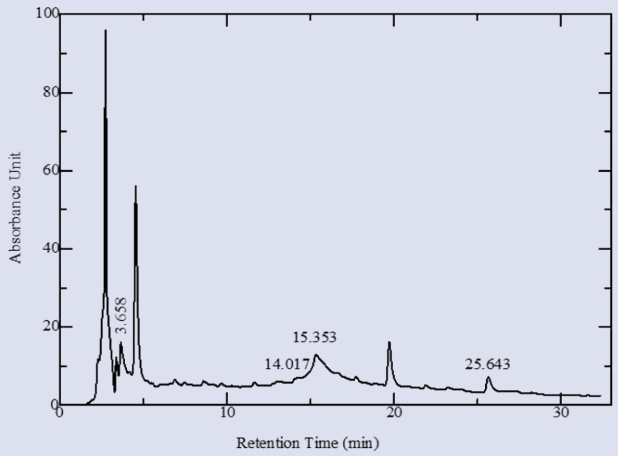

Figure 1: Chromatograms of gallic acid, rutin, ellagic acid, and kaempferol in guava fruits extract

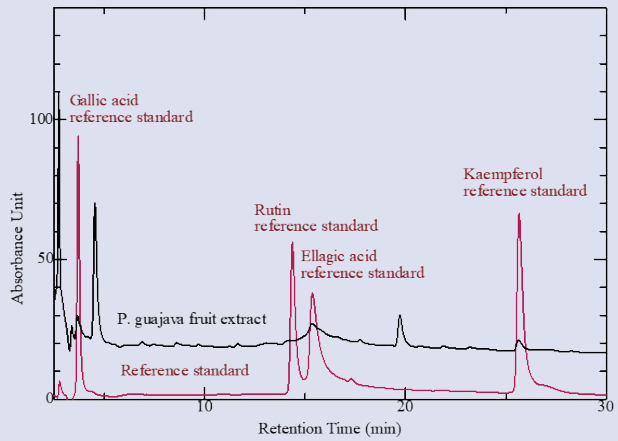

Figure 2: Chromatograms of references standard of gallic acid, rutin, ellagic acid, and kaempferol and the guava fruits extract

Gallic acid, ellagic acid, rutin and kaempferol content in the extract were identified from its chromatogram data (retention times). Then, chromatogram data of extract was compared with chromatogram data of gallic acid, ellagic acid, rutin and kaempferol references standard. Comparison between two chromatograms data was performed with the aid of sma4w148e, the result was shown in Figure 2.

From data in Tables 1 to 3 and chromatogram in Figure 1 to 2 were obtained similarity of all retention times among gallic acid, ellagic acid, rutin and kaempferol reference standards were 3.709 min, 14.871 min,14.317 min and 25.595 min, respectively, while gallic acid, ellagic acid, rutin and kaempferol in guava fruit extract were 3.658 min, 15.353 min. 14.017 min and 25.643 min, respectively.

Quantification of gallic acid, ellagic acid, rutin and kaempferol by HPLC method

Reference standard curves of gallic acid, ellagic acid, rutin and kaempferol were made by various concentrations: 10, 20, 30, 40, and 50 ppm, respectively. The resulted areas of all reference standards in various concentrations above, limits of detection (LOD), and limits of quantification (LOQ) were shown in Table 2, while the reference standard curves were shown in Figure 3 to Figure 6, respectively.

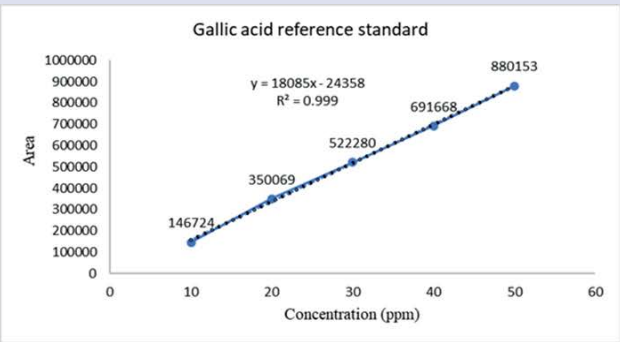

Figure 3 : The standard curve of gallic acid reference standard in various concentration of 10, 20, 30, 40 and 50 ppm

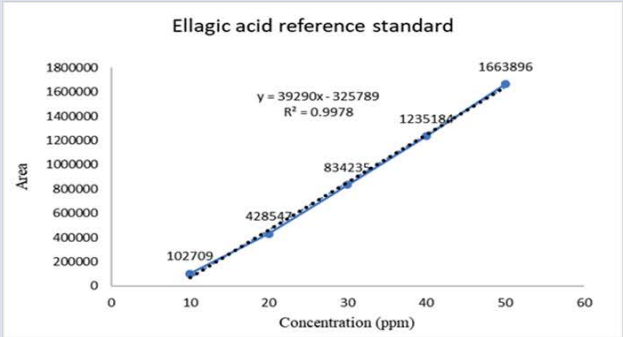

Figure 4 : The standard curve of ellagic acid reference standard in various concentration of 10, 20, 30, 40 and 50 ppm

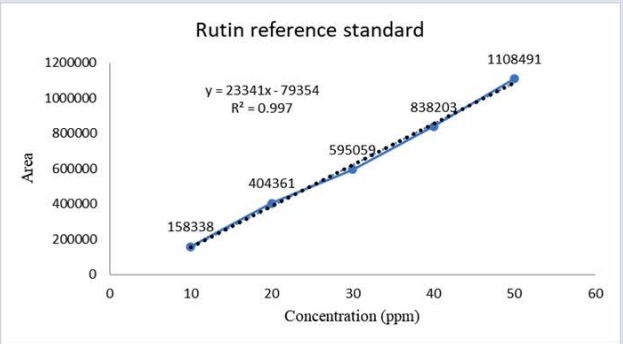

Figure 5 : The standard curve of rutin reference standard in various concentration of 10, 20, 30, 40 and 50 ppm

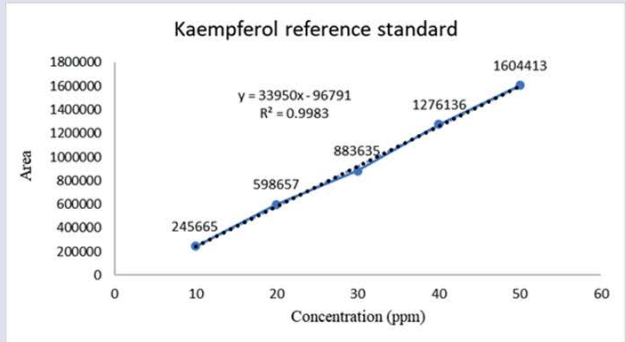

Figure 6 : The standard curve of kaempferol reference standard in various concentration of 10, 20, 30, 40 and 50 ppm

Equation of the reference standard curve of gallic acid was y = 18085x – 24358, ellagic acid was y = 39290x – 325789, rutin was y = 23341x – 79354, and kaempferol was y = 33950x – 96791.

All equations were utilized to calculate the concentration of gallic acid, ellagic acid, rutin and kaempferol, respectively. Percentage of gallic acid, ellagic acid, rutin and kaempferol content in guava fruits extract were shown in Table 3.

From data in Table 3, the percentage concentration of gallic acid, ellagic acid, rutin and kaempferol content in guava fruits extracts were 0.77, 1.37, 0.41, and 0.35 %, respectively.

Quantification of total tannin in guava fruits extract[17]

The calculations of absorbances of the extract, blank of extract, gelatine blank, respectively, are needed to determine tannin content in the fruit extract. The absorbances were required to calculate the tannin absorbance in the guava fruits extract. Then, the tannin absorbance in the guava fruits extract and the equation of calibration curve of tannic acid reference standard were used to calculate the total tannin content in the extract. The absorbances of guava fruits extract, blank of extract, and gelatine blank were presented in Table 4.

Based on the data in Table 4 above and the following equation

Anbs = Abbs - Agbs

At = As - Anbs

The result: the absorbance of the tannins in the sample = 0,608

Table 2: Area of gallic acid, ellagic acid, rutin and kaempferol reference standards in concentrations of 10, 20, 30, 40, and 50 ppm, and limits of detection (LOD) and limits of quantification (LOQ).

Standard concentration (ppm)	Area of reference standards			
	Gallic acid	Ellagic acid	Rutin	Kaempferol
10	146724	102709	158338	245665
20	350069	428547	404361	598657
30	522280	834235	595059	883635
40	691668	1235184	838203	1276136
50	880153	1663896	1108491	1604413
LOD	1.4178	0.3386	0.9786	0.8727
LOQ	4.2963	1.0262	2.9656	2.6444

Table 3: Percentage of gallic acid, ellagic acid, rutin and kaempferol content in guava fruits extract.

Parameters of guava fruit extract	Compound			
	Gallic acid	Ellagic acid	Rutin	Kaempferol
The retention time of the reference standard	3.709	14.871	14.317	25.595
Area of compound in the sample	114200	211663	16519	21295
The concentration of a compound in the sample (%)	0.77	1.37	0.41	0.35

Table 4: Absorbance of guava fruits extract, blank of extract, and gelatine blank.

Sample	Absorbance
Guava fruits extract	0,620
Blank ofguava fruits extract	0,082
Blank of gelatine	0,070

The Calibration curves of tannic acid reference standard in various concentrations were 0.5, 1.0, 2.0, 3.0 and 4.0 µg/mL, respectively, and the equation of calibration curve was shown in Table 5 and Figure 7.

The equation of calibration curve of tannic acid reference standard showed y = 0.1738x + 0.0892. Then, the guava fruit tannin content was calculated from the equation of calibration curve of tannic acid reference standard and the absorbance of the tannins in the sample.

The total tannin content as equivalent of tannic acid in guava fruits extract = 1.20 % (TAE).

Quantification of total flavonoid in guava fruits extract[18,19]

Calculation of flavonoid content required measurements of absorbances of the extract, the equation of calibration curve of flavonoid reference standard, namely rutin as a reference standard, and the following equation,

$$F1 = \frac{C \times V \times F \times 100\%}{m}$$

The calibration curve of the rutin reference standard was shown in Table 6 and Figure 8.

The flavonoid content in guava fruits extract was counted with a linear regression equation from the standard curve of rutin reference standard, y = 0.0014x + 0.0589 and the following equation,

$$F1 = \frac{C \times V \times F \times 100\%}{m}$$

The percentage of flavonoid content in guava fruits extract was 1.18%.

Table 5: Calibration curve of tannic acid reference standard.

Concentration ppm	Absorbance
1	0.233
2	0.469
3	0.623
4	0.783
5	0.945

Table 6: The absorbance of the Rutin reference standard in the various concentrations.

Concentration of Rutin reference standard (ppm)	Absorbance (average)
50	0.102
100	0.228
150	0.284
200	0.359
250	0.394

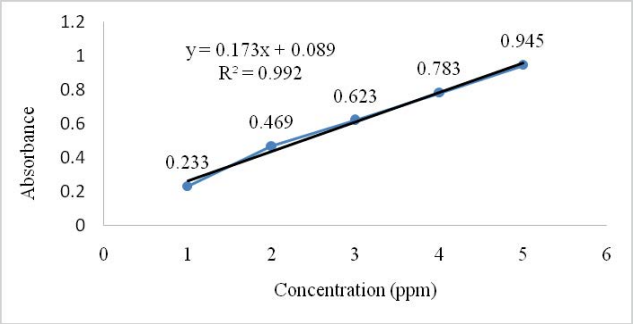

Figure 7: Calibration curve of tannic acid reference standard.

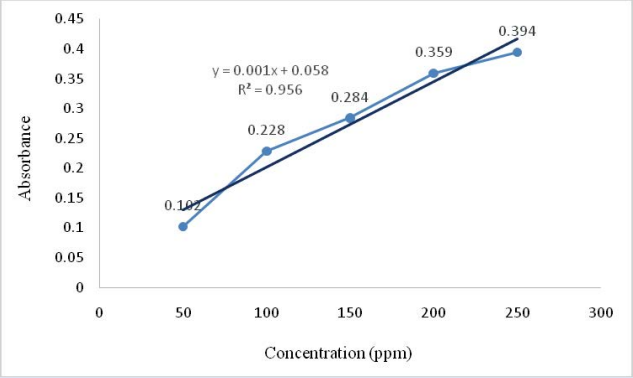

Figure 8: The standard curve of the rutin reference standard in various concentrations of 50, 100, 150, 200 and 250 ppm.

DISCUSSION

Thrombocytopenia caused bleeding problems and thrombosis, although the correlation of severity of thrombocytopenia and bleeding problems is still unknown.[20] This condition caused the researchers to explore the plants that have an activity to increase platelet count. Platelets are produced by two-step processes namely megakaryopoiesis and thrombopoiesis.[16]

The guava plant is used as a traditional medicine to manage many diseases. A previous study has proven that guava fruits extract from Dukuhwaluh has an activity to increase the number of megakaryocytes in thrombocytopenic rats. The acute toxicity test has been shown to be safe and non-toxic to the liver and kidneys of the mice. The phytochemical screening showed the extractcontained flavonoids, tannins, quinone, saponins, and triterpenoid/steroid.[1,2]

Therefore, the study of the guava fruit extract from Dukuhwaluh was developed by scientific research to prove its phytochemicals content, namely ellagic acid, gallic acid, rutin, kaempferol, total tannins and total flavonoids contents.

Other previous studies reported that ellagic acid derivates, namely 3,3',4-tri-O-methylellagic acid-4'-O-ß-D-xyloside or 3,3',4-tri-O-methylellagic acid were reported to induce megakaryopoiesis.[13] It also described that ellagic acid rendered the increased platelet number *via* overexpression of the cyclooxygenase pathway.[16] Another activity of ellagic acid inhibits the dengue virus (DENV), whereas DENV infection can cause thrombocytopenia.[21] Gallic acid derivatives, (-)-epigallocatechin gallate (EGCg) and (-)-epigallocatechin (EGC) inhibited severe fever with thrombocytopenia syndrome (SFTSV) infection[12] and gallic acid itself has activity against the dengue virus,[12,16] by which thrombocytopenia is one of the major clinical manifestations and laboratory abnormalities caused by SFTSV infection.[25]

Tannin had several physiological effects, such as speeding up the blood clotting.[14] As well as tannic acid, it had acted to protect megakaryocytes from apoptosis that is caused by ionizing radiation.[15] Gallic acid, ellagic acid, quercetin, rutin and kaempferol have activity against the dengue virus.[16]

The relation among gallic acid, ellagic acid and tannin were explained by Agrawal *et al.* and Harbon. Gallic acid structure has a relationship with ellagic acid structure. Gallic acid can form ellagic acid by dimerization of gallic acid by oxidative aromatic coupling with intramolecular lactonization.[26] Therefore, the gallic acid content in guava fruit extract may correlate with the ellagic acid content in the extract.

Both gallic acid and ellagic acid have relationship with tannin structure. Tannins are generally divided into two main groups: condensed tannins and hydrolysable tannins. Hydrolysable tannins are mainly of two classes, namely gallotannins and ellagitannins.[26,27] Gallotannins is an ester of gallic acid and ellagitannins is an ester of hexahydroxydiphenic acid with glucose. On hydrolysis both gallotannins and ellagitannins

produce gallic acid and ellagic acid, respectively, and glucose.[26,27] Condensed tannins or flavolans are oligomers of catechins and flavan-3,4-diols.[27]

Previous studies reported guava fruits contained gallic acid, +(-) catechin, (-)-epicatechin,[22] and (-)-epigallocatechin gallate (EGCg) which is an ester of epigallocatechin and gallic acid.[23,24]

Based on previous studies on gallic acid, ellagic acid and tannins as described above, hydrolysable tannins on hydrolysis can decompose to form gallic acid and ellagic acid. The condensed tannins are formed from catechins, and catechin with gallic acid formed esters, as well (-)-epigallocatechin gallate (EGCg) is an ester of epigallocatechin and gallic acid.[23,24] Thus, tannins are useful as a source of gallic acid, ellagic acid and their derivatives.

The HPLC analysis on guava fruit extract from Dukuhwaluh proved that the guava fruit extract contained ellagic acid, and gallic acid. Both gallic acid and ellagic acid have a relationship with tannin. Therefore, the study was developed by investigating the tannin content of the guava fruits extract.

The total tannin content in the guava fruits extract was 1.20 % (TAE).

The guava fruit extract from Dukuhwaluh contained gallic acid (0.77 %), ellagic acid (1.37 %), and tannin content 1.20 % (TAE).

Findings in the study are in accordance with previous studies that reported guava fruits contained gallic acid and its derivates, ellagic acid and its derivates, and tannin. All compounds were reported to increase the platelet number in thrombocytopenia and against dengue virus (DENV). Thus, the content of gallic acid, ellagic acid, and tannins in a guava fruit extract from Dukuhwaluh has supported the increased number of platelets.

Many studies report that flavonoids have pharmacological and biological activities, such as antioxidants. The antioxidant activity depends on its structure. The substitution, configuration, and a total number of hydroxyl groups will influence the antioxidant activities, such as free radical scavenging, inactivation of peroxides and other reactive oxygen species, chelation of metals ability.[28,29] Chemically, basic flavonoid structure is built by three rings, two benzene rings as A and B rings, and a heterocyclic pyrane ring as C ring. [27-29] Structural groups of flavonoids associate with the radical scavenging properties, such as the hydroxyl configuration in ring B is known to be the most significant determinant in scavenging ROS and RNS. Ring B donates electrons and hydrogen to peroxyl, hydroxyl and peroxyl nitrite radicals. This activity results a form relatively stable flavonoids radical. [28,29]

Flavonoids have general structure as aglycones, glycosides, and methylated derivatives.[27-29]

Rutin and kaempferol are flavonol, by which rutin is a glycoside of quercetin. [27-29]

The previous study reported both flavonols, rutin and kaempferol had antioxidant activity,[30,31] then, the other study reported that the natural compounds with antioxidant activities, especially flavonoids, could increase platelet production, besides quersetin, rutin and kaempferol had activity against dengue virus (DENV).[16] The antioxidant activity is supported by the theory as described above.

The HPLC analysis on guava fruit extract from Dukuhwaluh proved that the guava fruit extract contained two flavonols, namely rutin and kaempferol. The percentage of rutin and kaempferol were 0.41, and 0.35 %, respectively. Both rutin and kaempferol are flavonol, by which falvonol is a class in flavonoid classification. Therefore, the study was developed by investigating the flavonoids content of the guava fruits extract.

The total flavonoids content in the guava fruits extract was 1.18 % (RE).

The guava fruit extract from Dukuhwaluh contained rutin (0.41 %), kaempferol (0.35 %), and total flavonoids content 1.18 % (RE).

Findings in the study are in accordance with previous studies that guava fruits contained rutin and kaempferol, quercetin and others. All compounds were reported to have antioxidant activity, especially flavonoids that could increase platelet production. Likewise, to increase the platelet number in thrombocytopenia and against dengue virus (DENV).

Thus, the content of rutin, kaempferol, and other flavonoids in a guava fruit extract from Dukuhwaluh has supported the increased number of platelets.

As described above, the guava fruits extract contained gallic acid, ellagic acid, rutin, and kaempferol, likewise tannins and flavonoids contents, and all of the compounds have supported the activity to increase the number of thrombocytes in thrombocytopenia.

Therefore, the presence of gallic acid (0.7661%), ellagic acid (1.3679%), rutin (0.4107%) and kaempferol (0.3478%), tannin 1.2 % (TAE), and flavonoid 1,18% (RE), can be used as a typical characteristic of a guava fruits extract which has activity to increase the number of thrombocytes in thrombocytopenia.

CONCLUSION

This study proved that the guava fruits extract contained gallic acid (0.7661%), ellagic acid (1.3679%), rutin (0.4107%) and kaempferol (0.3478%), tannin 1.2 % (TAE), and flavonoid 1,18% (RE).

The presence of these compounds in previous studies were reported to have an activity to increase the number of thrombocytes in thrombocytopenia.

Thus, the presence of these phytoconstituents in the extract could support the activity of the increased number of platelets in thrombocytopenia and can be used as phytoconstituents parameters of the guava fruits extract which have an activity to increase the number of platelets in thrombocytopenia.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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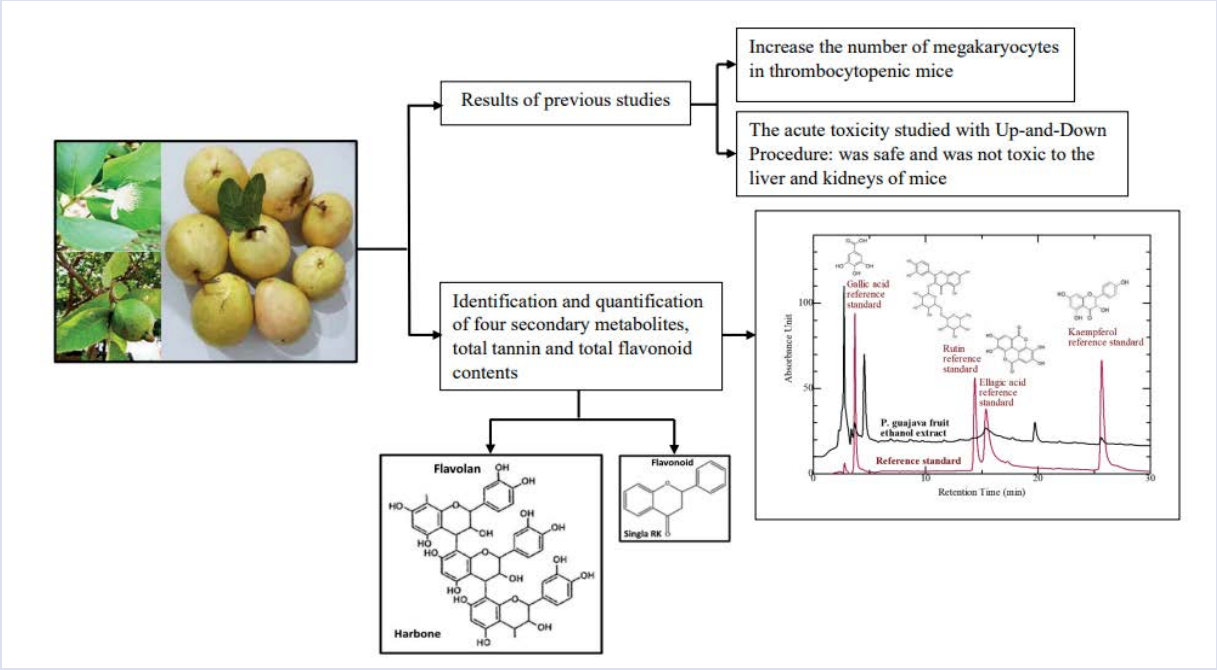
GRAPHICAL ABSTRACT



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