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Determination of Total Tannin of White and Red Rind Pomegranate (*Punica granatum* L.) by Colorimetry Method Using Reagent 1, 10 Phenantroline

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Abstract

Determination of the total tannin of white and red rind pomegranate (*Punica granatum* L.) has been carried out by colorimetry method using reagent 1,10 phenantroline. This method is based on reduction of iron (III) into iron (II) by tannin at temperature 80^0C for 20 min. Then the formed of iron (II) was reacted with 1,10 phenantroline to form orange red colour complex that could be measured by spectrophotometer visible at maximum absorption wavelength of 508 nm. The limit of detection (LOD) and the limit of quantitation (LOQ) obtained were 0.34 µg/mL and 1.14 µg/mL, respectively. This result was found to be linier with R value of 0.9984; accuracy as percent recovery was 84.69 ± 0.85 % and coefficient of variant (KV) was 1.003 % for white rind pomegranate while red rind pomegranate percent recovery was 84.38 ± 0,45% and coefficient of variant (KV) was 0.53 %. The total tannin of white rind pomegranate was 18.28 ± 0.072 %b/b and red rind pomegranate was 17.33 ± 0.081%b/b

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Keywords: Tannin, 1,10 phenantroline, colorimetry, white and red rind pomegranate, *Punica granatum* L

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1. Introduction

Malaria Pomegranate has been used in various regions and traditional medical systems as a a medicine because of its enormous compounds with lots of activities and without toxicity. Tannin is one of the active compounds of secondary metabolites known to protect against intestinal mucosal lining of the intestinal contents stimulation and can precipitate toxic. Tannins are widespread in the plant parts such as bark, leaves, fruit and root. The development of standardized herbal medicine requires one standardisation product contains a chemical compound used as a

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marker. Therefore, the method of analysis of the content of the required medicinal plants, one in the analysis of the assay tannin[1]. Determination total amount of tannins done using colorimetric reagents 1.10 phenantrolin and a solution of iron (III), because it has a better sensitivity value, the reaction happened to running quickly and specifically so that measurements can be effectively and efficiently[2,3].

2. Experiments

2.1. Material

White and red rind pomegranate, distilled water, Iron (III) solution, tannic acid, kaolin, acetate buffer solution pH 4.4, Ethylenediaminetetraacetic acid (EDTA) solution, 1,10 phenantrolin solution, gelatin solution, sodium chloride solution.

2.2. Methods

2.2.1. Preparation of sample solutions

A rind pomegranate sample (0.05 g) was boiled with 80 ml of distilled water for 1 hour. The mixture was cooled, filtared and the filtrate was made up to 100 ml with distilled water in a calibrated flask.

2.2.2. Determination

Calibration graphs. standard tannic acid solutions were pipetted separately into a series of 25 ml calibrated flasks so that the concentration of tannic acid in the final solutions ranged from 1 - 6 μg ml^{-1}. To each of these flasks were added 2.5 ml of the iron (III) solution and the mixtures were heated on a water-bath at 80°C for 20 min. Then, a mixture containing 2.5 ml of acetate buffer, 5.0 ml of 1,l0 phenanthroline solution and 0.50 ml of EDTA solution was added to each flask. After the flasks had been cooled to room temperature, the solutions were made up to the mark with distilled water. The absorbance of the solutions was measured against a reagent blank at 508 nm. The calibration graph was prepared by plotting absorbance *versus* concentration of tannic acid[1]

. *Sample and blank determinations.*1.0 ml of the sample solution were treated as described above. The sample blank was obtained as follows. A 10-ml volume of the sample solution was pipetted into a 100 ml beaker containing 5.0 ml of gelatin solution. To the mixture were added 10.0 ml of acidic sodium chloride solution followed by 2.0 g of kaolin and the whole was shaken for several minutes. The precipitate was allowed to settle and the mixture was filtered. Then, 10.0 ml of the filtrate, 6.0 ml of distilled water, 3.0 ml of gelatin solution and 6.0 ml of acidic sodium chloride solution were pipetted into a 100 ml beaker followed by the addition of 2.0 g of kaolin. After shaking for several minutes, the mixture was filtered and 1.25-2.5 ml of the filtrate were treated as described under Calibration graphs. The procedure for determining the gelatin blank was the same as that for the sample blank except that distilled water was used instead of the sample solution. The difference in absorbance between the sample blank and the gelatin blank gave the net sample blank. The difference in absorbance between the sample and net sample blank was due to tannins in the sample and their concentration was deduced from the calibration graph.

3. Results and Discussion

The determination of the level of tannin had been done by colorimetric method by using reagent 1,10 phenantrolin and a solution of iron (III). The principal methode based on reduction of iron (III) to become iron (II) because of tannin. By the addition of reagent 1,10-phenantrolin is to be formed complex iron (II) that is colored red orange.

The water was used as solvent for sample extraction because of the nature of tannin which dissolves easily in water. The extraction was done by heating for 1 hour at a temperature of 80^{o}C. With this condition was expected that

tannin can be extracted perfectly so that it acquired levels of tannin maximally. The first experiment was to optimize the weight of simplisia started from 250 mg, 100 mg and 50 mg. It was done because the more the simplisia used absorption obtained even higher. 50 mg Then the weight rind pomegranate white and red used was 50 mg. The Extract was added $FeCl_3$ solution and it was a reaction reduction of iron (III) become iron (II). Reduction to reaction can run perfectly by warming the extract for about 20 minutes. Then iron (II) was formed and reacted with reagent 1,10 phenantroline. After a warm-up then added acetate buffer pH 4.4, 1.10-Na phenantroline and EDTA. The function of buffer acetate pH 4.4 was to stabilize the pH to acidic atmosphere while Na-EDTA serves as a complexing agent for other metals contained in solution so that the formation reaction of iron (II) with 1,10 phenantroline not disturb with the existence of other metals. In addition to the measurement of samples, sample measurement was done without in order to see tannin other compounds that enter the measured compounds other than that which is tannin can react with iron (III) chloride so get involved in the process of the reduction of iron (III) iron (II), in which the measurement of the sample without added gelatin solution tannin 0.3% prior to extract is reacted with iron (III) chloride 0, 01M. A solution of gelatin was used to bind the tannin. Tannin was bound by gelatin, when react with iron (II) chloride. A solution of gelatin was appended twice with a view to the tannin completely bound perfect by gelatin so, when measurements have not been there is a compound of tannin. To ascertain the tannin filtrat drops are bound at last, a solution of gelatin when it has not happened feculence; tannin already properly bound. Result of measuring absorption here in the form of absorption in addition to the tannin namely polifenolat because tannin including a compound polifenolat. So, absorption of tannin total obtained is absorption samples reduced absorption blanko samples. From extraneous matter obtained levels of tannin total to the rind of a pomegranate white is 18,28 % ± 0,072 (result see table 3.1) and rind of a pomegranates red is 17,33 % ± 0,081 (table 2).

Table 1. Concentration of Tannin In White Rind Pomegranate

Nr.	As	ABL(s)	AT	C (%)
1	1,692	0,5035	1,1885	18,32
2	1,695	0,5028	1,1922	18,40
3	1,690	0,5036	1,1864	18,27
4	1,685	0,5026	1,1824	18,18
5	1,689	0,5031	1,1859	18,26
6	1,691	0,5038	1,1872	18,29
	X ± Sb			18,28 ± 0,072

Table 2. Concentration of Tannin in Red Rind Pomegranate

Nr	As	ABL(s)	AT	C (%)
1	1,681	0,5396	1,1441	17,33
2	1,676	0,5372	1,1388	17,21
3	1,678	0,5368	1,1412	17,29
4	1,680	0,5370	1,1430	17,31
5	1,684	0,5367	1,1473	17,40
6	1,686	0,5371	1,1483	17,44
	X ± Sb			17,33 ± 0,081

The wavelength of maximum absorbance was 508 nm. The linier equation of curve calibration y = 0,225x + 0,364 to r = 0,9984. This equation was used to determine the concentration of tannin that will be sought. In addition, the value of linearity was also determined. The limit of detection (LOD) and the limit of quantization (LOQ)[4]. The value of LOD obtained is 0,34µg/mL and LOQ is 1,14µg/mL. Determination of the limits detection and bounds quantization can be computed from curves calibration by counting value $Sy/x = \sqrt{\sum(y-\hat{y})^2 / n-2}$, Where value LOD = 3sy / / b x and LOQ = sy / 10 / b x with sy / x = by way raw residual and b = slant a line (table 3.3).

Table 3. Determination of tannin concentration at λ maximum 508 nm

Concentration (µg/mL)	Absorbance (A)	y^	y-y^	(y-y^)²
1	0.611	0.589	0.022	0.000484
2	0.814	0.814	0	0
3	1.025	1.039	-0.014	0.000196
4	1.238	1.264	-0.026	0.000676
5	1.475	1.489	-0.014	0.000196
6	1.747	1.714	0.033	0.001089
			Total =	0.002641

Table 4. Accuracy as Percent Recovery and Coefficient of Variant (KV) for White Rind Pomegranate

Nr.	A s+a	A BLs+a	As	A BL(s)	Ast	Recovery (%)
1	1,958	0,2598	1.692	0,5035	0,619	84,28
2	1,956	0,2601	1,695	0,5028	0,619	83,47
3	1,960	0,2594	1,690	0,5036	0,619	84,84
4	1,962	0,2605	1,685	0,5026	0,619	85,94
5	1,964	0,2596	1,689	0,5031	0,619	84,41
6	1,961	0,2592	1,691	0,5038	0,619	85,24
			X±Sb			84,69 ± 0,85
			%KV			1,003 %

Table 5. Accuracy as Percent Recovery and Coefficient of Variant (KV) for Red Rind Pomegranate

Nr.	A s+a	A BLs+a	As	A BL(s)	Ast	Recovery (%)
1	1,958	0,2992	1.681	0,5396	0,619	83,96
2	1,956	0,2986	1,676	0,5372	0,619	84,60
3	1,960	0,2988	1,678	0,5368	0,619	84,82
4	1,962	0,2993	1,680	0,5370	0,619	84,72
5	1,964	0,2991	1,684	0,5367	0,619	84,43
6	1,961	0,2989	1,686	0,5371	0,619	83,71
			X±Sb			84,38 ± 0,45
			%KV			0,53 %

4. **Conclusion**

In the measurement of the calibration curve obtained line equation y = 0.225 x + 0.364 with r = 0.9984.The limit of detection (LOD) and the limit of quantitation (LOQ) obtained were 0,34 µg/mL and 1,142µg/mL, respectively. Accuracy as percent recovery were 84,69 ± 0,85 % and coefficient of variant (KV) were 1,003 % for white rind pomegranate while red rind pomegranate percent recovery were 84,38 ± 0,45% and coefficient of variant (KV) were 0,53 %. The total tannin of white rind pomegranate was 18,28 ± 0,072 %w/w and red rind pomegranate was 17,33 ± 0,081%w/w. The proposed method can be used for quantitave determination of total tannin in white and red rind pomegranate (*Punica granatum* L.).

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