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Formulation and Test of Antioxidant Activity of Serum Active Fraction of Red Pomegranate Skin Extract (*Punica granatum* L.) by FRAP Method (*Ferric Reducing Antioxidant Power*)

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Abstract: Aging of the body occurs with age, which can be seen in the skin. Skin aging is a physiological process that occurs in the human body. Free radicals are one of the causes of skin aging. By using antioxidant compounds, you can stop the increase in free radicals. Red pomegranate peel extract (*Punica granatum* L.) is one of the natural ingredients for a natural antioxidant, cause it's contains polyphenols such as tannins. The aim of this research was to determine the fraction of pomegranate peel extract with the highest antioxidant activity and to determine the optimal serum formula for the red pomegranate peel fraction. The antioxidant activity testing method used in this research is the FRAP method (*ferric reducing antioxidant power*). The sample extract is fractionated, and then the fraction that has the highest antioxidant activity is made into a serum preparation formulation and preparation evaluation test. Based on the results of total tannin levels in all samples also showed positive results with a change in the color of the solution to bluish. The results of the antioxidant activity of all samples were categorized as very strong antioxidants as stated in the IC value$_{50}$ < 50 ppm. Optimizing the antioxidant serum formulation used HPMC concentrations of 0.5%, 1% and 2% respectively. The results obtained from testing antioxidant activity obtained IC values$_{50}$ the best was 0.368 ppm for the ethyl acetate fraction of red pomegranate peel. Optimizing the serum formula used a HPMC concentration of 1%, with an IC value$_{50}$ amounting to 133,903 ppm.

Keywords: red pomegranate peel extract, antioxidant, FRAP, fraction, serum

INTRODUCTION

Skin aging is a physiological process that occurs in living things. Free radicals are the main cause of skin aging, which is formed from reactions within the body and the environment and is due to excessive UV exposure. Free radicals are atoms or electrons that are unpaired and highly reactive. Free radicals can be inhibited from increasing using antioxidant compounds. Antioxidants are compounds that can remove, purify, and prevent the formation of reactive oxygen and free radicals from the body (Wulandari *et al*., 2017).

One of the uses of antioxidants that can prevent premature aging is in cosmetic products. Serum dosage forms are considered quite convenient because their water content moisturizes the skin and spreads easily during use. In addition, treatments with antioxidant serums have shown improvements in the appearance of facial aging, supporting their use as a treatment to reduce free radical skin damage (Liandhajani *et al*., 2022). Due to their carcinogenic nature, it is feared that synthetic antioxidants may harm human health. Therefore, the use of natural ingredients as antioxidants is considered healthier and safer than synthetic antioxidants.

Red pomegranate peel extract (*Punica granatum* L.) is one of the natural ingredients that has the potential as an antioxidant. According to previous research, pomegranate peel extract contains polyphenolic compounds, such as tannins, flavonoids, gallic acid, ellagic acid, and punicalagin (Hidayah *et al*., 2016). Where this compound has antioxidant properties (Wulandari *et al*., 2017). In another study, it was mentioned that the IC_{50} value of pomegranate peel extract measured using the DPPH method was 4.53 ppm (Wahyuni *et al*., 2023). It can be stated that pomegranate peel extract is one of the most powerful antioxidants.

Based on the above, this study will formulate serum preparations containing fractions of red pomegranate peel extract (*Punica granatum* L.) and test its antioxidant activity using the FRAP (Ferric Reducing Antioxidant Power) method. FRAP is one of the antioxidant measurement methods. The advantage of the FRAP method is that it is cheap, the reagents are easy to prepare, and it is quite simple and fast to carry out (Maryam *et al*., 2016). This method has the advantage that total plasma antioxidant activity can be determined directly and does not rely on enzymatic and non-enzymatic methods to generate free radicals before evaluating the antiradical efficiency of plasma (Kumar, 2012).

Pomegranate skin was extracted using the maceration method with 96% ethanol solvent. Then diffraction with nonpolar (N-hexane) and polar (ethyl acetate) solvents. The three resulting fractions (n-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction) were tested for antioxidant activity using the FRAP (Ferric Reducing Antioxidant Power) method. Next, the fraction that has the highest antioxidant activity is formulated into

a serum preparation. The serum preparations that have been made are tested for antioxidants again to determine their antioxidant activity.

METHODS

This research was conducted from February to August 2024. This research was conducted at the Research and Organic Chemistry Laboratory at the Faculty of Mathematics and Natural Sciences and the Organic Chemistry Laboratory at the Faculty of Medicine, Universitas Negeri Semarang. The materials used in this research were red pomegranate peel powder (*Punica granatum* L.), 96% ethanol, n-hexane, ethyl acetate, distilled water, $FeCl_3$, NaOH, KH_2PO_4, $K_3Fe(CN)_6$, TCA, oxalic acid, ascorbic acid, Mayer's reagent, MG powder, HCl, acetic acid, concentrated sulfuric acid, tannic acid, Follin-Ciocalteu, Na_2CO_3, HPMC, Glycerin 10%, methyl paraben 0.1%, and Na_2EDTA 0.07%.

Making Simplicia and Extracts

The red pomegranate peel that has been obtained is cleaned and washed thoroughly, then cut into small pieces, then put into the oven to dry. Furthermore, the dried simplicia was pulverized into powder. Using 96% ethanol solvent, extraction was carried out through the maceration method. The collected extract was then evaporated with a vacuum rotary evaporator at a temperature of 50°C to evaporate 96% ethanol with solutes until a thick extract was obtained which was ready for fractionation.

Fractionation

Fractionation was carried out by liquid-liquid method with n-hexane, and ethyl acetate solvents. The extract was weighed as much as 1 g, put into a test tube and dissolved with 5 ml of distilled water, then added 4 ml of n-hexane. Then the solution was vortexed for 5 minutes until the solution separated into 2 parts, the solution was then centrifuged to take the dissolved part. The insoluble part can be added again with ethyl acetate with the same procedure to get the dissolved part. The ethyl acetate insoluble part will become ethyl acetate insoluble fraction. All fractions that have been obtained (n-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction) are collected to be tested for antioxidant activity.

Phytochemical Screening

Alkaloid Test

In a test tube, 500 mg samples of the ethanol extract fraction of red pomegranate peel (n-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction) were added, then dissolved with 1 ml of HCl and 9 ml of distilled water. Then heated for 2 minutes on a water bath. Then cooled and filtered. Put 3 drops of filtrate on the watch glass, then add 2 drops of Mayer's reagent. The formation of a white or cream precipitate indicates a positive alkaloid test.

Flavonoid Test

In a test tube, 500 mg of fraction samples (n-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction) from the ethanol extract of red pomegranate peel were added, 1 ml of P ethanol, 0.1 g of Mg powder and 10 drops of HCl P were added. If a red color appears, the flavonoid identification will be positive.

Saponin Test

In a test tube, 500 mg samples of the ethanol extract fraction of red pomegranate peel (n-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction) were added, then dissolved with 10 ml of hot distilled water and filtered. After that, the filtrate was shaken for 1 minute, then 1 drop of 2N HCl was added. If a layer of foam remains for no less than 10 minutes, the Saponin compound test will be positive.

Tannin Test

In a test tube, 500 mg samples of the ethanol extract fraction of red pomegranate peel (n-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction) were added, 10 ml of distilled water was added, then heated for 5 minutes and filtered. 1-2 $FeCl_3$ 1% solutions were dropped into the filtrate. A greenish-brown or blackish-blue color change allows identification of positive tannin compounds.

Steroid and Terpenoid Test

In a test tube, 40 mg of the ethanol extract fraction of red pomegranate peel (n-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction) was added, then 10 drops of acetic acid were added. Leave it for a few minutes before adding 2 drops of concentrated sulfuric acid. The formation of an orange or purple color indicates a positive test for terpenoids and a blue color indicates a positive test for steroids.

Determination of Total Tannin Content

The three fractions of ethanol extract of red pomegranate peel that have been obtained are tested with certain reagents to determine the total amount of tannin contained in them. Determination of the total tannin content in red pomegranate peel was tested using the total phenol method using *Follin-Ciocalteu reagent* and tannic acid standard replicated 3 times (Malangngi *et al*., 2012).

Preparation and Measurement of Comparative Solutions

A 1000 ppm stock solution was prepared by dissolving 10 mg of tannic acid with 10 ml of methanol. Then 2.5 ml of the solution was pipetted into a 25 ml measuring flask, then diluted using methanol to the mark to produce a concentration of 100 ppm. Next, 1,2,3,4, and 5 ml of the solution were pipetted, then diluted in a 10 ml measuring flask to the mark using methanol to produce concentrations of 10, 20, 30, 40, and 50 ppm (Ahmad *et al*., 2015).

Take 0.1 ml of each concentration, then add 7.5 ml of distilled water, 0.1 ml of Follin-Ciocalteu and 2 ml of Na_2CO_3. After that, it was incubated at a temperature of 37°C for 30 minutes, then the maximum wavelength was measured in the wavelength range 400-800 nm with one of the standard samples of tannic acid. Next, absorbance measurements were carried out at the maximum wavelength that had been determined (Malangngi *et al*., 2012).

Testing and Determination of Total Tannin Content

A total of 10 mg of pomegranate peel fraction was dissolved in 10 ml of methanol (Ahmad *et al*., 2015). Then 0.1 ml was taken, then added 7.5 ml of distilled water and 0.1 ml of Follin-Ciocalteu and 2 ml of Na_2CO_3. After that, it was incubated at a temperature of 37°C for 30 minutes, then the absorbance was measured at the maximum wavelength that had been determined. The results of the total tannin content obtained were expressed in mg TAE/g fraction (Malangngi et al., 2012). The tannin level is determined using the formula.

$$\text{Total tannin} = \frac{C \times V \times fP}{g}$$

Where:

C : Tannin concentration (X value)
V : Extract volume (L)
fP : Dilution factor
g : Sample weight (grams)

Antioxidant Test of Extracts and Fractions using the FRAP Method

Phosphate buffer solution 0.2M pH 6.5

The solution was prepared by weighing 1 gram of NaOH and dissolved with CO^{2-} free distilled water to exactly 25 mL in a measuring flask. Then as much as 0.0681 grams of KH_2PO_4 was dissolved with 10 mL CO^{2-} free distilled water in a measuring flask. Then dripped NaOH is included in KH_2PO_4, then measured until pH 6.5.

$K_2Fe(CN)_6$ 0.1 % solution

The solution was prepared by dissolving 1 g of potassium ferricyanide in distilled water and diluted in a 100 mL volumetric flask. Then 1 mL of $K_3Fe(CN)_6$ solution was diluted with 9 mL of distilled water.

0.03% $FeCl_3$ solution

The solution was prepared by dissolving 0.1 g of $FeCl_3$ in distilled water and diluted in a 100 mL volumetric flask. then 3 mL of 0.1% $FeCl_3$ solution was diluted with 7 mL of distilled water.

TCA solution 10%

The solution was prepared by dissolving 1 gram of TCA (trichloroacetic acid) in distilled water and diluted in a 10 mL volumetric flask.

Blank solution and wavelength determination

Pipetted 1 mL phosphate buffer and added 1 mL $K_3Fe(CN)_6$ 0.11% then incubated at 50°C for 20 minutes. Added 1 mL of 10% TCA and then centrifuged at 3000 rpm for 10 minutes. The top layer was taken 1 mL, added 1 mL of distilled water and 0.5 mL of FeCl3 0.03%, and let stand for 10 minutes. The absorbance was measured with a UV-Vis spectrophotometer that had been set wavelength from 400-800 nm until the maximum wavelength is obtained.

Ascorbic acid standard solution

The 1000 ppm stock solution was prepared by dissolving 1 mg ascorbic acid with 1 mL ethanol. Furthermore, from the 1000 ppm stock solution, 0.1 mL was taken and diluted with ethanol to 1 mL, forming a solution with a concentration of 100 ppm. The 100 ppm solution was taken 0.02; 0.04; 0.06; 0.08; and 0.1 mL each and placed in different eppendorf and diluted with ethanol to 1 mL and homogenized. The concentration series of ascorbic acid standard solution is 2, 4, 6, 8, and 10 ppm.

1 mL of phosphate buffer was pipetted and 1 mL of $K_3Fe(CN)_6$ 0.1% was added and incubated at 50°C for 20 minutes. Added 1 mL of 10% TCA and then centrifuged at 3000 rpm for 10 minutes. The top layer was taken 1 mL added 1 mL of distilled water and 0.5 mL of $FeCl_3$ 0.03%, and let stand for 10 minutes. Then the absorbance was measured at the maximum wavelength.

Antioxidant Activity using FRAP Method

The 1000 ppm stock solution was prepared by dissolving 1 mg of serum base and serum fraction of ethanol extract of red pomegranate peel from the formula dissolved with ethanol up to 1 mL. Furthermore, from the 1000 ppm stock solution, 0.02; 0.04; 0.06; 0.08; and 0.1 mL were taken respectively and placed in different *eppendorf* and diluted with ethanol up to 1 mL and homogenized. The concentration series of the sample solution is 20, 40, 60, 80, 100 ppm.

Pipetted 1 mL of phosphate buffer and added 1 mL of $K_3Fe(CN)_6$ 0.1% then incubated at 50°C for 20 minutes. Added 1 mL of 10% TCA and then centrifuged at 3000 rpm for 10 minutes. The top layer was taken 1 mL added 1 mL of distilled water and 0.5 mL of $FeCl_3$ 0.03%, and let stand for 10 minutes. Then the absorbance was measured at the maximum wavelength. Do the same to all fractions of ethanol extract of red pomegranate peel. Then the antioxidant activity was calculated.

Calculation of Antioxidant Activity

The antioxidant power of the sample was determined by finding the IC_{50} value based on the percent reduction of free radicals in the sample and ascorbic acid. The result of absorbance measurement using a UV-Vis spectrophotometer was used to calculate the percent inhibition. Percent (%) inhibition was calculated using the formula:

$$\%\text{inhibition} = \frac{(A_{sample} - A_{blank})}{A_{blank}} \times 100\%$$

Where:
A blank = Absorbance of FRAP radicals and solvent
A sample = Radical absorbance of FRAP containing sample

The sample and the comparator, vitamin C, each calculated the IC_{50} value using linear regression analysis with the formula:

$$y = ax + b$$

Where:
y = Percentage of antioxidant activity
x = sample antioxidant content
b = Regression coefficient/slope
a = intercept/constant

Calculation of IC_{50} is carried out using (50-a)/b, and the level of antioxidant strength is determined. Determination of the strength of antioxidant activity can be seen in Table 1, where the smaller the IC_{50} value, the higher the antioxidant activity (Tristantini *et al.*, 2016).

Table 1. Strength of Antioxidant Activity

IC_{50} Value	Category
< 50	Very strong
50-100	Strong
100-150	Currently
> 150	Weak

Antioxidant Serum Preparation

The serum formulation in this study used the formula from Liandhajani *et al.* (2022) with modifications. The serum base optimization used is to modify the concentration of HPMC as a serum base with concentrations of 0.5%, 1%, and 2%, for other serum compositions such as 10% Glycerin, 0.1% Methyl Paraben, 0.07% Na_2EDTA, and distilled water up to 30 ml using a fixed concentration. This serum base then added with a 1% fraction of ethanol extract of red pomegranate peel. The serum formula design of the active fraction of red pomegranate peel extract is shown in Table 2.

Table 2. Formulation design for serum fraction ethanol extract of red pomegranate peel

Material	F1 (%)	F2 (%)	F3 (%)	F4 (%)	Function
Active fraction of red pomegranate peel extract	-	-	-	1	Active substance
HPMC	0,5	1	2	1	Base Serum
Glycerin	10	10	10	10	Humectant
Methyl Paraben	0,1	0,1	0,1	0,1	Preservative
Na_2EDTA	0,07	0,07	0,07	0,07	Chelating agent
Aquadest (until)	30	30	30	30	Solvent

Where:
F1 : Serum formulation with 0.5% HPMC base
F2 : Serum formulation with 1% HPMC base

F3 : Serum formulation with 2% HPMC base
F4 : Serum formulation with the addition of active substances.

Evaluation of Serum Preparations

Evaluation testing was carried out on all serum formulas that had been made and the test was replicated 3 times. Evaluation of serum preparations carried out, namely Organoleptic Test, Homogeneity, Viscosity, Spreadability, Adhesiveness, and pH Test. The organoleptic test measures the color consistency, and aroma of the serum to determine its physical condition.

A homogeneity test was performed by placing 0.1 gram serum preparation on transparent glass and leveled. If there are no coarse grains, the serum preparation is declared homogeneous. Serum viscosity testing using an Ostwald Viscometer. The serum is put into the viscometer, then the serum is sucked until the upper limit mark. Record how long it takes for the serum to reach the lower limit mark. Viscosity is calculated by the following formula.

$$\eta = \eta 1 \frac{t2\ \rho 2}{t1\ \rho 1}$$

Where:
η : Sample Viscosity
η1 : Water Viscosity
t1 : Water Flow Time
t2 : Sample Flow Time
p1 : Density of Water
p2 : Sample Density

Spreadability testing was done by placing 0.5 grams in the center of a transparent glass and then placing another transparent glass on top of it. After settling for one minute, the diameter of the spreader was recorded. The adhesion test is carried out by placing a 0.1 gram serum preparation on the glass available on the tool, then placing another glass on top of the glass that has been given serum. Apply a 1 kg weight on top of the glass stack and let it stand for 5 minutes. Afterward, remove the 1 kg weight and the 50 gram weight holder, calculate how long it takes for the two glasses to come off.

The pH measurement was carried out using a pH meter. A total of 1 gram of serum preparation was dissolved in 10 ml of water at room temperature. For one minute, the electrode was left in contact with the surface of the solution.

Antioxidant Test of Serum Preparations using the FRAP Method

Blank solution

1 mL of phosphate buffer was pipetted and 1 mL of $K_3Fe(CN)_6$ 0.1% was added and incubated at 50°C for 20 minutes. Added 1 mL of 10% TCA and then centrifuged at 3000 rpm for 10 minutes. The top layer was taken 1 mL added 1 mL of distilled water and 0.5 mL of $FeCl_3$ 0.03%, and let stand for 10 minutes. Then the absorbance was measured at the maximum wavelength.

Garnier serum standard solution

The 1000 ppm stock solution was prepared by dissolving 1 mg of Garnier serum with 1 mL ethanol. Furthermore, from the 1000 ppm stock solution, 0.1 mL was taken and diluted with ethanol to 1 mL, forming a solution with a concentration of 100 ppm. The 100 ppm solution was taken at 0.02; 0.04; 0.06; 0.08; and 0.1 mL each and placed in different eppendorf and diluted with ethanol to 1 mL and homogenized. The concentration series of ascorbic acid standard solution is 2, 4, 6, 8, and 10 ppm.

Antioxidant Activity using FRAP

The 1000 ppm stock solution was prepared by dissolving 1 mg of serum base and serum fraction of ethanol extract of red pomegranate peel from the formula dissolved with ethanol up to 1 mL. Furthermore, from the 1000 ppm stock solution, 0.02; 0.04; 0.06; 0.08; and 0.1 mL were taken respectively and placed in different eppendorf and diluted with ethanol up to 1 mL and homogenized. The concentration series of the sample solution is 20, 40, 60, 80, 100 ppm.

RESULT AND DISCUSSION

Extraction and Fractionation Analysis

This research began with the main ingredient used, namely red pomegranate peel powder (*Punica granatum L.*) will be extracted using the maceration method using 96% ethanol solvent. The maceration process begins by soaking 1 kg of red pomegranate peel powder (*Punica granatum* L.) with 1 liter of 96% ethanol for 2 days with occasional stirring. After 2 days, the process continues with filtration using filter paper to separate the macerate from the dregs. The existing dregs are remacerated with 500 ml of 96% ethanol, then filtered again. The resulting macerate is

evaporated using a tool *vacuum rotary evaporator* at a temperature of 30°C and a thick extract weighing 240 grams is produced. With the resulting yield of 24%. The results of this rendition can be said *poor* or bad based on research Wibowo *et al*. (2018) The ideal yield is 100%, if the yield of a compound is above 90% then it is called *excellent*, for yield values above 80% it is called *very good*, then if the yield value is more than 70% it can be called *good*, above 50% is called *fair* and in less than 50% called *poor*. This is in line with the statement that the higher the yield value produced, the higher the active compound that can be demonstrated (Subaryanti *et al*., 2022). However, according to the Indonesian Kementerian Kesehatan RI (2017), this thick extract of pomegranate peel still meets the requirements, where the requirement for the yield value of thick extract of pomegranate peel according to the 2nd edition of the herbal pharmacopeia is not less than 19.9%.

The next step is fractionation of the thick extract that has been obtained by dissolving the thick extract with 5 ml of water, then adding 5 ml of n-hexane, until 2 layers are formed which will then be separated and the top layer will be collected. The n-hexane solvent is a non-polar solvent that can filter chemical compounds such as wax, lipids and volatile oils. Fractionation using n-hexane solvent was carried out several times until it was felt that there were no more compounds that could be transported by the solvent. Next, 5 ml of ethyl acetate was added. Just like fractionation with n-hexane, ethyl acetate fractionation will form 2 layers where the top layer will be separated and collected in a porcelain cup. The fractionation process with ethyl acetate was carried out several times until it was felt that no more compounds were being transported. Semi-polar solvents such as ethyl acetate are able to extract phenolic compounds, terpenoids, alkaloids, aglycones and glycosides (Hidayah *et al*., 2016). The remainder of the fractionation of ethyl acetate is called the insoluble fraction of ethyl acetate, where it is assumed that the compounds contained in this compound are compounds that cannot be transported by ethyl acetate.

The fractionation process also produced a yield, where the yield of the n-hexane fraction was 4.042%, the ethyl acetate fraction was 10.001%, and the insoluble ethyl acetate fraction was 79.842%. The n-hexane fraction and ethyl acetate fraction are included in the yield category *poor*, while the ethyl acetate insoluble fraction is categorized in the yield *good*. It can be assumed that there are still many compounds in the ethyl acetate insoluble fraction that can be transported by n-hexane or ethyl acetate. Another assumption that can explain is that the presence of water in the fractionation process can increase the yield of the ethyl acetate insoluble fraction.

Table 3. Yield of Pomegranate Peel Extract Fractions

Fraction	Extract Weight (grams)	Fraction Weight (grams)	%Yield	Fraction Color
n-Hexane	76	3,0719	4,042	Yellowish Green
Ethyl Acetate	46	4,6005	10,001	Brownish red
Insoluble Ethyl Acetate	46	36,7272	79,842	Deep Brownish Red

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Phytochemical screening was performed to identify sample-dependent compounds. This analysis was carried out to determine the presence of alkaloids, flavonoids, saponins, tannins, as well as steroids and terpenoids (Agustina *et al*., 2017). The following are the results of phytochemical screening obtained from the extract, n-hexane fraction, ethyl acetate fraction, and ethyl acetate insoluble fraction.

The phytochemical screening results obtained in the extract samples were positive for alkaloids, flavonoids, saponins and tannins and contained terpenoid compounds. In the n-hexane fraction, positive results were obtained only in the tannin group and contained terpenoid group compounds. Similar to the ethyl acetate fraction, this fraction is only positive for the tannin and terpenoid groups. However, there were differences in the two fractions, where the ethyl acetate fraction showed more concentrated tannin results than the n-hexane fraction. Meanwhile, the insoluble fraction of ethyl acetate showed positive results for the flavonoids, saponins, tannins and terpenoids. From Table 4, it can be seen that the ethyl acetate fraction shows more concentrated tannin compounds. Therefore, it is necessary to determine the total tannin content.

Table 4. Phytochemical Screening Results

	Extract	F. N-hexane	F. Ethyl Acetate	F. Insoluble Ethyl acetate	Positive
Alkaloid	+	-	-	-	A white/creamy precipitate is formed
Flavonoid	+	-	-	+	Formed red color
Saponin	+	-	-	+	Constant foam
Tannin	+++	++++	+	+++	Formed greenish brown/blackish blue
Steroid	-	-	-	-	Blue color is formed
Terpenoids	+	+	+	+	An orange/purple color forms

Determination of Total Tannin Content

Determination of total tannin content is done by adding reagents *Folin-Ciocalteu* The absorbance was then calculated using a UV-Vis spectrophotometer with the maximum wavelength used being 744 nm. The reaction that occurs is that the hydraulic group contained in the phenolic compound reduces heteropoly acid (*fosfomolibdat-fossotungstat*) contained in the reagent *folin-ciocalteu* into a blue complex *molybdenum-tungsten* in alkaline conditions with the help of Na_2CO_3 solution (Shinta Cania Maiza *et al.*, 2022).

Table 5. Results of Standard Absorbance Measurements for Tannic Acid

Concentration (ppm)	Absorbance			Average absorbance	Linear Line Equations
10	0.147	0.157	0.162	0.155	
20	0.208	0.218	0.206	0.211	
30	0.366	0.261	0.258	0.295	$y = 0.0077x + 0.0662$ $R^2 = 0.9916$
40	0.355	0.386	0.354	0.365	
50	0.458	0.468	0.468	0.465	

The results obtained from measuring standard solutions of tannic acid are linear regression equations, where this equation is used to calculate total tannin levels. The resulting linear regression equation is y = 0.0077x + 0.0662, with an R^2 value obtained was 0.9916. Table shows the results of the tannin content of the extract at 10.308 mg TAE/g extract, the n-hexane fraction at 3.988 mg TAE/g extract, the ethyl acetate fraction at 15.529 mg TAE/g extract, and the insoluble ethyl acetate fraction at 9.183 mg TAE/g extract. Positive results can also be seen by changing the color of the solution from clear to bluish.

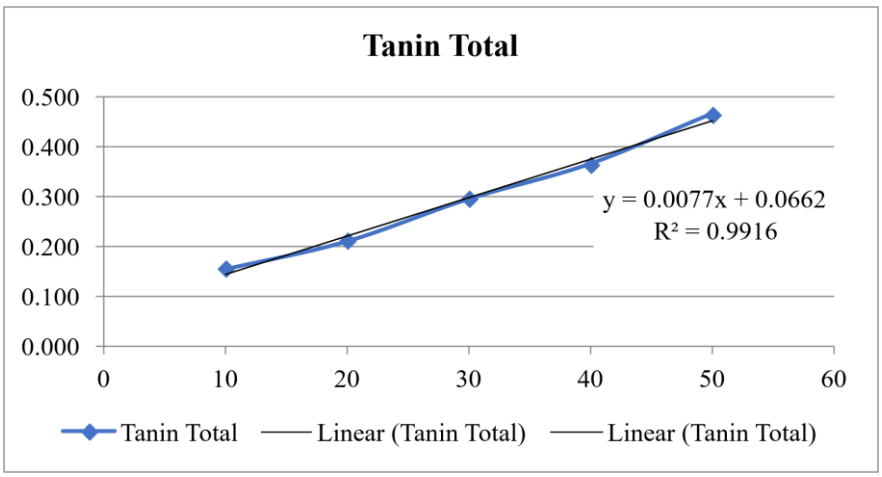

Figure 1. Tannic Acid Standard Absorbance Curve

Table 6. Results of Determination of Total Tannin Content Extract and Red Pomegranate Peel Fraction (*Punica granatum* L.)

Sample Weight (grams)	Sample	Absorbance			Average absorbance	Equivalent Concentration (mg/L)	Total Tannin Content (mg TAE/g extract
0.01	Extract	0.332	0.335	0.325	0.331	9729.167	9.729
0.01	N-Hexane	0.087	0.086	0.087	0.087	3645.833	3.646
0.01	Ethyl Acetate	0.531	0.540	0.524	0.532	14754.167	14.754
0.01	Insoluble	0.259	0.324	0.279	0.287	8645.833	8.646

Antioxidant Test

In this study, antioxidant testing used the FRAP method (*Ferric Reducing Antioxidant Power*), by adding several solutions such as $K_3Fe(CN)_6$, TCA, dan $FeCl_3$ into the sample under acidic conditions. The standard solution used is ascorbic acid or vitamin C. The addition of TCA aims to make the complex $K_3Fe(CN)_6$ settles. In addition, the addition of $FeCl_3$ functions to form a berlin blue complex. An indicator of the potential of an antioxidant compound is reducing power, which in the FRAP method is measured by the ability of a compound to convert Fe^{3+} to be Fe^{2+}. It can be assumed that compounds that have reducing power can act as antioxidants, this is because they can stabilize radical compounds by donating electrons or hydrogen atoms so that radical compounds can become more stable (Maryam *et al*., 2016).

Table 7. Antioxidant Activity Test

Concentration (ppm)	% Inhibition	IC_{50}	Linear Line Equations
Vit C			
1	27.747	4.660	y = 5.5495x + 24.139 R^2 = 0.9786
3	42.766		
5	51.099		
7	66.392		
9	71.429		
Extract			
10	86.996	6.313	y = 9.5879x - 10.531 R^2 = 0.972
8	64.835		
6	49.817		
4	19.597		
2	13.736		
N-hexane fraction			
10	124.451	3.091	y = 10.606x + 17.214 R^2 = 0.9728
8	98.077		
6	87.996		
4	52.564		
2	41.150		
Ethyl Acetate Fraction			
2	71.154	0.368	

1.5	65.201		y = 13,803x + 44,914 R^2 = 0.959
1	61.630		
0.5	53.205		
0.1	43.773		
Ethyl Acetate Insoluble Fraction			
2	56.685	1.511	y = 12.388x + 31.283 R^2 = 0.9963
1.5	48.968		
1	43.584		
0.5	37.832		
0.1	32.522		

Calculation of antioxidant activity was carried out by calculating the % inhibition of each sample concentration, then creating a standard curve. Next, we get the linear equation y = ax+b, this equation is needed to calculate the IC value$_{50}$ by means of (50-a)/b. As for the IC value$_{50}$ What was produced was 4,660 ppm of Vitamin C, 6,313 ppm of extract, 3,091 ppm of the n-hexane fraction, 0.368 ppm of the ethyl acetate fraction, and 1,511 ppm of the ethyl acetate insoluble fraction. Where is the IC value$_{50}$ of all samples were categorized as very strong antioxidants as stated in the IC value$_{50}$ < 50 ppm is categorized as very strong, 50-100 ppm is categorized as strong, 101-150 ppm is in the medium category, 151-200 ppm is a weak antioxidant, and > 200 ppm is assumed to have no antioxidant activity (Tristantini *et al*., 2016). Determination of the active substance for making serum is seen from the IC_{50} value. The lowest is the ethyl acetate fraction.

Red Pomegranate Skin Serum

Red pomegranate skin has potential as an antioxidant seen from the results of previous antioxidant activity. Antioxidant activity can be determined by the IC value$_{50}$. Nilai IC_{50} is the concentration at which a substance can inhibit 50% of free radicals. The smaller the value of the IC_{50} the stronger the antioxidant activity (Loe WE *et al*., 2022). Therefore, the ethyl acetate fraction was chosen as the active substance of red pomegranate skin serum. However, before that, optimization of the serum formula can be done to get a better serum. Serum optimization in this study was carried out by modifying the concentration of the serum base itself, namely HPMC, but the other ingredients used fixed or the same concentrations. The formula is made into 3 types, namely formula 1 with a concentration of 0.5% HPMC, formula 2 with 1% HPMC, and finally formula 3 with 2% HPMC. The ideal determination of a good serum formula is determined from the results of the serum evaluation of the three existing formulas.

Table 8. Results of Organoleptic Evaluation and Serum Optimization Homogeneity

	F1	F2	F3
Texture	Liquid	A bit thick	Thick
Color	Clear	Clear	Clear
Aroma	Odorless	Odorless	Odorless
Homogeneity	Homogeneous	Homogeneous	Homogeneous

Table 9. Results of Serum Optimization Adhesion Evaluation Results

	F1	F2	F3	Standard
Rep 1	1	4	28	> 4 min
Rep 2	1	2	29	
Rep 3	2	6	30	
Average	1.3333	4	29	

Table 10. Results of Serum Optimization Spreadability Evaluation

	F1	F2	F3	Standard
Rep 1	9.8	6.1	4.6	5-7 cm
Rep 2	10.1	6.4	4.8	
Rep 3	10.2	7.2	4.4	
Average	10.033	6.567	4.600	

The result of optimization of the serum chosen was the second formula, namely with an HPMC concentration of 1%. After obtaining the optimal formula, a pomegranate peel serum preparation was made by adding 1% ethyl acetate fraction. Making serum is done by pouring hot distilled water into a mortar, then sprinkling HPMC on top, then letting it sit for 5 minutes. In another mortar, add Na_2EDTA and methyl paraben, grind and add distilled water, homogenize. After 5 minutes, grind the HPMC serum base slowly, then add it to the Na_2EDTA mixture and methyl paraben, homogenize. Once homogeneous, add glycerin little by little while still grinding, then add the remaining distilled water, homogenize. Pomegranate skin serum was also evaluated.

Table 11. Results of Serum Optimization pH Evaluation

	F1	F2	F3	Standard
Rep 1	**4.89**	5.14	5.15	4,5-6,5
Rep 2	**4.89**	5.12	5.16	
Rep 3	**4.85**	5.15	5.17	
Average	**4.88**	5.14	5.16	

Table 12. Results of Serum Optimization Viscosity Evaluation

F1	F2	F3	Standard
199.0412	3699.8386	5059.5981	2000-4000

Table 13. Evaluation Results of Red Pomegranate Peel Serum Preparations

	Standard	Rep 1	Rep 2	Rep 3	Average
Texture	-	A bit thick			
Color	-	Yellow			
Aroma	-	Odorless			
Homogeneity	Homogeneous	Homogeneous			
Adhesion	> 4 min	5	6	4	5
Spread Power	5-7 cm	6.9	5.9	5.96	6.23
pH	4,5-6,5	4.25	4.26	4.26	4.26
Viscosity	2000-4000	3619.2410			

The prepared red pomegranate skin serum was also tested for its antioxidant activity. The comparison solution used was Garnier Vitamin C serum. Apart from the serum that has been made, the optimal base is also tested for antioxidant activity to find out whether the base alone has antioxidant activity or not. Based on Table 9, the serum fraction of pomegranate peel has an IC value$_{50}$ amounted to 133,903, where the activity was better than the base alone which was 155,018 ppm. IC_{50} value Pomegranate peel fraction serum is categorized as a medium antioxidant, while the serum base is categorized as a weak antioxidant.

Table 14. Results of Serum Antioxidant Activity

Concentration (ppm)	% Inhibition	IC_{50}	Linear Line Equations
Garnier			
10	30.128	22.264	y = 2.5641x + 5.0366 R^2 = 0.9933
8	25.549		
6	21.429		
4	15.476		
2	9.524		
Basis			
100	34.878	155.018	y = 0.2776x + 6.9671 R^2 = 0.9998
80	28.981		
60	23.589		
40	18.113		
20	12.553		
Pomegranate Skin Serum			
100	38.922	133.903	y = 0.3593x + 1.9292 R^2 = 0.9943
80	29.823		
60	22.662		
40	16.259		
20	9.773		

CONCLUSION

Red pomegranate peel fraction (*Punica granatum L.*) which has the highest antioxidant activity is the ethyl acetate fraction which has an IC_{50} value the lowest was 0.368 ppm. The best serum formula base based on the evaluation results of serum preparations is serum with a HPMC concentration of 1% with a texture that is neither too thick nor too liquid. Nilai IC_{50} the serum fraction of red pomegranate peel was 133,903 ppm, with an IC value$_{50}$ the base is 155,018 ppm. It is hoped that irritation and toxicity testing will be carried out on the red pomegranate skin fraction serum preparation (*Punica granatum L.*). and further research was carried out regarding the utilization of red pomegranate peel fractions (*Punica granatum L.*) in the manufacture of other pharmaceutical preparations as an antioxidant.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest

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