Antioxidant Activity Potential of Red Pomegranate *(Punica granatun L.) Peel as Herbal Tea*

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Abstract: Red pomegranate (*Punica granatum* L.) contains punicalagin (elagitanin) which has strong antioxidant activity. This study aims to examine the antioxidant activity of steeping tea from red pomegranate peel. This research is observational with red pomegranate peel as the research object. The red pomegranate peel is processed into simplicia, then some of the simplicia is brewed in boiling water at 100 °C and the other part is made of ethanol extract. Steeping tea and ethanol extract at a dose of 1.25; 2.5; 5; 10; and 20 ppm were measured for its antioxidant activity by the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method. As a control, ascorbic acid was used. The antioxidant activity is expressed in IC₅₀. Data were analyzed by one-way Analysis of Variance. The results showed that the IC₅₀ of steeping tea was 17.59 ppm, ethanol extract was 9.58 ppm, and ascorbic acid was 2.98 ppm. There was no significant difference in the IC₅₀ between brewed tea, ethanol extract and ascorbic acid (p = 0.05). It was concluded that *P. granatum* L. peel in the form of tea infusion had lower antioxidant activity than the ethanol and ascorbic acid extracts, but statistically all three showed the same strong antioxidant activity.

Keywords: antioxidant, DPPH, *Punica granatum* L., ethanol extract, IC₅₀, tea.

INTRODUCTION

As we are getting older, the body’s function and ability to adapt to organs damage will decrease.¹ There are two factors which play a role in the aging process namely internal and external factors. Internal factors include free radicals, reduced hormones, glycosylation processes, apoptosis, decreased immune system, and genes.
External factors are an unhealthy lifestyle, including unhealthy diets, wrong habits, environmental pollution, radiation, ultraviolet (UV) rays, cigarette smoke, and stress. 1-3 Continuous exposure to UV rays can cause damage to the structure and function of the skin, thereby accelerating the skin aging process. 4,5 One of the most developed theories of aging is the theory of free. This theory explains, an organism gets old because of the accumulated damage by free radicals in the cells over time. Free radicals will damage molecules whose electrons are attracted by these free radicals, causing cell damage, impaired cell function, and even cell death. 5 Free radicals that cause skin aging come from solar UV radiation. In living cells, solar UV radiation generates free radicals that can cause various chemical photo risks such as photo isomerization and photo oxidation. Photo oxidation reactions occur due to the release of Reactive Oxygen Species (ROS) in the form of superoxide anions (O2·−), hydrogen peroxide (H2O2) and hydroxyl radicals (OH) by chromophores that absorb ultraviolet light. 6

Free radicals can be inhibited by antioxidants. The inhibition process is carried out by reducing the level of free radical reactivity by compounds that acts as an antioxidant. 7 Red pomegranate (Punica granatum L.) is one of the natural antioxidants. Red pomegranate is a source of polyphenol antioxidants, especially punicalagin (elagitanin) which has been shown to have strong antioxidant, anti-inflammatory, and anti-carcinogenic activity in several studies. 8,9 Various diseases, that are the target of research to find out the benefits of pomegranate, are various types of inflammatory diseases, antioxidants, degenerative diseases, various types of cancer and the regulation of the fibrosis process. 10 Pomegranates juice and peel have been shown to have antioxidant and anti-cancer activity. 11

Tea is a famous beverage among Indonesians. The ingredients used to make tea also vary with the respective benefits and advantages. Tea products can not only be produced from tea leaves but also from other plants such as red pomegranate (P. granatum L). This study aims to examine the antioxidant activity of steeping tea from P. granatum peels.

MATERIALS AND METHOD

This research was an observational study with red pomegranate peel made of tea and ethanol extract. Pomegranate peel was dried, cut into small pieces, and crushed to form simplicia. Most of the simplicia was brewed with boiling water at 100 °C to form tea, some of the simplicia was macerated with 70% ethanol and filtered, the filtrate was concentrated, and the red pomegranate peel extract was obtained. Steeping tea and ethanol extract dose of 1.25; 2.5; 5; 10; 20 ppm of antioxidant activity was measured by using the DPPH method and ascorbic acid was used as a comparison. The antioxidant activity was expressed by the IC50 value. Data were analyzed by oneway Anava test.

Making Tea from Red Pomegranate Peel. Red pomegranate peel powder that has been mashed in a blender, separated between the smooth and the coarse. As much as 33 mg of fine powder, put into a beaker glass, and poured with 33 mL of boiling water at 100 °C, the mixture was stirred at 900 rpm for 5 minutes, then filtered using filter paper to obtain a stock solution of the simplicia test with a concentration of 1000 ppm.

Preparation of 70% Ethanol Extract from Pomegranate Peel. 105 g of red pomegranate peel powder was put into a 500 mL Erlenmeyer flask, then 500 mL of 70% ethanol solvent was added, centrifuged for 6 hours using a shaker, and filtered to obtain the filtrate. 500 mL of ethanol solvent was added back into the Erlenmeyer flask containing the residue and left to stand for 24 hours. Then done the same way for 3 days. The filtrate was evaporated with a vacuum rotary evaporator at a temperature of 500 °C to obtain a thick extract. 11,12 A sample of 10 mg extract was dissolved with methanol up to 10 mL into a 1000 ppm stock solution.

Preparation of DPPH Solution. DPPH with a concentration of 160 mg / L was prepared by weighing the substance as much as 4.0 mg and dissolving it in 25 mL of methanol. The solution was then stored in a dark room and protected with aluminum foil.

Preparation of Ascorbic Acid Solution. Ascorbic acid was used as a positive control. As much as 11 mg of ascorbic acid and dissolved in 11 mL of methanol to obtain a stock solution of ascorbic acid with a concentration of 1000 ppm. Furthermore, 4 mL of ascorbic acid were taken with the respective concentrations of 0 ppm, 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm from the stock solution. Each tube was added with 1 mL of DPPH solution, incubated, then inserted into a 1x1 cm2 cuvette and the antioxidant activity was read on a UV-Vis spectrophotometer.

Measurement of Antioxidant Activity. Measurement of antioxidant activity began by entering a stock solution of 4 mL of simplicia with a concentration of 0 ppm, 1.25 ppm, 2.5 ppm, 5 ppm, 10 ppm, 20 ppm.
10 ppm, and 20 ppm into the tube. Each tube was added with 1 mL of DPPH solution and incubated for 30 minutes. The antioxidant activity was read by inserting each tube into a 1x1 cm² cuvette and reading it on a UV-Vis spectrophotometer with a wavelength of 517 nm. The same treatment was carried out on the ethanol extract of red pomegranate peel with the same concentration.

Preparation of Red Pomegranate Peel Powder. A total of seven red pomegranates were washed with running tap water, peeled, peeled, then cut the red pomegranate peel to a size of 1x1 cm² so that the weight of the red pomegranate peel was 420 grams. The pieces of red pomegranate peel were dried for 3 days at room temperature to obtain a dry weight of 280 g, then mashed in a blender until they became a powder. The fine powder was separated from the coarse powder. The fine powder of red pomegranate peel was obtained as much as 105 grams.

Preparation of Red Pomegranate Peel Ethanol Extract. The extraction of red pomegranate peel was carried out using the maceration method with 70% ethanol. The filtrate obtained was 4,000 mL in each filtering process. Filtering was carried out 3 times so that a total of 12,000 mL of brown filtrate was obtained. The filtrate obtained was concentrated using a vacuum rotary evaporator. The ethanol solvent evaporation was carried out at 50 °C. The results of the evaporation process obtained a weight of 30.434 g of red pomegranate peel so that the yield of red pomegranate peel ethanol extract was 73.14%.

Determination of percent inhibition of antioxidant activity. Starting with the dilution of the test sample with DPPH which was carried out in accordance with Table 1. All solutions were put into a test tube, then incubated at room temperature for 30 minutes, counting from the addition of the DPPH solution. Next, measure the absorbance at λ DPPH which is 517 nm. The percentage of inhibition of antioxidant activity can be calculated using equation (1). The test was carried out 3 times repetition.

\[ \% \text{ inhibition} = \left( \frac{A_0 - A_i}{A_0} \right) \times 100 \] .................................(1)

Note: \( A_0 \) = absorbance blank (methanol)
\( A_i \) = absorbance of sampel (extract)

Table 1. Simplicia Dilution Test with DPPH

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Test</th>
<th>Water (µL)</th>
<th>DPPH 160 ppm (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>4000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Antioxidant Activity Test. This test was to measure the antioxidant activity quantitatively by measuring the DPPH radical scavenging using a UV-Vis spectrophotometer. The value of free radical reduction activity was expressed by IC\(_{50}\). The IC\(_{50}\) describes the concentration of the simplicia that can capture 50% free radicals through a linear regression equation. The linear regression equation states the relationship between the concentration of the test compound (x) and % inhibition (y).

The regression equation is \( y = \frac{50-a}{b} \).................(2)

RESULT

The percentage of inhibition in each group is presented in Table 2. Table 2 shows that the percentage of inhibition of red pomegranate peel tea ranged from 4.12%-35.51% with an average absorbance of 0.503-0.780. The ethanol extract of red pomegranate peel has the percentage of inhibition ranging from 5.16%-94.87% with an average absorbance ranging from 0.047-0.911. It means that the red pomegranate peel ethanol extract has better potential as an antioxidant than the red pomegranate peel tea steeping.

Table 2. Percentage Inhibition of Steeping Tea, Ethanol Extract of Pomegranate Peel and Ascorbic Acid

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (ppm)</th>
<th>Absorbance in Average (nm)</th>
<th>Percentage Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steeping</td>
<td>1.25</td>
<td>0.747 ± 0.070</td>
<td>4.12</td>
</tr>
<tr>
<td>P. granatum</td>
<td>2.5</td>
<td>0.709 ± 0.075</td>
<td>9.06</td>
</tr>
<tr>
<td>peel Tea</td>
<td>5</td>
<td>0.664 ± 0.081</td>
<td>14.92</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.503 ± 0.064</td>
<td>35.51</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.359 ± 0.042</td>
<td>53.98</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0</td>
<td>0.911 ± 0.017</td>
<td>5.16</td>
</tr>
<tr>
<td>P. granatum</td>
<td>1.25</td>
<td>0.864 ± 0.023</td>
<td>5.16</td>
</tr>
<tr>
<td>peel</td>
<td>2.5</td>
<td>0.799 ± 0.014</td>
<td>12.26</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.626 ± 0.004</td>
<td>31.27</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.344 ± 0.017</td>
<td>62.26</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.047 ± 0.006</td>
<td>94.87</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>0</td>
<td>0.874 ± 0.000</td>
<td>22.34</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.679 ± 0.012</td>
<td>22.34</td>
</tr>
</tbody>
</table>
The determination of antioxidant properties based on the IC$_{50}$ by making a regression equation between the % inhibition value (Table 2; $y$) and the amount of concentration ($x$). The regression results are shown in Figure 2.

![Figure 2. Regression Result between the % Inhibition ($y$) and the amount of concentration ($x$)](image)

Table 3. Results of One Way ANOVA for the Percentage of Inhibition

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test Sample</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Inhibition</td>
<td>Pomegranate peel tea</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Ethanol Extract of Pomegranate</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Ascorbic acid</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 4. Result of Duncan Test for the Percentage of Inhibition

Table 3 shows that there is a significant difference in antioxidant activity between the test samples ($p < 0.05$), and is followed by the Duncan test whose results are shown in Table 4. In Table 4, it appears that each addition in the concentration of the test sample results in a significant difference in the percentage of inhibition. In the form of ethanol extract, red pomegranate peel has an average% inhibition greater than steeping tea at all concentrations, but the greatest is at a concentration of 20 ppm (95.02%).

Analysis was also carried out to determine the significance of the difference in the IC$_{50}$ among the test samples. The result of oneway Anova showed that there was no significant difference in the IC$_{50}$ among the test samples ($p > 0.05$). These results indicate that the antioxidant activity of steeping and red pomegranate peel extract is as great as ascorbic acid.

**DISCUSSION**

This study proves that the red pomegranate peel both in the tea preparation and in the extract has the same antioxidant activity as ascorbic acid. Antioxidants are compounds that can reduce the level of reactivity of a substrate. The mechanism of antioxidant compounds in reducing reactivity includes transfer of hydrogen atoms, transfer of single electrons, and the ability to chelate transition metals.18 Antioxidant molecules available naturally are divided into two categories, namely non-enzymatic antioxidants and enzymatic antioxidants. Compounds classified as enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase, and catalase. In general, compounds classified as non-enzymatic antioxidants include vitamin C, vitamin E, vitamin A, glutathione, and flavonoids.

The efficacy of red pomegranate is due to the presence of various polyphenol compounds which have very high antioxidant activity.20 Many studies have revealed the efficacy of red pomegranate. According to Afaq (2006),7 polyphenolic compounds possessed by red pomegranates are punicalagin (elagitanin), which has been shown to have strong antioxidant, anti-inflammatory, and anti-carcinogenic activity. Apart from being anti-carcinogenic, red pomegranate can also prevent premature aging due to exposure to UV-A and UV-B rays.7 The anti-carcinogenic properties are probably due to the antiproliferative and apoptotic properties of red pomegranate due to the presence of punicalagin which can inhibit peroxidation lipids which are equivalent to synthetic antioxidants.9 Other studies have shown that fresh red pomegranate fruit extract and its fermentation can prevent the proliferation of HL-60 leukemia cells.9

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Steeping Red Pomegranate Peel Tea</th>
<th>Ethanol extract of Red Pomegranate Peel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>$4.120^a$</td>
<td>$5.160^c$</td>
</tr>
<tr>
<td>2.5</td>
<td>$9.060^d$</td>
<td>$12.263^d$</td>
</tr>
<tr>
<td>5</td>
<td>$14.923^c$</td>
<td>$31.276^c$</td>
</tr>
<tr>
<td>10</td>
<td>$35.153^b$</td>
<td>$62.600^b$</td>
</tr>
<tr>
<td>20</td>
<td>$53.983^a$</td>
<td>$95.023^a$</td>
</tr>
</tbody>
</table>
Elagitanin contained in red pomegranate can have antioxidant properties. Under in vivo physiological conditions, elagitanin will be hydrolyzed to elagic acid. Administration of elagic acid to human peel fibroblasts in vitro showed increased expression of collagen type 1 m-RNA and could inhibit MMP-1 (matrix metalloproteinase-1), an enzyme that degrades collagen. The process of inhibiting collagen-degrading enzymes can inhibit the effects of premature aging. The effect of inhibiting aging is also due to the presence of anthocyanins. Anthocyanins contained in red pomegranate consist of delphinidin-3-glucoside, cyanidin-3-glucoside, delphinidin-3,5-diglucoside, cyanidin-3,5-diglucoside, and pelargonidin. Anthocyanins protect collagen through an inhibitory mechanism of phosphorylation of tyrosine kinase, an enzyme inactivation of epidermal growth factor (EGF).  

In this study, it also appears that the red pomegranate peel on the ethanol extract preparation has a higher antioxidant activity than steeping tea. This is due to the level of the punicalagin compound in each sample. The extract sample is a sample that has a better punkalagin level than samples derived from processed tea brewed products. This has implications for the effectiveness of punicalagin activity as an antioxidant. The total content of punicalagin derived from pomegranate extract ranged from 98.70–118.60 in the Desi, Kandhari, and Badana varieties. In addition, the samples derived from tea brewing had low punicalagin purity, so the activity of punicalagin was less effective in these samples. Some of the polyphenol compounds contained in red pomegranates include elagic acid, gallic acid, punicalagin A, and punicalagin B. 

It can be summarized that pomegranate, including its peel, can be used as antioxidants, anti-inflammatory, anti-aging and anti-cancer properties. The presentation can be in various forms such as fresh fruit, fermentation, extract or brew.

**CONCLUSION**

Steeping red pomegranate peel tea and red pomegranate peel ethanol extract have the same strong antioxidant activity as ascorbic acid. The ethanol extract of red pomegranate peel has higher antioxidant activity than steeping tea from red pomegranate peel.

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