Radical catcher activities free of methanol fruit leather and pomegranate seeds (*punica granatum. L*)

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Abstract. Research on the activity of free radical scavenger of pomegranate peel and seed (Punica granatum) methanol extract was carried out with the aim of determining the free radical scavenger activity of methanol extract of pomegranate peel and purple pomegranate seeds, and determining the IC50. The results showed that: The free radical scavenger activity of the methanol extract of pomegranate peel and seed was 61.44% and 64.95% at a concentration of 250mg / ml, respectively. The IC50 value of the methanol extract of purple pomegranate rind was 1.6869 g / g DPPH, while the seeds were 1.3569 g / g DPPH.

1. Introduction

Pomegranate or what is often referred to as pomegranate (French) which means apples with many seeds, is not very popular for Indonesians. People plant it only as an ornamental plant, even though this sexy looking fruit has many benefits. In Indonesia, there are three types of pomegranate, namely red pomegranate, white pomegranate, and purple pomegranate [1].

You can get the intake of polyphenol antioxidant compounds, which function to paralyze cancer cells and overcome the hardening of the artery walls, which can be obtained by consuming pomegranates. A research report submitted by cancer experts from the American Association for Cancer Research at a national conference in November 2003 stated that pomegranate extract can help cure and prevent skin cancer [2].

One of the phenolic members that are relatively new and not widely known in Indonesia is ellagic acid, which has the potential to be a strong antioxidant. The antioxidant content in pomegranate is mediated by the activity of the phenol hydroxyl group which includes tannins and flavonoids. [3] Apart from being potential as a strong antioxidant, it also has the potential to be anti-inflammatory, antibacterial, antimutagenic, and inhibit tumor growth. [2] Other medicinal properties of pomegranate include being an anticancer due to its high antioxidant content, cardiovascular disease, antidiabetic, bacterial infection, and antibiotic resistance, repairing skin damage caused by ultraviolet light induction, Alzheimer's, obesity, and antiplasmodial. [4] and also from the research results have activity as Selective Estrogen Receptor Modulators (SERMs). [5]

Ellagic acid is a complex compound found in many fruits, vegetables, and nuts. Besides being found in various kinds of berries, it turns out that ellagic acid is also found in many fruits such as pineapple, longan, apple, guava, and pomegranate. In general, if we consume pomegranate, the skin and seeds of the pomegranate are immediately discarded without being used. In fact, from the skin and
seeds of a pomegranate, many benefits can still be taken, one of which is as a source of ellagic acid. [6]

The yield of ellagic acid obtained is a recovery in which solid waste that is wasted can be reprocessed to produce a useful and economically valuable product. For this reason, this study was conducted to determine the activity of free radical scavengers from methanol extract of the skin of the fruit and purple pomegranate seeds, and determining the IC$_{50}$

2. Methodology

2.1 Tools and Materials

Tools: The tools used are analytical balance, desiccator, rotary evaporator, oven, mini UV spectrophotometer, micropipette, measuring flask, measuring pipette, glassware, UV light, reflux.

Material: The samples used were fresh purple pomegranate (Punica granatum L) rind and seeds. The chemicals used are methanol, distilled water, TCA 2 N

2.2 Method

2.2.1 Extraction of Ellagic Acid

A sample of 250 grams was dissolved in 400 ml of methanol, refluxed for 24 hours, then evaporated to dryness (as a crude extract). The extract was hydrolyzed with Trichloro Acetic (TCA) 2 N in methanol for 2 hours (containing total ellagic acid). Then methanol: water = 4: 1 solvent was used to separate the ellagic acid compound from other compounds. The isolated results were dissolved with methanol and placed in a sample bottle. The purity was measured using HPLC. [18]

2.2.2 Measurement of Free Radical Capture Activity Using the DPPH Method [19]

1 ml of sample with a concentration of 250 $\mu$g / ml was added with 3 ml of DPPH 0.1 mM in 95% ethanol. The solutions were incubated at room temperature and in the dark for 30 minutes. The absorbance was measured at a wavelength of 517 nm. as a blank sample solvent is used as a substitute for the sample. Ellagic acid was used as a comparison.

$$% \text{ inhibition} = \frac{Absorbance \text{ Blanks} - Absorbance \text{ Sample}}{Absorbance \text{ Blanks}} \times 100\%$$

Each sample was determined by Inhibition Concentration (IC$_{50}$), namely the ability to capture free radicals in the sample to inhibit 50% of DPPH free radicals. IC$_{50}$ calculations with various sample concentrations (25, 50, 75, 100, 125, 150, 175, 200, 225, 250 $\mu$g / ml).

2.2.3 Data Analysis

The data obtained were analyzed using the t-test with a confidence level of 5% and repeated 5 times.

3. Result and Discussion

3.1 Activity of Free Radical Capture of Methanol Extract of Fruit Skins and Purple Pomegranate Seeds using the DPPH Method

The percentage of inhibition of the methanol extract of pomegranate peel and seeds at a concentration of 250$\mu$g / ml ranged from 61.44 ± 2.80 - 64.95 ± 6.16.

Table 1. Free Radical Capture Activity of Methanol Extract of Fruit Skins and Purple Pomegranate Seeds by Method
| Sample (250µg/ml) | % inhibition ± SE |
|-------------------|--------------------|
| Fruit Skins       | 61.44 ± 2.80 (a)   |
| Seeds             | 64.95 ± 6.16 (a)   |

Note: Numbers followed by the same letter show no significant difference, while numbers followed by different letters show significant differences (t-test with a = 5%).

The results showed that the free radical scavenger activity of the methanol extract of pomegranate peel was 61.44% and not significantly different (a = 5%) with the seed extract (64.95%). This is presumably because the fruit peel samples used in this study were fresh samples with high water content (78.08% w / w). As a result, the weight or number of samples used in the test is much smaller or less when compared to the weight or number of dry fruit peels, so that the free radical scavenger activity is not as large as the activity in dry samples. According to Singh et al [2], which stated that the free radical scavenging activity of methanol extract of fruit peels and dried pomegranate seeds at a concentration of 50 ppm was able to inhibit DPPH free radicals by 81% and 23.2%. The water content in the sample affects the amount of free radical scavenger activity. This proves that dry samples are more effective than fresh samples in testing free radical scavenger activity using the DPPH method.

The activity of the methanol extract has the property to block free radical oxidation and donate hydrogen from the phenolic hydroxyl group into a stable product without undergoing an initiation or propagation process in the antioxidant mechanism [6].

Ellagic acid as a reference compound showed an inhibitory percentage of 94.55 ± 2.69 at a concentration of 250µg / ml, while the methanol extract of fruit peels and seeds at the same concentration showed an inhibition percentage of 61.44 ± 2.80 and 64 respectively. 95 ± 6.16 (Figure 1.).

Figure 1. Histogram of Free Radical Capture Activity of Methanol Extract of Fruit Skins and Purple Pomegranate Seeds using DPPH and Ellagic Acid Methods as Comparison.

3.2 IC\textsubscript{50} Determination of Methanol Extract of Purple Pomegranate Peels and Seeds by the DPPH Method

The results of IC\textsubscript{50} determination of the methanol extract of pomegranate rind and seeds can be seen in Figure 2, and Table 2. The IC\textsubscript{50} value is the concentration of purple pomegranate peel and seed extract needed to capture 50% of DPPH free radicals. A low IC\textsubscript{50} value indicates a high free radical scavenger activity.

The method used is based on the stable DPPH free radical reduction reaction. DPPH has unpaired electrons and has a maximum absorbance at 517 nm with a spectrophotometer (purple color). The unpaired electrons become paired in the presence of a hydrogen donor, which is a free radical scavenger antioxidant so that the absorbance decreases and results in a decrease in purple color equivalent to the number of electrons captured [20]. This reaction has been widely used to test the...
ability of a compound as a free radical scavenger or hydrogen donor and to evaluate antioxidant activity in plant extracts [12].

One class of compounds that can be hydrogen donors is phenolic. The number of hydrogen donors that can be donated is influenced by the number and position of the OH group on the phenolic compound. Ellagic acid has a favorable chemical structure because of the large number of OH groups that can bind relatively large amounts of DPPH free radicals [25][26].

Table 2. IC50 Value of Methanol Extract of Purple Pomegranate Peels and Seeds using the DPPH Method

| Sample     | IC50 ± SE (g/g DPPH) |
|------------|----------------------|
| Fruit Skins| 1,6869 ± 0,063 (a)   |
| Seeds      | 1,3569 ± 0,1283 (b)  |

Note: The numbers followed by the same letter show not significantly different, while the numbers followed by different letters indicate significantly different between samples (t-test with α = 5%).

The IC50 value of purple pomegranate seed extract was 1.3569 g / g DPPH lower than the fruit peel extract (1.6869 g / g DPPH). This means that the free radical scavenger activity of 50% in the methanol extract of the seeds is higher than the skin of the fruit. This is thought to be related to the content of compounds found in the skin of the pomegranate fruit and seeds. Punicalagin is one of the tannin compounds contained in the skin of pomegranate fruit and seeds. The punicalagin content in pomegranate seeds is more than the skin of the fruit [20]. Punicalagin has a significant IC50 value of 1.6 ppm [14]. While pomegranate peel contains more anthocyanins than the seeds, characterized by a purple color from the fruit skin extract.[21] Punicalagin is an ellagitannin group that has higher antioxidant activity when compared to anthocyanins.

According to Talcoot et al. [23] the ellagic acid in pomegranate peel and seed extract functions as a powerful antioxidant. If the free radical scavenger activity in the methanol extract of purple pomegranate peel and seeds is compared to ellagic acid with an IC50 of 0.0025 g/g DPPH, it appears that the free radical scavenger activity of the methanol extract of purple pomegranate peel and seeds is much lower than the free radical scavenger activity in ellagic acids (Figure 2). This is presumably because, in the methanol extract, the skin of the fruit and seeds is still a mixture (such as anthocyanins, punicalagin, punicalagin, and alkaloids), not pure ellagic acid so that its activity is not as big as pure ellagic acid.

![Figure 2](image)

**Figure 2.** Histogram of IC50 Value of Methanol Extract of Fruit Skins and Purple Pomegranate Seeds with DPPH and Ellagic Acid Methods as Comparison
When compared to IC$_{50}$ ellagic acid (0.33 ppm) with IC$_{50}$ punicalagin (1.6 ppm), it can be seen that ellagic acid can scavenge free radicals higher than punicalagin [22]. However, in this study the samples used were methanol extract of fresh pomegranate peel and dried pomegranate seeds, so that at the same concentration (250 g/ml) between the skin of the fruit and pomegranate seeds, it showed the same activity but the resulting IC$_{50}$ value. by the skin of the fruit is higher than the seed.

Other compounds besides ellagic acid that are thought to act as free radical scavengers in the extract are anthocyanins. Anthocyanins have high free radical scavenging activity [24]. However, when the extract was obtained, heating was used, so it was suspected that the anthocyanins had degraded, resulting in a decrease in free radical scavenger activity.

4. Conclusion
The conclusions that can be drawn from this research are:
1. The free radical scavenger activity of the methanol extract of pomegranate peel and seed was 61.44% and 64.95% at a concentration of 250 μg/ml.
2. The IC$_{50}$ value of the methanol extract of purple pomegranate rind is 1.6869 g/g DPPH, while the seeds are 1.3569 g/g DPPH.

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