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Research Article

Studies on antioxidant activity of red, white, and black pomegranate (*Punica granatum* L.) peel extract using DPPH radical scavenging method

Uswatun Chasanah[1]*
[1] Department of Pharmacy, Faculty of Health Science, University of Muhammadiyah Malangg, Malang, East Java, Indonesia
* Corresponding Author's Email: Uswatun@umm.ac.id

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ABSTRACT

Pomegranate (*Punica granatum* L.) has high antioxidant activity. In Indonesia, there are red pomegranate, white pomegranate, and black pomegranate. The purpose of this study was to determine the antioxidant activity of red pomegranate peel extract, white pomegranate peel extract, and black pomegranate peel extract. The extracts prepared by ultrasonic maceration in 96% ethanol, then evaporated until thick extract was obtained and its antioxidant activity was determined using the DPPH radical scavenging method. This study showed that all pomegranate peel extract varieties have potent antioxidant activity and the black pomegranate peel extract has the highest antioxidant power.

1. INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the Puricaceae family, a plant originating from the Middle East (Rana, Narzary & Ranade, 2010). All parts of the pomegranate, such as fruit (fruit juice, fruit seeds, peel fruit), leaves, flowers, roots, and bark, have therapeutic effects such as neuroprotective, antioxidant, repair vascular damage, and anti-inflammatory. The clinical application of this plant used in cancers, atherosclerosis, hyperlipidemia, carotid artery stenosis, myocardial perfusion, periodontal disease, bacterial infections, ultraviolet radiation, erectile dysfunction, male infertility, neonatal hypoxic-ischemic brain injury, Alzheimer's disease, and obesity (Jurenka, 2008; Mackler, Heber & Cooper, 2013). it is also used as cosmetic ingredients (Aslam, Lansky & Varani, 2006). The bioactive contained in the pomegranate peel is triterpenoids, steroids, glycosides, flavonoids, tannins, carbohydrate & Vitamin C. Much of bioactivity pomegranate peel and one of them is antioxidant activity (Derakhshan et al., 2018).

There are red pomegranate, white pomegranate, and purple/black pomegranate. Red pomegranate has a sweeter and fresher taste, white pomegranate more chewy and coarse and less sweet, and black pomegranate has a sweeter flavor than this red variety. Interested in the three varieties of pomegranate, a study will be conducted to compare the ethanol extract's antioxidant activity of red pomegranate peel, white and black pomegranate peel.

2. MATERIALS AND METHODS

Material

The materials used in this study was red, white, and black pomegranate from Situbondo, East Java, Indonesia, ethanol (technical grade), methanol (pro analysis, MERCK©), 2,2-Diphenyl-1-picrylhydrazyl (Sigma-Aldrich©), ascorbic acid (CSPC Weisheng Pharmaceutical (Shijiazhuang) Co. Ltd.©)

Instruments

This study's instruments were ultrasonic bath, rotary vacuum evaporator, Universal oven memmert UN 75, and Spectrophotometer UV-VIS (Shimadzu).

Methods

Preparation of Pomegranate Peel Extract

Fresh pomegranate fruit was harvested from Situbondo, East Java, Indonesia, in December 2019. The peels of fruit that had cut pieces dan cleaned were drying at 40 ^{o}C for three days and then crushed into powder. As much as 50 gr of red, white, and black pomegranate peel powder (mesh 60) extracted by ultrasonic macerated for 45 minutes at an amplitude of 20-40 Hz in 96% ethanol using the ratio of 1:10 (pomegranate:solvent) (Baihaqi, Budiastra & Darmawati, 2018). The extract was pressed, filtered, and the ethanol removed by a rotary vacuum evaporator. Furthermore, the remaining ethanol was evaporated in the oven for three days at 40^{o} C until a thick consistency was obtained.

Evaluation of Antioxidant Activity by DPPH Radical Scavenging Method

Free radical scavenging activity of red, white, and black pomegranate cortex was measured by the DPPH method (Molyneux P, 2004; Alam, Bristi & Rafiquzzaman, 2013). The 200 µg/mL solution of DPPH in methanol, 20 µg/mL solution of extract in methanol, and ascorbic acid solution of 20 µg/mL in methanol were prepared. The 1.0 mL of DPPH solution added to 0.5;1.0;2.0;3.0;4.0; and 5.0 mL of extract solution or ascorbic acid, as standard sample, then added methanol to 10.0 mL. The mixture was shaken vigorously and allowed to stand at 37 ^{o}C for 30 minutes, then absorbance is measured at 514-515 nm by a spectrophotometer UV-VIS. The lower absorbance of the sample indicated a higher free radical activity. The percentage of inhibition calculated by the following equation:

$$Percentage\ Inhibition = (A_0\text{-}A_1/A_0) \times 100 \quad (1)$$

A_o = absorbance of the control reaction

A_1 = absorbance in the presence of a test or standard sample.

An inhibitor concentration of 50% (IC_{50}) is used to express the activity of the antioxidant. The sample's IC_{50} was defined as the concentration of the sample required to reduce DPPH free radical by as much as 50%. The linear regression equation is used from the extract's concentration range to the immersion % DPPH to determine the extract concentration that reduces 50% of DPPH. The value of 50% was obtained from the x value after substituting y = 50. From the equation y = a + bx, the IC50 value calculated using the following formula:

$$IC_{50} = (50\text{-}a)/b \quad (2)$$

A comparison of the IC_{50} value of pomegranate peel extract and ascorbic acid to determine the pomegranate peel extract sample's antioxidant activity.

Data Analysis

From the gradient of the Linear inhibition curve determined of IC_{50}, then it is analyzed using One-way Anova with a degree of confidence of 95%.

3. RESULTS AND DISCUSSIONS

The Pomegranate Peel Extract

The pomegranate peel extracts obtained (**Figure 1**), are a thick dark extract sequentially from the white, red, and black pomegranate peel extracts, which have an increasingly dark color intensity.

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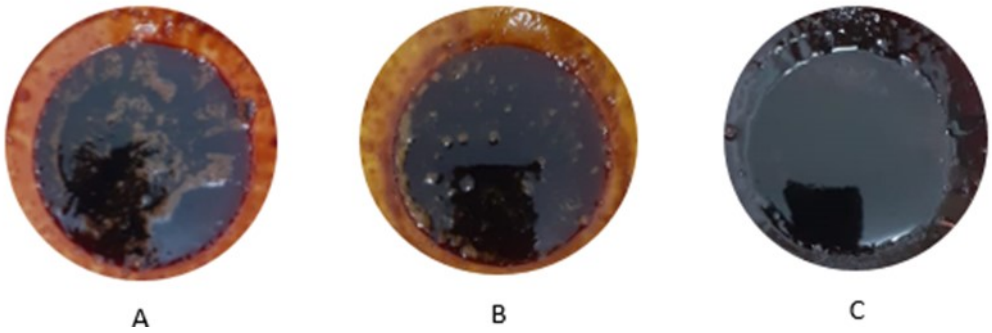

Figure 1. Red (A), white (B), and black pomegranate peel extract ©.

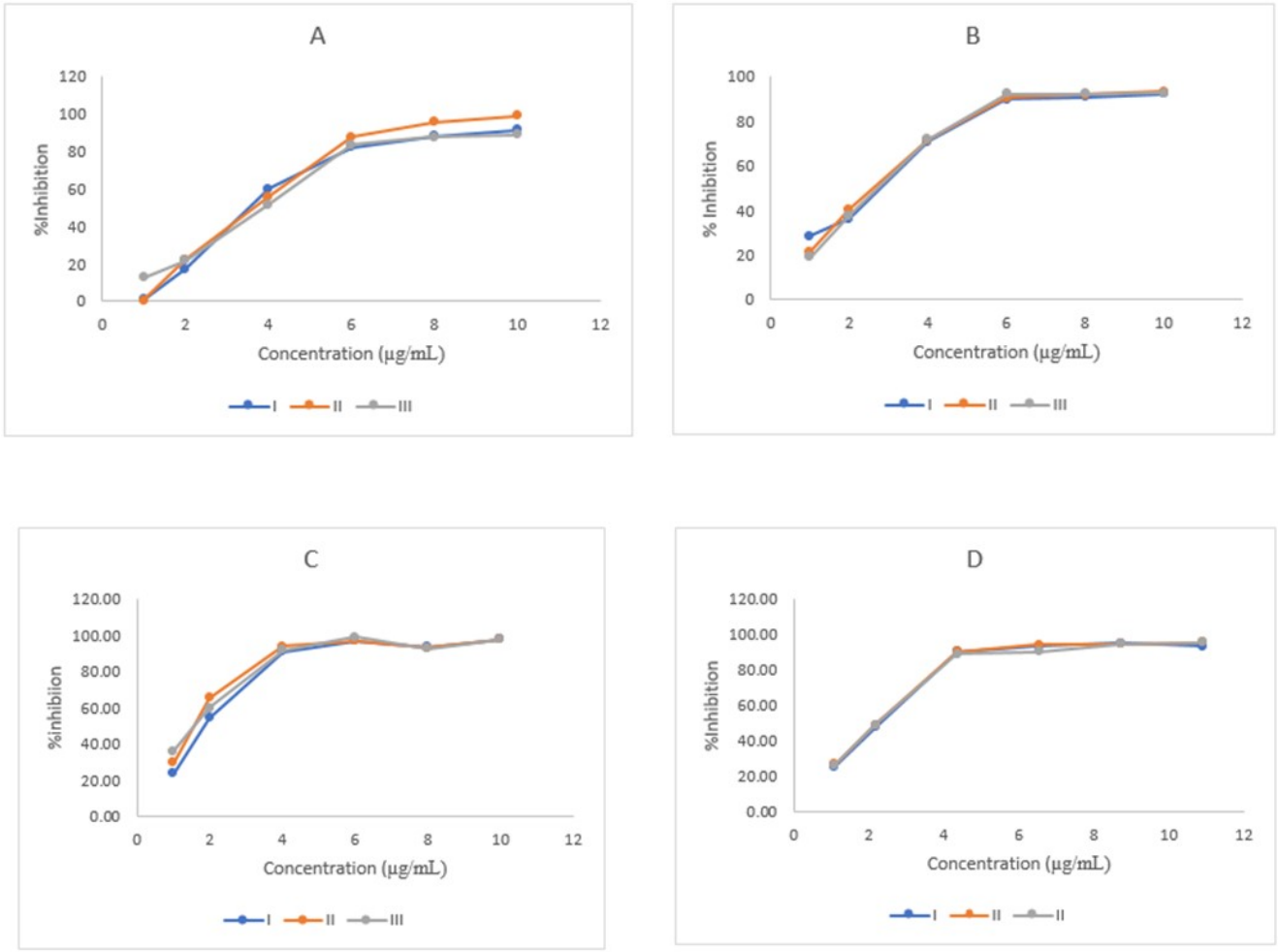

Figure 2. This figure is the Percentage inhibition of red pomegranate peel extract (A), white pomegranate peel extract (B), black pomegranate peel extract (C), and Ascorbic acid as a control (D).

The bioactive contained in the pomegranate peel is triterpenoids, steroids, glycosides, flavonoids, tannins, carbohydrate, and vitamin C. The peel's brilliant red color is attributed to anthocyanidins and flavan-3-ol (Bhandary, Kumari, Bhat, Sharmila & Beka, 2012). From the intensity of the color obtained, black pomegranate may have the highest anthocyanidins and flavan-3-ols.

Antioxidant Activity

The results of determining the maximum wavelength achieved by the DPPH solution in methanol are 514 - 515 nm. The percentage inhibition of the red, white, and black pomegranate peel extract is shown in **Figure 2**. The gradient obtained from this curve was used to determine IC_{50} of pomegranate peel extract (**Table 1**).

The sample concentration of red, white, and black pomegranate peel extract made of 1.0;2.0;4.0;6.0;8.0;

Table 1. The IC50 of pomegranate peel extract

Pomegranate	IC 50 (µg/mL) Replication			Mean of IC50 (µg/mL)	SD (µg/mL)
	I	II	III		
Red pomegranate	3.88	3.78	3.80	3.82	0.05
White pomegranate	2.75	2.82	2.89	2.82	0.07
Black pomegranate	2.02	1.69	1.63	1.78	0.21
Ascorbic acid	2.30	2.25	2.27	2.27	0.02

and 10.0 µg/mL, as well as the concentration of ascorbic acid, too. **Figure 2** shown the horizontal curve of the black pomegranate peel extract starts at a concentration of 4.0 µg/mL; the same thing is shown by the ascorbic acid curve, while the red and white pomegranate peel extract the horizontal curve line starts at a concentration of 6.0 µg/mL. The IC_{50} obtained from the gradient concentration of this curve; therefore, the IC_{50} calculation of black pomegranate peel extract and vitamin C uses a concentration of 1.0;2.0; and 4.0 µg/mL., while red and white pomegranate peel extract uses a concentration of 1.0;2.0;4.0; and 6.0 µg/mL.

The IC_{50} of red, white, and black pomegranate peel extract is 3.82;2.82; and 1.78 µg/mL, while ascorbic acid as control has an IC_{50} is 2.27 µg/mL. The IC_{50} of all pomegranate peel extract is less than 50 µg/mL, so that their antioxidant activity is very strong. The IC_{50} of black pomegranate peel extracts less than ascorbic acid. Otherwise, red and white pomegranate pell extract having an IC_{50} more than ascorbic acid. The highest score of IC_{50} was owned by black pomegranate, followed by white and red pomegranate. This result, contrary to the research conducted by Andriyani & Suharyanto (2015), that reported the antioxidant activity of black pomegranate has the highest score followed by red and white pomegranate. The pomegranate peel ethanolic extract contained flavonoids, tannins, and carbohydrates (Bhandary et al., 2012). The pomegranate peel's flavonoids with antioxidant activity are catechin, cyanidin, kaempferol, luteolin, quercetin, and rutin, while significant pomegranate tannins peel is casuarinin, methyl gallate, granatin A, granatin B, pedunculagin, punicalagin, and punicalin (Middha, Usha & Pande, 2013). Further research is needed to determine the total content of flavonoids and tannins from red, white, and black pomegranate peel.

4. CONCLUSIONS

The antioxidant activity of red pomegranate pell, white pomegranate peel, and black pomegranate peel are potent.

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