Antioxidant Activities in Different Parts of Pulasan (Nephelium mutabile Blume) from East Borneo

I Hairunisa1, I A Mentari1, T Julianti1, E R Wikantyasning2, Z Cholisoh2, S C Ningsih1, M R F Muslim1

1 Department of Pharmacy, Faculty of Health and Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Indonesia
2 Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Indonesia

*Corresponding author: ih787@umkt.ac.id

Abstract. Nephelium mutabile Blume (Traditionally known as Pulasan or Kapulasa) is a plant resembling the rambutan fruit that grows mostly on the islands of Sumatra and Kalimantan. Pulasan has a unique characteristic, the skin of this fruit was hairless and had very bright color. The aim of this study was to determine the antioxidant activity of different parts of the Pulasan, including the peels, seeds and leaves. Antioxidant activity was determined by IC50 values using the DPPH method (2,2-diphenyl-1-picrylhydrazyl) with Vitamin C as a positive control. Each part of the plant was extracted using 96% ethanol then the total phenolic compound was tested using a gallic acid as the standard. The results showed that ethanol extract of pulasan leaves had the highest antioxidant activity with the value 20.99 µg/mL (very strong antioxidant), while the seeds and peels of pulasan showed antioxidant activity of 520.68 µg/mL and >1000 µg/mL (not active as antioxidants) respectively. This result shows that ethanol extract from the leaves of Pulasan has potential to be developed as a source of natural antioxidants.

Keywords. Antioxidant, Nephelium mutabile Blume, Pulasan, East Borneo

1. Introduction

East Kalimantan is the largest Indonesian islands and known as of high level the diversity of natural potential sources. Meanwhile Indonesian people especially in East Kalimantan usually used plants as medicine, utilization the potential of natural sources used to cure diseases in the form of traditional medicine. One example of potential natural sources as a medicine is pulasan (Nephelium mutabile Blume) (Figure 1).

Based on research conducted by Fadhli et al. (2018), Pulasan has strong antioxidant activity. This activity makes it possible to fight free radicals in the body system [1]. This activity also can be used as a preventive treatment to reduce degenerative diseases caused by free radicals. Some diseases caused by free radical include atherosclerosis, cancer, stroke, trauma, asthma, heart attack, arthritis, age pigment, cataracts, hepatitis, and periodontitis [2]. Free radicals are normal products of cellular metabolism, which is our cells routinely produce free radicals and reactive oxygen species (ROS) or commonly called reactive oxygen groups [4][5]. When there is an imbalance between the production of free radicals and antioxidants as a defense in the body, then the body will produce oxidative stress. Oxidative stress is defined as a disturbance in the balance between free radicals reactive oxygen
species (ROS) and endogenous defence mechanisms. Reactive oxygen species (ROS) can multiply production in a pathological state [2][5][6].

![Figure 1. The appearance of Pulasan (Nephelium mutabile Blume) leaves (A) and fruits (B)](image)

Pulasan (Nephelium mutabile Blume) contain active component such as tannin (geraniin and corilagin), phenolics and saponins (7R-methoxyerythrodiol, erythrodiol, maniladiol), flavonoid [1][3]. All that component have potential to act as antioxidant. Antioxidant responsible for prevention of the damaging effects of free radicals and toxic products of their metabolism which is have four possibility mechanism they are reduce the rate of oxidation of fats and oils, electron and hydrogen donation by antioxidant, reduce the rate of oxidation of fats and oils [7]

Thus, we conducted to evaluate the antioxidant activity and analyze total phenolic compound in Pulasan (Nephelium mutabile Blume). This plants are chosen based on their use as traditional medicine in the region East Kalimantan. This research is very important, cause by determine the antioxidant activity of Pulasan, we can ensure the safety of its use.

2. Material and Method

2.1 Materials and Equipment

Pulasan fruits and leaves, Ethanol 96%, Methanol 96% PA (Merck), DPPH (2,2-diphenyl-1-picrylhydrazyl) (TCI), Ascorbic Acid PA (Merck), Gallic Acid PA (Merck), Folin-Ciocalteu Reagent (Merck), Sodium Carbonate, Distilled water, Micropipette (Dragonlab), UV-Vis Spectrophotometer (Genesys 10s), Rotary Evaporator (RV 10), Vortex (B-One), Glassware (Iwaki).

2.2 Sample Preparation

Pulasan fruits and leaves were taken from the local trees located in Samarinda, East Kalimantan. Pulasan fruits then separated into peels and seeds. Seeds, peels and leaves are then wet sorted to remove impurities then dried under the indirect sunlight. The dry sample is then cut smaller (1 cm x 1 cm) for further extraction.

2.3 Sample Determination

The validity of the plants used was confirmed through the determination of samples by the Laboratory of Ecology and Conservation of Tropical Forest Biodiversity at the University of Mulawarman through letter number 7/UNI7.4.08/LL/2020 stating that the plants used were Pulasan (Nephelium mutabile Blume).

2.4 Sample Extraction

Sample extraction was carried out using maceration techniques. Each sample was macerated using 96% ethanol with a ratio of sample and solvent 2:3. Maceration is done for 3 days with stirring every
24 hours. Re-maceration was carried out on the sample 2 times with stirring every 24 hours. The maceration results are then concentrated using a rotary evaporator at 60°C until a concentrated extract is obtained.

2.5 Determination of Total Phenolic Compound (TPC)

2.5.1 Preparation of Gallic Acid (1 mg/mL) Twenty (20) mg of gallic acid was carefully weighed and then dissolved with 1 mL 96% methanol. After dissolving then added with 19 mL distilled water to obtain a gallic acid solution with a concentration of 1 mg/mL.

2.5.2 Preparation of Na₂CO₃ (10%) Ten (10) gram of Na₂CO₃ were weighed and then dissolved with 100 mL distilled water in a volumetric flask to obtain a solution concentration of 10%. Furthermore the solution is incubate for 24 hours before use.

2.5.3 Preparation of Sample (10 mg/mL) One hundred (100) mg samples were dissolved with 1 mL methanol 96%. Furthermore distilled water is added up to 10 mL in volumetric flask. The samples then vortex for 10 minutes and filtered. This procedure is carried out for samples of peels, seeds and leaves extract.

2.5.4 Determination of Gallic Acid Standard Curve : Standard curve of gallic acid is made using concentrations of 3-50 µg/mL. Gallic acid solution pipetted then added with 0.5 mL of folin-ciocalteu reagent, shaken and incubated for 8 minutes. Subsequently added 3 mL of 10% Na₂CO₃ solution and distilled water to 10 mL in volumetric flask. The sample is then incubated for 2 hours in a dark condition. The sample is then read on a UV-Vis spectrophotometer at the wavelength of 765 nm.

2.5.5 Determination of Phenolic Content : Five hundred (500) µL samples were pipetted into the volumetric flask. Samples then added with 0.5 mL of Folin-Ciocalteu reagent, shaken and incubate for 8 minutes. Next, add 3 mL of 10% Na₂CO₃ solution and distilled water to 10 mL. The sample then incubate for 2 hours in a dark condition and read on a UV-Vis spectrophotometer at a wavelength of 765 nm. Results are expressed as mg gallic acid/g extract

2.6 Determination of Antioxidant Activity

2.6.1 Preparation of DPPH (100 µg/mL) Five (5) mg of DPPH were weighed carefully and added with methanol 96% up to 50 mL in volumetric flask to obtain a DPPH concentration of 100 µg/mL. DPPH solution then stored in a dark condition.

2.6.2 Preparation of Ascorbic Acid (1 mg/mL) Five (5) mg of ascorbic acid were weighed carefully then added with methanol 96% up to 5 mL in volumetric flask to obtain ascorbic acid concentration of 1 mg/mL.

2.6.3 Preparation of Sample (10 mg/mL) Five hundred (500) mg extracts were weighed and added with 96% methanol up to 5 mL in volumetric flask to obtain a sample concentration of 10 mg/mL. The sample was then shaken for 5 minutes and filtered. This procedure is carried out for samples of extract of seeds, peels and leaves of Pulasan.

2.6.4 Determination of antioxidant activity for Ascorbic Acid and Samples : Determination of antioxidant activity is carried out using DPPH reagents. Ascorbic acid (positive control) was made with a concentration series of 3.125-12.5 µg/mL, pulasan seeds and peels extract samples with a concentration series of 62.5-1000 µg/mL, while pulasan leaves extract using a concentration series of 6.25-100 µg/mL. Ascorbic acid and samples were pipetted and then added 3 mL of DPPH solution and
96% methanol to 10 mL in volumetric flask. The sample was then incubate for 30 minutes in dark conditions for further absorption readings using UV-Vis spectrophotometry at a wavelength of 517 nm with 96% methanol as a blank. The reading results are used to determine % inhibition. Each sample was tested three times.

3. Result and Discussion

3.1 Sample Extraction Result

Pulasan (*Nephelium mutabile* Blume) is a plant that has many similarities with the rambutan plants (*Nephelium lappaceum*). Not much research is done on pulasan, this is because these plants only bear fruit once a year and grow wild. Based on studies that have been conducted on three parts pulasan (peels, seeds, and leaves) obtained extraction yield (in percentage) and organoleptic data (Table 1). Previous research has also been done by Sukemi, *et al* (2015) and Fadhli *et al* (2018) using various kinds of solvents and yields vary from 2-24%. As a comparison of the same solvents, a study by Sukemi *et al* (2015) obtained a yield of 6.04% for extraction using ethanol [8] [9]. The difference in the extraction yield can be caused by several things including the type of solvent used, the extraction method, the extraction time, and the origin of the material/sample [10][11][12]. In the research conducted, there were differences in the solvent and the origin of the sample. The sample used in the previous study was a sample from South Kalimantan, while the research was conducted using a sample from East Kalimantan. Meanwhile, the sample preparation process is also different. In previous studies using fresh samples, while in research conducted using samples that have been dried.

| Type of extract | Yield % (w/w) | Organoleptic                  |
|-----------------|---------------|-------------------------------|
| Peels Extract   | 3.22%         | Dark Brown                    |
| Seeds Extract   | 1.28%         | Light Brown                   |
| Leaves Extract  | 4.65%         | Dark Green                    |

Table 1. The extraction yield and organoleptic data of pulasan peels, seeds and leaves extract after extraction by maceration using ethanol 96%.

Based on the results of secondary metabolite tests showed that the peels extract of pulasan is thought to contain steroid compounds, terpenoids, alkaloids and phenolics [8] and saponins, tannins [13]. While the seeds extract of pulasan contain alkaloids and steroids [13]. The leaves extract of Pulasan has never been investigated before, so there are no secondary metabolite data from this part.

3.2 Determination of Total Phenolic Compound (TPC)

The aim of this study was to determine the antioxidant activity of the peels, seeds and leaves of Pulasan. The antioxidant activity is generally related to the total phenolic compound (TPC) in a sample [14][15]. Where in general, the higher the total phenolic compound, the higher the antioxidant activity. This total phenolic compound is usually related to the content of active ingredients that will have good activity as antioxidants, structural polymers (lignin), attractants (flavanoids and carotenoids), UV screens (flavanoids), signal compounds (salicylic acid and flavonoids) and defense response chemicals (tannins and phytoalexins) [16].

To determine the compound from the sample used, a total phenolic compound (TPC) was carried out using gallic acid as a standard. This study is performed on both peels, seeds and leaves extract of Pulasan. The results showed that each sample contained phenolic compounds, with the highest phenol content found in peels extracts of Pulasan with the value 0.35 mg gallic acid/g extract. While the leaves and seeds extract of Pulasan by 0.15 mg gallic acid/g extract and 0.09 mg gallic acid/g extract. Based on these results, it is suspected that peels extract of Pulasan will provide the most powerful
antioxidant results, this is because antioxidant activity is closely related to the content of phenolic compounds.

3.3 Determination of Antioxidant Activity
Antioxidant activity study was carried out by the DPPH (1,1-diphenyl-2-pircrilhidrazil) method. The DPPH method is the method most often used because the components of the tool used are easy to obtain, the samples used are few, more sensitive, simple and less time consume. The antioxidant activity assay using DPPH was carried out using a UV-Vis spectrophotometer at a wavelength of 517 nm, this wavelength itself is a wavelength that can detect changes in purple color of the sample [17]. This change in purple intensity occurs due to the reduction of free radicals produced by the reaction of DPPH molecules with hydrogen atoms released by molecules of antioxidant compounds sampled to form stable compounds and cause DPPH color changes from purple to yellow (Figure 3). This change will provide a change in absorbance at the maximum wavelength of DPPH so that free radical scavenging activities are known as expressed by IC$_{50}$ (Inhibitory Concentration) values.

As a comparison or positive control used Ascorbic acid (vitamin C) solution because it has been proven as strong antioxidant. This comparison aims to see whether the sample has potential as an antioxidant and is able to match the activity of vitamin C. Vitamin C is an antioxidant that is soluble in water and is well known and widely used. In this study, The results of antioxidant activity of vitamin C...
C get a very strong antioxidant activity with an IC$_{50}$ value of 1.39 µg/ml (Figure 4; Table 2). These results show similarities to claims that vitamin C is a strong antioxidant activity.

![Figure 4](image.png)

**Figure 4.** The curve for the antioxidant activity of Ascorbic acid (Vitamin C). Antioxidant activity tests were carried out using DPPH reagents. Vitamin C was used as a positive control at a test concentration of 3.125-12.5 µg/mL.

Antioxidant assay were also performed on pulasan samples, both on peels, seeds and leaves extract of Pulasan (Figure 5; Table 2). Based on a study conducted on these three samples, it was found that the leaves extract of Pulasan has the highest antioxidant activity with the value 20.99 µg/mL. The antioxidant activity of leaves extract of pulasan can be categorized as strong antioxidants [17]. While the antioxidant activity of the peels and seeds extract of Pulasan showed the opposite with the value 520.68 µg/mL and >1000 µg/mL. Both of these samples are categorized as not active antioxidants.

![Figure 5](image.png)

**Figure 5.** The curve for antioxidant activity of peels (A), seeds (B) and leaves (C) extract of Pulasan (*Nephelium mutabile* Blume). Antioxidant activity assay were carried out using DPPH reagents. Each
extract was tested with various concentration series, 62.5-1000 µg/mL for peels and seeds extract of Pulasan and 6.25-100 µg/mL for leaves extract of Pulasan.

Previous research conducted by Sukemi, et al (2015) and Fadhli et al (2018) obtained different results, Sukemi et al (2015) reported that methanol extracts of pulasan peels had weak antioxidant activity with IC$_{50}$ 190 µg/mL, whereas Fadhli et al (2018) reported that methanol extract of Pulasan peels was categorized as a strong antioxidant with IC$_{50}$ 57.389 µg/mL. This difference is possible because in this study using dried sample, while in other studies using fresh peels of Pulasan.

| Sample type       | Concentration (µg/mL) | % inhibition (in average) | SD  | Persamaan Regresi Linear | IC$_{50}$ (µg/mL) |
|-------------------|-----------------------|---------------------------|-----|--------------------------|------------------|
| Vitamin C         | 3.125                 | 51.7                      | 0.23| $y = 4.4823x + 43.85$    | 1.39             |
|                   | 6.25                  | 81.1                      | 0.11| $y = 0.0368x + 0.6601$   | >1000            |
| (Positive Control)| 12.5                  | 96.8                      | 0.13| $R^2 = 0.8734$           |                  |
| Peels extract of  | 1000                  | 35.59                     | 1.03| $y = 0.0368x + 0.6601$   | 520.68           |
| Pulasan           | 500                   | 23.05                     | 1.09| $R^2 = 0.97$             |                  |
|                   | 250                   | 9.90                      | 1.89| $R^2 = 0.9797$           |                  |
|                   | 125                   | 5.33                      | 2.82|                          |                  |
|                   | 62.5                  | 0.73                      | 1.60|                          |                  |
| Seeds extract of  | 1000                  | 84.65                     | 0.59| $y = 0.0793x + 8.7097$   | 20.99            |
| Pulasan           | 500                   | 55.22                     | 1.01| $R^2 = 0.9797$           |                  |
|                   | 250                   | 29.94                     | 2.58|                          |                  |
|                   | 125                   | 17.58                     | 0.35|                          |                  |
|                   | 62.5                  | 9.88                      | 1.05|                          |                  |
| Leaves extract of | 100                   | 93.91                     | 0.80| $y = 1.6201x + 16.001$   |                  |
| Pulasan           | 50                    | 93.49                     | 0.72| $R^2 = 0.9663$           |                  |
|                   | 25                    | 64.51                     | 7.24|                          |                  |
|                   | 12.5                  | 36.83                     | 5.69|                          |                  |
|                   | 6.25                  | 21.05                     | 3.33|                          |                  |

The results of the antioxidant activity obtained were slightly different from the results of the TPC test. At the TPC test, the highest yield was obtained on the peels extract of Pulasan which was then estimated to have the highest antioxidant activity. It turned out that the results were the opposite, the peels extract of Pulasan showed inactive results as an antioxidant. Based on the test results, the total phenolic content of the leaves extract of Pulasan has a lower TPC when compared to the sample of peels extract of Pulasan, but when testing the antioxidant activity, the leaves extract of Pulasan shows very good results, which has a very strong antioxidant activity. This result may be due to the fact that the antioxidant activity of the leaves extract of Pulasan may be caused not only by phenolic compounds but possibly from other classes of compounds such as flavonoids. From this result, it would be even better if future research focuses on a deeper study of leaves extract of Pulasan.

4. Conclusion
Based on the results obtained from this study it can be concluded that the leaves extract of Pulasan has the highest antioxidant activity compared to the peels and seeds extract of Pulasan with the IC$_{50}$ value of 20.99 µg/mL.
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