The antioxidant activity of white kapul (Baccaurea macrocarpa) fruit rinds

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Abstract. The antioxidant activity of white kapul (Baccaurea macrocarpa) fruit rinds was investigated in this research. Baccaurea macrocarpa fruit rinds were extracted with n-hexane, ethyl acetate, and methanol, consecutively. All extracts were determined for their antioxidant activity based on the DPPH method. The yields from hexane, ethyl acetate, and methanol extract were 0.14%, 0.64%, and 0.94%, respectively. The highest antioxidant activity was observed in methanol extract (IC50 22.968 ppm), followed by the activity from ethyl acetate extract (IC50 29.741 ppm), and hexane extract (IC50 141.931 ppm). As a comparison, the IC50 of vitamin C was 5.019 ppm.

1. Introduction
The genus Baccaurea (Euphorbiaceae) is a tropical fruit tree that spreads from India, Borneo, Sumatra, Peninsular Malaysia, Thailand, Philippines, to the Pacific island [1]. Baccaurea genus consists of 43 species, such as B. motleyana (rambai), B. angulata (belimbing darah), B. macrocarpa (kapul or tampoi), B. brevipes (rambai tikus), B. velutine, B. reticulata, B. lanceolata (limpasu), B. polyneura (jentik), and B. ramiflora.

There is a lot of research on Baccaurea genus. Nutrient composition of B. angulata on whole fruit, skin, and berry i.e moisture, ash, protein, total fat, carbohydrate, and crude fiber have reported [2]. Skin or fruit rind’s of B. angulata contained the highest total phenol (16.58 mgGAE/g), total flavonoid (31.05 mgQE/g) dan total antocyanin (0.72 mgc-3-g/100g) than the whole fruit and berry of B. angulata [2]. Fruit seed of B. macrocarpa contained fiber 2.2%, fat 1.1%, carbohydrate 34.6%, protein 1.5%, moisture 61.9% and vitamin C 1.5% [3]. The pericarp contained the highest of total phenol (60.04±0.53 mgGAE/g), total flavonoid (44.68± 0.67 mgQE/g), total antocyanin (1.23±0.20 mgc-3-gE/100g), and total carotene (0.81±0.14 mgCE/g) than the flesh and seed of B. macrocarpa [4].

The whole fruit, skin, and berry of B. angulata have antioxidant activity by DPPH method, TEAC/ABTS assay and FRAP assay [2]. The juice of pulp, whole fruit, and skin of B. angulata inhibits lipid peroxidation and induces the increase in antioxidant enzyme activities [5]. Methanol extract of flesh, pericarp, and seed of B. macrocarpa and B. lanceolata showed antioxidant activity [4].

B. macrocarpa or known as white kapul produces edible fruits (pulp or berry) and their fruit rinds are not used. Therefore, the research aimed to evaluate the antioxidant activities of B. macrocarpa fruit
rind’s extract. Fruit rinds were extracted with n-hexane, ethyl acetate, and methanol, consecutively. Antioxidant activity was evaluated by the DPPH method.

2. Materials and methods

2.1 Materials
Fruits of *B. macrocarpa* were collected from Banjar, South Kalimantan, methanol p.a (Merck), 2,2-Diphenyl-1-Picryl Hydrazil (Aldrich), methanol (technical), ethyl acetate (technical), n-hexane (technical). All technical solvents were distilled prior to use. Ultra Violet-Visible Spectrophotometer (GENESYS 10S), rotary vacuum evaporator, and glasses apparatus were used.

2.2 Preparation of fruit rind’s
Fruits of *B. macrocarpa* were washed with water and the fruit rinds were separated from the edible parts. The fruit rinds were cut into small pieces and dried up at room temperature. The air-dried fruit rinds were powdered with the milling machine.

2.3 Extraction of fruit rind’s
Approximately, 500 g of the air-dried powdered fruit rinds were extracted with n-hexane (ratio solvent 1:5) for 24h and filtered. Remaceration was repeated 2 times. The residue was extracted with ethyl acetate for 24h and filtered. Remaceration was repeated 2 times. The residue was extracted with methanol and filtered. Remaceration was repeated 2 times. The extracts were concentrated with a rotary vacuum evaporator to get 0.68 g of hexane extract, 3.21 g of ethyl acetate extract, and 4.71 g of methanol extract. The percentage yield was determined as the following equation 1.

\[
\text{Yield (\%)} = \frac{\text{mass of crude extract (g)}}{\text{mass of fine powder (g)}} \times 100
\]  

(1)

2.4 Antioxidant activity
The antioxidant activity based on the free radicals of DPPH was determined according to the method described by Molyneux [6] with slight modification. Different concentrations of hexane, ethyl acetate, and methanol extract in the methanol solvent (2 mL) were mixed with 2 mL DPPH 0,1mM. After incubation for 30 minutes in the dark at room temperature, the absorbance was recorded at a maximum wavelength (516 nm). Ascorbic acid was used as a standard. The controls contained all reagents except the extract or standard (ascorbic acid). The decreased absorbance DPPH indicates an increase of DPPH radical scavenging activity. The scavenging of free radical DPPH was estimated based on the following equation 2.

\[
\text{Inhibition of free radical (\%)} = \frac{A_0 - A_s}{A_0} \times 100\%
\]  

(2)

Ao was the absorbance of control reaction and As was the absorbance of solutions with the sample extract or standard. The value was presented in triplicates analysis. The IC50 was the inhibition concentration that could scavenge 50% DPPH. The IC50 value for each extract was graphically determined by plotting the inhibition of free radical DPPH percentage (y-axis) and extract concentration (x-axis).

3. Results and discussions

3.1 Extract yields
Fruit rinds of *B. macrocarpa* were extracted with solvents with different polarities, consecutively. Extraction was done at room temperature. The extraction of fruit rinds of *B. macrocarpa* initiated with
n-hexane, followed by ethyl acetate, and terminated by methanol. The gradual extraction would extract compounds with different polarities.

![Figure 1. Yields of various extracts.](image)

| Extract/Standard | Concentration (ppm) | Inhibition of free radical (%) | IC50 (ppm) |
|------------------|---------------------|--------------------------------|------------|
| n-Hexane         | 40                  | 4.716                          | 141.931    |
|                  | 60                  | 12.301                         |            |
|                  | 80                  | 20.650                         |            |
|                  | 100                 | 28.298                         |            |
|                  | 120                 | 39.771                         |            |
|                  | 140                 | 51.052                         |            |
| Ethyl acetate    | 15                  | 18.986                         | 29.471     |
|                  | 20                  | 31.169                         |            |
|                  | 25                  | 45.207                         |            |
|                  | 30                  | 52.938                         |            |
|                  | 35                  | 57.514                         |            |
| Methanol         | 5                   | 11.975                         | 22.968     |
|                  | 10                  | 23.949                         |            |
|                  | 15                  | 35.142                         |            |
|                  | 20                  | 45.406                         |            |
|                  | 25                  | 54.888                         |            |
|                  | 30                  | 62.219                         |            |
| Ascorbic acid    | 1                   | 7.279                          | 5.019      |
|                  | 2                   | 15.342                         |            |
|                  | 3                   | 27.436                         |            |
|                  | 4                   | 38.634                         |            |
|                  | 5                   | 50.504                         |            |
Figure 1 shows that methanol extract has the highest yield of the other extracts. This means that there were more polar chemical compounds than non-polar compounds. Total yields from various extracts were 1.72%. The obtainable total yields from *B. macrocarpa* rinds were less than the yield from methanol extract of *B. angulata* rinds, i.e 31.0% [7]. This is due to different extraction methods and species of *B. angulata* fruit rinds were extracted in an oven shaker set at 250 rpm and 37°C [7] while *B. macrocarpa* in this research was extracted at room temperature.

### 3.2 Antioxidant activity

Antioxidant activity was measured at a wavelength of 516 nm, which was its maximum absorption of DPPH. Free radical inhibition of various extracts and reference standard (ascorbic acid) are shown in table 1. All extracts had an antioxidant activity with IC$_{50}$ vary from 22.968 to 141.931 ppm, and IC$_{50}$ ascorbic acid was 5.019 ppm. Methanol extract had the highest antioxidant activity, followed by ethyl acetate extract, and n-hexane extract. The antioxidant activity of all extracts was lower than ascorbic acid as a standard. The extract was a mixture of various compounds while the ascorbic acid was a pure chemical compound.

Methanol and ethyl acetate extract might contain polar and semipolar phytochemical compounds, and n-hexane extract contained non-polar phytochemical compounds. Some polar phytochemical compounds that might be in methanol extract and responsible for antioxidant activity were phenolic glycoside and phenols because it is suspected that *B. macrocarpa* also had phenolic glycoside compounds as well as *B. ramiflora*. Some phenolic glycosides have been isolated from *B. ramiflora* were 4’-O-(6-O-vanyloil-β-D-glucopiranosyltachioside D, 6’-O-vanyloilicarisida B$_5$, 6’-O-vanilloylisotachioside and 6’-O-vanilloyltachioside [8,9]. Some phenols compounds in *B. ramiflora* were epicatechin, aviculin [9].

### 4. Conclusion

The highest antioxidant activity was observed in methanol extract, followed by the activity from ethyl acetate extract and hexane extract.

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