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FULL PAPER

Baccaurea racemosa (Reinw. ex Blume) Müll. Arg. pulp: a potential natural antioxidant

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Abstract

Baccaurea racemosa (Reinw. ex Blume) Müll. Arg. is a fruit widely grown in Indonesia locally known as 'kepundung'. Some of genus Baccaurea has potential to be developed as a natural antioxidant. However, the antioxidant activity of *B. racemosa* pulp has not been published. This study is aimed to (i) explore the antioxidant activity, (ii) measure the total phenolic and flavonoid contents, and (iii) investigate the correlation of antioxidant activity of extract and fractions of *B. racemosa* pulp with their total phenolic and flavonoid contents. Fresh *B. racemosa* pulp was macerated with methanol to obtained methanolic extract. The methanolic extract was partitioned using n-hexane followed dichloromethane and ethyl acetate. This study revealed that ethyl acetate fraction has the highest antioxidant activity with the scavenging activity of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azinobis(3-etylbenzthiazoline)-6-sulfonic acid (ABTS) with values IC_{50} 127.155±4.311ug/mL and 108.155±6.455 mM trolox equivalent/100 mg dry extract respectively. However, the highest antioxidant activity using β-carotene bleaching (BCB) assay is the methanolic extract with value 36.898±13.240%. Furthermore, the ethyl acetate fraction has the highest total phenolic content (TPC) and total flavonoid content (TFC) with values 42.975±1.978 mg gallic acid equivalent/g dry extract (mg GAE g^{-1}dry extract) and 122.813±1.604 mg rutin equivalent/g dry extract (mg RE g^{-1}dry extract) respectively. TPC and TFC of *B. racemosa* pulp have a significant correlation with ABTS free radical scavenging activity. In this current study, it was discovered that ethyl acetate fraction of *B. racemosa* pulp can be developed to be a natural antioxidant.

1. Introduction

Free radical is a reactive and unstable compound because it can attract the electron from another molecule (Lobo *et al*., 2010). It will cause a chain reaction and increase the amount of free radical. The high level of free radical in our body is dangerous for health because it can cause many kinds of diseases, such as Alzheimer, rheumatoid arthritis, aging, cataract and even cancer (Pham-Huy *et al*., 2008). Free radical can be overcome with antioxidant. Naturally, the human body produces antioxidant such as superoxide dismutase (SOD), glutathione peroxidase, and catalase (Zadak *et al*., 2009). Nevertheless, antioxidant from the outside is needed due to unbalance amount of antioxidant and free radical in the body. Synthetic antioxidant such as Buthyl Hydroxy Toluene (BHT) and Buthyl Hydroxy Anisole (BHA) are developed. However, both of them was reported to cause cancer in mouse (Race, 2009; Fitri, 2013). Because of this problem, there is a need to identify more effective dan safety antioxidant from a natural resource.

Kepundung, *Baccaurea racemosa* a fruit widely grown in Indonesia. The fruit of *B. racemosa* ripens from December until March. The leaves of *B. racemosa* have reported containing the activity of antioxidant with 91.23±0.02% scavenging of DPPH free radical (Wulansari and Chairul, 2011). Leaves and bark of *B. racemosa* have reported not containing alkaloid but containing flavonoid (Ismail *et al*., 2010). Other genus of Baccaurea have the potential as a medicine plant because it contains secondary metabolites such as alkaloid, flavonoid, antosianin, carotenoids, and phenolic which was commonly used as antioxidant, anticancer and antimicrobial (Gunawan *et al*., 2016). The pulps of *Baccaurea angulata* contain total phenolic and flavonoid that has a high correlation with its antioxidant activity with a correlation coefficient >0.99. However, there is no report about activity antioxidant from *B. racemosa* pulp and its correlation with phenolic and flavonoid total

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contents.

The purpose of our study was to explore for the first time the antioxidant activity of the methanolic extract, n-hexane, dichloromethane, ethyl acetate and water fraction of *B. racemosa* pulp through β-Carotene Bleaching (BCB) assay, scavenging activity of free radical of DPPH and ABTS. Besides that, this study was aimed to quantify the total phenolic and flavonoid content of extract and fractions of *B. racemosa* pulp and to investigate the correlation between their total phenolic and flavonoid content with their antioxidant activity.

2. Materials and methods

2.1 General experimental procedures

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), Trolox, quercetin, rutin, gallic acid, linoleic acid, β-carotene and Tween 20 were obtained from Sigma (Aldrich, USA). Folin-Ciocalteu, methanol, chloroform, dichloromethane, n-hexane and other pro-analytical grade solvent and reagent were purchased from Merck (Darmstadt, Germany). The *B. racemosa* pulp was obtained from Limbungan village, Gunung Sari, Lombok Barat, West Nusa Tenggara, Indonesia. *B. racemosa* was authenticated by I Gde Merthe (senior lecturer in Plant Taxonomy) in Laboratory of Biology, Faculty of Mathematics and Science, Mataram University, West Nusa Tenggara, Indonesia.

2.2 Preparations of sample

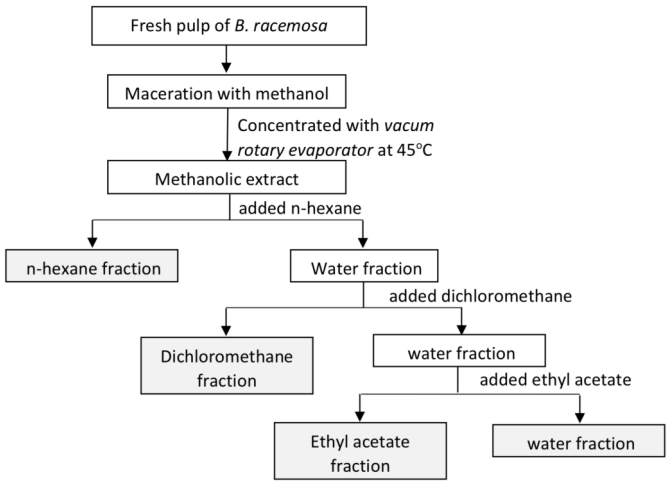

Figure 1. Extraction and fractionation step of *B. racemosa* pulp

The fruit of *B. racemosa* was washed with tap water. Seed and peel were separated from the pulp. The pulp was then stored in the freezer for 24 hrs. The 4.89 kg *B. racemosa* pulp was blended with 4 L analytical grade of methanol and was extracted by maceration method for 3 days and re-maceration for 2 days. The mixture was filtered and concentrated using vacuum rotary evaporator at 45°C to obtain the methanolic extract of *B. racemosa* pulp. It was then fractioned using n-hexane, followed by dichloromethane and ethyl acetate to obtain n-hexane, dichloromethane, ethyl acetate and water fractions. The extract and all fractions were subjected in the following assays. The scheme of extraction and fractionation of *B. racemosa* pulp was illustrated in Figure 1.

2.3 DPPH free radical scavenging assay

The method to determine DPPH free radical scavenging activity was according to Kikuzaki *et al*. (2002) with slight modifications. The solution of DPPH 0.4 mM was prepared by dissolving 15.8 mg of DPPH with 100 mL of methanol p.a and then 1 mL of this solution was mixed with 4 mL of extract, fractions and quercetin (standard) at different concentration. A control was prepared with adding 1 mL of DPPH 0.4 mM with 4 mL of methanol p.a. They were shaken and stand at room temperature for 30 mins. The absorbance of the solutions were measured at 515.5 nm with methanol as blank using UV-Visible spectrometer (Shimadzu 1800). The DPPH free radical scavenging activity of the sample was calculated using the following equation:

$$\text{DPPH Scavenging Activity (\%)} = \frac{\text{Control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%$$

2.4 ABTS radical scavenging activity

The method to determine ABTS free radical scavenging activity was according to Aktumsek *et al*. (2013) with slight modifications. Solutions of ABTS 7 mM and natrium persulphate 2.45 mM were mixed with ratio 1:1. The mixture was left for 12-16 hrs at room temperature. Before assay, the mixture was diluted with methanol until it absorbance of 0.700±0.02 at 734 nm. Samples (300 µL) and 3 mL of ABTS solution were mixed and were left for 30 mins at room temperature. Their absorbance was read at 734 nm using UV-Visible spectrometer (Shimadzu 1800). The ability of samples to scavenge the ABTS radical was expressed as mM Trolox equivalent/100 mg dry extract (mM TE/100 mg dry extract).

2.5 β-Carotene Bleaching (BCB)

The method of measurement activity samples of BCB was according to Maisarah *et al*. (2013) with modifications. The β-carotene (0.2 mg/mL in chloroform) 1 mL, 0.2 mL tween 20, and 0.02 mL linoleic acid were mixed. The chloroform in the mixture was evaporated using vacuum rotary evaporator at 40°C and followed by the addition 100 mL deionized water with vigorous shaking to form an emulsion of β-carotene. The solution (3.5 mL) was added with 200 µL sample, quercetin (standard) and methanol (control). The

mixture was incubated in water bath at 50°C for 80 mins. The absorbance was measured at 20 mins interval at 470 nm using UV-Visible spectrometer (Shimadzu 1800) with emulsion without β-carotene as a blank. The antioxidant activity of samples was calculated using the equation:

$$\text{dr sample} = \frac{(\ln[\frac{Ao}{At}])}{t}$$

$$\%AA = \frac{\text{dr control} - \text{dr sample}}{\text{dr control}} \times 100$$

Where dr = degradation rate of β-carotene; Ao= absorbance sample at time 0; At= absorbance sample at 80 mins of incubation; and AA= antioxidant activity.

2.6 Total phenolic content (TPC)

Sample (30 uL) was added with 0.4 mL Folin-Ciocalteu reagent and was left for 5 mins. The mixture was added with 4.0 mL of Na_2CO_3 7% and was followed by adding aquabidest. The solution was left for 2 hrs and was read the absorbance at 750 nm using UV-Visible spectrometer (Shimadzu 1800) (Chun *et al*., 2003). The total phenolic contents were revealed as mg gallic acid equivalent/ g dry extract (mg GAE g^{-1}dry extract).

2.7 Total flavonoid content (TFC)

Sample (300 µL), aquabidest 4.0 mL and 0.30 mL $NaNO_2$ 10% were mixed and they were left for 6 mins. The mixture was added with 0.30 mL $AlCl_3$ 10% and then left during 5 mins. The solution was added with 4.0 mL NaOH 10% and 1.1 mL aquabidest. The absorbance was read at 510 nm using UV-Visible spectrometer (Shimadzu 1800) after incubation for 15 mins at room temperature. The flavonoid total contents of samples were revealed as mg RE g^{-1} extract (Chang *et al*., 2002).

2.8 Statistical analysis

Statistical analysis was conducted using the windows software of Statistical Package for Social Sciences (SPSS, version 22). The statistical analysis of antioxidant activity, TPC and TFC of *B. racemosa* were using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant different (HSD). The significances of samples were accepted at $p<0.05$. Meanwhile, the correlation of various result of antioxidant activity of *B. racemosa* pulp with their TPC and TFC was revealed using Pearson correlation test with significances $p<0.05$ and $p<0.01$.

3. Results and discussion

3.1 DPPH free radical scavenging activity

Samples showed dose-dependent inhibition on DPPH free radical. Elevating the concentration of *B. racemosa* pulp was clearly increasing antioxidant activity on inhibiting the DPPH free radical (Table 1). IC_{50} values of scavenging activity of DPPH free radical of extract and fractions can be ranked as ethyl acetate fraction < methanol extract < water fraction < n-hexane fraction < dichloromethane fraction (Table 1). Ethyl acetate fraction had the best results of antioxidant activity among the methanolic extracts and several fractions. The scavenging of DPPH radical estimated that there was a hydroxyl group in the components of ethyl acetate fraction of *B. racemosa* pulp which can donate its electron to the DPPH radical. Ethyl acetate is the semi-polar solvent that tends to extract aglycon, glycoside compounds, and flavonoid. These compounds have hydroxyl groups that are directly connected to chromophore so they can stabilize these compounds when they donate its electron to DPPH free radical. Meanwhile, n-hexane has low DPPH free radical scavenging activity because n-hexane is the non-polar solvent which extracts the lignin, lipid, less alkaloid and sterol (Widyawati *et al*., 2015). Dichloromethane is the lowest scavenging activity of DPPH free radical because it dissolves the fatty acid (Cequier-Sánchez *et al*., 2008). These compound does not have hydroxyl group so the DPPH radical cannot be stabilized. The DPPH radical scavenging activity was reported in the several species of genus Baccaurea, such as pulp of *Baccaurea lanceolata* and leaves of *Baccaurea ramifolia* (Dey and Pal, 2015; Hadi *et al*., 2015).

3.2 ABTS free radical scavenging activity

The ABTS was oxidated by potassium persulphate to generated cation radical of ABTS ($ABTS^{\cdot+}$). ABTS radical scavenging activity was calculated with trolox equivalency activity calculated (TEAC) (Cerretani and Bendini, 2010). The trolox equivalency values of extract and fractions of *B. racemosa* pulp were in order of ethyl acetate fraction > n-hexane fraction > dichloromethane fraction > methanol extract > water fraction (Figure 2). The higher the trolox equevalency of the sample, the higher the capacity of the sample to scavenge the activity of ABTS radical. The ABTS radical scavenging activity of the extract was reported that the ethyl acetate fraction had the highest antioxidant activity with the value of 108.155±6.455 mM trolox equivalent/100 mg dry extract (Figure 2). The capacity of ethyl acetate fraction of *B. racemosa* pulp to scavenge the ABTS free radical was due to the compounds extracted in ethyl acetate fraction to have hydrogen that can stabilize the ABTS free radical. Meanwhile, the n-hexane fraction has the highest ABTS free radical scavenging activity compared to methanol extract and water fraction because the n-hexane fraction contained a lipophilic compound which can participate to neutralize the free radical (Gali and

Table 1. DPPH free radical scavenging activity of *B. racemosa* pulp

B. racemosa fraction	Concentration (µg/mL)	Percent inhibition (% ± SD)	IC_{50} $\overline{X}$ ± SD (µg/mL)
n-hexane fraction	40	9.040±3.261	948.710±29.344[d]
	280	19.191±3.022	
	520	30.125±2.502	
	760	42.065±2.954	
	1000	51.813±1.159	
Dichloromethane fraction	80	9.268±3.112	1141.0698±151.184[e]
	560	33.659±3.549	
	1040	49.918±1.544	
	1520	60.610±3.189	
	2000	66.666±4.103	
Ethyl acetate fraction	40	26.349±1.263	127.155±4.311[b]
	80	37.410±1.242	
	120	50.321±1.342	
	160	61.193±0.880	
	200	65.949±1.903	
Water fraction	90	15.037±3.138	402.228±8.748[c]
	180	28.608±4.086	
	270	38.680±2.611	
	360	45.856±0.595	
	450	53.019±1.160	
Methanolic extract	60	16.706±4.289	391.790±6.130[c]
	180	32.225±0.480	
	300	44.299±0.943	
	420	54.394±1.133	
	540	60.570±1.684	
Quercetin	1.5	24.446±0.615	2.995±0.080[a]
	2	32.602±2.085	
	2.5	43.881±1.586	
	3	49.143±0.737	
	3.5	58.365±2.314	

Values are expressed as mean ± SD, n=3. Same alphabet superscripts within the same column indicate no significant difference (p>0.05) which measured with Tukey's HSD test. Quercetin as the positive control.

Bedjou, 2019). The other species of Baccaurea, the *Baccaurea angulata* was reported to have ABTS radical scavenging activity (Ahmed *et al*., 2015).

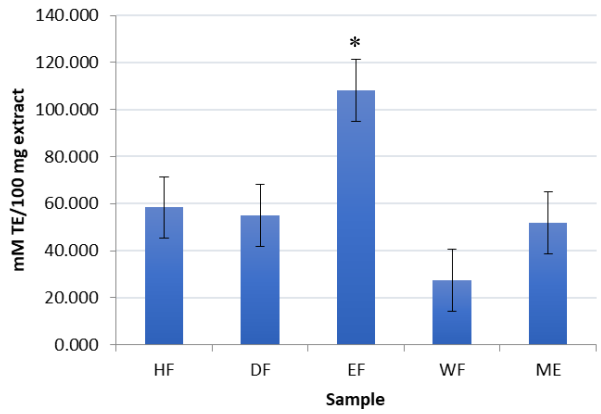

Figure 2. Scavenging activity of Baccaurea racemosa pulp on ABTS free radical. n-hexane fraction (HK), dichloromethane fraction (DK), Ethyl Acetate Fraction (EF), Water Fraction (WF), Methanolic extract (ME).
*significantly different with other sample (p>0.05) which measured with Tukey's HSD test. Values are expressed as means ± SD (n=3).

3.3 BCB

Lipid membrane commonly consists of linoleic acid that is unsaturated fatty acid. Linoleic acid is a target of lipid peroxidation which can describe the lipid peroxidation of lipid membrane (Tchimene *et al.,* 2016). The curve of the degradation rate of β-carotene after 80 mins incubation was shown in Figure 3. It showed that quercetin has a high capacity to inhibit BCB followed by n-hexane fraction and methanolic extract. They exhibited a stabilization of β-carotene absorbance after incubation for 80 mins. The slower the degradation rate of the sample, the higher the antioxidant activity of the sample. The values of degradation rate and percentage of antioxidant activity of BCB were tabulated in Table 2. It showed that the percentage of antioxidant activity of extract and fractions were in the following order of methanolic extract > n-hexane fraction > ethyl acetate fraction > dichloromethane fraction > water fraction. Methanolic extract of *B. racemosa* pulp has a higher percentage of antioxidant activity than other fraction because of polyphenol compounds tends to dissolve in

Table 2. Degradation rate and percentage of antioxidant activity of various *B. racemosa*

Extract	dr (degradation rate)	Percentage of Antioxidant Activity (%AA)
n-hexane fraction	0.015±0.0039^{ab}	31.276±14.085^{ab}
Dichlorometane fraction	0.017±0.0039^{abc}	22.538±15.207^{ab}
Ethyl acetate fraction	0.016±0.0002^{abc}	23.938±4.786^{ab}
Water fraction	0.019±0.0008^{bc}	10.604±8.524^{b}
Methanolic extract	0.014±0.0030^{a}	36.898±13.239^{a}
Quercetin	0.012±0.0033^{a}	44.116±14.750^{a}
Control	0.021±0.0012^{c}	-

Values are expressed as mean ± SD, n=3. Same alphabet superscripts within the same column indicate no significant difference (p>0.05) which measured with Tukey's HSD test. Quercetin as the positive control.

Table 3. Total phenolic and flavonoid content of *B. racemosa* pulp

Extract	TPC (mg GAE g^{-1} dry extract)	TFC (mg RE g^{-1} dry extract)
n-hexane fraction	27.481±0.304^{b}	NA
Dichlorometane fraction	26.615±1.393^{b}	NA
Ethyl acetate fraction	42.975±1.978^{a}	122.813±1.604^{a}
Water fraction	13.538±0.473^{c}	32.798±2.500^{c}
Methanolic fraction	25.288±1.502^{b}	55.468±2.768^{b}

Values are expressed as mean ± SD, n=3. Same alphabet superscripts within the same column indicate no significant difference (p>0.05) which measured with Tukey's HSD test.

the methanol extract. These compounds can stop chain reactions of free radical by donating their electron to free radical compounds. The n-hexane fraction has a higher percentage of antioxidant activity than ethyl acetate fraction because n-hexane fraction has lipophilic compounds. Based on the research by Gali and Bedjou (2019), BCB assay has high specificity toward lipophilic compounds as these compounds can participate to stabilize the free radical. In Table 2, the percentage value of antioxidant activity statistically showed methanol extract, ethyl acetate fraction, n-hexane fraction and dichloromethane fraction did not have significant difference.

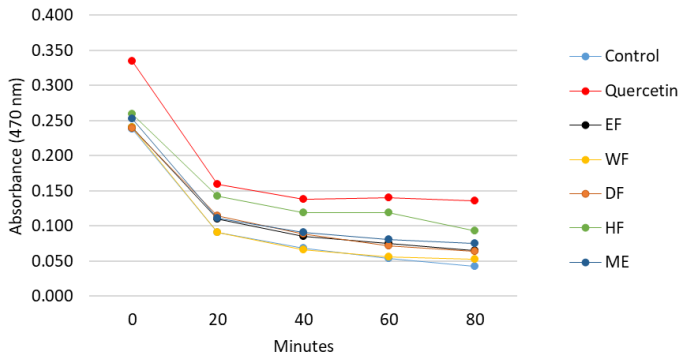

Figure 3. Degradation rate of various fraction of Baccaurea racemosa pulp assayed by β-carotene bleaching test. Quercetin is used to be a standard. N-hexane fraction (HK), dichloromethane fraction (DK), Ethyl Acetate Fraction (EF), Water Fraction (WF), Methanolic extract (ME). Values are expressed as means ± SD (n=3).

3.4 TPC and TFC

Flavonoids, including flavones, flavonols and catechins, are included class of phenolic compounds which contain hydroxyl groups (Afshar *et al*., 2012). These will destroy the free radical through donating their hydrogen atom to radical (Bajalan *et al*., 2017). Therefore, the quantification of TPC and TFC are important to determine the activity of antioxidant in plants. TPC and TFC were determined using gallic acid calibration curves (Y = 109.65x + 0.0276, r^2= 0.9927) and rutin calibration curves (Y = 10.038x – 0.0151, r^2= 0.9978). The data showed in Table 3 exhibited that ethyl acetate fraction had the highest TPC and TFC with 42.975±1.978 mg GAE g^{-1} dry extract and 122.813±1.604 mg RE g^{-1}dry extract respectively. Flavonoid, belonging to phenolic compounds, are soluble in ethyl acetate which resulted a high total flavonoid and phenolic contents in the ethyl acetate fraction. Their values were statistically significant different with other fraction.

The correlation of TPC and TFC with antioxidant activity displayed variation (Table 4). The TPC and TFC of various fraction of *B. racemosa* pulp have no significant correlation with their values of DPPH free radical scavenging activity and BCB which indicates that the DPPH free radical scavenging activity and BCB of *B. racemosa* were not influenced by their total phenolic and flavonoid content. β-carotene method has a high specificity on lipophilic compounds and therefore its antioxidant activity has low correlation with its total flavonoid and phenolic contents. Meanwhile, TPC and TFC have significant correlation with ABTS free radical scavenging activity with a correlation coefficient of 0.989 and 0.999 respectively. This results showed that the phenolic and flavonoid compounds of *B. racemosa*

contributed highly to the ABTS free radical scavenging activity.

Table 4. Correlation coefficients between antioxidant activity and total phenolic and flavonoid content of *B. racemosa* pulp

	TPC	TFC	ABTS	DPPH	BCB
TPC	1	0.987	0.989**	-0.257	0.367
TFC		1	0.999*	-0.978	0.25
ABTS			1	-0.357	0.249
DPPH				1	0.202
BCB					1

Values of correlation coefficient from Pearson correlation.
** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 (2-tailed)

4. Conclusion

This study evaluated the antioxidant activity of the extract and fractions of *B. racemosa* pulp. The partition of *B. racemosa* pulp with ethyl acetate was the best solvent to extract the compound with the highest antioxidant activity. This fruit had the potentiality as antioxidant that can be developed to be as a medicinal plant. The compounds in *B. racemosa* had the potential to scavenge the free radicals as well as to inhibit lipid peroxidation. The phenolic and flavonoid compounds contributed to the antioxidant activity of *B. racemosa.*

Conflict of Interest

Authors declare no conflict interest.

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