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In Vitro Antioxidant Activity of Unused Parts of Jackfruit (*Artocarpus heterophyllus*)

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Abstract

BACKGROUND: Free radical is unstable and highly reactive, which may lead to oxidative stress that causes various diseases, that is, diabetes mellitus. Antioxidant can prevent oxidation process by scavenging free radicals. Jackfruit (*Artocarpus heterophyllus*) is a native tropical fruit that can easily be found in Indonesia. When the flesh is commonly eaten, the unused parts – such as the leaves, fruit peels, and pulps will be considered waste to be thrown away. However, these unused parts of Jackfruit are rich in antioxidant compounds that potentially can work as therapeutic agents.

AIM: The aim of the study was to determine the antioxidant properties of leaves, peels, and pulps of *A. heterophyllus* by calculating their antioxidant activity index (AAI) with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Cupric Ion-Reducing Antioxidant Capacity (CUPRAC) method; total phenolic content (TPC) and total flavonoid content (TFC); observing the correlation between TPC and TFC with AAI DPPH and CUPRAC; as well as the correlation between AAI DPPH and CUPRAC.

MATERIALS AND METHODS: Extraction process was carried out using reflux method using three different polarity solvents. UV-visible spectrophotometer was used to determine the TPC, TFC, AAI DPPH, and AAI CUPRAC. Pearson's method was used to observe the correlation between TPC and TFC with AAI DPPH and CUPRAC, as well as the correlation between both methods.

RESULTS: The AAI in DPPH method were varied from 0.0310 to 36.8852, while CUPRAC from 0.1156 to 1.2503. Ethanol leaves extract gave the highest TPC value (5.53 g GAE/100 g) and n-hexane peels extract exposed the highest TFC value (16.07 g QE/100 g). The correlation between TPC and AAI of leaves, peels, and pulps extracts with DPPH method, as well as between TFC and AAI CUPRAC of peels extracts was positive and significant. Rutin was determined as the marker compound, valuing at 0.0106%.

CONCLUSION: Phenols and flavonoids (including rutin) content contributed to DPPH and CUPRAC antioxidant activity. The antioxidant property between both methods was not linear in leaves, peels, and pulps extracts. Unused parts (peels and leaves) of *A. heterophyllus* might be potential to be developed as natural antioxidant sources.

Introduction

Free radical is an atom or molecule with unpaired electrons on the outer orbit, making the radical unstable and highly reactive [1]. Oxygen metabolism can produce free radical which was named as reactive oxygen species (ROS). High concentration of ROS can cause oxidative stress, resulting to various diseases such as diabetes mellitus, cardiovascular disease, and respiratory disease [2]. Antioxidant can prevent oxidation process by neutralizing free radicals, making the radical become more stable. Natural antioxidant can be found in plants, that is, *Artocarpus heterophyllus* (Jackfruit) which its fruits are commonly consumed in Indonesia. The antioxidant activity of *A. heterophyllus* (Jackfruit) is due to its compounds such as phenols and flavonoids which are able to scavenge free radicals [3]. Flavonoids that can be obtained were catechin in its leaves, peels, and pulps [4]. Besides phenols and flavonoids, tannins in leaves and peels of *A. heterophyllus* contributed as radical scavenging compounds [5], [6].

2,2-diphenyl-1-picrylhydrazyl (DPPH) and CUPRAC (Cupric Ion-Reducing Antioxidant Capacity) can be used as methods to determine antioxidant activity of several plants [7], [8], [9]. Some studies mostly used DPPH method to evaluate the antioxidant activity of *A. heterophyllus* [10], [11], [12]. Before the antioxidant activity was determined, an extraction process was preceded. This research used reflux as a method to extract nonpolar, semipolar, and polar compounds of leaves, peels, and pulps of *A. heterophyllus* in this research. Solvents with increasing polarity were used to extract those compounds, namely, n-hexane, ethyl acetate, and ethanol. The objectives of this research were to determine the antioxidant properties of leaves, peels, and pulps of *A. heterophyllus* by calculating their antioxidant activity index (AAI) with DPPH and CUPRAC method; total phenolic content (TPC) and total flavonoid content (TFC); identifying and determining the marker

compound, observing the correlation between TPC and TFC with AAI DPPH and CUPRAC; as well as the correlation between AAI DPPH and CUPRAC.

Materials and Methods

Materials

Reagents used for antioxidant activity determination were consisted of DPPH, neocuproine, and cupric chloride. LiChrospher® 100 RP-C18 (100 × 4 mm, 5 μm with 20 mm precolumn), and phosphoric acid were utilized for the chromatographic analysis. Other pro analytical grade extraction reagents and solvents were also used (methanol, ethanol, ethyl acetate, and n-hexane). All of the solvents were obtained from Merck. Luteolin 7-O-glucoside, rutin, quercetin, kaempferol, and apigenin were dissolved in ethanol for standard stock solutions. This research was facilitated with Shimadzu's high performance liquid chromatography (HPLC) LC-20AD, UV/Vis Spectrophoto Densitometry SPD-20A, CTO-20A oven, and CTO-20A pump injector.

Collection of plant samples

The leaves (LV), peels (PE), and pulps (PU) of *A. heterophyllus* were collected from Kuningan, West Java, Indonesia. Semi-ripe fruits were used in this research. The leaves that were used are obtained from near the fruit. The plant was determined and identified by official botanists in Herbarium Bandungense, School of Life Sciences and Technology, Bandung Institute of Technology. The sample was cleaned from contaminants with water. Subsequently, the sample was sorted, cut, dried, and milled into a powder. Drying cabinet was operated at 45°C–50°C. Knife mill grinder equipment and 20 mesh size sieves were used to ensure the uniformity of the sample.

Extraction of leaves, peels, and pulps of A. heterophyllus

Extraction of sample was carried out sequentially using the reflux method with increasing polarity of solvents. The solvent used were n-hexane as the nonpolar solvent, ethyl acetate as the semipolar solvent, and ethanol as the polar solvent. Each part of the sample was extracted in the amount of 300 g and a cycle of extraction performed in 2–3 h after the solvent boiled. Therefore, data were collected from n-hexane leaves extracts (LV1), n-hexane peels extracts (PE1), n-hexane pulps extracts (PU1), ethyl acetate leaves extracts (LV2), ethyl acetate peels extracts (PE2), ethyl acetate pulps extracts (PU2), ethanol leaves extracts (LV3), ethanol peels extracts (PE3), and ethanol pulps extracts (PU3). Triplicate determination was conducted in each solvent of each part of the sample.

Determination of the total phenolic content

Total content of phenolic content in ethanol, ethyl acetate, and n-hexane extracts was evaluated using a modified Folin–Ciocalteu reagent [13]. Primarily, 0.5 mL of extract and 5 mL of Folin–Ciocalteu 10% were mixed. The mixture was added with 4 mL of sodium carbonate 1 M afterwards. The solvent was incubated for 15 min then the absorbance measured at λ 765 nm using a UV-vis spectrophotometer Beckman Coulter DU 720. Gallic acid of different concentrations (40–160 μg/mL) was used as the standard. The TPC result was expressed as gallic acid equivalent per 100 g extract (g GAE/100 g). The test was done in triplicates for each extract.

Determination of the total flavonoid content

TFC was determined using Chang's method [14]. Quercetin was used as standard. Absorbance of quercetin (30–120 μg/mL) was determined using UV-vis spectrophotometer to obtain a calibration curve. Quercetin was dissolved in methanol (0.5 mL in 1.5 mL solvent). Aluminum (III) chloride 10% (0.1 mL), sodium acetate 1 M (0.1 mL), and water (2.8 mL) were added to the solution. Incubation of the solution was carried out for 30 min at room temperature. After that, the absorbance of the sample was determined at λ 415 nm. The same procedure was conducted for each extract. TFC was expressed as quercetin equivalent per 100 g of extract.

Identification and quantitative determination of the marker compounds

Shimadzu's LC-20AD HPLC instrument was used for the determination of marker compounds (Shimadzu, Japan). Detection was performed in tandem with Shimadzu's UV/Vis SPD-20A detector at 360 nm (Shimadzu, Japan). Separation was performed on LiChrospher® 100 RP-C18 (100 × 4 mm, 5 μm) as the stationary phase that was connected to 20 mm pre column (Merck). The column temperature was 30°C and determined with a CTO-20A oven (Shimadzu, Japan). Mobile phases used consisted of 0.01% H_3PO_4 (A) and methanol (B). The sample was injected to the injector (20 μL volume), eluted in 1 mL/min flow rate pumped with CTO-20A pump (Shimadzu, Japan). The linear gradient system was performed with initial B eluent 40–60% (min 0–5), 60–70% (min 6–10), and 60–40% (min11–15). Rutin as standard was injected at 5 ppm and ethanol extract of jackfruit pulps at 10.000 ppm. Rutin content in *A. heterophyllus* pulps extract was calculated with the following equation.

$$RCE = \frac{AUC\,RE}{AUC\,RS} \times \frac{CRS}{CE} \times 100\%$$

RCE: Rutin content in extract, AUC RE: AUC rutin in extract, AUC RS: AUC rutin in standard, CRS: Concentration of Rutin Standard, CE: Concentration of extract.

Determination of antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl

Antioxidant activity was determined using modified Blois' method [15]. Ascorbic acid was used as standard. Absorbance of ascorbic acid was determined using UV-vis spectrophotometry. The DPPH solution was dissolved in methanol. Incubation was carried out for 30 min at room temperature. Then, the absorbance was determined at λ 515 nm. Each extract was determined triplo in various concentrations and diluted with DPPH solution (50 μg/mL) with ratio volume 1:1. Free radical scavenging by antioxidants was indicated by the shifting purple color of DPPH solution to yellow. IC_{50} stands for inhibitory concentration 50%, where it can be determined with a calibration curve in plot of DPPH scavenging activity (%) and concentration.

Determination of antioxidant activity by CUPRAC

The antioxidant activity was determined using CUPRAC method [16]. First, 100 μg/mL of CUPRAC solution was made by dissolving the CUPRAC solution in ammonium acetate buffer pH 7. Ammonium acetate buffer pH 7 and 100 μg/mL of CUPRAC solution were applied as blank and control, consecutively. The absorbance of the solution was read at λ 450 nm using a UV-vis spectrophotometer. Ascorbic acid was diluted in methanol to attain a stock solution which was used as standard. Then, various concentrations of ascorbic acid solution were mixed with CUPRAC solution (1:1). The absorbance was calculated repeatedly using UV-vis spectrophotometer at 450 nm after incubation for 30 min. The absorbance values were transformed into calibration curve to calculate the exhibitory concentration 50% (EC_{50}) of ascorbic acid. The test was performed in triplicate for each extract and standard.

Measurements of the antioxidant activity index

AAI of each extract in DPPH scavenging activity and CUPRAC capacity were estimated with the following equation.

$$AAI = \frac{Final\ concentration\ of\ radical\ solution\ (\mu g\ /\ mL)}{IC_{50}\ or\ EC_{50}\ (\mu g\ /\ mL)}$$

Statistical analysis

Statistical analysis was conducted for each extract in tri-replication using IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. Results were expressed as means ± standard deviation. The significance of each statistic data was described using one-way analysis of variance method ($p < 0.05$). *Post hoc* test conducted with Tukey test. The correlation of TPC, TFC, and each antioxidant activity method was determined with Pearson's correlation method.

Results

Antioxidant activity index of leaves, peels, and pulps extracts of A. heterophyllus

Antioxidant activities of *A. heterophyllus* leaves, peels, and pulps extracts data were collected as results of the research.

Table 1: Antioxidant activities of *Artocarpus heterophyllus* extracts by 2,2-diphenyl-1-picrylhydrazyl assays

AAI DPPH	Sample		
	1	2	3
LV	1.0776 ± 0.0190[a]	9.3550 ± 0.3697[b]	19.3904 ± 1.2410[c]
PE	0.0583 ± 0.0029[a]	36.8852 ± 2.7737[b]	7.6035 ± 0.2524[c]
PU	0.0310 ± 0.0019[a]	22.8462 ± 1.0749[b]	21.7936 ± 0.8622[b]
Ascorbic acid	64.1409 ± 2.5465		

DPPH: 2,2-diphenyl-1-picrylhydrazyl, AAI: Activity index, LV: Leaves, PE: Peels, PU: Pulps.

Before testing the sample, the DPPH and CUPRAC method should be verified with a standard. To verify DPPH and CUPRAC methods, ascorbic acid was used as standard. The AAI of ascorbic acid in the DPPH method was 64.1409 ± 2.5365 and in the CUPRAC method 9.2954 ± 0.0832. Both standards indicated a very strong antioxidant. AAI results of the standard with both methods are shown in Tables 1 and 2.

Table 2: Antioxidant activities of *Artocarpus heterophyllus* extracts by Cupric Ion-Reducing Antioxidant Capacity assays

AAI CUPRAC	Sample		
	1	2	3
LV	0.5898 ± 0.0053[a]	0.1960 ± 0.0038[b]	0.7177 ± 0.0072[c]
PE	0.9409 ± 0.0222[a]	0.8680 ± 0.0181[b]	0.2467 ± 0.0045[c]
PU	1.2503 ± 0.0209[a]	0.1156 ± 0.0013[b]	0.2243 ± 0.0026[c]
Ascorbic acid	9.2954 ± 0.0832		

[a-c]Different letters in the same column show the significant difference ($p<0.05$). 1: n-hexane extract, 2: Ethyl acetate extract, 3: Ethanol extract. LV: Leaves, PE: Peels, PU: Pulps, DPPH: 2,2-diphenyl-1-picrylhydrazyl, AAI: Activity index.

The AAI in DPPH method was varied from 0.0310 to 36.8852, where ethyl acetate extract contributed to give the highest number of antioxidant activity followed by ethanol extract and n-hexane extract. The highest number of yields on the DPPH method was given by the peels in ethyl acetate extract (36.8852), followed by the ethanol extract (7.6035), and n-hexane extract (0.0583). Meanwhile, the moderate number of yields was given by the pulps in ethyl acetate extract (22.8462), followed by the ethanol extract (21.7936), and n-hexane extract (0.0310). The leaves of jackfruit tend to have the highest yields in ethanol extract (19.3904), followed by ethyl acetate extract (9.3550), and n-hexane extract (1.0776).

The AAI in the CUPRAC method varied from 0.1156 to 1.2503, where n-hexane extract contributed to give the highest number of antioxidant activity followed by ethanol extract and ethyl acetate extract. The highest number of yields on the CUPRAC method was given by the pulps in n-hexane extract (1.2503), followed by the ethanol extract (0.2243), and ethyl acetate extract (0.1156). Meanwhile, the moderate number of yields was given by the peels in n-hexane extract (0.9409), followed by the ethyl acetate extract (0.8680), and ethanol extract (0.2467). The leaves of jackfruit tend to have the highest yields in ethanol extract (0.7177), followed by n-hexane extract (0.5898), and ethyl acetate extract (0.1960). Complete results of these extracts are shown in Tables 1 and 2.

Total phenolic content and total flavonoid content in leaves, peels, and pulps extracts of A. heterophyllus

The value of TPC in n-hexane, ethyl acetate, and ethanol extracts was determined by using a linear regression equation (y = 0.0053x + 0.0368, R^2 = 0.9989) with gallic acid as standard, thus resulting as gallic acid equivalent. Jackfruit had diverse TPC values, ranging from 0.10 to 5.53 g GAE/100 g (Figure 1). The lowest TPC value (0.10 g GAE/100 g) was obtained from pulps extracts of jackfruit in n-hexane extract (PU1). On the other side, ethanol extract of jackfruit leaves gave the highest TPC value (5.53 g GAE/100 g). In contrast, quercetin as standard and different linear regression equation (y = 0.0065x + 0.0232, R^2 = 0.9961) was used to obtain the value of TFC in n-hexane, ethyl acetate, and ethanol extract. Various TFC value from 0.21 to 16.07 g QE/100 g was obtained. Ethanol pulps extract (PU3) exposed the lowest TFC value (0.21 QE/100 g), meanwhile n-hexane peels extract (PE1) showed the highest (16.07 g QE/100 g) (Figure 1).

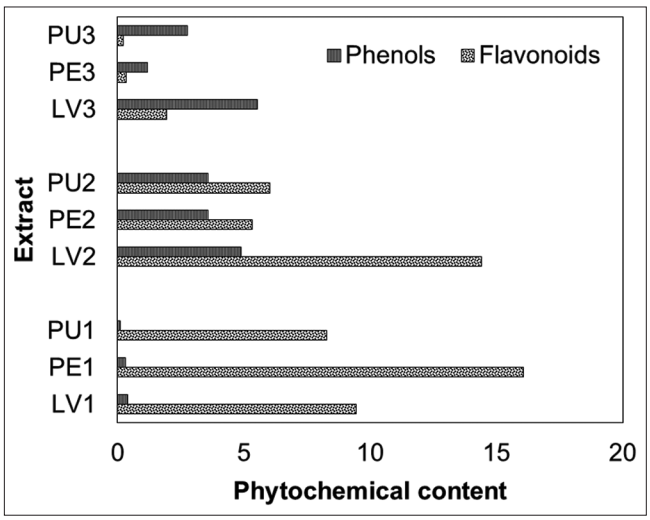

Figure 1: Phytochemical content in Artocarpus heterophyllus extracts. LV = leaves, PE = peels, PU = pulps, 1 = n-hexane extract, 2 = ethyl acetate extract, and 3 = ethanol extract, total phenolic content in g GAE/100 g extract, total flavonoid content in g QE/100 g extract

Correlation between the total phenolic content and total flavonoid content with antioxidant activity index of leaves, peels, and pulps extracts of A. heterophyllus

The largest amount of AAI defined the strongest capacity of antioxidant. Pearson's method was used to determine the correlation between TPC and TFC with AAI DPPH and CUPRAC. If the correlation was positive and significantly different, thus the TPC and TFC gave contribution to antioxidant activity (Table 3).

Table 3: Correlation of the total phenolic content and total flavonoid content of *Artocarpus heterophyllus* extracts with activity index 2,2-diphenyl-1-picrylhydrazyl and Cupric Ion-Reducing Antioxidant Capacity

Antioxidant parameter	Pearson's correlation coefficient (*r*)	
	TPC	TFC
AAI DPPH LV	0.892**	−0.633*
AAI DPPH PE	0.997**	−0.390 (NS)
AAI DPPH PU	0.981**	−0.661*
AAI CUPRAC LV	−0.168 (NS)	−0.917**
AAI CUPRAC PE	0.167 (NS)	0.804**
AAI CUPRAC PU	−0.987**	0.632*

**Significant at p < 0.01, *Significant at p < 0.05, NS: Not significant, LV: Leaves, PE: Peels, PU: Pulps, DPPH: 2,2-diphenyl-1-picrylhydrazyl, AAI: Activity index, TPC: Total phenolic content, TFC: Total flavonoid content, CUPRAC: Cupric Ion-Reducing Antioxidant Capacity.

Five compounds (luteolin 7-O-glucoside, rutin, quercetin, kaempferol, and apigenin) were used as standard to determine the marker compound in jackfruit ethanolic pulps extract (Figure 2). Based on the result (Table 4), the highest AUC was obtained from rutin (17,020). Therefore, rutin was the most dominant compound, resulting in rutin as the marker compound of *A. heterophyllus*. After being calculated, rutin content in ethanolic pulps extract was 0.0106%.

When different assays were used to analyze the antioxidant activity, they could run a different mechanism. For that reason, Pearson's method was applied to determine the correlation between DPPH and CUPRAC method (Table 3). This method was used to define if the AAI of DPPH and CUPRAC method showed a linear or nonlinear result. If the correlation was significant and positive, meaning linear results can be obtained from both methods. According to Table 3, leaves, peels, and pulps extracts of *A. heterophyllus* did not give linear results in both methods.

Table 4: Retention time and AUC of five standards

Standard (5 ppm)	Standard peak		*A. heterophyllus* pulps extract (ethanol extract)	
	Retention time (min)	AUC	Retention time (min)	AUC
Luteolin 7-O-glucoside	4.958	154,918	-	-
Rutin	5.466	80,185	5.563	17,020
Quercetin	8.345	218,622	8.423	12,935
Kaempferol	10.109	66,757	-	-
Apigenin	10.564	65,485	10.618	5853

A. heterophyllus: Artocarpus heterophyllus, AUC: Area Under Curve

Discussion

Prior study of antioxidant activity found that there is no previous evidence regarding to leaves,

Table 5: Correlation between activity index 2,2-diphenyl-1-picrylhydrazyl and Cupric Ion-Reducing Antioxidant Capacity

Antioxidant parameter	Pearson's correlation coefficient (*r*)		
	AAI CUPRAC LV	AAI CUPRAC PE	AAI CUPRAC PU
AAI DPPH LV	0.292 (NS)		
AAI DPPH PE		0.231 (NS)	
AAI DPPH PU			−0.997**

**Significant at *P*<0.01. NS: Not significant, CUPRAC: Cupric Ion-Reducing Antioxidant Capacity, LV: Leaves, PE: Peels, PU: Pulps, DPPH: 2,2-diphenyl-1-picrylhydrazyl, AAI: Activity index

peels, and pulps of *A. heterophyllus* which was grown in Kuningan, West Java, Indonesia in various extracts (n-hexane, ethyl acetate, and ethanol) using DPPH and CUPRAC methods.

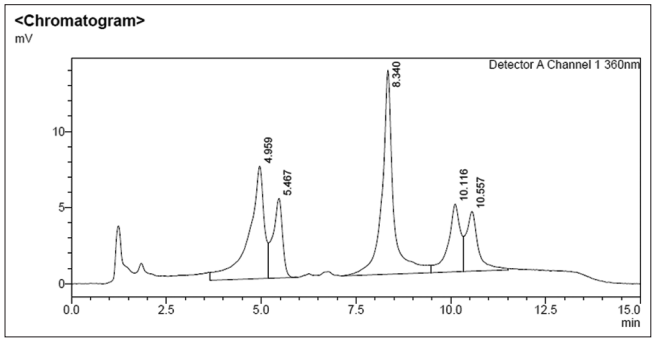

Figure 2: Retention time chromatogram of five standards (luteolin 7-O-glucoside, rutin, quercetin, kaempferol, and apigenin)

Extraction is a separating method of compounds based on their polarity. To get the highest yield of each compound polarity, extraction was carried out with n-hexane, ethyl acetate, and ethanol in chronological order. As a nonpolar solvent, n-hexane selectively extracted only nonpolar compounds. As a semipolar solvent, ethyl acetate mostly extracted semipolar compounds and remains nonpolar that weren't extracted before. As polar solvent, ethanol mostly extracted polar compounds and remains nonpolar and semipolar that was not extracted before.

Antioxidant is a compound that can reduce oxidation in a free radical by delaying initiation or propagation of oxidation chain reaction, free radical scavenging, chelating, and inhibit singlet oxygen forming [17]. In this research, antioxidant activity was estimated using DPPH and CUPRAC methods.

DPPH is a free radical that undergoes a complete delocalization of residual electrons, where the maximal absorbance happens in 515 nm wavelength. A hydrogen donor will reduce the absorbance and the original color (purple to transparent) in the same wavelength. Ascorbic acid was commonly used as standard. The decreasing number solution of DPPH and sample absorbance was comparable to the concentration of free radicals that have been inhibited [18]. IC_{50} is described as the sample concentration which can decrease 50% DPPH absorbance. The smaller IC_{50} value, the stronger the antioxidant activity [19]. This method is commonly used due to its sensitivity, accuracy, and ease of use.

Neocuproine is a reagent that was combined with cupric chloride in ammonium acetate buffer pH 7. Neocuprine acted as a chromophore which helped Cu^{2+} identified in UV-vis spectrophotometry at 450 nm wavelength. The sample that could be analyzed must act as a reductor, oxidized by $Cu^{2+,}$ and have a reduction potential lower than the Cu^{2+}/Cu^{+}. Reduction potential of Cu^{2+}/Cu^{+} is measured as 0.159 V [20]. The reducing number of Cu^{2+} to Cu^{+} increasing number of sample absorbance was comparable to the concentration of free radicals that have been inhibited. EC_{50} is described as the sample concentration which can increase 50% CUPRAC absorbance. The smaller EC_{50} value, the stronger the antioxidant activity. This method is commonly used for antioxidant activity assay in Vitamin C, polyphenols, and Vitamin E [21].

Both DPPH and CUPRAC methods can contribute to AAI estimation, where antioxidant activity could be classified as poor antioxidant with AAI < 0.05, moderate antioxidant with AAI in range of 0.5 ≤ AAI < 1, strong antioxidant with AAI in range of 1 ≤ AAI ≤ 2, and very strong antioxidant with AAI > 2 [22].

A general trend for both DPPH and CUPRAC methods gave a result where the highest AAI number in the DPPH method was given by ethyl acetate extract (peels); meanwhile, in CUPRAC method was given by n-hexane extract (pulps). As results from DPPH method, *A. heterophyllus* was concluded as a very strong antioxidant for both ethyl acetate and ethanol extract of all plant parts. In addition, n-hexane extract of all plant parts was also concluded as strong antioxidant. On the other side, using CUPRAC method, *A. heterophyllus* showed a strong antioxidant activity for n-hexane pulps extracts and moderate antioxidant activity for both leaves and peels extracts of n-hexane, ethanol leaves extracts, and ethyl acetate peels extract. The other plant parts in ethyl acetate and ethanol extracts indicated a poor antioxidant activity. Based on the results in Table 1, it can be suggested that the unused parts of jackfruit (peels and leaves) had good prospective as source of natural antioxidant. The prior study showed that water extract of *A. heterophyllus* with DPPH method have the strongest antioxidant activity (219.9 μg/mL) followed by ethyl acetate extract (235.8 μg/mL) [23]. Methanolic and aqueous extracts of *A. heterophyllus* flower and peels parts proved a higher activity as an antioxidant [24]. Ethanolic pulps extract of *A. heterophyllus* obtained a lower IC_{50} value than the leaves extract [25]. Jackfruit pulps were also mentioned as a strong antioxidant potential with scavenging abilities in the range of 21.82–69.64% [26]. The previous determination with FRAP methods also demonstrated a good reducing antioxidant ability of *A. heterophyllus* stem bark. Bound phenolics compounds have the better potential compared to free phenolics [27].

Based on the TPC and TFC results, ethanol leaves extracts had the highest TPC value (5.53 g GAE/100 g); meanwhile, n-hexane peels extracts had the highest TFC value (16.07 g QE/100 g). In the previous research [28], the TPC result of ethanol leaves

extracts (404.903 μg GAE/mg) has higher value than ethyl acetate leaves extracts (177.187 μg GAE/mg). The present research showed a similar result, where the TPC of ethanol leaves extract (5.53 g GAE/100 g) was higher than ethyl acetate leaves extract (4.89 g GAE/100 g). It was stated that high antioxidant capacity could be contributed by a high amount of TPC [29]. In another study by Zhu *et al*. [26], it was resulted that amongst pulps, flakes, and seeds extracts; methanol peels extracts had the highest TFC value which was 48.04 mg GAE/g DM. In this research, the highest TFC value was also given by peels extract (16.07 g QE/100 g). The TFC value of the two studies has different amounts because the solvent that was used to extract the peels was different. Previous research [11] used the seeds extract of *A. heterophyllus* from India to determine the total phenolic and flavonoid content. The study revealed that the highest TPC and TFC value were obtained from the ethanolic extract which was 4.16 mg GAE/g and 4.05 mg QE/g, consecutively. Consequently, the present study had a similar result for the phenolic content where the highest TPC value was obtained from ethanolic extract (5.53 g GAE/100 g). However, after being calculated, the TPC value in the present study was higher than the previous research (4.16 mg GAE/g). On the other hand, these studies had different result for the TFC value. Being compared with the previous research [11] that demonstrated the highest TFC value in ethanolic extract (4.05 mg QE/g), while the present study showed higher TFC value (16.07 g QE/100 g) in n-hexane extract. The leaves and peels of the plant were uncovered to outside environment like radiation by the sun and ultraviolet or extreme temperature [26]. Hence, those compounds were accumulated in the outer part of the plant, resulting a high TPC and TFC value in leaves and peels of jackfruit.

The correlation between TPC and AAI of leaves, peels, and pulps extracts with DPPH method was positive and significant ($0.892 \leq r \leq 0.997$). However, the correlation between TFC and AAI was positive and significant only in peels extracts with CUPRAC method. Hence, TPC contributed to DPPH free radical scavenging activity of leaves, peels, and pulps extracts. On the other hand, TFC only contributed to CUPRAC antioxidant capacity of peels extracts. It was similar with a previous study, where the correlation between phenolic content and DPPH was positive; meanwhile, there was no correlation with TFC. It might be due to flavonoids had various activities, thus did not contribute to high level of antioxidant activity [6]. In similarity with other study, the TPC which was obtained from ethanol extract was prevalent in contributing antioxidant activity using DPPH method [12]. In research by Eve *et al*. [30], the presence of tannins could contribute to antioxidant capacity. Another study reported the antioxidant activity of *A. heterophyllus* in IC_{50} value [31]. If the antioxidant activity was determined using IC_{50}, then the Pearson's correlation must be significantly negative [32].

As stated previously, phenols and flavonoids exhibited diverse antioxidant activities due to their structures. The hydroxyl groups on the structure of flavonoids aromatic ring allowed them to inhibit free radicals through redox reaction [33]. Likewise, hydroxyl groups of phenolic compounds could donate hydrogen to react with reactive species to scavenge free radicals [34]. Extracts that possessed many hydroxyl groups in their compounds could give bigger antioxidant activity [32]. It has been apprised that the presence of OH at C-3 and C-5 in the ring structure of flavonoids gave stronger radical scavenging activity [17]. The TPC in LV3 (5.53 g GAE/100 g) was higher than TPC in LV2 (4.89 g GAE/100 g); moreover, the AAI DPPH and AAI CUPRAC of LV3 was also higher than LV2. Hence, it could be assumed that LV3 had more hydroxyl groups in their phenolic compounds, thus increasing the antioxidant capacity (Table 5). In comparison with TPC, the TFC in PE1 (16.07 g QE/100 g) was higher than PE3 (0.33 g QE/100 g), but the AAI DPPH of PE1 was lower than PE3. It may be presumed that PE1 did not have ortho di OH at C-3' and C4' or OH at C-3, resulting in a low antioxidant capacity.

In the previous research, rutin as bound phenolic compounds contributed the most to antioxidant activities of Mulberry leaves [35]. Araujo *et al*. reported that rutin was used as control and showed DPPH scavenging activity which was 62.6% [36]. Being similar with other flavonoid compounds, rutin presented unsaturated 2,3 in conjugation with 4-keto group in C-ring and had a catechol group in B-ring that was able to stabilize free radicals by donating hydrogen [37]. Hence, rutin as flavonoid glycosides contributed to the antioxidant properties.

Conclusion

To determine the antioxidant activity of extracts, various methods should be performed to obtain diverse results. Ethyl acetate peels extract gave the highest antioxidant activity using DPPH, meanwhile n-hexane pulps extract showed the highest antioxidant activity in CUPRAC method. The TPC in leaves, peels, and pulps extracts had positive and significant correlation with AAI of DPPH. In contrast, the TFC in peels extracts had positive and significant correlation with AAI of CUPRAC. Therefore, phenols and flavonoids content gave contribution to the antioxidant activities. In addition, rutin as the marker compound also contributed to the antioxidant properties. DPPH and CUPRAC methods didn't give linear results in jackfruit extracts. Unused parts of *A. heterophyllus* (peels and leaves) might be potential to be used as natural antioxidant resources, however further studies can be done to improve this research.

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