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Determination of antiradical activity and phenolic and flavonoid contents of extracts and fractions of jackfruit (*Artocarpus heterophyllus* Lamk) seeds

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Abstract

Jackfruit (*Artocarpus heterophyllus* Lamk) is a plant from the Moraceae family that is widespread in Indonesia. Empirically, jackfruit can be used to cure hypertension, diabetes, cancer, asthma, dermatosis, coughs, wounds, acne, and diarrhea. The bioactive compounds in jackfruit include phenolics and flavonoids, which function as natural antioxidants. This research investigated the antiradical activity of jackfruit seed extracts and fractions using DPPH (2-2-diphenyl-1-picrylhydrazyl) and examined the total phenolic and flavonoid contents which may be developed as functional medicines and foods. The jackfruit seed powder was extracted using the maceration method. Radical scavenging activities were measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Total phenolic contents were determined using the Folin-Ciocalteu method. Meanwhile, the contents of flavonoids were determined using the aluminum chloride complex colorimetric method. The IC_{50} value of ethyl acetate fraction, methanol extract, *n*-hexane fraction, and water fraction was 5.435±0.064 µg/mL, 5.639±0.302 µg/mL, 7.201±0.475 µg/mL, and 9.134±0.2911 µg/mL, respectively. The phenolic and flavonoid contents of ethyl acetate fraction, methanol extract, *n*-hexane fraction, and water fraction were 49.597±1.589, 47.949±1.966, 41.214±4.354 and 35.504±0.913 g GAE/100 g of sample, respectively for phenolic, and 70.199±0.458, 65.228±0.615, 59.907±0.719, and 54.234±0.351 g of quercetin equivalent/100 g of sample, respectively of flavonoids, with a correlation value (R^2) to antiradical power of 0.4582 for phenolic and 0.5281 for flavonoids. The ethyl acetate fraction of jackfruit seeds can be further developed as an anti-radicals and functional food.

1. Introduction

Jackfruit (*Artocarpus heterophyllus* L) is a plant of the Moraceae family, which is widely grown in Indonesia and other tropical countries such as Brazil, Thailand, the Philippines, and Malaysia. The seeds can usually be consumed as snacks by roasting, boiling, or steaming them (Burci *et al*., 2015). Jackfruit can be consumed directly and also in the form of a jackfruit salad, which has high nutrition. In addition, jackfruit is also reported to have the antioxidant compound of prenylflavonoids (Gupta *et al*., 2011). Empirically, jackfruit can help to cure hypertension, diabetes, cancer, asthma, dermatosis, coughs, wounds, acne, and diarrhea (Moke *et al*., 2017; Ilmi *et al*., 2020).

In order to explore local food sources that have been used from generation to generation as medicine and have economic value, it is necessary to investigate the bioactive compounds in these foods. Among the bioactive compounds that are abundant in plant foods and have antioxidant functions are phenolic compounds. Oxidants are reactive oxygen species (ROS), which are species that are produced continuously by animals and humans that have the dual function of damaging and repairing the body's biological systems (Burci *et al*., 2015). Antioxidants are needed by the body because they have the ability to delay substrate oxidation by inhibiting initiation and propagation caused by oxidation reactions from free radicals (Azlim Almey *et al*., 2010; Widodo *et al*., 2020). In addition, ROS also has a role in the process of food spoilage through lipid autoxidation and enzymatic oxidation that occurs during the storage process of fats, oils and foods containing fat (Matthaus, 2002).

Synthetic antioxidants, such as butyl hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ), propyl gallate (PG), and butylated hydroxytoluene (BHT) have been

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widely used in the world, but synthetic antioxidants have side effects, such as cytotoxicity to the heart and lungs, and are carcinogens (Azlim *et al*., 2010; Sulastri et al., 2018). In addition, butyl hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) also have low solubility and have moderate antioxidant power (Sannigrahi *et al*., 2010). Therefore, a lot of recent research has been attempting to discover antioxidant compounds from natural ingredients, which are believed to have a high activity level and lower toxicity than synthetic compounds (Rohman *et al*., 2010), for example, rambutan peel (Mistriyani *et al*., 2018), *Moringa oleifera* leaves (Fitriana *et al*., 2016), and extracts and fractions from the peel of avocado (*Persea Americana* Mill), which were observed in vitro (Antasionasti *et al*., 2017). Therefore, the objectives of the research were to investigate the potential of jackfruit seed extract and fraction as antiradical and examine its total phenolic and flavonoid content.

2. Materials and methods

2.1 Materials

The materials used in this research were ripe jackfruit that was obtained from Pebaoa Village, North Buton Regency, Southeast of Sulawesi Province, methanol, ethyl acetate, *n*-hexane, DPPH radicals (Sigma -Aldrich®), Folin-Ciocalteu reagent (Sigma-Aldrich®), aluminium chloride, gallic acid, and quercetin.

2.2 Extraction

Jackfruit seeds were covered with a black cloth and dried in the sun. The dried seed of the jackfruit was pulverized into powdered form. After that, jackfruit seed powder (350 g) was macerated using methanol for 3 x 24 hours. The solvent was replaced every 24 hours. The extract that was obtained from maceration was concentrated using a rotary evaporator in order to obtain methanol extract.

$$Extract\ yield = \frac{Weight\ of\ the\ extract}{Weight\ of\ the\ simplicia} \times 100\%$$

2.3 Fractionation

A total of 20 g of methanol extract was dissolved with 200 mL of warm water and then fractionated using *n*-hexane solvent in order to obtain the *n*-hexane fraction and remaining water. The remaining water was fractionated again using ethyl acetate solvent so that the ethyl acetate fraction was obtained from the remaining water. The *n*-hexane, ethyl acetate, and water fractions were concentrated using a rotary evaporator as shown in Figure 1.

$$Fraction\ yield = \frac{Weight\ of\ the\ fraction}{Weight\ of\ the\ extract} \times 100\%$$

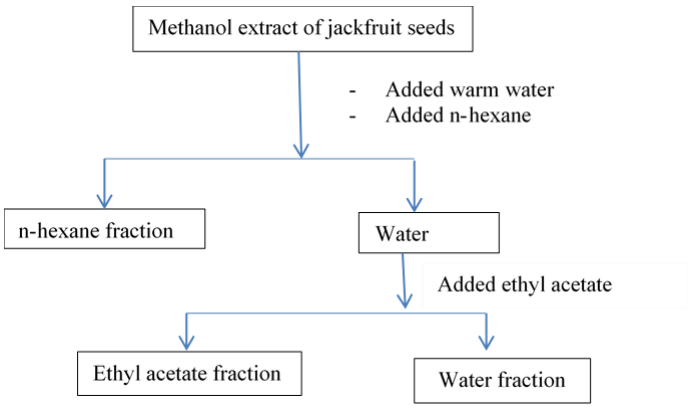

Figure 1. Schematic of fractionation of the methanol extract of jackfruit seeds

2.4 Phytochemical Screening

Phytochemical screening was conducted as in the phytochemical screening on Raghu bark (Dracontomelon dao (Blanco) Merr) extracts conducted as follows (Yamin *et al*., 2020).

2.4.1 Alkaloids

A total of 1 mL of each dissolved extract and fraction was taken and then put into a test tube. Two or three drops of Dragendorff's reagent were added. The formation of a red precipitate indicates the presence of alkaloids.

2.4.2 Tannins

The dissolved extracts and fractions were taken as much as 1 ml then put into a test tube and then 1 mL Fe (III) chloride 1% was added. Positive results are indicated by blue-black or green colour.

2.4.3 Flavonoids

Jackfruit seed extract and fractions were put into test tubes as much as 1 mL, then heated for 5 mins. Concentrated HCl was added, then 0.2 g of magnesium powder was added. Positive results are indicated by the formation of a deep red to magenta colour within 3 mins.

2.4.4 Saponins

A total of 1 mL of each Jackfruit seed extract and fraction was put into a test tube, then 4 mL of water was added and boiled for 2-3 mins and then cooled and shaken vigorously. The positive result is indicated by white foam that is stable for at least 10 mins.

2.4.5 Terpenoids

A total of 1 mL of each extract and fractions was put into a test tube. Acetic anhydride (1 mL) was added and then cooled. After it cooled down, concentrated H_2SO_4 was added. A reddish-brown colouration indicates positive results for the presence of terpenoids.

2.5 Measurement of anti-radical activity using the DPPH method

The antiradical activity was determined by following the modified Garcia method (Garcia *et al*., 2012). Each test sample was taken as much as 1 mL, then 3 mL absolute methanol and 1 mL DPPH radical (2,2-diphenyl -1-picrylhydrazyl) were added. The mixture was shaken to homogeneity, then incubated in a dark room for 30 mins. The absorbance was measured using a UV-Vis spectrophotometer at 513 nm. The antioxidant capacity of extract and fraction samples to inhibit DPPH radical can be calculated using the equation below.

$$\%\ inhibition = \frac{A_c - A_s}{A_c} \times 100\%$$

Where % inhibition = DPPH radical inhibition percentage, A_c = Absorbance of control and A_s = Sample concentration.

Antioxidant activity (IC_{50}) was calculated based on the linear regression equation between percent inhibition and the concentration of the sample or fraction, where the *x*-axis was the concentration, while the *y*-axis was the percent inhibition. So, the regression equation $y = bx + a$ was obtained. After that, the *y* value was replaced by 50. IC_{50} is defined as the concentration of the sample that is needed to inhibit 50% of DPPH radicals (Mistriyani *et al*., 2018).

2.6 Determination of total phenolic content

The total phenolic content of jackfruit seeds and fractions were determined by the Folin-Ciocalteu method according to John *et al*. (2014). However, in this research, the method was slightly modified. In short, 1 mL of each concentration series from the sample was taken and then 0.4 mL of the Folin-Ciocalteu reagent was added. After that, it was shaken, left for 8 mins, and then 4 mL of Na_2CO_3 7% was added. The mixture was shaken to homogeneity, then was added methanol to make 10 mL. The mixture was allowed to stand for 30 mins. After that, absorbance was measured using UV-Vis spectrophotometer at a wavelength of 647 nm. Measurement of each sample concentration series was conducted three times for replication. Total phenolic content is expressed as grams of Gallic acid equivalent (GAE)/100 g sample.

2.7 Determination of total flavonoid content

Total flavonoid content was measured using the colorimetric method (John *et al*., 2014; Vyas *et al*., 2015). However, in this research, the method was slightly modified where 10 mg of sample was dissolved with methanol p.a. to 10 mL. Then take 1 mL and added 3 mL of methanol p.a., then 0.2 mL of 10% aluminium chloride and 0.3 mL of 1 M potassium acetate were also added. Then, sufficient volume to 10 mL with aqudest. The mixture was incubated for 30 mins. Then, the absorbance was measured using a UV-Vis spectrophotometer at 439 nm. Measurement was conducted three times. Total flavonoid content is expressed as grams of quercetin equivalent (QE)/100 g sample.

2.8 Statistical analysis

All data in this research were analysed by using the Microsoft Excel program (Microsoft Inc. USA). Data analyses were replicated three times. Values were expressed in average data±standard deviation (SD).

3. Results and discussion

In this research, the extraction method used was maceration. With maceration, it is expected that metabolite compounds that are not heat-resistant will not be damaged due to high temperatures. Then, the process was continued with fractionation using solvents with different polarity. The purpose of using a solvent with a different polarity is to completely extract constituent compounds—not only non-polar compounds but also semipolar and polar compounds. A compound can be discovered in different solvents, but the level of these compounds in each solvent is different, depending on the distribution coefficient of the compound (Yamin *et al*., 2010). The results of Jackfruit seeds extraction and fractionation are presented in Table 1. The data showed that the most metabolites in the methanol extract of jackfruit seeds were polar with a yield of 66.15%. This is because a compound will be easily attracted to a suitable solvent, in accordance with the "like dissolves like" principle where the compound will be attracted to a solvent with the same polarity (Amaro *et al*., 2015).

Table 1. Yield of extract and fraction of jackfruit seeds

Sample	Simplicia/Extract weight (g)	Extract/Fraction weight (g)	Yield (%)
Methanol extract	350	36.2	10.34
n-hexane fraction	20	2.36	11.8
Ethyl acetate		4.41	22.05
Water fraction		13.23	66.15

3.1 Phytochemical screening

The results indicated that the jackfruit seed extract and fractions positively contained alkaloids, flavonoids, terpenoids, tannins, and saponins. However, the water and n-hexane fractions displayed negative results for saponins. These results are shown in Table 2.

Table 2. Phytochemical screening extract and fraction of jackfruit (*A. heterophyllus* Lamk.) seeds

Sample	Test				
	Alkaloid	Flavonoid	Tannin	Terpenoid	Saponin
Methanol extract	+	+	+	+	+
n-hexane fraction	+	+	+	+	-
Ethyl acetate fraction	+	+	+	+	-
Water fraction	+	+	+	+	-

3.2 Anti-radical activity test using DPPH (2-2-diphenyl-1-picrylhydrazyl)

DPPH radical (2,2-diphenyl-1-picrylhydrazyl) was used in this research because DPPH is one of the radicals that are widely used in a preliminary test to test antioxidants in plants (Jamuna *et al*., 2012). In addition, DPPH is a stable radical that accepts electron or hydrogen radical to become a stable diamagnetic molecule (Arina and Rohman, 2013) and measurement using DPPH requires a very short time (Sharma and Bhat, 2009; Shekhar and Anju, 2014). In addition, the DPPH assay is also considered as a standard colorimetric technique for measuring the antioxidant activity of plant extracts and pure compounds (Mishra *et al*., 2012). The antioxidant activity of the jackfruit extract and fractions was measured based on the scavenging activity of the stable 2,2-diphenyl- 1-picrylhydrazyl (DPPH) free radical. In this research, the standard used was vitamin C and methanol was used to dissolve DPPH. Methanol is used as a solvent in the DPPH test because methanol provides a very high sensitivity result compared to other solvents (Rohman *et al*., 2017)

The reaction between DPPH radicals and antioxidants is marked by a change in colour, from purple to yellow. This colour change is due to the DPPH radicals react with the hydrogen radicals released by compounds that act (Ningsih *et al*., 2016; Rizkayanti *et al.,* 2017).

Table 3 shows antiradical activity (IC_{50}) of jackfruit seed extract and fractions with vitamin C as a positive control. The IC_{50} values shown in Table 4 indicated that the ethyl acetate fraction had very strong antiradical activity compared to methanol extract, *n*-hexane fraction, and water fraction. The IC_{50} value of ethyl acetate fraction, methanol extract, *n*-hexane fraction, and water fraction was 5.435±0.064 µg/mL, 5.639±0.302 µg/mL, 7.201±0.475 µg/mL, and 9.134±0.2911 µg/mL, respectively. It indicated that semipolar compounds had very strong antiradical properties. This is in line with the results of previous studies which discovered that the ethyl acetate fraction had stronger antioxidant activity, including ethyl acetate fractions of peels of two rambutan species, namely, rambutan fruits cultivar Binjai and Aceh (Rohman *et al*., 2017), ethyl acetate fraction of *Oroxylum indicum* (Linn.) (Trang *et al*., 2014), ethyl acetate fractions of *Polygala sabulosa* and *Cyathea phalerata* (Brighente *et al*., 2008), ethyl acetate fraction of bark of Pacific walnut (*Dracontomelon dao* (Blanco) Merr) (Yamin *et al*., 2020). The antiradical power of a sample, both extract and fraction of a plant, is influenced by the number of hydroxyl groups (—OH) of phenolic and flavonoid compounds in the plant (Brighente *et al*., 2008)

Table 3. Antiradical activity of jackfruit peel that is described by the IC_{50} Value (µg/mL)

Sample	IC_{50} (µg/mL)			Average (µg/mL)
	I	II	III	
Methanol extract	5.963	5.59	5.366	5.639±0.302
n-hexane fraction	7.606	7.318	6.678	7.201±0.475
Ethyl acetate fraction	5.497	5.438	5.369	5.435±0.064
Water fraction	8.839	9.142	9.421	9.134±0.2911
Vitamin C	4.304	4.275	4.245	4.275±0.029

Values are expressed as mean±SD.

Table 4. Total phenolic content of extract and fraction of jackfruit seed

Sample	Total phenolic content (g GAE/100 g sample)			Average (g GAE/100 g sample)
	I	II	III	
Methanol extract	36.309	44.622	42.712	41.214±4.354
n-hexane fraction	50.113	47.459	46.274	47.949±1.966
Ethyl acetate fraction	51.38	49.081	48.329	49.597±1.589
Water fraction	36.451	35.432	34.63	35.504±0.913

Values are expressed as mean±SD.

3.3 Determination of total phenolic and flavonoid contents

Phenolic compounds and flavonoids are the most abundant compounds in plants and responsible for antioxidant activity. Flavonoid and phenolic compounds will donate hydrogen radical to bind to free radicals so that free radicals that enter the body will be stable (Hamid *et al*., 2010). The phenolic hydroxyl groups in plant substances are responsible for the scavenging of free radicals. Phenolic contents in plant extracts and fractions were measured using the Folin-Ciocalteu method (Aryal *et al*., 2019). Based on the data in Table 5, the ethyl acetate fraction of jackfruit seeds had a higher phenolic content than the n-hexane fraction, ethanol extract, and water fraction with values of 49.597±1.589, 47.949±1.966, 41.214±4.354 and 35.504±0.913 g GAE/100 g of sample, respectively.

The basic structure of flavonoids is a flavan nucleus, which consists of fifteen carbon atoms arranged in three rings, C6-C3-C6, labelled A, B and C. Several types of flavonoids have different degree of oxidation and

saturation in ring C. Meanwhile, several flavonoids in the same class have different substitution patterns in rings A and B. The differences in structure and substitution patterns affect the antioxidant properties of flavonoids (Wojdyło *et al*., 2007).

Table 5. Total flavonoids content of extract and fraction of jackfruit seed

Sample	Total flavonoids content (g QE/100 g sample)			Average (g QE/100 g sample)
	I	II	III	
Methanol extract	59.474	59.509	60.737	59.907±0.719
n-hexane fraction	65.263	65.825	64.596	65.228±0.615
Ethyl acetate fraction	70.351	70.561	69.684	70.199±0.458
Water fraction	54.211	54.596	53.895	54.234±0.351

Values are expressed as mean±SD.

The total flavonoid content was determined using the aluminium chloride colorimetric method. In principle, aluminium chloride will form stable complexes with a C-4 keto group and either the C-3 or C-5 hydroxyl groups of flavonols and flavones. In addition, it can also form unstable complexes with hydroxyl groups in the ortho position in the B-ring of flavonoids (Chang *et al*., 2002; Al-matani *et al*., 2016; Sembiring *et al*., 2018). Flavonoid is needed by the human body to maintain good health (Shi *et al*., 2019).

Based on the data presented in Table 5, the ethyl acetate fraction of jackfruit seeds had a higher content of flavonoids than the n-hexane fraction, methanol extract, and water fraction, with values of 70.199±0.458, 65.228±0.615, 59.907±0.719, and 54.234±0.351 g of quercetin equivalent/100 g of sample, respectively.

In relation to the data on antiradical activity (IC_{50}) in Table 3, data on phenolic and flavonoid contents presented in Table 4 and Table 5 indicated that there was a correlation between phenolic and flavonoid contents and antiradical power in the ethyl acetate fraction. However, the opposite happened to methanol extract and n-hexane fraction. This was due to the complexity of the compounds in the extract. Thus, the possibility was that the antioxidant power was not solely influenced by phenolic compounds and flavonoids, but was also by other compounds, such as ascorbic acid, tocopherols, pigments, and others. Another possibility was that the phenolic and flavonoid structures in the sample also affected the antiradical power of the material (Kaur and Mondal, 2014).

The correlation of phenolic and flavonoid content with the anti-radical activity of the substance can be determined based on the R^2 value of the regression equation between flavonoid or phenolic levels on the *x*-axis and antiradical power on the *y*-axis (Arnous *et al*., 2001). Figure 2 and Figure 3 presents the R^2 value of phenolic, which was 0.4582, illustrating that 45.82% of the anti-radical power of jackfruit seed extract and fraction was influenced by the phenolic compound. The R^2 value of flavonoids was 0.5281, indicating that the effect of the flavonoid in the extract and fraction of jackfruit seeds was 52.81%.

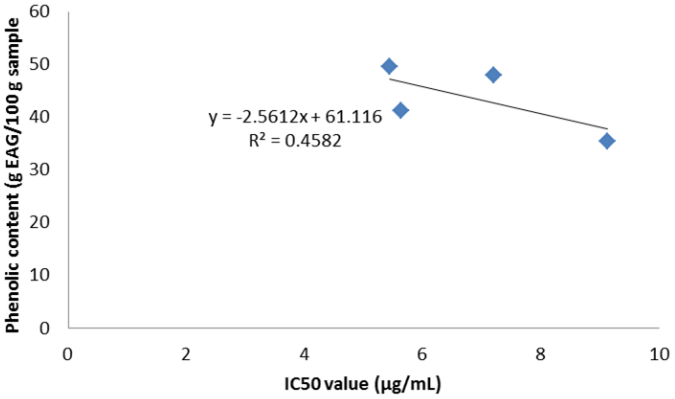

Figure 2. Correlation of phenolic content with IC50 value of jackfruit seeds

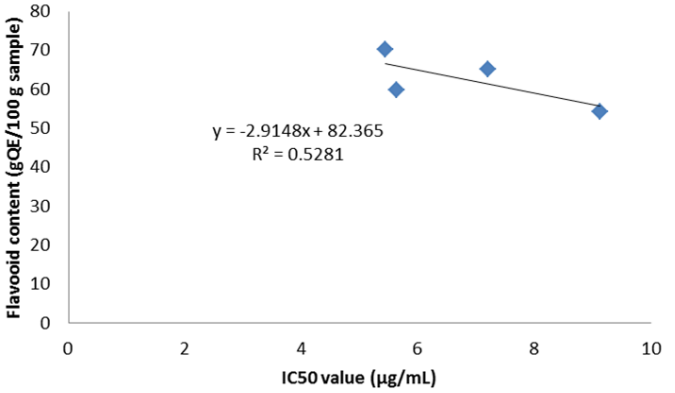

Figure 3. Correlation of flavonoids content with IC50 value of jackfruit seeds

4. Conclusion

Methanol extract and fractions of jackfruit seeds were tested for their anti-radical activity using DPPH and they displayed strong anti-radical activity. Ethyl acetate fraction displayed the strongest antiradical activity with an IC_{50} value of 5.435±0.064 µg/mL. The ethyl acetate fraction had a phenolic content of 49.597±1.589 g GAE/100 g of sample and flavonoid content of 70.199±0.458 g/100 g of quercetin equivalent/100 g of sample. The correlation value of R^2 of phenolic was 0.4582, while the correlation value of R^2 of flavonoid was 0.5281. Thus, the ethyl acetate fraction can be further developed as natural antioxidant and as a functional food.

Conflict of interest

The authors declare no conflict of interest.

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