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Determination of total phenolic and flavonoid contents of jackfruit peel and in vitro antiradical test

*Yamin, Ruslin, Mistriyani, Sabarudin, Ihsan, S., Armadany, F.I., Sahumena, M.H. and Fatimah, W.O.N.

Faculty of Pharmacy, Halu Oleo University, Kendari City, Indonesia

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Abstract

Free radical is any molecular species that have unpaired free electrons in their outer orbital shell that make radicals highly reactive, resulting in pathogenesis conditions such as cellular injury, premature aging, cancer, hepatic disorders, neurodegenerative diseases, cardiovascular disease, and kidney disease. One source of natural antioxidant is jackfruit. The purpose of this research was to determine the phenolic and flavonoid contents in the extracts and fractions of jackfruit peel and their potential as antioxidants. Jackfruit peel powder was extracted from maceration. The total phenolic content was determined by the Folin-Ciocalteu method. Meanwhile, flavonoid content was determined using the aluminium chloride complex colorimetric method. Measurements of antioxidant activity were conducted using the 2,2-diphenyl-1-picrylhydrazyl (DPPH). The ethyl acetate fraction had high phenolic and flavonoid contents, which were 49.667±1.508 g GAE/100 g of sample and 70.199±0.374 g of quercertin equivalent/100 g. The ethyl acetate fraction had the strongest antioxidant activity with IC_{50} value of 4.539±0.201 µg/ mL and correlation value (R^2) of 0.5881 for phenols and R^2 of 0.7241 for flavonoids. Ethyl acetate fraction of jackfruit peel is very potential to be developed as a natural antioxidant and functional food.

1. Introduction

Research on antioxidants as therapeutic agents in order to prevent damage caused by free radicals in the human body and research on natural antioxidant sources have been conducted for years by researchers. This is because natural antioxidants are believed to have relatively small side effects compared to synthetic antioxidants, which tend to be unstable and may be carcinogenic (Chandra *et al*., 2014). Free radical is any molecular species that have unpaired free electrons in their outer orbital shell that make radicals highly reactive, resulting in pathogenesis conditions such as cellular injury, premature aging, cancer, hepatic disorders, neurodegenerative diseases, cardiovascular disease and kidney disease (Aryal et al., 2019). Free radicals can be derived from environmental pollution, radiation, chemicals, fried foods and spicy foods and stress. If someone is exposed to sources of free radicals continuously, there will be a lack of antioxidants in their body (Agrawal *et al*., 2011). Therefore, antioxidants that are derived from outside the body are needed. One source of natural antioxidants is a biological material. Indonesia is one of the countries that are rich in natural materials. There are around 30,000 plant species in Indonesia and 7,000 of them can be used as medicinal ingredients (Jumiarni and Komalasari, 2017).

One of the plants that can be used as medicine is jackfruit. Jackfruit (*Artocarpus heterophyllus* L.) is a plant of the family Moraceae, which is widely grown in tropical countries such as Indonesia, Brazil, Thailand, the Philippines, and Malaysia (Burci *et al*., 2015). Jackfruit leaves and bark are traditionally used to treat hypertension, diabetes, cancer, asthma, dermatosis, cough, wounds, acne, and diarrhea (Moke *et al*., 2017, Ilmi *et al*., 2020). Jackfruit leaves contain metabolites, such as alkaloid, saponins, phenols, and flavonoids (Ilmi *et al*., 2020). Therefore, this research aims to determine the phenolic and flavonoid contents in extracts and fractions of jackfruit peel and their potential as antioxidants. This research also aims to observe the correlation of phenolic content and flavonoid content with the strength of antioxidant activity.

*Corresponding author.
Email: *yamintaeri@uho.ac.id*

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2. Materials and methods

2.1 Material

The materials used in this study were jackfruit that was obtained from Pebaoa Village, North Buton Regency, Southeast Sulawesi Province, methanol, ethyl acetate, n-hexane, DPPH (2,2-diphenyl-1-picryhidrazyl) radicals (Sigma-Aldrich®), Folin-Ciocalteu reagent (Sigma-Aldrich®), aluminium chloride, gallic acid (Sigma-Aldrich®), and quercetin (Sigma-Aldrich®).

2.2 Extraction

Jackfruit, that was obtained from Pebaoa Village, North Buton Regency, Southeast Sulawesi Province, was covered with a black cloth and dried in the sun. The dried peel of the jackfruit was pulverized into powdered form. After that, jackfruit peel powder (350 g) was macerated using methanol for 3 x 24 hrs. The solvent was replaced every 24 hrs. The extract that was obtained from maceration was concentrated using a rotary evaporator in order to obtain methanol extract.

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 remaining water fractions were concentrated using a rotary evaporator as shown in Figure 1.

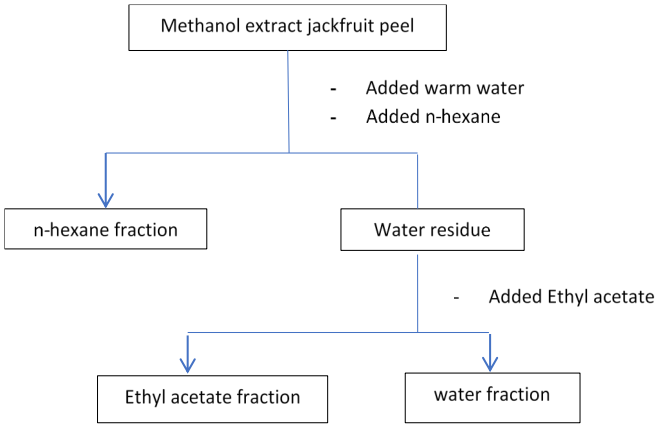

Figure 1. Schematic of the fractionation of moringa leave methanol extract

2.4 Phytochemical screening

Phytochemical screening was conducted as in the phytochemical screening on moringa plant extracts conducted by Yamin *et al.* (2020) as follows:

2.4.1 Alkaloids test

The extract and fractions of jackfruit peel were inserted separately into 1 mL test tubes and added with three drops of Dragendorff's reagent. The formation of brown precipitate indicated presence alkaloid.

2.4.2 Flavonoids test

The extract and fractions of jackfruit peel were inserted separately into test 1 mL tubes at and added with 0.2 g of magnesium powder and 2 mL of concentrated HCl. The formation of red, orange and green solutions indicated the flavonoid presence.

2.4.3 Terpenoid test

The extract and fractions of jackfruit peel were inserted separately into 1 mL test tubes and added with 0.5 mL of acetic acid anhydride and 2 mL of concentrated sulfuric acid. The formation of green, bluish, and brown solutions indicated the terpenoid presence.

2.4.4 Tannin test

The extract and fractions of jackfruit peel were inserted separately into 1 mL test tubes and added with 1 mL of 1% Fe (III) chloride solution. The formation of blue to black solution indicated the presence of tannin.

2.4.5 Saponin test

The extract and fractions of jackfruit peel were inserted separately into 1 mL test tubes and added with 2 mL of hot water, then cooled and shaken for 10 s. It was declared positive for saponin if the fume generated stabilized in less than 10 mins.

2.5 Determination of total phenolic content

Total phenolic concentrations of pumpkin leaf extracts 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, then 4 mL of Na_2CO_3 7% was added. The mixture was shaken to homogeneity, then methanol was added 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 in Gallic Acid Equivalents (GAE).

2.6 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. Next, 1 mL and 3 mL of pure methanol were added, then 0.2 mL of 10% aluminium chloride and 0.2 mL of 1 M potassium acetate were also added. Distilled water was added to make 10 mL. The mixture was incubated for 30 mins. Then, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 439 nm. Measurement was conducted three times. Total flavonoid content is expressed in Quercetin Equivalents (QE).

2.7 Determination of antiradical activity using DPPH methods

Radical 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 methanol p.a and 1 mL DPPH radical were added. The mixture was shaken to homogeneity, then incubated in a dark room for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 513 nm. 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 is the concentration, while the *y*-axis is 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).

2.8. Statistic analysis

All data in this research were analyzed by using the Microsoft Excel program (Microsoft Inc. USA). Data analyses were replicated 3 times. Values were expressed as mean±standard deviation.

3. Results and discussion

Phytochemical screening results of jackfruit peel extracts and fractions showed that both extract and fraction contained metabolites, such as alkaloids, flavonoids, tannins, and terpenoids. However, saponin metabolites were only found in methanol extract and the n-hexane fraction, as shown in Table 1.

Table 1. phytochemical screening results of jackfruit (*A. heterophyllus* Lamk.) peel

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

Phenolic compounds have redox potentials and can donate hydrogen atoms to the free radicals, so they can act as antioxidant compounds (Soobrattee *et al*., 2005). The total phenolic content can be used to conduct rapid screening of antioxidant activity (Baba and Malik, 2015). and in this study, it was used to know the phenolic and flavonoid contents in jackfruit peel extracts and fractions. Table 2 shows the phenolic content in jackfruit peel extracts and fractions.

Phenolic compounds had a direct contribution in reducing free radicals. Based on data in Table 2, the ethyl acetate fraction had a greater total phenolic content than the n-hexane fraction, methanol extract, and water fraction. The total phenolic content values of ethyl acetate fraction, n-hexane fraction, methanol extract, and water fraction were 49.667±1.508, 48.029±1.866, 41.214±4.254 and 35.504±1.913 g GAE/100 g of sample, respectively.

Table 2. Total phenolic contents (g EAG/100 g sample) of jackfruit extracts and fractions

Sample	Dataset I	Dataset II	Dataset III	Mean±SD
Methanol extract	36.309	44.622	42.712	41.214±4.254
n-hexane fraction	50.113	47.459	46.514	48.029±1.866
Ethyl acetate fraction	51.38	49.081	48.541	49.667±1.508
Water fraction	36.451	35.432	34.63	35.504±1.913

The high content of phenols in a sample will affect the strength of antioxidant activity in the sample. The high and low capability of an antioxidant is influenced by high and low phenolic contents in the sample (Duh *et al*., 1999). This is because phenolic compounds are free radical terminators that contribute directly to antioxidant activity (Shahidi *et al*., 2009).

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 groups 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).

Table 3 shows the total flavonoid content of the extracts and fractions of jackfruit peel. The ethyl acetate fraction showed higher flavonoid levels compared to the s-hexane fraction, methanol extract, and water fraction with values of 70.199±0.374, 65.228±0.502, 59.907±0.587 and 54.134±0.412 g QE/100 g of sample, respectively.

Table 3. Total flavonoid content (g EK/100 g sample) of jackfruit extracts and fractions

Sample	Dataset I	Dataset II	Dataset III	Mean±SD
Methanol extract	59.474	59.509	60.737	59.907±0.587
n-hexane fraction	65.263	65.825	64.596	65.228±0.502
Ethyl acetate fraction	70.351	70.561	69.684	70.199±0.374
Water	54.211	54.596	53.595	54.134±0.412

One method for determining the anti-radical capacity of a material is by measuring the absorbance value of DPPH solution. This method was used because DPPH is a radical that is widely used in preliminary tests for antioxidants in plants (Jamuna *et al*., 2012), This method is also widely applied in order to measure the anti-radical capacity of a compound, both pure compound and multi-compound that are derived from natural substances (Mishra *et al.*, 2012; Oliveira *et al*., 2014; Permatasari and Rohman, 2016). In addition, DPPH radicals are used to measure antioxidant activity because DPPH is a stable radical and measurement using DPPH requires a very short time (Sharma and Bhat, 2009; Shekhar and Anju, 2014).

DPPH radicals also have a high sensitivity to polar solvents, especially to methanol and ethanol solvents. In a study conducted by Sarma and Bhat (2009), sensitivity testing for methanol buffer, methanol and ethanol solvents was conducted. The results of the study indicated that methanol and methanol buffer had higher sensitivity compared to ethanol solvent (Sharma and Bhat, 2009). Therefore, the methanol solvent was used in this research.

The reaction between DPPH radicals and antioxidants is characterized by color change from purple to yellow. This is due to the DPPH radical reaction that forms hydrogen bondings with substances that act as antioxidants (Ningsih *et al*., 2016; Rizkayanti *et al*., 2017).

Table 4 shows the IC_{50} values of extracts and fractions of jackfruit peel with vitamin C as standard. The IC_{50} data in Table 4 shows that the ethyl acetate fraction showed greater strength of antioxidant activity compared to methanol, n-hexane, and water extracts. The values of ethyl acetate fraction, methanol extract, n-hexane extract, and water extract were 4539±0.201 µg/ mL, 6.13±0.209 µg/mL, 7.156±0.240 µg/mL, 8.859±0.104 µg/mL, respectively.

Table 4. Anti-radical activity of jackfruit peel that is illustrated by IC_{50} value (µg/ mL)

Sample	Dataset I	Dataset II	Dataset III	Mean±SD
Methanol extract	6.065	5.962	6.364	6.130±0.209
n-hexane fraction	6.912	7.392	7.165	7.156±0.240
Ethyl acetate fraction	4.755	4.502	4.359	4.539±0.201
Water fraction	8.853	8.759	8.967	8.859±0.104
Vitamin C	4.304	4.275	4.245	4.275±0.029

This is in line with the results of research showing that ethyl acetate fractions had stronger antioxidant activity, for example, ethyl acetate fraction of peel of rambutan (*Nephelium lappaceum* L.) (Permatasari and Rohman, 2016), ethyl acetate fraction of bark of *Albizia adianthifolia* (Tamokou *et al*., 2012), ethyl acetate fractions of peels of two rambutan species, namely, rambutan fruits cultivar Binjai and Aceh (Rohman *et al*., 2017), ethyl acetate fraction of Raghu (Yamin, *et al*., 2020), ethyl acetate fraction of senggani (*Melastoma candidum* D. Don) leaves (Marjoni and Zulfisa, 2017) and ethyl acetate fraction of jackfruit seeds (Burci *et al*., 2015).

The strength of antioxidant activity correlates with phenolic and flavonoid contents in the sample. The more hydroxyl groups are in the sample, the stronger the sample will be in counteracting free radicals. The correlation of phenolic and flavonoid contents with the strength of antioxidant activity can be seen in Figure 2 and Figure 3. Correlation values are indicated by R^2. The R^2 value of phenolic content was 0.5881, which illustrated that as much as 58.81% of the strength of antioxidant activity was influenced by the content of phenolic compounds in the extracts and fractions of jackfruit peel. Meanwhile, the value of R^2 that showed the correlation between flavonoid content and antioxidant activity was 0.7241. It meant that 72.41% of the strength of antioxidant activity is influenced by the

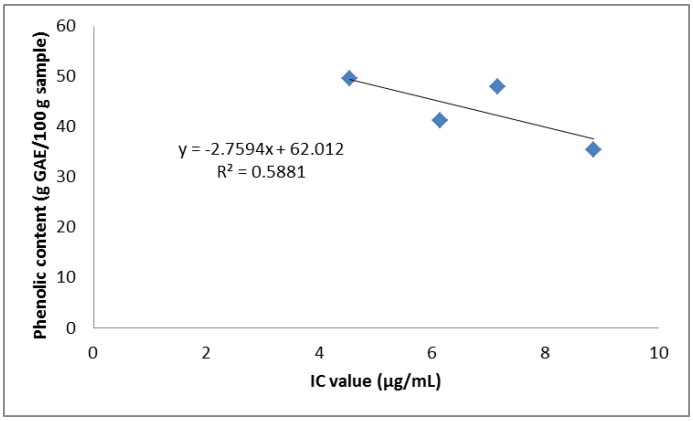

Figure 2. Correlation of phenolic contents (g GAE/100 g of sample) with IC_{50} value (µg/mL)

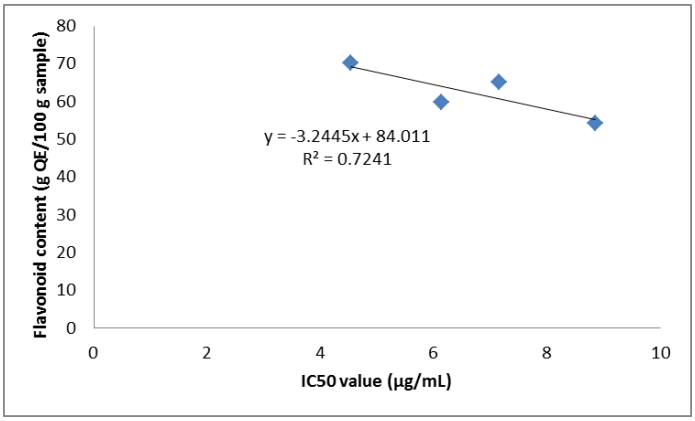

Figure 3. Correlation of flavonoid content (g QE/100 g of sample) with IC_{50} value (µg/mL)

content of phenolic compounds in the extracts and fractions of jackfruit peel.

4. Conclusion

The ethyl acetate fraction of jackfruit peel had a very strong antiradical activity compared to other extracts and fractions of jackfruit peel. The IC_{50} value of ethyl acetate fraction of jackfruit peel was 4.539±0.201 µg/ mL. Similarly, phenolic and flavonoid contents of ethyl acetate fraction were the highest, namely, 49.667±1.508 g GAE/100 g of sample and 70.199±0.374 g QE/100 g of sample, respectively. The correlation value between phenolic content and antiradical activity was 0.5881, while the correlation value of flavonoid content with antiradical activity was 0.7241. The results of this research indicate that jackfruit extract and peel can be developed as a functional food.

Conflict of interest

The authors declare no conflict of interest.

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