International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Special Issue 3, 2022

Original Article

# QUALITY PARAMETERS AND DETERMINATION OF TOTAL FLAVONOID LEVELS FROM THE HIGHEST ANTIOXIDANT ACTIVITY OF ETHANOL 70% EXTRACT JACKFRUIT PEEL (*ARTOCARPUS HETEROPHYLLUS* L.) BY MACERATION, REFLUX, AND ULTRASONIC METHODS

**YUNAHARA FARIDA[1], RAHMATUL QODRIAH[1*], SANAM NILESH[1]**

[1]Faculty of Pharmacy, Pancasila University, Srengseng Sawah, Jagakarsa, South Jakarta, 12640, Indonesia
[*]Email: rahmatulqodriyah@gmail.com

*Received: 25 Dec 2021, Revised and Accepted: 27 Mar 2022*

**ABSTRACT**

**Objective**: The aim of this study was to investigate and determination of total flavonoid levels antioxidant activity of ethanol 70% extract jackfruit peel (*Artocarpus heterophyllus* L.) by maceration, reflux, and ultrasonic methods

**Methods:** Jackfruit peel was extracted by using maceration, reflux, and ultrasonic methods with 70% of ethanol as the solvent. Antioxidant activity using DPPH free radical scavenging and determination of total flavonoid levels using colorimetric methods.

**Conclusion:** The results of phytochemical screening indicates that jackfruit peel contains flavonoids, saponins, tannin, and triterpenoids; the best antioxidant activity is found in the maceration method. The extract of Jackfruit peel fullfill the quality parameters requirements.

**Results:** The determination was carried out at the Center for Plant Conservation and Botanical Gardens-LIPI, Bogor. The results of this determination indicate that the material studied belongs to the species *Artocarpus heterophyllus* L.

**Keywords**: Jackfruit peel, Extraction methods, Antioxidant activity, Quality parameters, Flavonoids


DOI: https://dx.doi.org/10.22159/ijap.2022.v14s3.21 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

## INTRODUCTION

Humans as living beings need energy obtained from intracellular chemical oxidation reactions. This reaction causes the formation of free radicals. Free radicals can form new radical compounds that produce excess free radicals in the body. This situation can be overcome with compounds that are antioxidants [1, 2].

In recent years, the popularity of herbal medicine has greatly increased. One of the interesting plants is jackfruit (*Artocarpus heterophyllus*). Jackfruit have been used by the Indonesian people to treat inflammation, malaria, ulcers, abscesses, dysentery, diarrhoea, and skin diseases. Jackfruit flesh can be useful as an antibacterial, antidiabetic, anti-inflammatory, and antioxidant. Jackfruit leaves have antibacterial activity. Jackfruit root has antioxidant and antimalarial activity [3, 4]. Based on research, the ethanolic extract of jackfruit rind contains compounds of alkaloids, flavonoids, phenols, and terpenoids. Activity test shows the $IC_{50}$ value 87.09 µg/ml. Jackfruit plants are thought to have abundant flavonoid content. Phenolic compounds such as flavonoids exhibit antioxidant activity. The complete the antioxidant compounds in jackfruit rind, flavonoid analysis is needed [5-7]. Traditional medicines in Indonesia market must be meet the requirements for quality, safety, and benefit. Therefore, it is necessary to develop standards for the quality, safety, and efficacy of medicinal plant extracts, so that the general standard parameters of medicinal plant extracts must first be determined so that they can be used in formal health services.

One of the factors that affect the quality of the extract is the extraction method used [8]. The extraction method in this study was carried out by cold, hot method, and extraction using ultrasonic waves. The purpose of this study was to determine the difference in antioxidant activity of ethanol extract in jackfruit rind from maceration, reflux, and ultrasonic methods. The highest antioxidant activity will determine the total flavonoid content and the quality parameters so that a standardized extract is obtained.

## MATERIALS AND METHODS

### Materials

Jackfruit peel (*Artocarpus heterophyllus* L.) was obtained from the Center for Spice and Medicinal Plants (Balittro, Indonesia). 70% ethanol, Karl fisher reagent 30%, ammonia, hydrochloric acid, Dragendorff's reagent, Mayer's reagent, 10% ammonia, ether, concentrated sulfuric acid, anhydrous acetic acid, 1% ferric (III) chloride, 30% formaldehyde, magnesium powder, petroleum ether, sodium hydroxide 1 N, chloroform, amylalcohol, glacial acetic acid P, acetone P, ethyl acetate P, aqua demineralisata, phosphoric acid buffer solution (LDF) pH 7.2, Potato Dextrose Agar (PDA), Nutrient Agar (NA), water-chloroform LP, citric acid 10% were purchased from Brataco, Indonesia. 1, 1-diphenyl-2-picrylhydrazil (DPPH) and methanol pro-analysis were purchased from Merck, Indonesia. Vitamin C was purchased from reference standard Pharmacopeia, Indonesia.

### Plant determination

The determination of this plant was carried out at the Indonesian Institute of Sciences (Plant Conservation Research Center and Botanical Gardens), Bogor. Research material collection. The research material used was jackfruit skin (*Artocarpus heterophyllus* L) which was obtained from the Research Institute for Medicinal Plants and Spices (Balittro).

### The jackfruit peel extraction

The jackfruit peel was extraction with kinetic maceration method. 250 g of jackfruit peel simplicia powder was put into a clean maceration container, added 70% ethanol then stirred with a mechanical stirrer for 6 h. Let stand for 1x24 h. at room temperature. Then filtered. The liquid extract obtained was evaporated using a rotary vacuum evaporator at a temperature of 40 °C, a speed of 60 rpm, and a pressure of 200 mBar to obtain a thick extract. Reflux extraction method: 250 g of jackfruit peel simplicia powder was put into a round bottom flask, added 70% ethanol and heated at 78 °C for 3 h, then filtered. The liquid extract obtained was evaporated using a rotary vacuum evaporator at a temperature of 40 °C, a speed of 60 rpm, and a pressure of 200 mBar to obtain a thick extract. Ultrasonic extraction method: 250 g of jackfruit peel simplicia powder was put into a glass beaker, added 70% ethanol, and then was extracted using ultrasonic waves at a frequency of 50 kHz for 15 min, then filtered. The liquid extract was evaporated using a rotary vacuum evaporator at a temperature of 40 °C, speed of 60 rpm, and a pressure of 200 mBar to obtain a thick extract. The yield and DER-

native yield were calculated from the 70% ethanol of jackfruit peel extract (*Artocarpus heterophyllus* L.).

**Phytochemical screening**

Phytochemical screening test was conducted according to Farnsworth's and Harborne's method [9,10] to identify compounds of samples such as alkaloids by Dragendorff's reagent/Mayer's reagent, flavonoids by the reduction test (Mg-HCl/amyl alcohol), saponins by the foam formation test, tannins by the iron (III) chloride reagent, quinone by the NaOH reagent, steroids/triterpenoids by the Liebermann-Burchard's reagent, coumarins by the fluorescence test with ammonia and essential oil by the odor test.

**Antioxidant activity**

Determination of the maximum wavelength of the DPPH solution. 1,0 ml of 0.4 mmol DPPH solution was prepared with methanol to 5.0 ml; then the DPPH sample was incubated for 30 min at room temperature. Then the absorption was measured using a UV-Vis spectrophotometer with a wavelength of 400-600 nm [11, 12].

Test for antioxidant activity 70% ethanol of jackfruit peel extract was using the stable DPPH. Five concentrations are made 30, 40, 50, 60, and 70 µg/ml 70% ethanol of jackfruit peel extract *Artocarpus heterophyllus*. Each sample was mixed with 1.0 ml of 0.4 mmol DPPH solution. All the solutions were prepared with methanol to 5.0 ml and then incubated at 37 °C for 30 min the absorbance was recorded at 516.5 nm using a UV-Visible spectrophotometer. The experiment was repeated for 3 times. Vitamin C was used as a standard control. $IC_{50}$ values denote the concentration of the sample, which is required to scavenge 50% of DPPH free radicals.

**Total flavonoid determination**

Aluminum chloride colorimetric method was used for total flavonoid content. Each plant extracts (0.5 ml of 1:10 g ml-1) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with UV/Visible spectrophotometer

**Specific quality parameters determination**

The quality parameter was determined organoleptic, measurement of soluble compound content, ethanol-soluble compound content, and water-soluble compound content. Organoleptic test was analyzed the consistency, color, smell of the extract that visually observed. Water-soluble compound was determined by macerated 1 g extract in 20 ml chloroform for 24 h. Filter and evaporate 4 ml of the filtrate to dryness, which has been preweighed, and heat the residue in an oven at 105 °C until constant weight. Calculate the water-soluble compound. Ethanol soluble compound was determined by macerated 1 g extract in 20 ml ethanol for 24 h. Filter and evaporate 4 ml of the filtrate to dryness, which has been preweighed, and heat the residue in an oven at 105 °C until constant weight.

**Non-specific quality parameters determination**

The non-specific quality parameters determination has analyzed the loss on drying, water content, total ash content, acid insoluble ash content, water-soluble ash content, residual solvent content, heavy metal, and microbial contamination (Total Plate Number and Molds and Yeasts Number). The loss on drying was determined by weighing about 1 g extract in the shallow weighing bottle, which has been heated at a temperature of 105 °C for 30 min and then dried at a temperature of determination to reach a constant weight. Residual solvent determined using gas-liquid chromatography with a flame ionization detector and a glass column 30 cm x 0.32 mm stationary phase flowed TR-WAX with a particle size of 100–200 mesh and nitrogen as a carrier gas. Water content was determined using Karl Fischer reactor. Total ash content was determined by weighed about 2 g extract and then put the extract in the silicate crucible, which has been heated at 450 °C until the weight is fixed and weighed. Calculate the ash content in comparison with the air-dried extract.

The acid-insoluble ash content was determined by boil the ash that had been obtained in the measurement of total ash content in 25 ml hydrochloride acid for 5 min. collect the acid-insoluble ash. Filter using an ash-free paper filter, wash with hot water and centrifuge the residue and paper filter under a temperature of 400–600 °C to produce a constant weight. Acid-insoluble ash content was determined in comparison with the air-dried extract. Microbial contamination determination was analyzed by the Total Plate Count (TPC) and Molds and Yeasts Count (MYC). The TPC was determined by the weighed amount of 1.0 g of extract carefully examined or measured, put into 10 ml volumetric flask, add phosphate buffer pH 7.0 up to 10 ml, mix. The mixture obtained form of a solution or clear liquids; continue the experiment by Total Plate Count. After the procedure above is done (a $10^{-1}$ dilution) and then pipette 1 ml of the sample that is mixed put 9 ml of phosphate buffer pH 7,2. It is a 10-2 dilution. Subsequent dilutions were made to 10-6. From each dilution pipette 1 ml in to a sterile petri dish and made triplicate. Into each petri dish, poured NA 15-20 ml of seed medium (45±1 °C) and then shaken and rotated until the suspension is spread evenly. Blank made on the petri dish filled with 1 ml of diluent and media in order. After the medium solidified, petri dishes were incubated at 35-37 °C for 24 h in the inverted position. The number of colonies that grew was observed and counted. The determination of molds and yeasts number procedure same with TPC method using Potato Dextrose Agar as media. The analyzed of heavy metal contamination (Cd and Pb) by using Atomic Absorption Spectrophotometry method [13].

**RESULTS AND DISCUSSION**

**Plant determination**

The determination was carried out at the Center for Plant Conservation and Botanical Gardens-LIPI, Bogor. The results of this determination indicate that the material studied belongs to the species *Artocarpus heterophyllus* L.

**Extraction**

Extraction using the kinetic maceration method. In this method, re-maceration was carried out three times. The amount of extract obtained was 88.89 g with a % yield of 35.55% and a native DER of 2.81. *The reflux method* was extracted at a temperature of 78 °C for 2 h 3 times. The amount of extract obtained was 67.29 g with a % yield of 26.92% and a native DER of 3.72. Ultra sonication method. The ultrasonic method was extracted using an ultra-sonicator with a frequency of 15 kHz for 15 min and resonated 3 times. The amount of extract obtained was 50.13 g with a % yield of 20.05% and a native DER of 4.98. The obtained macerate was collected and concentrated by means of a rotary evaporator. Each extraction method produces a different yield. The maceration method gave the highest yield percentage and the ultrasonic extraction method produced the lowest extract yield. One of the factors that affect the extraction is time. Maceration requires the longest extraction time, which is 3 d, so that more samples are extracted. Extract with ultrasonic method produces the lowest yield because the time used is very short, namely 45 min. In addition, the factors that can affect the extraction process are temperature. The higher the extraction temperature, the easier the penetration of the solvent into the material so that more samples are extracted. Extract with reflux method produces a lower yield than maceration and higher than ultrasonic.

**Phytochemical screening**

The results of the phytochemical screening of ethanol 70% Extract Jackfruit Peel (*Artocarpus Heterophyllus* L.) with methods maceration, reflux, and ultrasonic contains alkaloids, tannins, flavonoids, saponins, steroids, triterpenoids, essential oils and coumarins. The results can be seen in table 1.

**Antioxidant activity test**

The results of antioxidant activity testing of ethanol 70% Extract Jackfruit Peel (*Artocarpus Heterophyllus* L.) can be seen in table 2. Based on the results of antioxidant activity tests, its shows that 70% ethanol extract has $IC_{50}$ value higher than other maceration extracts, which was 88.38 ppm, which indicates that its activity is strong Vitamin C as positive control has the highest antioxidant activity that Vitamin C has the power of attenuation against free radicals with

$IC_{50}$ value of 4.93 ppm. According to Molyneux (2004), antioxidant activity is very strong when the $IC_{50}$ is less than 50 ppm, strong if the $IC_{50}$ is 50-100 ppm, moderate if the $IC_{50}$ is 101-150 ppm and weak when the $IC_{50}$ value is 151-200 ppm

**Table 1: Results of phytochemical screening of powdered and extracts**

| No | Group of chemical compounds | Powdered | Maceration extract | Reflux extract | Ultrasonic extract |
|---|---|---|---|---|---|
| 1 | Alkaloids | - | - | - | - |
| 2 | Flavonoids | +++ | + | + | + |
| 3 | Saponins | ++ | ++ | ++ | ++ |
| 4 | Tannins | +/++ | +/+ | +/+ | +/+ |
| 5 | Quinone | - | - | - | - |
| 6 | Steroids | + | + | + | + |
| 7 | Triterpenoids | ++ | ++ | ++ | ++ |
| 8 | Essential oils | - | - | - | - |
| 9 | Coumarins | - | - | - | - |

Note: (+) indicates present, (-) indicates absent/not detected

**Table 2: Antioxidant activity of jackfruit peel with DPPH free radical scavenging**

| Sample | $IC_{50}$ (ppm) |
|---|---|
| Vitamin C | 4.93±0.01 |
| Maceration extract | 88.38±0.09 |
| Reflux extract | 106.73±0.64 |
| Ultrasonic extract | 139.88±3.76 |

Date was given in mean+SD, n=3

**Total flavonoid content**

The principle of determining total flavonoid content using the aluminum chloride method is that is the sample is added $AlCl_3$ will form stable complexes between $AlCl_3$ with a carbonyl group at C-4 and hydroxyl group at C-3 or C-5 (flavones/flavonols) so that there is a shift in the wavelength toward visible light which is characterized by the solution producing a more yellow color.

Determination of total flavonoid content using the colorimetric method with the addition of $AlCl_3$. Maximum wavelength optimization is carried out to determine the wavelength that has the highest absorption. The sample must be measured at the maximum wavelength for maximum sensitivity. Maximum wavelength measurements were carried out in the range of 200-600 nm. The maximum wavelength obtained was 437.5 nm with an absorbance of 0.6870 for quercetin with the addition of reagents and 370 nm with an absorbance of 0.6070 for quercetin without the addition of reagents [14, 15]. In determining the total flavonoid content of 70% ethanolic extract of jackfruit peel made by refluxing the thick extract, then filtered and the filtrate obtained was extracted with ethyl acetate using a separating funnel three times, then the ethyl acetate phase was collected as a test solution, the test solution will be measured. Absorption at the maximum wavelength. Determination of the total flavonoid content of the 70% ethanol extract of jackfruit by maceration method, the results obtained was 1.72±0.11%. These findings are supported by a study by McDonald (2001), which found that in general, extracts with a high amount of phenolic content could significantly contribute to high antioxidant activity [16]. The higher TPC values obtained for the rind may be explained by the higher accumulation of phenolic compounds in the external part compared to the inner part since their formation is a light-dependent process [17]. It has also been reported that the highest levels of phenolic compounds, especially the phenolic acids group, are often found in the external parts of the ripe fruit and these could contribute to high antioxidant capacity [18].

**Table 3: Specific parameters determination of the 70% ethanol extract**

| Specific parameters | Result |
|---|---|
| Organoleptic | |
| Form | Thick Extract |
| Color | Brown to yellow |
| Smell | Aromatic odor |
| Taste | Bitter |
| Measurement of soluble compound content | |
| Ethanol soluble compound content (%) | 16.97±0.03 |
| Water-soluble compound content (%) | 61.19±0.06 |

Date was given in mean+SD, n=3

**Table 4: Non-specific parameters determination of the 70% ethanol extract**

| Non-spesific parameters | Result |
|---|---|
| Loss on drying (%) | 6.58±0.37 |
| Water content (%) | 4.09±0.06 |
| Total ash content (%) | 2.48±0.06 |
| Acid insoluble ash content (%) | 0.44±0.02 |
| Water-soluble ash content (%) | 4.53±0.03 |
| Solvent residual content (%) | 0.22 |
| **Heavy metal contamination** | |
| Pb Metal contamination (mg/kg) | 0.37±0.02 |
| Cd metal contamination (mg/kg) | 0.47±0.04 |
| **Microbial contamination** | |
| Total Plate Number (colony/g) | No-detection |
| Molds and Yeasts Number (colony/g) | No-detection |

Date was given in mean+SD, n=3

Determination of water content is important pharmaceutical preparation, especially extract preparations because the presence of will be a good medium for fungal growth and also a medium for the chemical reaction. The water contained in the extract which a lower water content, is more stable to be stored in the long term. From the results, the water content that meet the quality requirement is<10%.

The results of the determination of residual solvent ethanol with gas-liquid chromatography in the extract obtained 0.22% ethanol content. The results showed that the residual content still fulfills the requirements of maximum residual solvent in the extract is less than 1% in accordance with the regulatory of BPOM, 2005. The extract met the requirements to be used as raw material for preparation because it contain low ethanol content. The content of Pb and Cd in the extracts can be derived from the environment the plants grow and the production process. The content of heavy metals such as Pb and Cd that into the body should be limited in number because it is dangerous for health. Based on the results showed levels of Pb in the extracts of 0.37±0.02 mg/kg, whereas Cd levels of 0.47±0.04 mg/kg.

## CONCLUSION

The results of phytochemical screening on the powder and 70% ethanol extract of jackfruit peel showed the presence of flavonoids, saponins, tannins, and triterpenoids. The 70% ethanol extract of jackfruit peel has antioxidant activity. The 70% ethanol extract of jackfruit peel in each extraction method has different antioxidant activity. The highest antioxidant activity results were found in the kinetic maceration extract, which was 88.38±0.09 ppm. The 70% ethanol extract of jackfruit peel by maceration method met the requirements of quality parameters in general. The total flavonoid content of the 70% ethanol extract of jackfruit peel was 1.72±0.11% calculated against the quercetin comparison standard.

## FUNDING

Nil

## AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

## CONFLICT OF INTERESTS

Declared none

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