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Article

The Effect of Extraction Methods on the Total Phenols and Total Flavonoids Content of Jackfruit (*Artocarpus heterophyllus* Lamk) Peels Extract

Dyan Wigati[1], Lindawati Setyaningrum[1*], Dwi Koko Pratoko[2]

[1]Bachelor in Pharmacy Program, Faculty of Health Science, Universitas dr Soebandi, Jember, Indonesia
[2]Drug Utilisation and Discovery Research Group, Faculty of Pharmacy, Universitas Jember, Jember, Indonesia

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Abstract. Jackfruit (*Artocarpus heterophyllus* Lamk), especially the flesh usually consumed in a ripe state. The jackfruit peel is a non-edible waste. The research aims to determine the effect of different extraction methods on the total phenolic content and total flavonoid content of jackfruit peel extract. Extraction methods are percolation, maceration, and digestion. The total phenolic content (TPC) and total flavonoid content (TFC) using a spectrophotometer with $AlCl_3$ and Folin-ciaocalteu reagents. Rutin and gallic acid as a standarts. The data from various extraction methods were analyzed statistically by One-Way Anova. The results showed TPC values by percolation, maceration, and digestion respectively 5.94 ± 0.92; 3.03 ± 0.16 and 2.72 ± 0.22 (GAE mg/g). The TFC respectively 13.04 ± 0.38; 5.49 ± 0.33 and 4.16 ± 0.28 (RTE mg/g). Statistical analysis showed that there were significant differences ($p < 0.05$) in the levels of flavonoids and total phenolics between various extraction methods from the ethanol extract of jackfruit peel. The percolation method showed the highest TPC, TFC, and yield rather than other methods.

Corresponding Author :
Lindawati Setyaningrum
Bachelor in Pharmacy Program, Faculty of Health Science, Universitas dr Soebandi, Jember, Indonesia
Email : linda.w.setyaningrum@uds.ac.id

1. Introduction

Indonesia is a country with a diversity of medicinal plants and has the potential to be developed as a source of new drugs. Secondary metabolic compounds extracted from plants have been reported effective in preventing or treating chronic diseases, such as natural antioxidants that can prevent

oxidative stress or cell damage [1-2]. The leaves are commonly used as traditional medicine, but the fruit, flowers, seeds, and even the peel also contain lots of bioactive compounds. Sundai lime peel ethyl acetate fraction has antioxidant activity against DPPH caused by flavonoids and phenolic compounds.

Jackfruit (*Artocarpus heterophyllus* Lamk) is a fruit that is usually consumed in a ripe state, especially the flesh. The unripe (young) jackfruit can also be cooked as a vegetable. The part of the jackfruit such as the peel and central axis is a non-edible waste [4-6]. The right way to reduce disposal costs of non-edible waste is the utilization of by-products, which will increase economic value and environmental protection [7-9]. The ethanol extract of jackfruit peel reported contains a group of alkaloids, flavonoids, phenols and flavonoids terpenoids. One of them is stigmasterol which is a flavonoid group. Jackfruit peel reportedly contains pectin, cellulose, ferulic acid, 4-hydroxybenzoic acid, tryptophan N-glucoside and caffeic acid [10-12].

Jackfruit peel extract has a higher concentration of total phenolics and flavonoids than pulp, flakes, or seed extract. It also has the strongest glucosidase inhibitor and DPPH and ABTS scavenging abilities [13]. The ethanolic extract of jackfruit peel had an antioxidant activity with an IC_{50} of 87.09 g/ml [14]. The total value of polyphenols and flavonoids is correlated with their antioxidant activity, mainly due to the redox properties that allow phenolic compounds to act as reducing agents or hydrogen donors [15-16]. The jackfruit peel was suggested as a new source of natural antioxidants.

The main step in obtaining bioactive compounds from natural materials is extraction. Extraction is the process of separating active secondary metabolites from plants using suitable solvents, commonly used to quantify or isolate compounds. Several factors affect the level of bioactive metabolites in the extract including the total phenolic and flavonoid content. The selection of extraction methods and solvents is a critical point in producing extracts with high levels of bioactive compounds [17-18]. To standardize extracts and increase yield, it is important to select an appropriate extraction method because the different extraction methods will produce different yields and bioactivity. Conventional methods like maceration, percolation, infusion, decoction, hot continuous extraction, etc., are often used for extraction [19-20].

Maceration is one of the simple methods commonly used to extract the metabolite bioactive and this method is commonly used for the extraction of thermolabile compounds. A modification of the maceration method is digestion where extraction involves churning and low heat (Kinetic maceration). The other method is percolation which is extraction using a percolator with constant solvent changes during the extraction process. Research on the effect of different extraction methods on the total phenol and total flavonoid content of jackfruit peel has never been reported. Therefore, to find the effect of different extraction methods to the yield, the total phenol and the total flavonoid content of jackfruit peel ethanolic extract, the present investigation was carried out.

2. Experimental Section

2.1. Materials

Jackfruit collected from a local market in Jember, East Java. Ethanol 96 %, rutin (Sigma), Gallic acid (Sigma), Follin ciaocalteu (Merck), $AlCl_3$, CH_3COONa anhydrous, sodium carbonate, methanol, distilled water.

2.2. Instrumentation

A macerator container, percolator, separating funnel, glassware, volumetric flask, filler, UV-Vis spectrophotometer, and rotary evaporator.

2.3. Methods

2.3.1. Sample Preparation

The jackfruit is peeled and separated between the skin, flesh, and seeds, then cut into small pieces and washed with clean water. The next process is to dry the peel in an oven at a temperature of 50 ℃ until dry then mashed and sieved through a 30 mesh. Dried jackfruit peel powder is ready to be extracted.

2.3.2. Extraction

2.3.2.1 Maceration

200 g jackfruit peel dried powder mixed with ethanol 96 % (1:10) in a macerator container, soaked for 3 days with continuous stirring. The liquid extract is separated from the residue by filtration and then concentrated using a rotary evaporator. The yield was calculated.

2.3.2.2 Percolation

Soaking 60 g of jackfruit peel dried powder with 96% ethanol for 24 hours then putting it into the percolator and percolating with 96% ethanol (1:10). The supernatant was concentrated with a rotary evaporator then the yield was calculated.

2.3.2.3 Digestion

Mixed 100 g of jackfruit peel dry powder with 1 L ethanol 96 % (1:10). Extraction was carried out at 50 ℃ with a rotation speed of 120 rpm (Kinetic maceration- digestion) [21]. The supernatants separated from the residue by filtration and then concentrated using a rotary evaporator, and calculated the yield.

2.3.3. Analysis of Total Phenolic Content

Total phenolic test using Folin-Ciocalteu reagent. The TPC value is expressed as milligrams of gallic acid equivalent (mg GAE/g extract) [22-23]. 0.1 ml from 1.000 ppm extract solution was mixed with 7.9 ml of distilled water and 0.5 ml of Folin-ciaocalteu reagent for 15 minutes before adding 1.5 ml of $Na2CO_3$ solution and incubating for 30 minutes. The absorbance was measured at 760 nm with a UV Vis spectrophotometer and compared to the gallic acid calibration curve. The test was three replicated

2.3.4. Analysis of Total Flavonoid Content

Total flavonoid levels were measured using a colorimetric method using $AlCl_3$ [24-25]. The sample was made at a concentration of 10,000 ppm and then 0.5 mL mixed with 1.5 mL methanol, 0.1 mL $AlCl_3$ solution, 0.1 mL 20% Na acetate anhydrous, and 2.8 mL of distilled water, homogenized. The mixture was incubated for 30 minutes and the absorbance was measured at 421 nm. The total flavonoid content was calculated as rutin equivalenst (mg rutin/g extract). This test was three replicated.

2.3.5. Statistic Analysis

Results are expressed as mean ± standard deviation (SD) of three replicates. Regression analysis to calculate the TPC and TFC. Data analysis between extracts with various methods was carried out using one-way ANOVA.

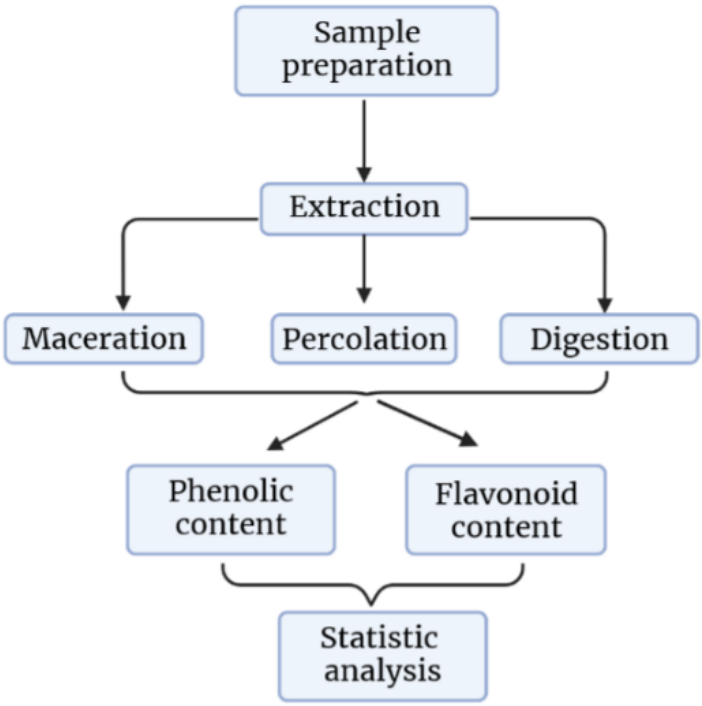

Figure 1. Design experimental

3. Results and Discussion

Biologically active substances are typically found in small amounts in plants. An effective extraction technique can produce high yields of extracts with minimal changes in the extracts' properties. Several studies reported that different extraction techniques influence the biological activity also the levels of compounds in the extract, including total phenolic and total flavonoid levels. Selecting the appropriate extraction method is necessary.

Total phenolics and total flavonoids are secondary metabolites that have the potential as antioxidants. Jackfruit peel has high total phenolic and total flavonoid content [13]. There was a high correlation between the extraction method and total phenolic content on antioxidant activity [26]. The higher the total phenolic content in extracts will increase an antioxidant activity.

The jackfruit peel extraction in this study used maceration, percolation, and digestion methods (kinetic maceration) with ethanol 96 % as solvent. Ethanol is a universal solvent and can dissolve a large number of secondary metabolites. It is also less toxic than other organic solvents and more volatile [27]. Other solvents such as water, methanol, ethanol, and acetonitrile even cyclohexane could be used in the extraction of phenolic compounds [28-30] or a combination of several solvents.

The highest yield was obtained from extraction using the percolation method, followed by maceration and digestion. The different extraction methods impacted the yield describes in figure 1.

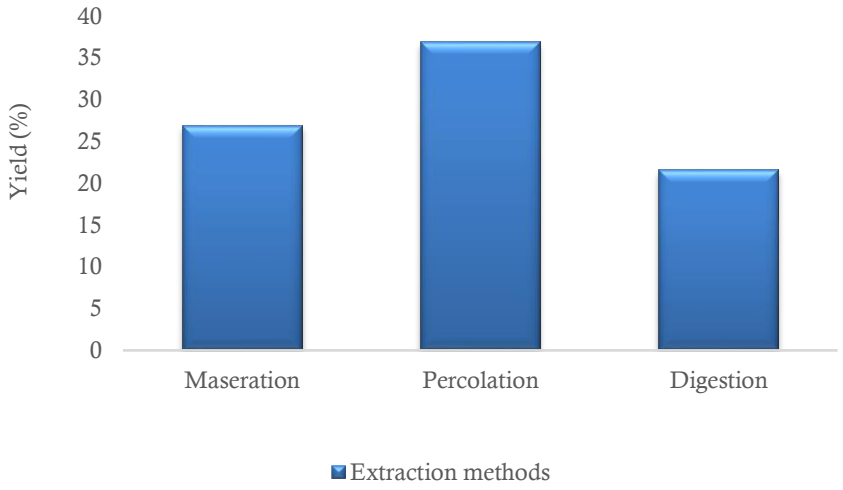

Figure 1. The yield of the jackfruit peel extract in various methods of extraction

In the percolation method, the sample is moistened with solvent several times and then put into the percolator and the solvent is allowed to flow from top to bottom until the active ingredients are extracted. Solvents can be used up to their saturation point [31-32]. The benefit of this percolation method is the extraction of secondary metabolites is more optimal because the solvent flows constantly and is always new, increasing the degree of concentration difference and the yield higher than other methods. Similar to Safitri et al [33] and Anwar et al, (2022) [34] who reported that the yield of extract by the percolation method produced the highest yield compared to other extraction methods.

Digestion is maceration with kinetic and heat presence at a low temperature. Digestion is an option when extraction at a high enough temperature will damage the compounds in the sample [35-36] but extraction time is faster than maceration so secondary metabolites may not be extracted completely. In this study, the digestion methods showed the lowest yield. A high yield value indicates that a large amount of extract is produced, but quantity does not always correlate with extract quality [37-38].

To determine the total phenolic content (TPC), the first step is to calculate the standard curve equation using gallic acid as a standard. The regression equation obtained is $y = 0.00402 x + 0.0095$ with the correlation coefficient ($r = 0.9993$) shown in Figure 2. Gallic acid is a phenolic compound that is stable, pure, cheap, and widely available [39-41]. Suitable for use as an assay standard. The Folin-Ciocalteu reagent was used to determine TPC levels. The principle is the reducing power of phenolic hydroxyl groups. A phenolic compound with an aromatic core can reduce phosphotungstic phosphorus to molybdenum blue [42]. The total phenolic content expressed in GAE is the number of milligram equivalents of gallic acid per gram of sample.

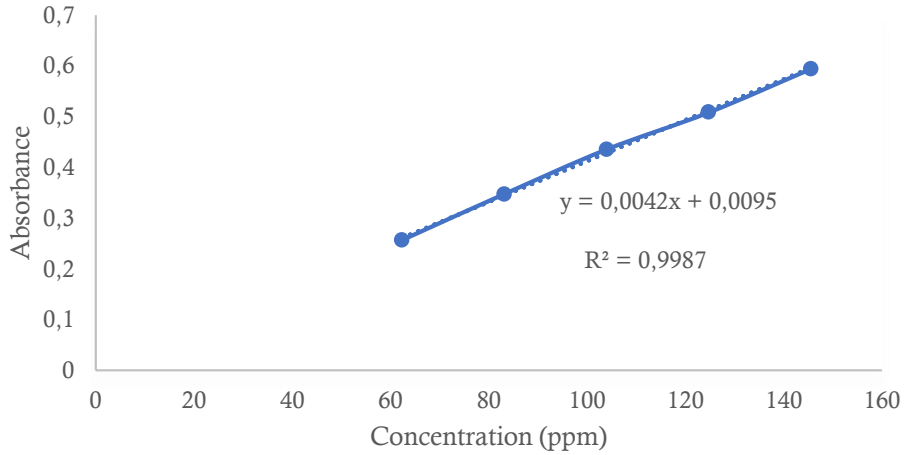

Figure 2. Standard curve equation of gallic acid

The total phenolic and total flavonoid content of ethanol extract of jackfruit (*Artocarpus heterophyllus*) peels with various extraction methods are described in Table 1.

Table 1. Total phenolic and total flavonoid content of ethanol extract of jackfruit (*Artocarpus heterophyllus*) peels with various extraction methods

Methods	Organoleptic	Total Phenolic (GAE mg/g)	Total Flavonoid (RTE mg/g)
Maceration	thick extract, brownish green, and specific aroma	3.03 ± 0.16 [a]	5.49 ± 0.33 [d]
Percolation		5.94 ± 0.92 [b]	13.04 ± 0.38 [e]
Digestion		2.72 ± 0.22 [c]	4.16 ± 0.28 [f]

GAE = gallic acid equivalent; RTE = rutin equivalent;
Values with different superscripts in one column show significant differences ($p < 0.05$).

Based on table 1, the highest TPC was obtained in the percolation method, followed by maceration and digestion. The results are in agreement with the TPC of bay leaf extract [39] and beluntas leaf methanol extract [33] that showed the percolation methods have the highest TPC rather than other methods. The lowest TPC value in the digestion method indicates that the phenolic compounds are thermolabile, and heating during the extraction process affects the degradation and destruction of these phenolic compounds. In the maceration method (cold extraction), the solvent does not change continuously, it is affected by solvent saturation and the phenolic in a sample may not be completely extracted [31].

Total flavonoid levels are measured by colorimetry, based on the formation of color complexes. $AlCl_3$ forms complexes with the hydroxyl group on flavonoids. The total flavonoid content (TFC) expressed in RTE, which is the number of milligram equivalents of rutin per gram of sample The total flavonoid content is calculated using the regression equation y = 0.0031 x + (-0.0151) with correlation coefficient = 0.9996 using rutin as a reference shown on Figure 3 below.

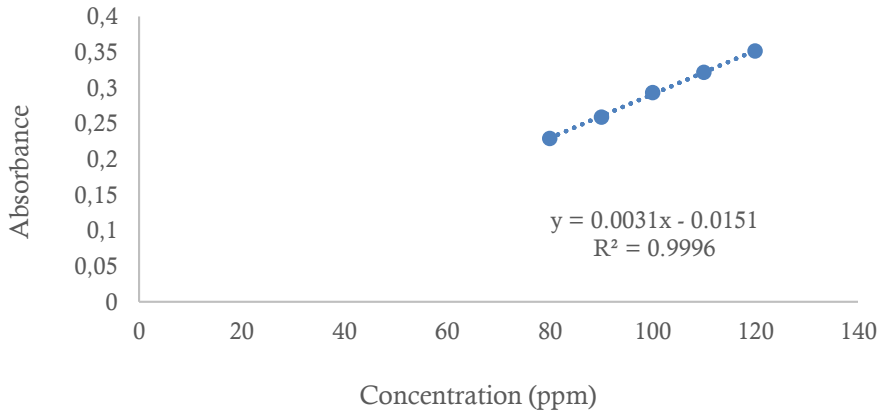

Figure 3. Standard curve equation of rutin

The result of the determination of TFC (table 1), showed relatively similar to TPC that the percolation method showed the highest TFC compared to other methods. The different extraction methods resulted in different total phenol and total flavonoid values. Data on TPC and TFC were then analyzed statistically using One-Way Anova. The results showed that the levels of flavonoids and total phenolic ethanol extract of jackfruit peel with various extraction methods have differences significant with a significance value <0.05.

Maceration and percolation are cold extraction methods, whereas digestion is a heating and stirring modification of maceration. Settharaksa et al [43] reported that temperature and heating during the extraction process can affect the levels of flavonoids and phenolic compounds. The heating process either directly or indirectly can affect the levels of these two compounds. This research showed a correlation between the levels of total phenol, total flavonoids, and the yield of each extract. Extracts by percolation method had the highest levels of TPC, TFC, and yield compared to the maceration and digestion methods. However, several studies have reported that the amount of yield with the levels of secondary metabolites extracted using different methods was unrelated [39-40]. Extraction method, extraction temperature, extraction time, and solvent selection are factors that affect the quality of the extract. Optimization is needed to get the expected active compounds.

Flavonoids may be phenolic compounds that contribute to various biological activities such as antioxidants [44] but there are many other phenolic compounds including saponins, terpenoids, etc. Phenolic compounds can prevent cell damage because of their ability to scavenge free radicals with reduction mechanisms and electron donors [45-46]. Selection of the appropriate extraction method is the first step for large-scale process implementation to produce high-quality active compounds.

4. Conclusion

The research shows that different extraction methods were applied on the same plant with the same solvent affect significantly in terms of the parameters evaluated. This might be useful in deciding on the best extraction technique for a later assessment of biological activity. The percolation method showed the highest TPC, TFC, and yield rather than maceration and digestion. This method can be developed for the extraction of Jackfruit peel.

5. Acknowledgement

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