Isolation of Flavonoid from Jackfruit Leaves (*Artocarpus heterophyllus* Lamk.) and its Antioxidant Activity

***Soraya Riyanti, Clara Sunardi, Sana Nurul Falah**

Departement of Pharmacy, University of Jenderal Achmad Yani,
Jalan Terusan Jendral Sudirman, PO BOX 148, Cimahi, Jawa Barat, Indonesia

Abstract

The flavonoid compound was successfully isolated from jackfruit leaves (*Artocarpus heterophyllus* Lamk.). Extraction was done by maceration with methanol-water (9:1), followed by methanol-water (1:1) as a solvent. Hydrolyzed extract was obtained by adding of 2NHCl, then extracted with ethyl acetate and monitored by thin layer chromatography (TLC) with n-hexane-ethyl acetate (3:5) as a mobile phase. Separation was performed by column chromatography, followed by preparative thin layer chromatography. Purity of isolates was determined by 2-dimensional thin layer chromatography. Characterization of isolate were carried out by ultraviolet-visible spectrophotometry and infrared spectrophotometry. The result showed a maximum absorbance of bands I at 328.4 nm and 271 nm for bands II. Characterization of isolate by infrared spectrophotometry showed the presence of functional groups OH, CH, C = C, C = O, CC, and CO. Based on the data, the isolate identified as a flavonoid compound. Antioxidant activity of ethyl acetate fraction and column fraction by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay showed EC_{50} at 60.53 mg/mL and 35.27 µg/mL respectively.

Key words: *Artocarpus heterophyllus* Lamk., flavonoid, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), EC_{50}.

Abstrak

Senyawa flavonoid berhasil diisolasi dari daun belimbing (*Artocarpus heterophyllus* Lamk.). Ekstraksi dilakukan dengan cara maserasi menggunakan metanol-air (9:1), diikuti dengan metanol-air (1:1) sebagai pelarut. Hasi dari ekstrak yang terhidrolisis didapatkan dengan menambahkan 2NHCl, kemudian diekstraksi dengan etil asetat dan diamati menggunakan kromatografi lapis tipis (KLT) dengan campuran n-heksana-etil asetat (3:5) sebagai fase gerak. Pemisahan dilakukan menggunakan kromatografi kolom yang dilanjutkan dengan kromatografi lapis tipis preparative. Kemurnian dari isolat ditentukan dengan cara kromatografi lapis tipis 2-dimensi. Karakterisasi isolat ditunjukkan dengan spektrofotometer UV-Vis dan inframerah. Hasil spektrum menujukkan adanya absorbansi maksimum dari pita I pada 328,4 nm dan 271 nm untuk pita II. Karakterisasi dari isolat menggunakan spektrofotometer inframerah menunjukkan adanya gugus fungsional OH, CH, C=C, C=O, CC, dan CO. Berdasarkan data tersebut, isolat teridentifikasi sebagai senyawa flavonoid. Aktivitas antioksidan dari fraksi etil asetat dan fraksi kolom menggunakan pengujian 1,1-difenil-2-pikril-hidrazil (DPPH) menunjukkan EC_{50} pada 60,53 mg/ml dan 35,27 µg/mL.

Kata kunci : *Artocarpus heterophyllus* Lamk., flavonoid, 1,1-difenil-2-pikril-hidrazil (DPPH), EC_{50}

Introduction

Indonesia is a country that has biodiversity, including a plant that can be used for medication. Efforts in caring for and maintaining the health of natural materials are increasingly in demand, so there was more natural ingredients introduced to the public.

One of the plants as a potential source of bioactive compound and abundance in Indonesia is *Artrocarpus heterophyllus* Lamk. Moraceae tribe. *Artrocarpus heterophyllus* Lamk. or known as jackfruit trees are widely in the yard, field, or sometimes growth on wild type. Leaves of jackfruit tree usually used for ulcer, abscess. Chemical content on leaves known as alkaloids, saponins, flavonoids, glucosides, tannins, and Ca oxalate (Chackrewarthy 2010; Heyne 1987; Robinson 1995).

Flavonoid compounds are very beneficial in food because belongs to phenolic compound groups, which are potential as antioxidants agent. Many condition of illness are known to get worse by the presence of free radicals such as superoxide and hydroxyl, and flavonoids have the ability to remove and effectively 'sweep up' free radicals known as the destructive oxidizing species. Therefore, foods which have flavonoids content is important to treat diseases, such as cancer and heart disease (Markham 1988).

Chandrika *et al*. (2006) interpretated that the jackfruit leaves effective as an antioxidant for diabetes. Ethyl acetate fraction of jackfruit leaves showed the effect to hyperglycemia by 49% higher compared with tolbutamide by 27%. This activity is mediated mainly by flavonoids, which have a higher solubility in ethyl acetate. In addition, based on preliminary test results

*Corresponding author. e-mail: soraya_riyanti@yahoo.co.id

showed the presence of flavonoids in crude drug of jackfruit leaf.

Methods

The research was carried out starting from the preparation of materials which including plant determination, collecting, processing into crude drug characterization phytochemical screening, extraction, hydrolysis, hydrolysis monitoring, and characterization of isolate, and antioxidant tested for ethyl acetate fraction and the column fraction (Harborne 1987; Gritter *et al.* 1991).

Extract preparation was done by maceration, using methanol water (9:1), followed by using methanol water (1:1) as a solvent and concentrated. Viscous methanol-water extract were obtained hydrolyzed with methanol HCl (1:1), and continuing with liquid-liquid extraction using the solvent ethyl acetate water (1:1) to obtain the fraction of water and ethyl acetate fraction.

Ethyl acetate fraction was purified by column chromatography with silica gel 60 as a stationary phase and gradient polarity of mobile phase (n-hexane-ethyl acetate). Characterization of isolates was done by using ultraviolet-visible spectrophotometry in the 200-400 nm, and infrared spectrophotometry.

The antioxidant activity the fraction known by determining maximum absorbance at a 516.0 nm of wavelength.

Results and Discussion

In this research, jackfruit (*Artocarpus heterophyllus Lamk*) obtained from the yard in Gunung Batu area, Cimahi. Taxonomic determination was done in the Herbarium Bandungense School of Life Sciences and Technology Institut of Teknologi Bandung (ITB). Results showed that the plant is jackfruit (*Artocarpus heterophyllus* Lamk.), belonging to the tribe and species *Artocarpus heterophyllus* Moraceae Lamk.

Column chromatography was carried out to obtain fractions. From the results obtained 128 column chromatography fractions (F1-F128) and then each fraction monitored by TLC and spots that has fluorescence blue with the same Rf fractions are combined in order to get combined. Fraction which has a spot that is F2, F3, and F4, while the fraction which has a dominant spot is F2. The mixture fraction F2 was carried out for further separation by preparative thin layer chromatography (PTLC). PTLC plate observe under 365 nm and showed fluorescence light blue band, Rf of 0.56. The purity test was done by two-dimensional TLC, with different systems mobile phase. The results showed one fluorescence blue spot on the chromatogram, which concluded that the isolate was pure.

Table 1. The Results of Screening Phytochemistry Jackfruit Leaf Extract and Fractions

Group of Compound	Result			
	Simplicia	Metanol Extract	Etil asetat Fraction	Water fraction
Alkaloid	-	-	-	-
Flavonoid	+	+	+	+
Quinon	+	+	+	-
Tannin	+	+	-	-
Polyphenol	+	+	+	+
Saponin	+	+	-	-
Steroid and Triterpenoid	+	+	-	-
Monoterpenoid and Seskuiterpenoid	+	+	+	+

Description: (+) Shows the components of the analyzed substance, (-) Shows no signs of component substances analyzed.

Table 2. Measurement of absorption spectra isolates with ultraviolet spectro-UV visible

Band	Wavelength of Isolates methanol (nm)	Range of Spectrum UV-Visible (nm)	Absorbance (A)	Type of Flavonoid
I	328,4	310-350	0,072	Flavon
II	271	250-280	0,13	

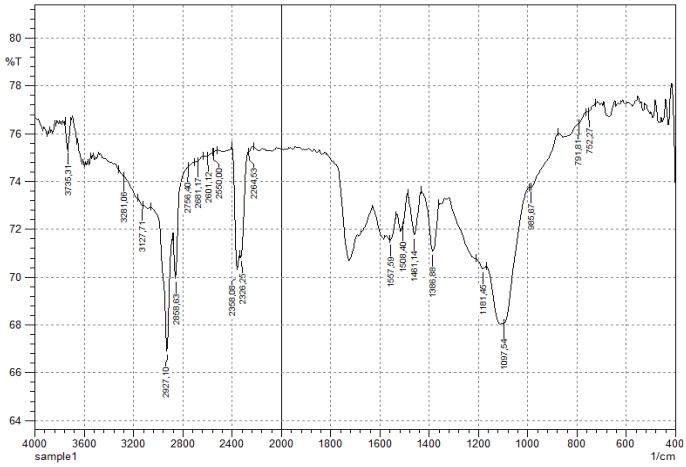

Figure 1. Infrared spectrum

Table 3. Results Characterization of Simplicia Jackfruit Leaves

Type Characterization	Values (b/b)
Determination of Ash:	
a. Total Ash	9.06±0.045 %
b. Water Soluble Ash	3.925±0.375 %
c. Acid Insoluble Ash	5.265±0.0636
Determination of Extract :	
a. Water Soluble Extract	11.52±0.88 %
b. Ethanol Soluble Extract	17.36±0.08 %
Water content	5.33±0.9428 % v/b

Characterization of isolates was done by using ultraviolet-visible spectrophotometry in the range of wavelenght at 200-400 nm and infrared spectrophotometry. The result showed maximum absorbance at 328.4 nm (bands I, A= 0.072) and 271 nm (band II, A = 0.13), that indicated a presence of flavonoid compound (Markham 1988).

Addition of NaOH reagent, showed bathochromic shifting from 328.4 nm to 387.2 nm, and the bathochromic shifting bands II from 271 nm to 294 nm. After 5 minutes, there were no wavelength shifting both bands I and II. Bathochromic shifting in NaOH showed the presence of OH group on the 4' of carbon.

There were no hipsochromic shifting of band I after adding of HCl, and hipsocromic shifting of band II from 271 nm to 256 nm that showed a presence of OH group on the prenyl group at 5 and 6 position of carbon.

There was no bathochromic shifting on band I by adding H_3BO_4. Bathochromic shifting from 271 nm to 274.6 nm showed on band II. This results showed a presence of ortho dihydroxy on ring A (at position 6, 7 or 7, 8).

Characterization by infrared spectrophotometry showed the presence of OH functional groups on the spectrum wave numbers 3127.71 (cm^{-1}), CH bond at 2927.10 spectrum wave numbers (cm^{-1}), C=C bond at 1557.50 spectrum wave numbers (cm^{-1}), the C=O at 1725.00 spectrum wave numbers (cm^{-1}), CC group at 1181.45 spectrum wave numbers (cm^{-1}), and carbonyl groups CO at 1097.54 spectrum wave numbers (cm^{-1}).

Antioxidant activity of ethyl acetate fraction and the column fractions showed EC_{50} value at 60.53 mg/mL and 35.27 mg/mL respectively.

Conclusion

Jackfruit leaves contained secondary metabolites that were identified as flavonoids, polyphenols, tannins, saponins, quinones, steroids, triterpenoids, monoterpenoid and sesquiterpenoid. Characterization of isolate were carried out by Ultraviolet-Visible Spectrophotometry and Infrared Spectrophotometry. The result showed maximum absorbance of band I at 328.4 nm and 271 nm for bands II. Characterization of isolate by infrared Spectrophotometry showed the presence of functional groups OH, CH, C=C, C=O, CC, and CO. Based on the data, the isolate was identified as

a flavonoid compound. Antioxidant activity of ethyl acetate fraction and column fraction by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay showed EC_{50} at 60.53 mg/mL and 35.27 mg/mL.

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