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Research Article

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Extraction and simple characterization of anthocyanin compounds from *Rubus rosifolius* Sm fruit

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ABSTRACT

Rubus rosifolius Sm is a species of the genus Rubus, Rosaceae family. Rubus rosifolius Sm fruit was thought to contain anthocyanin from phytochemical test results. Extraction of Rubus rosifolius Sm fruit has been performed with maceration method by acidified ethanol with acetic acid to pH 1. Characterization of anthocyanins was carried out with UV-Vis spectroscopy method, to observe the stability of anthocyanin against pH and temperature, and to characterize of anthocyanins by HPLC-DAD method. Rubus rosifolius Sm fruit was thought to contain dominant anthocyanin compounds pelargonidin type, anthocyanin compounds in the fruit of Rubus rosifolius Sm can be applied as an alternative as dyes in foods that have acidic pH. Extraction of anthocyanins in Rubus rosifolius Sm fruit with acidified ethanol with acetic acid was more stable compared acidified ethanol with citric acid.

Keywords: *Rubus rosifolius* Sm, anthocyanin, extraction, characterization, HPLC-DAD

INTRODUCTION

Rubus rosifolius Sm is a species of the genus *Rubus*, Rosaceae family, has the form of thorns, edge serrated leaves, white flowers and red fruit. The leaves are oval with pointed tip. Local names of plants *Rubus rosifolius* Sm are bereretean (Java), gunggung (Bali), and sickle (Borneo). In general, *Rubus rosifolius* Sm grows in open areas, forest edge, or the edge of the river [1].

Figure 1. Picture of *Rubus rosifolius* Sm Plant

Rubus rosifolius Sm fruit was thought to contain anthocyanin because the color of the fruit is red. The possibility of anthocyanin compounds contained in the *Rubus rosifolius* Sm fruit was also confirmed by the positive results of the test on the flavonoid phytochemicals. Based on phytochemical test data, *Rubus rosifolius* Sm fruit was expected to be a source of considerable potential of anthocyanin.

Anthocyanin is a water-soluble compounds and one class of flavonoids compounds. Generally, the color of anthocyanin pigments are red, blue, and violet. They are usually found on flowers, fruits, and vegetables [2]. Anthocyanin extraction process from the plant was usually perfomed by using a solvent extraction process [3], solvent extraction has become the most common method for the extraction of many compounds found in fruits, including flavonoids [4].

In this study, we investigated the major anthocyanin compounds in the fruit of *Rubus rosifolius* Sm with two solvents, ethanol-acetic acid and ethanol-citric acid, based on the properties of anthocyanins. Anthocyanin structure was identified by UV-VIS spectroscopy method, the effect of pH stability and analytical HPLC-DAD method.

EXPERIMENTAL SECTION

Sample plant material

Rubus rosifolius Sm fruit was obtained from Hiang village, Kerinci, Jambi, Indonesia. The identification was carried out in Herbarium Laboratory of Andalas University (ANDA). The identification number is 267/K-ID/ANDA/X/2014.

Chemicals

HPLC-grade water, methanol, ethanol, acetonitrile, acetic acid, citric acid, hydrocholric acid, formic acid was obtained from Merck, Germany. All other chemicals used in this study were of analytical grade.

Instrumentation

pH meter, Rotary Evaporator (Buchi), UV/VIS Spectrophotometer (Evolution 201), water bath (Buchi), HPLC (UFLC-Shimadzu), thermometer, aluminum foil and glassware commonly used in laboratories.

Procedures

1. Extraction of anthocyanins

Extraction from *Rubus rosifolius* Sm fruit used maceration method by acidified ethanol with acetic acid to pH = 1.5. 200gr sample was macerated with 250 mL solvent, and was kept for 24 hours. The result of maceration was filtered to separate the filtrate and residue. Extraction was repeated using the same solvent and the same treatment. The filtrate was combined and then the solvent was evaporated with rotary evaporator at 30°C. Furthermore, the extract was separated and stored at 4^{o}C.

2. Characterization of anthocyanin with spectrophotometer uv-vis

Characterization of the spectrophotometer was carried out by measuring the maximum wavelength of anthocyanins with spectrophotometer uv-vis. Maximum wavelength measurement was performed using a double beam spectrophotometer at wavelength of 200-800 nm.

3. Characterization of anthocyanins with the stability measurement of anthocyanin extracts

Measurement of color intensity of anthocyanin extracts was performed on six different pH conditions (1, 3, 5, 7, 9 and 11). Each solution was measured at the maximum wavelength with spectrophotometer in the area between 200-800 nm.

4. Characterization of anthocyanins by HPLC-DAD analysis

Mobile phase A: 2% formic acid and mobile phase B: acetonitrile:water:formic acid (49:49:2, v/v/v). HPLC elution system was performed by a linear gradient as follows: 0 ~ 4 min, mobile phase B increased from 6 to 10%; 8 ~ 12 min, mobile phase B increased from 10 to 25%; 12 ~ 13 min, mobile phase B (isocratic) fixed at 20%; 13 ~ 20 min, mobile phase B increased from 25 to 40%; 20 ~ 35 min, mobile phase B increased from 40 to 60%; 35 ~ 40 min, mobile phase B increased from 60 to 100% and 40 ~ 45 min, mobile phase B back to 5%. Flow rate of mobile phase was 1 mL/min and the injection volume was 100 mL, with a detection wavelength of 516 nm. UV-VIS absorption spectra was measured on-line during HPLC analysis [5].

5. Antioxidant activity test

Measurement of the activitywas determined based on Brand-Williams method [6] with slight modifications. Mixed solution containing 20 µL, 40 µL, 60 µL and 80 µL of extract and 2.0 ml of 0.1 mM DPPH solution was prepared,

homogenized and then and then left in the dark environment for 30 minutes. A control sample containing the solvent was used to measure the maximum absorption of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The absorbance was measured at wavelength of 517 nm.

The antioxidant activity of the extract sample was calculated by the formula:

$$\text{antioxidant activity } \% = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \text{ x } 100\ \%$$

Of the activity percentage of antioxidant or free radical scavenging percentage, it was inserted into the linear regression equation for determining the EC50 values.

RESULTS AND DISCUSSION

1. Extraction of anthocyanins

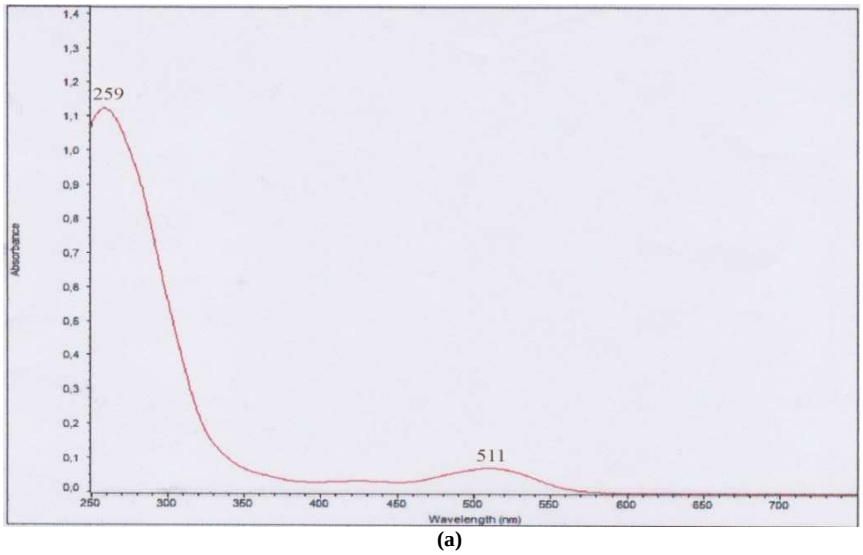
Extraction of anthocyanin from *Rubus rosifolius* Sm fruit maceration was carried out by using acidified ethanol with citric acid and acidified ethanol with acetic acid to pH 1. Extraction was performed by maceration because this method is relatively simple and does not need heating, because anthocyanin vulnerable to high temperature. Maceration process was done in dark environment because anthocyanins are very easily oxidized in the presence of light.

Solvent extraction of anthocyanins was the first step in the determination of total and individual anthocyanins before quantification, purification, separation, and characterization of [7] and generally involves the use of acidified methanol or ethanol. The use of acid will stabilize anthocyanin in flavylium cation form, which is red at low pH [8]. Selection of ethanol in this process because the nature of ethanol are non-toxic when later applied in food. The use of organic acids such as acetic acid, citric acid or tartaric acid in the extraction of anthocyanins intends to avoid damage to anthocyanin because of hydrolysis of glycoside bond and acylated in anthocyanins [9].

2. Characterization of anthocyanin with a spectrophotometer

From the UV-Vis spectra results of measurements of the sample extract with ethanol - acetic acid and ethanol - nitric acid with a pH of 1, it was obtained maximum peak at wavelength of 259 nm and 511 nm in the visible area (fig. 2).

The appearance of two absorbance peaks from extracts of the samples showed the presence of anthocyanins. Anthocyanins as a group of flavonoid indeed had the same absorption pattern with the flavonoid. Anthocyanins showed two absorbance peaks at wavelengths of 200-800 nm. They were identified in the uv range (250-280 nm) which represents the benzoyl group and in the visibel range (490-550 nm) which represents cinnamoyl groups [10]. Concentration of anthocyanins in the extract was calculated by differential pH method [11]. The total concentration of anthocyanin obtained from ethanol - acetic acid extract was 86,83 mg/L and from ethanol-citric acid extracts was 57,78 mg/L. The result obtained from ethanol with acetic acid was higher than ethanol with citric acid. It was caused by the acidity of the acidified ethanol with acetic acid was higher than the acidity of thenacidified ethanol with citric acid.



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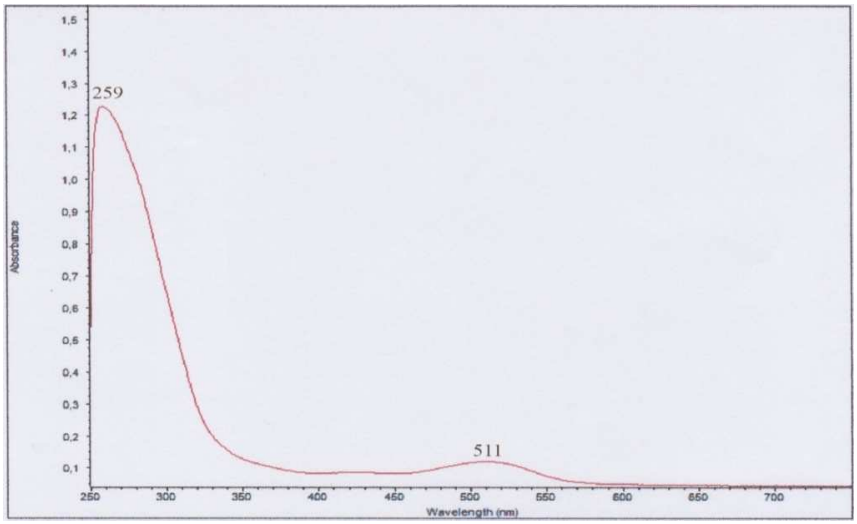


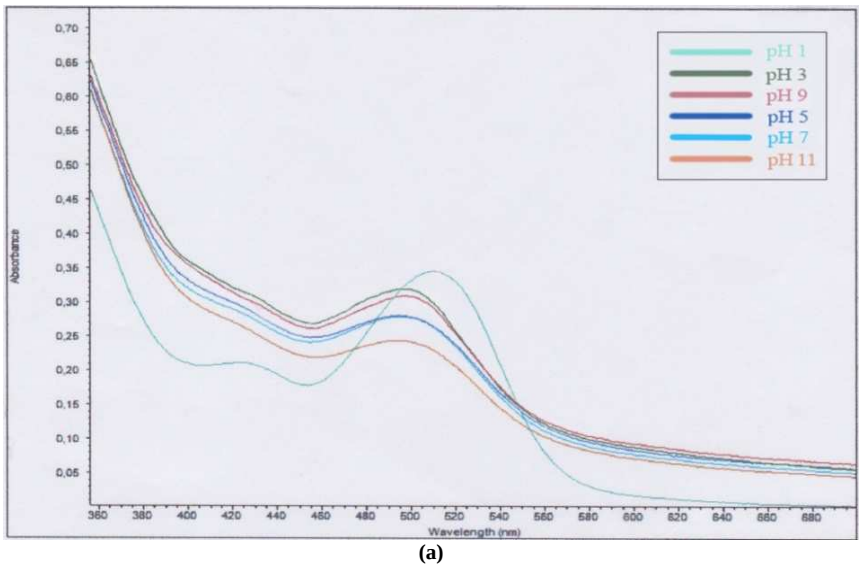
(b)

Figure 2. UV-Visible absorption spectra of anthocyanins from *Rubus rosifoliu* Sm fruit (a) ethanol-acetic acid solvent, (b) ethanol-acetic acid solvent

3. Characterization of anthocyanins with measurement stability of anthocyanin extracts

Characterization of anthocyanins by the measurement of anthocyanin extracts stability was conducted on the effect of pH. pH treatment given was pH 1, 3, 5, 7, 9 and 11. The color of anthocyanin extracts at various pH conditions provided different colors, which followed by the difference in the maximum wavelength and absorbance of the extract. Observations from each pH treatment can be seen in Figure 3. In the sample, max λ for ethanol - acetic acid pH 1 was at 511 nm with absorbance of 0.344, whereas at pH 3 – 11, max λ shifted to 500 nm, reduction of maximum wavelength shift was caused by the resonance of electrons that shortened, while the absorbance obtained at pH 3, 5, 7, 9, and 11 were 0.320, 0.276, 0.276, 0.309 and 0.240, respectively. As for the citric acid - ethanol extract the max λ obtained equal to ethanol - acetic acid extract but had difference in absorbance, for pH 1-11 absorbance obtained were 0.270, 0.215, 0.152, 0.147, 0.161 and 0.120, respectively. At pH ≤ 2, anthocyanins were in the most stable condition, the main anthocyanin structure was in the form of red flavilium cation [12].

Wavelength of *Rubus rosifolius* Sm fruit anthocyanins at pH 1 is 511 nm. At pH 3, 5, 7, 9, and 11, the wavelengths down to 500 nm, with increasing pH from pH 3, 5, 7, and 11, there was a decline in the value of absorbance at *Rubus rosifolius* Sm fruit anthocyanins. It showed the degradation of the anthocyanin structure. In addition to the changes in absorbance values, the degradation was also known from color change of the sample under acidic condition (pH 1) which was red, but the more alkaline condition was, the more the color will fade, on this condition, red flavilium cation degraded to colorless carbinol structure form [12].



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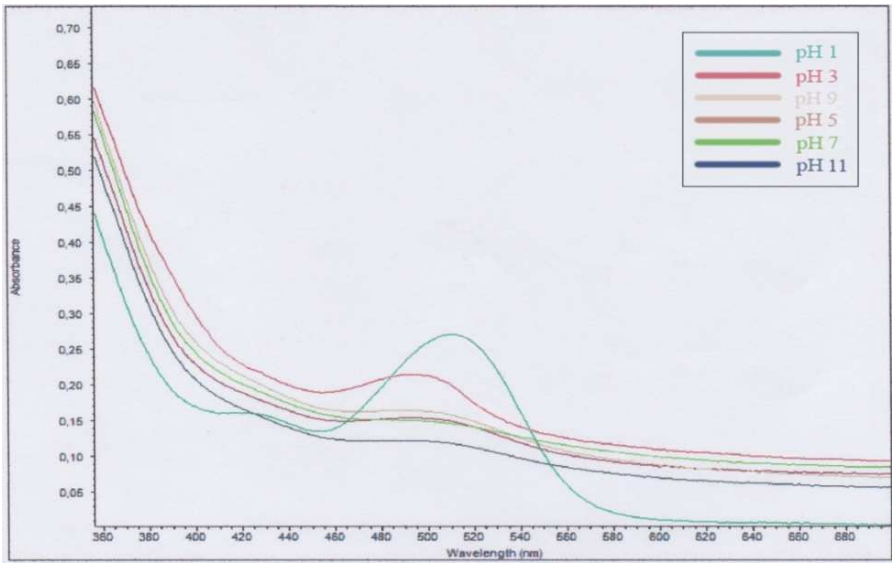
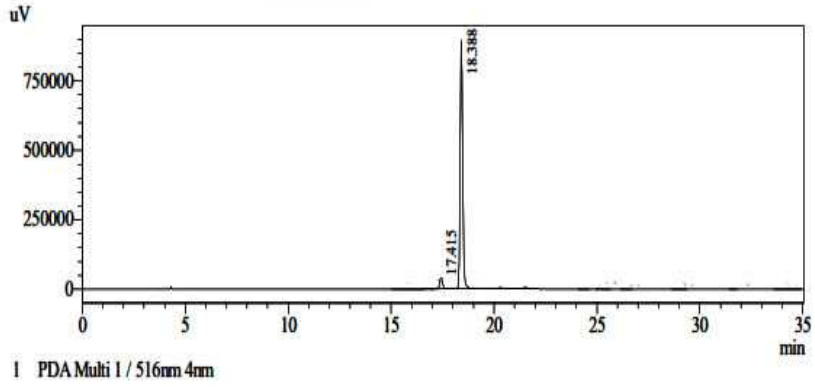


(b)

Figure 3. UV-Vis spectra of the pH effect on the anthocyanin extract of *Rubus rosifolius* Sm fruit (a) ethanol - acetic acid solvent, (b) ethanol - citric acid solvent



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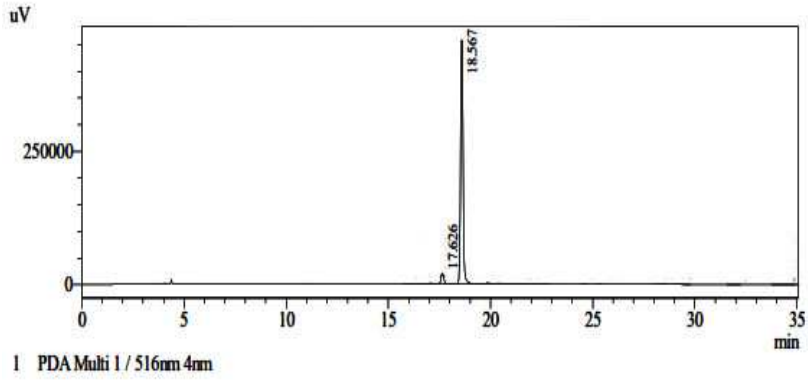

(b)

Figure 5. HPLC-DAD Chromatogram of anthocyanin extract of *Rubus rosifolius* Sm fruit (a) ethanol - acetic acid solvent, (b) ethanol - citric acid solvent

4. Characterization of anthocyanins by HPLC-DAD analysis

Based on observation from the HPLC chromatogram analysis result obtained from extracts of Rubus rosifolius Sm (fig. 5), it was identified peaks expected two anthocyanin compounds, the first peak appeared at a retention time of 17.415 minutes with a smaller size (4.8%) than the second peak which appeared at a retention time of 18.388 min (92.8%), for ethanol - acetic acid extract. As for ethanol - citric acid extract, the first peak appeared at a retention time of 17.626 minutes with a smaller size (4.6%) than the second peak appeared at a retention time of 18.567 min (93.2%). Based on the retention time of the both anthocyanin peaks, it can be predicted that they have almost the

same polarity, this might be due to the differences in sugar or acyl groups bonded to anthocyanidins (aglycone) of the anthocyanin compounds [13].

Of the dominant retention times obtained as follows: 18.388 minutes (92.8 %) and 18.567 minutes (93.2 %), it was estimated that the anthocyanins contained in *Rubus rosifolius* Sm fruit was type of pelargonidin. It was based on comparison with anthocyanin extracted from *Ficus padana* Burm.f fruit which had retention time of 18.993 minutes, contained pelargonidin 3-(6"-p-coumarylglucoside)-5-(4"-Malonylglucoside) anthocyanin compound [14]. In addition, UV-Vis data can also shown that the sample had a maximum absorbance peak at wavelength of 511 nm, equal to the wavelength of anthocyanins contained in the mulberry fruit, which is from 507 nm to 516 nm [15].

5. Antioxidant activity test

Scavenging activity of DPPH free radical from the extract samples can be expressed by EC50 parameter (Efective Concentration). EC50 is concentration of the sample that led to the capture of the free radicals by 50%. EC50 value was determined from the linear regression equation between the concentration of the sample and the average free radical scavenging percentage of each concentration.

Specifically, a compound was said to be a very strong antioxidant if EC50 value was less than 50 µg/mL, strong for EC50 worth 50-100 µg/mL, moderate if EC50 worth 151-200 µg/mL [16]. The antioxidant activity of *Rubus rosifolius* Sm fruit extracts with ethanol - citric acid gave EC50 value of 75.25 µg/mL (75.25 ppm), while for ethanol - acetic acid gave EC50 value of 49 µg/mL (49 ppm). This showed that the ethanol - acetic acid extract had antioxidant activity stronger than the ethanol - citric acid extract.

CONCLUSION

From the data obtained, it can be concluded that the fruit of *Rubus rosifolius* Sm contained anthocyanins expected type of pelargonidin. Extraction of anthocyanins in the fruit of *Rubus rosifolius* Sm by ethanol - acetic acid was more stable compared to ethanol - citric acid. Anthocyanin concentration obtained were 86,83 mg/L for ethanol - acetic acid extract and 57,78 mg/L for ethanol - citric acid extract. Anthocyanins in the fruit of Rubus rosifolius Sm can be applied as an alternative to dyes in foods that have an acidic pH, because it can provide a clear color and has a very strong antioxidant activity.

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