



Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Characterization of pelargonidin compound from raspberry fruit (*Rubus rosifolius* Sm) with mass spectroscopy method

Adlis Santoni*, Hilda Amanda and Djaswir Darwis

Department of Chemistry, Andalas University, Padang, West Sumatra, Indonesia

ABSTRACT

Pelargonidin is one of the 19 anthocyanin types which have been found. Raspberry (*Rubus rosifolius* Sm) is a species of the *Rubus* genus from family of *Rosaceae*. In this research, characterization pelargonidin contained in extract of raspberries has been carried out by using HPLC-DAD-ESI-MS and also to apply the extract for drinks with different pH. The main pelargonidin contained in extract of raspberries is pelargonidin-3-rutinoside-5-malonylglucoside with a molecular ion of m/z 843 and base peak of 578 (pelargonidin-3-rutinoside). Anthocyanins from raspberry fruit extract can be applied as an alternative dyes in foods that have an acidic pH, as it gives a clear color.

Keywords: Anthocyanins, pelargonidin, *Rubusrosifolius*Sm, HPLC-DAD-ESI-MS

INTRODUCTION

Pelargonidin is one of 19 types of anthocyanin compounds that have been discovered. But there are only six well known anthocyanidins: pelargonidin, peonidin, cyanidin, malvidin, petunidin, and delphinidin. General difference between these six anthocyanidins structure is based on a variation of hydroxyl and methoxy-substituent groups at two positions in the ring-B at position 3 'and 5' [1]. Anthocyanidins are glycosides which are polihidroksiflavilium salts [2]. Anthocyanin is a water-soluble compound. Generally, anthocyanin pigment colors are red, blue, and violet, is usually found in flowers, fruits, and vegetables [3]. Anthocyanins are derived glycosides flavonoid compounds, which consists of a group of sugar (glikon), a of group of non-sugar (aglycone), and there are some containing acyl group [4].



Figure 1. Raspberry fruit(*Rubus rosifolius*Sm)

Raspberry (*Rubus rosifolius* Sm) has a form of thorns, serrated leaf edges, white flowers and red fruit with a diameter of 1.5 cm. Raspberries is known as the fruit of a group that contains a small seed with cavity on the inside of the fruit [5]. The shape of the fruit raspberry can be seen in Figure 1.

Amanda et al, (2015) reported that pelargonidin compounds had been found from the extract of raspberry which analyzed by using UV-Vis spektrosfotometer and HPLC-DAD. The maximum wavelength of UV-Vis spektrosfotometer is 511 nm while the dominant peaks in HPLC-DAD appeared at a retention time of 18.388 minutes. Pelargonidin obtained has an excellent antioxidant activity with EC50 value of 49 µg/ml (49 ppm) [5].

In this research, characterization pelargonidin contained in extract of raspberries has been carried out by using HPLC-DAD-ESI-MS and also to apply the extract for drinks with different pH.

EXPERIMENTAL SECTION

Plant sample

Rubus rosifolius Sm fruit was obtained from Hiang village, Kerinci, Jambi. Plant identification had been performed at Herbarium Laboratory of Andalas University (ANDA) with identification number 267/K-ID/ANDA/X/2014. Raspberry (*Rubus rosifolius* Sm) was sorted, the white part inside was removed, then weighed 200 g and chopped until smooth.

Chemicals

HPLC-grade water, methanol, ethanol, acetonitrile, acetic acid, hydrochloric acid, and formic acid were obtained from Merck, Germany, mineral water, soda water and all the other chemicals used in this study were analytical grade.

Instrumentations

Rotary evaporator (Buchi), HPLC-DAD-ESI-MS (Shimadzu, Lc 8030), aluminium foil, filter paper, and glassware commonly used in laboratories.

Procedures

1. Extraction of anthocyanins

Extraction of raspberry fruit was using maceration method by ethanol solvent acidified with acetic acid to pH = 1. 200 g sample was macerated with 250 mL solvent, and left for 24 hours, then the result was filtered to separate the filtrate and the residue. Extraction was repeated by using the same solvent and the same treatment. The filtrate was combined and then the solvent was evaporated by rotary evaporator at 30 °C. Afterward, the extracts were separated, 20 ml of the extract was filtered with a 0.2 micron syringe filter for the HPLC-DAD-ESI-MS analysis and the rest was stored for the others analysis.

2. Characterization of anthocyanins by HPLC-DAD-ESI-MS

At this step, agilent 6100 series HPLC-DAD-ESI-MS with 10 cm sized C18 column had been used. MS conditions used were: ESI interface, positive ions mode, nebulizer pressure of 35 psi, a dry gas flow rate of 10 L/min, a dry gas temperature of 350 °C and m/z scanning from 100 to 1000. The mobile phase used were 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 with linear gradient as follows: 0 ~ 4 min, mobile phase B increased from 6 to 10%; 8 ~ 12 minutes, mobile phase B increased from 10 to 25%; 12 ~ 13 minutes mobile phase B fixed (isocratic) at 20%; 13 ~ 20 minutes mobile phase B increased from 25 to 40%; 20 ~ 35 minutes mobile phase B increased from 40 to 60 %; 35 ~ 40 minutes mobile phase B increased from 60 to 100% and 40 ~ 45 min mobile phase B back to 5%, with a mobile phase flow rate of 1 mL/min and injection volume of 2.5 mL.

3. Application of anthocyanin pigments

The extract was applied to several soft drinks which have different pH. The soft drinks were drink with an acidic pH (5) and alkaline pH (9). Treatment of this application intended to see the changes in the color of the anthocyanin pigment in the drinks.

RESULTS AND DISCUSSION

1. Extraction of anthocyanins

Extraction of anthocyanin compounds from raspberries had been performed by maceration method by using ethanol solvent acidified with citric acid to a pH of 1. 200 g of sample was macerated with 250 ml of solvent, and left for 24 hours, the result was filtered to separate the filtrate and the residue, the filtrate was stored and then the residue was extracted again using the same solvent and the same treatment. The filtrate was combined and then the solvent was

evaporated with a rotary evaporator at 30 °C. It was retrieved concentrated extract as much as 200mL, stored at a temperature of 40 °C for the next procedure.

Anthocyanin solvent extraction is the first step in the determination of total and individual anthocyanins before quantification, purification, separation, and characterization [6] and generally involve the use of methanol or ethanol acidified. The use of acid will stabilize anthocyanin in the form of flavyliumcation, red at low pH [7]. The use of organic acids such as acetic acid, citric acid or tartaric acid intends to avoid damage to the anthocyanin which can be caused by the process of hydrolysis in glycoside bond and acylation [8].

2. Characterization of anthocyanins by HPLC-DAD-ESI-MS

Characterization of anthocyanins by HPLC-DAD-ESI-MS had been done by comparing the m/z data of the sample with the m/z data obtained from the previous anthocyanins research. The fragmentation pattern of the MS data of the raspberry extract can be seen in Figure 2.

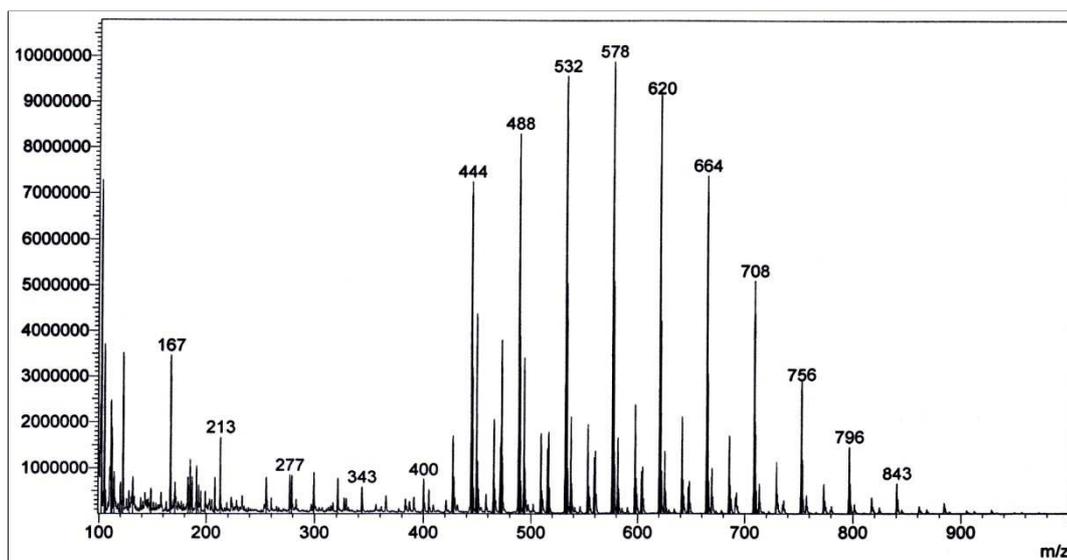


Figure 2. The data of anthocyanin compounds in extract of raspberries

MS data fragmentation pattern of raspberry fruit extract gave a molecular ion value of m/z 843 with a base peak of 578. Based on the data obtained, it shows that the anthocyanin compounds which have a molecular weight of value m/z 843 is pelargonidin-3-rutinoside-5-malonylglucoside. The structure of pelargonidin-3-rutinoside-5-malonylglucoside compound can be seen in Figure 3.

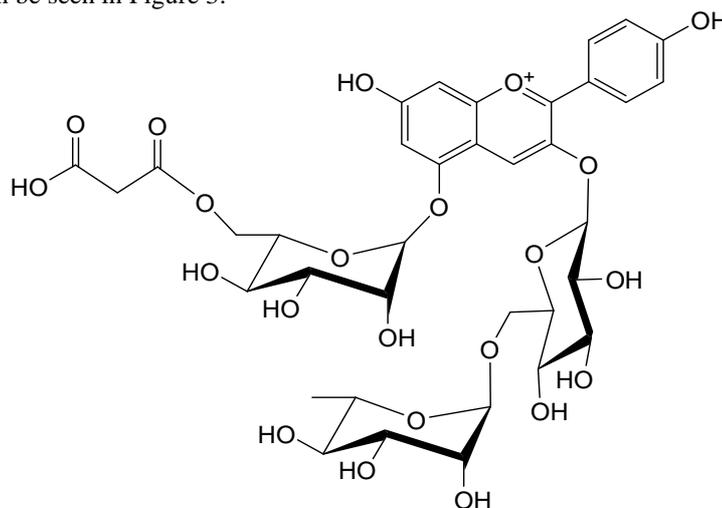


Figure 3. The structure of 3-rutinoside pelargonidin-5-malonylglucoside compound

In figure 4, it was shown the fragmentation pattern of the MS data on the compounds with the m/z value of 843, the appearance of peak 756 shows the release of Malonyl group (-87), then peak 664 indicates the release of a sugar (-

179), and the release of Malonylglucoside (-264) was shown on the peak base of 578 (pelargonidin 3-rutinoside) which can be seen in Figure 4, the fragmentation supports the proposal that the compound obtained is pelargonidin-3-rutinoside-5-malonylglucoside. In general, anthocyanin compounds contained in fruits with genus of rubus is pelargonidin 3-rutinoside and cyanidin 3-rutinoside compound [9].

Characterization of the anthocyanin structure in the extracts of by HPLC-DAD-MS method provides information about the allegation of the existing structure, to further strengthen the allegation, an NMR analysis characterization should be carried on to obtain a more accurate structure of anthocyanin.

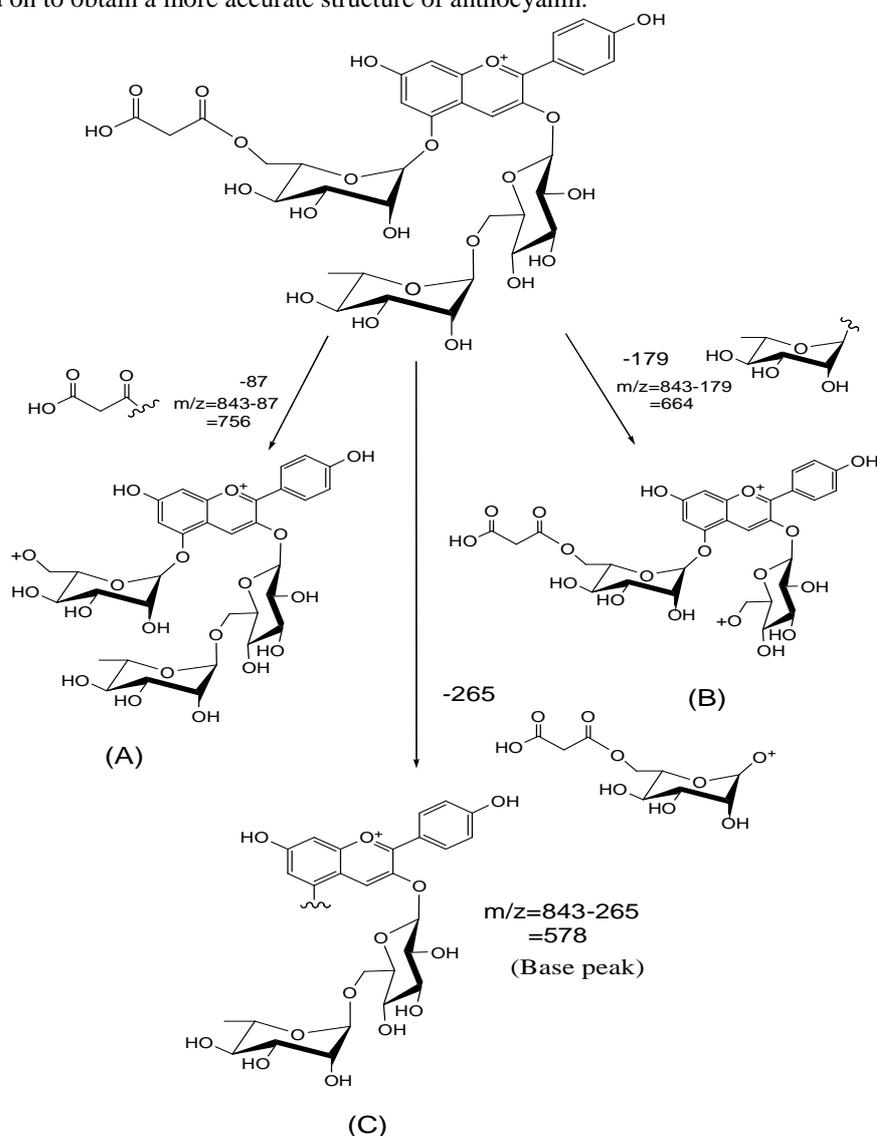


Figure 4. Structure of pelargonidin 3-rutinoside-5-malonylglucoside compound (A) The release of Malonyl group, (B) The release of a sugar group, (C) The release of Malonylglucoside

3. Applications anthocyanin pigment

For applications, the extract was used to two types of beverages which have different pH, ie pH 6 and pH 9. The result of anthocyanin addition into two beverages was the change of color, for the pH 6 beverage turned into pink, while for the pH 9 beverage turned into orange, which can be seen in Figure 5 and 6.



Figure 5. Application of anthocyanin on pH 6 beverages



Figure 6. Application of anthocyanin on pH 9 beverages

An important factor in the application of anthocyanin as a natural pigment in foods and drinks is its stability against pH. There are several advantages of using anthocyanin as dyes in foods or beverages, such as safe for human body because the dye used comes from natural ingredients. Moreover, anthocyanin is as an antioxidant which can counteract free radicals [4].

CONCLUSION

From the data obtained can be concluded that the main pelargonidin contained in extract of raspberries (*Rubus rosifolius* Sm) is pelargonidin-3-rutinoside-5-malonylglucoside with a molecular ion value of m/z 843 and base peak of 578 (pelargonidin 3-rutinoside). Anthocyanins from raspberry fruit extract can be applied as an alternative dye in foods that have an acidic pH, as it gives a clear color.

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