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Research Article

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Characterization of cyanidin 3-(6-acetylglucoside)-5-(3''-coumaryl-6''-malonylglucoside) compound from cinnamon bud leaves (*Cinnamomum burmanni* (Ness & T. Ness) Blume) by HPLC-DAD-ESI-MS

Dina Dwilistiani*, Djaswir Darwis and Adlis Santoni

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

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ABSTRACT

Cyanidin is a particular type of anthocyanidin. It is ethanol soluble pigment more than water. Cinnamon (Cinnamomum burmanni (Ness & T. Ness) Blume) is a species of the Cinnamomum genus from family of Lauraceae. In this research, characterization cyanidin contained in extract of cinnamon leaves tip have been carried out by using UV-Visible spectrophotometer and HPLC-DAD-ESI-MS. The main cyanidin contained in extract of cinnamon leaves are cyanidin 3-(6-acetylglucoside)-5-(3''-coumaryl-6''-malonylglucoside) with a molecular ion of m/z 885.

Keywords: Anthocyanins, cyanidin, *Cinnamomum burmanni* (Ness & T. Ness) Blume, characterization

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INTRODUCTION

Most of anthocyanins are found in six forms of anthocyanins, such as pelargonidin, cyanidin, delphinidin, peonidin, malvidin and petunidin. Chemical structure of cyanidin is similar to the other types of anthocyanins. The differences are spotted in the bond with substitution group. Sugar group in anthocyanin varied, but mostly in the form of glucose, ramnosa, galactose or arabinose. The sugar group can be found in the form of mono or disaccharide and acylated with phenolic acids or aliphatic. Chemically, all of anthocyanin is a derivative of a single aromatic structure cyanidin with the addition or subtraction of hydroxyl groups, methylation or glycosylation, thus other types of anthocyanin is formed [1]. Cyanidin commonly found in several parts of the plants; from leaves, fruit to flower.

Cinnamon (*Cinnamomum burmanni* (Ness & T. Ness) Blume) has pale red color leaves and finely hairy when young [2]. Based on initial test, cinnamon leaves contain anthocyanin based on the colors of red leaves and containing flavonoids after phytochemical test. In this research, the characterization of cyanidin contained in the extract of cinnamon leaves have been carried out by using UV-Visible spectroscopy and HPLC-DAD-ESI-MS.

EXPERIMENTAL SECTION

Plant samples

Cinnamomum burmanni (Ness & T. Ness) Blume bud leaves were obtained from the region Andalas University, Padang, West Sumatra. The plant was identified at Herbarium Laboratory Department of Biology with identification number 047/K-ID/ANDA/II/2015.

Chemicals

HPLC-grade water, methanol, ethanol, acetonitrile, acetic acid, hydrochloric acid, and formic acid were obtained from Merck, Germany. All other chemicals used in this study were analytycal grade.

Instrumentations
Rotary evaporator (Buchi), UV-Visible spectrophotometer (Evolution 201), HPLC-DAD-ESI-MS (Shimadzu, Lc 8030), aluminium foil, filter paper and glassware commonly used in laboratories.

Procedures
1. Extractions of anthocyanins
Extraction of 50 g cinnamon bud leaves were macerated method by 200 ml ethanol solvent acidified with acetic acid to pH 1 at room temperature for 24 hours in dark environment, and then filtered to separate the filtrate and the residue. The filtrate was evaporated by rotary evaporator at 30°C. The filtrate were analyzed by UV-Visible spectrophotometer and HPLC-DAD-ESI-MS.

2. Characterization of anthocyanins by UV-Vis spectroscopy
Characterization was done by spectrophotometer measurements performed with a maximum wavelength of anthocyanin in UV-Visible spectrum. Maximum wavelength measurements performed using a double beam spectrophotometer with an area measuring wavelengths between 200-800 nm.

3. 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 temperature 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.

RESULTS AND DISCUSSION

1. Extraction of anthocyanins
Extraction method by maceration was used because it was relatively simple and did not use heat. Heat treatment could degrade the anthocyanin pigment and formed colourless chalcone. Maceration process was done in dark bottles because anthocyanin compounds were very easily oxidized by light. In addition, the results of maceration should be stored in a refrigerator at a certain temperature 4°C because of its vulnerability caused by the heat. The solvent was ethanol that was acidified with acetic acid until pH 1. Ethanol was used as solvent because of its polarity and less toxic than methanol so it can be applied in food stuff. The purpose of using organic acids such as acetic acid, citric acid or tartaric acid as acidifier was to prevent any damage to the anthocyanin because of hydrolysis of glycoside bond and acylation of anthocyanin[3].

2. Characterization of athocyanins by UV-Vis spectroscopy
Based on UV-Vis spectrum measurements of cinnamon (*Cinnamomum burmanni* (Ness &T.Ness) Blume) bud leaves extract with ethanol-acetic acid pH 1 as solvent, the results obtained was the peak with wavelength region at UV λ max 266 nm, 340 nm and 530 nm. The spectrum can be seen in Figure 1.

The absorption peak emerged at wavelength 266 nm and 530 nm indicate that the extract contain anthocyanin compounds. According to Lee [4], anthocyanin will show two absorption peaks when identified in the 200-800 nm wavelength i.e. the emergence of absorption peak in the UV region (250-280 nm) which represents the benzoyl group and the absorption peak in the Visible region (490-550 nm) which represents cinnamoyl group. In UV-Visible spectrum also appears absorption area at a wavelength 340 nm. According to Hong and Wrolstad [5], the spectra of anthocyanin peaks can also provide information about the presence of acylating groups in the wavelength range 310-340 nm. It suggests there's acyl group in the anthocyanin molecule that were obtained.

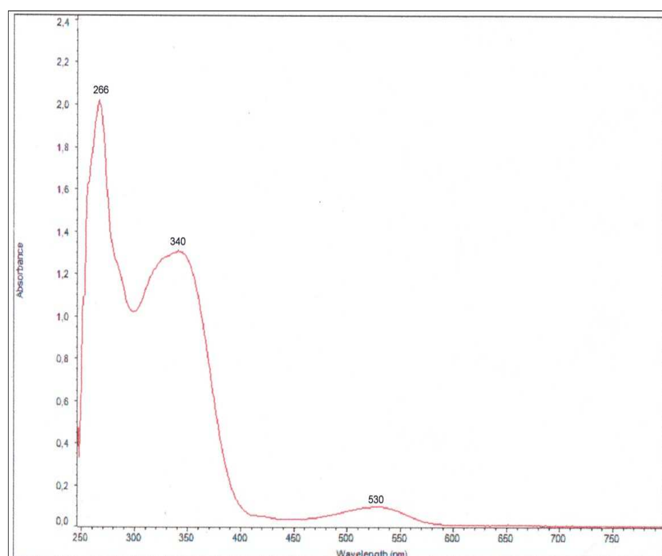

Figure 1. UV-Visible absorption spectra of anthocyanin from extract of cinnamon bud leaves

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

The column used was the type of column C-18. The mobile phase used was; solvent A = 2% solution of formic acid, solvent B = acetonitrile : water (1:1) containing 2% of formic acid. The detection was done at wavelength 516 nm. In figure 2, the ethanol-acetic acid extract has 12 peaks and there was a dominant peak at retention time 14.542 minutes (84.202%).

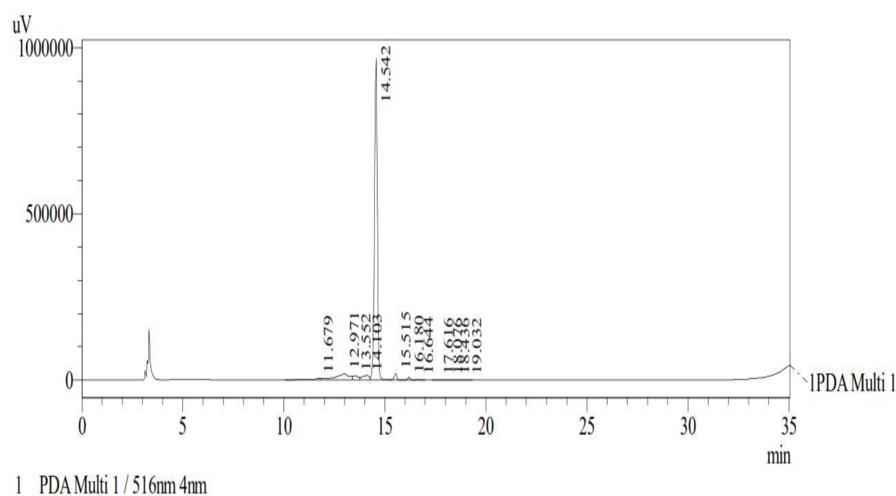

Figure 2. HPLC-DAD chromatogram of anthocyanin from extract of cinnamon bud leaves

The MS data of extract with ethanol-acetic acid solvent can be seen in Figure 3.

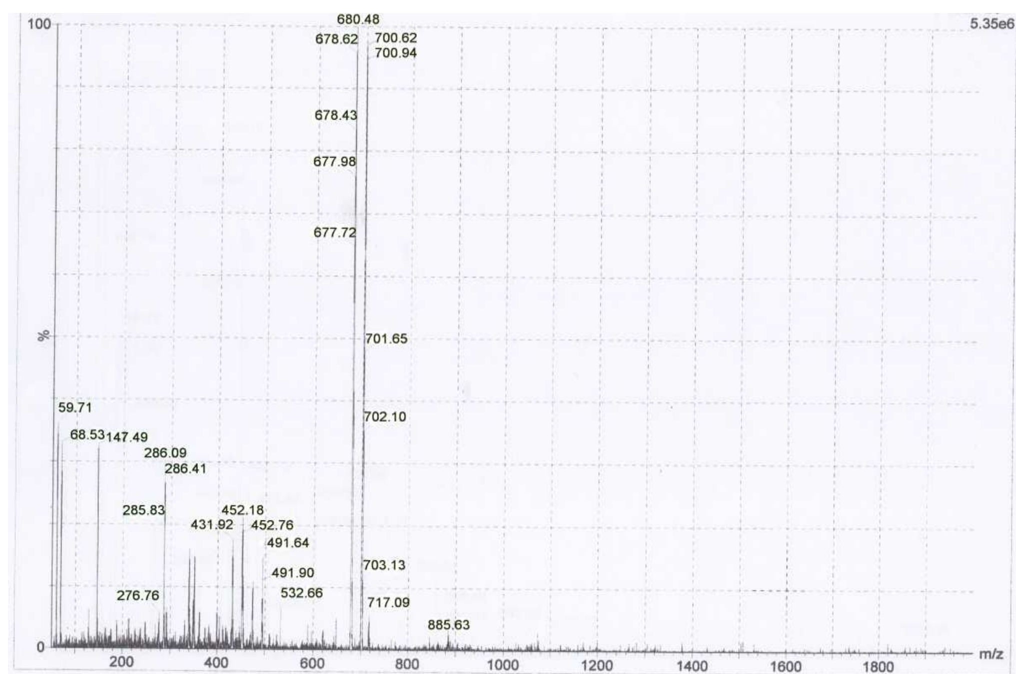

Figure 3. The data of anthocyanin compounds in extract of cinnamon

Based on the MS data above, it can be identified the anthocyanin compounds obtained were cyanidin group by the presence of fragment m/z 286 which indicated anthocyanin coumpound. MS fragmentation pattern data has molecul ion m/z 885. The emergence of base peak fragments m/z 680 showed the release of the acetylglucoside group (-205). The peak for the release of coumaryl-6"malonylglucoside (-394) showed us there's fragment m/z 491. The fragment m/z 286 showed the release of acetylglucoside group and coumaryl-6"-malonylglucoside (-599) group. Fragment m/z 147 was coumaryl group. Based on the fragmentation pattern, anthocyanin compound can be identified as cyanidin 3-(6-acetylglucoside)-5-(3"-coumaryl-6"-malonylglucoside) with the molecular formula $C_{41}H_{41}O_{22}$. The structure and fragmentation patterns of the compounds can be seen in figure 4 and 5.

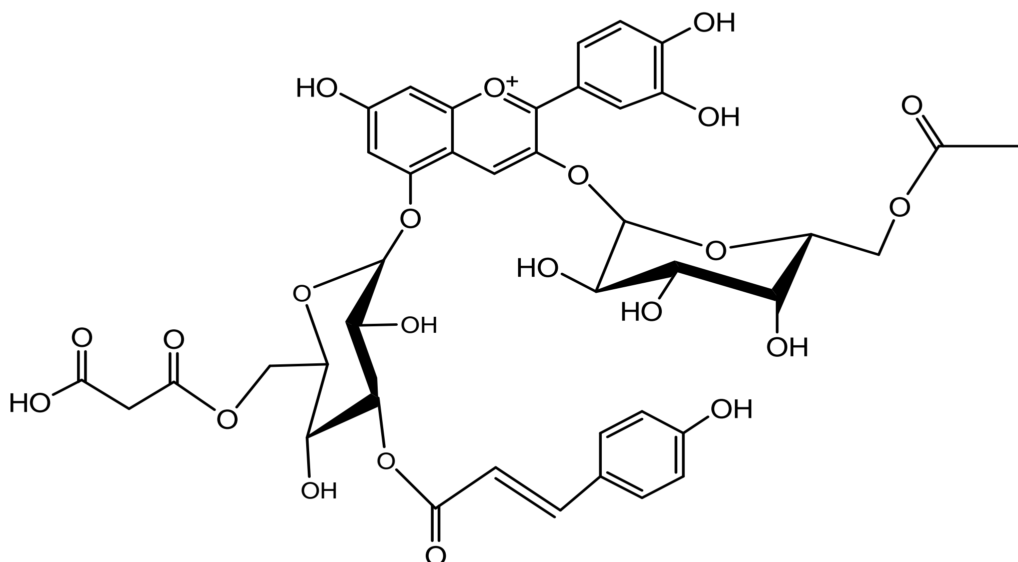

Figure 4. The structure of cyanidin 3-(6-acetylglucoside)-5-(3"-coumaryl-6"-malonylglucoside)

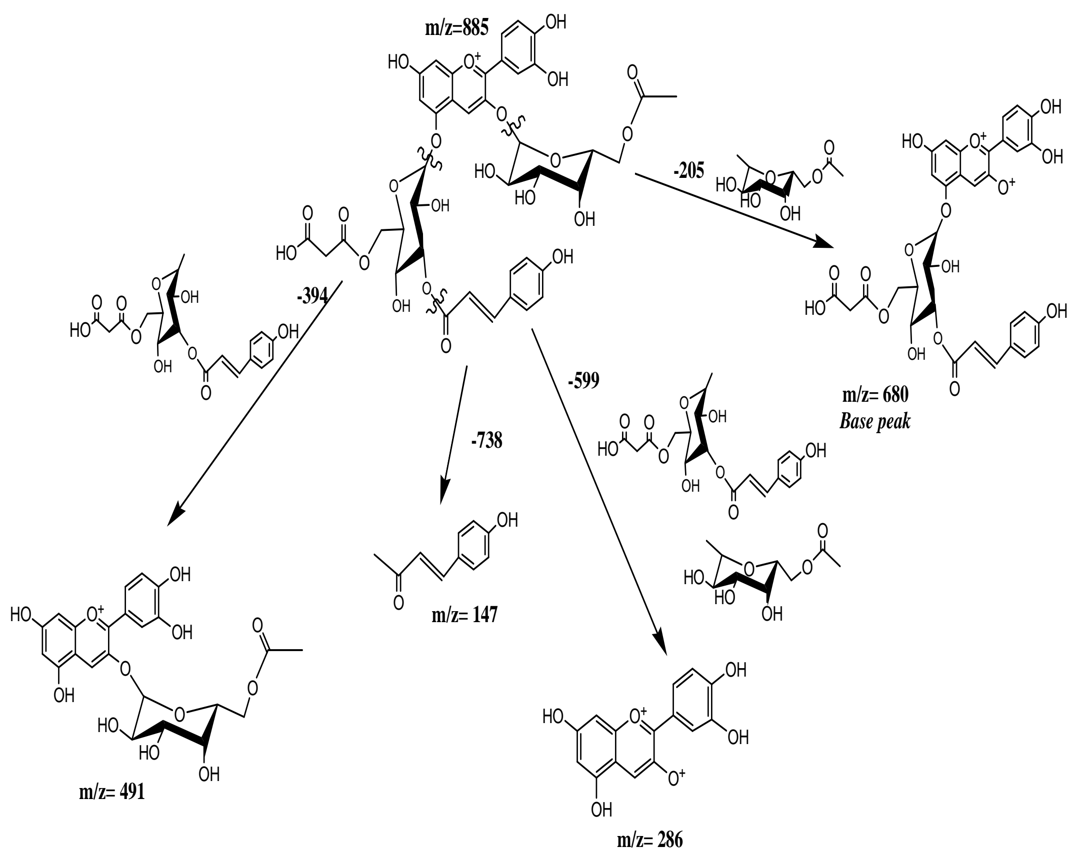

Figure 5. Fragmentation of cyanidin 3-(6-acetylglucoside)-5-(3”-coumaryl-6”-malonylglucoside)

CONCLUSION

Anthocyanin compounds that was contained in the extracts of cinnamon bud leaves (*Cinnamomum burmanni* (Ness &T.Ness) Blume) can be predicted as cyanidin 3-(6-acetylglucoside)-5-(3"-coumaryl-6"-malonylglucoside) with molecular ion m/z 885 and base peak m/z 680.

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