http://ejournal.seaninstitute.or.id/index.php/healt
Jurnal eduhealth, Volume 13, No 02, 2022
E-ISSN., P-ISSN. 2087-3271

Standardization of Anthocyanin Compounds in Plants in Indonesia: Literature Review

Vriezka Mierza[1], Bunga Nur Annisa[2], Annastya Eka Agustin[3] , Cantika Nurul Sa'adah[4], Fajar Prasetyo[5], Christina Febiola Lubis[6]
[1,2,3,4,5,6] Department Of Pharmacy Faculty of Health Sciences Univerty of Singaperbangsa Karawang, Jawa Barat,Indonesia

ARTICLE INFO

Keywords:
Antocyanins,
Standardization, Plants

ABSTRACT

Anthocyanins are secondary metabolites belonging to the flavonoid group which act as pigments or coloring agents in plants that give red, blue and purple colors to flowers, fruits and vegetables. Anthocyanins have several pharmacological activities such as antioxidants, anti-carcinogenic, preventing impaired liver function, anti-hypertensive, and anti-diabetic. The anthocyanin structure consists of an aglycone form which is sterified by several sugar groups (glycones) and acyl groups in several anthocyanin compounds. In an effort to maintain the standard of a drug made from natural ingredients, the process of standardization of active compounds can be carried out to guarantee and maintain safety, quality uniformity and efficacy. This review was conducted to identify the anthocyanins contained in an extract by means of thin layer chromatography (TLC), UV-Vis spectrophotometer, thin layer chromatography-densitometry, and high performance liquid chromatography (HPLC). The research methodology used is in the form of literature review with reference sources related to the review topic obtained from various sources, such as Pubmed, Science Direct, and Google Scholar.

Email :
Vriezka.mierza@fikes.unsika.ac.id
1910631210060@student.unsika.ac.id
1910631210025@student.unsika.ac.id
1910631210062@student.unsika.ac.id
1910631210006@student.unsika.ac.id
1910631210063@student.unsika.ac.id

1. INTRODUCTION

A compound in a plant that is used as an active substance in medicine should meet quality standards in order to guarantee its efficacy and safety. In an effort to maintain the standard of a drug made from natural ingredients, the process of standardization of the active compound can be carried out. Standardization is the stage of fulfilling the requirements of an active compound that is to be used as a raw material for medicine to guarantee and maintain safety, quality uniformity and efficacy. One example is the standardization process for determining specific and non-specific parameters for simplicia or plant extracts containing active and efficacious compounds to be used as a drug [1].

Anthocyanins are secondary metabolites that belong to the flavonoid group. Anthocyanin compounds are stored in plant cell vacuoles and act as pigments or coloring agents in plants that give red, blue and purple colors to flowers, fruits and vegetables [2]. Natural anthocyanins have the ability to protect plants from damage caused by biotics such as fungi and bacteria and abiotics such as UV rays and extreme temperatures. Based on its structure, anthocyanin compounds have several pharmacological activities such as antioxidants, anti-carcinogenic, preventing impaired liver function,

anti-hypertensive, and anti-diabetic [3]. Until now, more than 540 anthocyanin compounds have been identified in nature, with the most common compounds being cyanidin, delfinidin, peonidin, and malvidin [4].

In general, the anthocyanin structure consists of an aglycone form which is sterified by several sugar groups (glycones) and acyl groups in several anthocyanin compounds. Anthocyanin compounds are more active in their aglycone form. This compound is easily soluble in polar solvents under acidic conditions, highly reactive, easily oxidized or reduced, its glycosidic bonds are easily hydrolyzed, and more stable in acidic solutions than in basic solutions. Anthocyanins are easily soluble in polar solvents such as water and in slightly acidic conditions. Increasing temperature and pH can cause anthocyanin degradation. Anthocyanins can change color reversibly along with changes in pH where at a very acidic pH in the range of 1-2, Anthocyanin is in the form of colored flavilium or oxonium cations, while at pH 4-5 a colorless hemiketal is formed which will affect its absorbance when reading with a visible spectrophotometer. Anthocyanins provide maximum absorption in the visible light region, namely in the region of 505-535 nm. To obtain good anthocyanin compounds, specific sources and methods are needed starting from the extraction process to isolation [5]. Based on the explanation that has been presented, writing this review article aims to provide an overview of how to standardize anthocyanin compounds in order to maximize the isolation process. To obtain good anthocyanin compounds, specific sources and methods are needed starting from the extraction process to isolation [5]. Based on the explanation that has been presented, writing this review article aims to provide an overview of how to standardize anthocyanin compounds in order to maximize the isolation process. To obtain good anthocyanin compounds, specific sources and methods are needed starting from the extraction process to isolation [5]. Based on the explanation that has been presented, writing this review article aims to provide an overview of how to standardize anthocyanin compounds.

2. METHOD

The research methodology used is in the form of literature review with reference sources related to the review topic obtained from various sources, such as Pubmed, Science Direct, and Google Scholar. This literature search included keywords such as "standardization of anthocyanin analysis" and "standardization of anthocyanin analysis". In addition, the keyword "anthocyanidin" is also used. This review aims to determine the methods that can be used for anthocyanin analysis both qualitatively and quantitatively. A literature search was carried out using data inclusion criteria in the form of journal years 2012-2022, research journals, and the selected literature is available in full-text as well as exclusion criteria, namely journals outside English and Indonesian and theses/thesis/dissertations, then processed and analyzed. Writing a bibliography in this article uses IEE.

3. RESULTS AND DISCUSSION

Table 1. Results of Standardization of Anthocyanins

References	Sample	Standardization Method	Information
[6]	Purple sweet potato ethanol extract (*Ipomoea batatas* L.)	Thin layer chromatography Stationary phase = Silica gel 60 GF_{254} 5.5 cm Mobile phase = acetic acid: hydrochloric acid: water (1:1:2)	Rf value of 0.5 with anthocyanin compounds including the type of cyanidin 3-rhamnoside
[5]	Butterfly pea flower extract (*Clitoria ternatea*)	Visible Spectrophotometry Qualitative =λ400 – 800 nm Quantitative =λ700 nm with a solution of pH = 1 and 4.5 Blank : aquadest	λmax 510 nm and the highest anthocyanin content is found in fresh butterfly pea flowers at 80ºC, namely 0.1927±0.0034

[7]	Green Coconut Coir (*Cocos Nucifera* L Var Varidis)	Thin layer chromatography Stationary phase = silica gel 60 F_{254} Mobile phase = butanol: acetic acid: water (3:1:1) Spot detection = NH_3	The Rf value is 0.96 and looks light yellow in visible light with the detection of ammonia spots; at UV 366 nm blue before and after detection of stains with ammonia
[8]	70% ethanol extract in acidic medium of purple sweet potato (*Ipomoea batatas* L.)	TLC – Spectrophotodensitometry Stationary phase = silica gel 60 F_{254} Mobile phase = n-butanol: glacial acetic acid: water (4:1:2) λ= 200-700 nm Spot detection and color reagent = NH_3, 5% $AlCl_3$, and 2% $FeCl_3$	λmax 540 nm is cyanidin; The Rf value of 0.1 with 2% $FeCl_3$ and 5% $AlCl_3$ stains detected flavonol compounds, while with NH_3 reagent no anthocyanins were detected
[9]	Purple sweet potato extract (*Ipomoea batatas* L.)	Thin layer chromatography The stationary phase = silica gel GF_{254} Mobile phase = butanol: acetic acid: water (4:5:1)	Rf value 0.41
[10]	Purple eggplant skin extract (*Solanum melongena*)	UV-Vis Spectrophotometry Qualitative =λ250-700 nm pH = 1 and 4.5 for Abs Blank = ethanol	Dilution twice the maximum wavelength of 598 nm and dilution ten times the maximum wavelength of 510 nm with anthocyanin content of 13.41%
[11]	Purple sweet potato extract (*Ipomoea batatas* L.)	UV-Vis Spectrophotometry - Quantitative =λ300-700 nm - pH = 1 and 4.5 for Absorbance - Blank = water	Total monomeric anthocyanin content of sweet potato extract purple Cilembu, Banjaran, and Pakembangan respectively is 3.78±0.08; 3.18±0.01; and 2.25±0.01 CyE/g (bk).
[2]	Tomi Fruit Extract – Tomi (*Flacourtia inermis*, Roxb)	Thin layer chromatography Stationary phase = Cellulose plate Mobile phase = • Concentrated HCl: formic acid: distilled water (2:5:3) • Concentrated HCl: acetic acid: distilled water (3:30:10) • N-butanol: acetic acid: distilled water (4:1:5) UV-Vis Spectrophotometry Blank = methanol – 0.1% HCl λ= 200-800 nm High Performance Liquid Chromatography Stationary phase = Eurosphere RPC-18	based on ni- Rf value on TLC and HPLC peak retention time, it was confirmed that the anthocyanidin was of the delphinidine type. However, the absorbance dataλmax on the TLC spot shows cyanidin. Maybe, this is because the extract is used too dilute or anthocyanidins have been degraded during the hydrolysis process. Based on the Rf value on TLC and Glycosidation pattern on UV-Vis spectrophotometer, suspected anthocyanin cyanidin 3-glucoside and delfinidin 3-glucoside.

		Mobile phase = 10% formic acid: acetonitrile Wavelength = 530 nm Injection volume = 20 ml Flow rate = 1.2 ml/min	
[12]	Black stars extract *(Solanum ningrum)*	High Performance Liquid Chromatography Stationary phase = Zorbax Stablebond Analytical SB-C18 column Mobile phase = • 87% water, 11% acetonitrile, 2% acetic acid • 40% water, 50% acetonitrile, 2% acetic acid Flow rate = 1 ml/min Injection volume = -	Anthocyanin level 18.215 mg/g
[13]	Purple sweet potato extract (*Ipomoea batatas* L.)	Thin layer chromatography Stationary phase = silica gel 60 F254 Mobile phase = • Ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:26) {A} • N-butanol: glacial acetic acid: water (4:1:5) {B} • N-butanol: glacial acetic acid: water (4:1:2) {C} Wavelength = 190-600 nm	Rf value 0.30 and 0.58 with NH_3 and reagent λmax 540 nm indicates anthocyanins; A good mobile phase ie N-butanol: glacial acetic acid: water (4:1:2)
[14]	Black glutinous rice ethanol extract (*Oryza Sativa* L.)	Thin layer chromatography Stationary phase = silica gel 60 F_{254} Mobile phase = • Forestal = concentrated HCl: acetic acid: water (3:30:10) • BAA = n – butanol: acetic acid: water (4:1:5)	The Rf value of the third stain was 0.467 in the BAA mobile phase with the anthocyanin pelargonidin 3-glucoside type
[3]	Red gedi leaf extract (*Abelmoschus manihot* (L.) Medik.)	UV-Vis Spectrophotometry λ= 250-700 nm pH = 1 and 4.5	Maximum wavelength 535 nm
[15]	Roselle flower extract (*Hibiscus Sabdariffa* L.)	Thin layer chromatography Stationary phase = silica gel GF_{254} Mobile phase = n-butanol: acetic acid: water (5:1:2) Spotting = AlCl3 and ammonia vapor	The color change in ammonia to blue and $AlCl_3$ to yellow. Rf values 0.9 and 0.6.

[16]	Freeze Dried Acai (*Euterpe oleraceae* Mart.)	High Performance Liquid Chromatography Stationary phase = Symmetry Mobile phase = 10% formic acid (A) and methanol (B) Flow rate = 5 l/min Injection volume = 2 ml	The levels obtained for the cyanidin-3-glucoside compound were 35.29±0.12/100 mg and cyanidin-3-rutinoside as much as 58.73±0.22/ 100 mg

Discussion

Anthocyanins from natural materials can be obtained by means of thin layer chromatography (TLC), UV-Vis spectrophotometer, thin layer chromatography-densitometry, and high performance liquid chromatography (HPLC).

Standardization of anthocyanins using TLC generally uses silica gel 60 F254 stationary phase and 1% HCl (97:3, v/v) as mobile phase and Butanol:acetic acid:water (BAA) in different ratios. In general, the spot reagents and color reagents used in TLC were NH3, 5% AlCl3 and 2% FeCl3.

Standardization using TLC aims to identify (qualitatively) anthocyanins contained in an extract such as research conducted by Febriani et al (2016) on purple sweet potato extract. In this study, the Rf value was sought by looking at the stains/spots produced during the elution process using the mobile phase on the TLC plate. The Rf value is obtained from the calculation of the stain separation distance divided by the eluent travel distance. The Rf value obtained in this study was 0.41. This value is in accordance with the literature that the Rf value of anthocyanin is 0.1 – 0.4. The TLC method is usually carried out on compounds with groups that show positive results on phytochemical screening, one of which is anthocyanins.

In addition, further standardization of anthocyanins is done by TLC-spectrophotodensitometry which uses several parameters, namely chromatogram, maximum wavelength and spectrum. Based on the anthocyanin spectrum data in band I (270-280 nm) and in band II (456-560 nm). The absorption in the band II region is the absorption of the aglycone group which includes cyanidin. The spectrum of 70% ethanol extract in acidic medium of purple sweet potato showed the presence of anthocyanin compound with a wavelength of 540 nm as the maximum wavelength of cyanidin, namely anthocyanin which has an acyl group. Based on the observed results, the Rf value obtained was 0.1 in the administration of 2% FeCl3 and 5% AlCl3 reagent, which was then visualized in 366 nm UV light, indicating that the extract contained flavonols, whereas in the NH3 spot appearance, no anthocyanin was detected [4].

The difference in color in the results of spot detection and color reagents is due to the influence of aglycone bonds with other substitutional groups in anthocyanins which can affect the type of anthocyanin compounds contained in an extract [11] . As in a study conducted by Wirasuta et al (2015), namely purple sweet potato extract, which detected cyanidin and peonidin. The difference between the two lies in R1, where cyanidin has an -OH group, while peonidin has an -OCH3 group. Based on several journals that have been reviewed, the selection of the mobile and stationary phases and the use of spot sights affect the results obtained from the TLC method.

Standardization of anthosinins using a UV-Vis spectrophotometer was carried out for qualitative (maximum wavelength) and quantitative (content) analysis. One study that used qualitative analysis was Purwaniati, et al (2020) with a sample of butterfly pea flower (Clitoria ternatea) extract. In that study, the maximum anthocyanin wavelength was sought and the result was 510 nm. The results of this study are in accordance with the literature that anthocyanins provide maximum absorption in the visible light region of 505-535 nm.

In quantitative analysis using a UV-Vis spectrophotometer, a search for anthocyanin levels or total anthocyanin levels was generally influenced by pH because the chemical structure of anthocyanins is less stable and thus easily degraded. Anthocyanins are more stable in acidic solutions than in basic solutions so that in determining the total anthocyanin content, the pH of the solution is measured first with a pH of 1 and 4.5. At a pH lower than 2, anthocyanins exist as cations (flavilium ions). Therefore, anthocyanins are safest when worked in slightly acidic solutions. The increasingly acidic conditions

approaching pH 1 will cause more and more anthocyanin pigments to be in the form of colored flavilium or oxonium cations so that they can be easily read on the absorbance spectrum [12]. In addition to pH 1 and 4.5, the anthocyanin structure changes at 7. Basically, the change in anthocyanin color is due to a change in the structure of the flavilium cation into carbinol, quinonoidal and chalcone hemiketal pseudobasics. In the study of Mahmudatussa'adah, et al (2014), at pH 1-2, anthocyanins were dominant in the form of flavilium cations which were red in color, at pH <6 they changed to carbinol and some became quinonoidals which were blue in color so that they were purple in color, at pH 6.5-9 dominant quinonoidals which are blue in color, and at pH >9 chalcones are yellow in color.

Based on the journals that have been reviewed, research using the pH optimization method for anthocyanin characteristics by looking at the absorption area is in the study of Sahil, et al (2020) with samples of red gedi leaf extract (Abelmoschus manihot (L.) Medik). The journal uses optimization of pH 1-5 and a wavelength of 250-700 nm. The color change of the anthocyanin extract of the red gedi plant to the influence of pH is an indicator of the anthocyanin because it can change color to factors of acidity and alkalinity. The color results in the red gedi leaf extract test solution were yellowish green with decreasing color saturation as the pH of the test solution was increased.

Another factor that affects the presence of anthocyanins is temperature. The temperature difference also affects the anthocyanin levels obtained in each plant, the higher the temperature the more anthocyanins obtained. This is in accordance with research conducted by Purwaniati, et al (2020) when brewing butterfly pea flower preparations with 3 types of temperature, namely room temperature (25ºC) to determine the levels of anthocyanins in normal conditions, temperature 50ºC to determine anthocyanin levels in warm conditions, and around 80ºC to determine the levels of anthocyanins in hot conditions. However, anthocyanin levels will decrease at 100ºC due to degradation of anthocyanins.

Determination of total anthocyanin levels uses the maximum wavelength that has been measured previously and optimizing pH 1. The formula for determining the total content of an anthocyanin is found in the 2nd Edition of the 2017 Indonesian Herbal Pharmacopoeia, namely

$$\% = \frac{A \times BM \times f \times 1000}{\varepsilon \times b \times w} \times 100$$

Information :
A = absorption of the solution that has been corrected with a blank
BM = molecular weight of cyanidin-3-O-glucoside (449)
f = dilution factor
ε= absorption of cyanidin-3-O-glucoside (25965 /cm.M)
b = thickness of the cuvette (1 cm)
w = sample weight (g)

Furthermore, standardization of anthocyanins using HPLC. The use of HPLC is usually used to determine the concentration, characterization and determinants (complementary data from other methods) of anthocyanin compounds. HPLC is a development of the classic columnar liquid chromatography, with the development of column technology, more sensitive and sensitive detectors and technological advances in high-pressure pumps. Therefore HPLC becomes a fast and efficient chromatography (separation) tool [17]. The stationary phase commonly used for analysis is silica. In several studies the combined use of the mobile phase consists of an organic phase and a buffer. Organic phases such as acetonitrile and methanol are often used in HPLC systems. While the buffers that are often used are acetate buffer, phosphate buffer, tetrahydrofuran (THF). The result of HPLC is a retention time chromatogram. Retention time is expressed as the length of time for sample analysis, where in the reverse phase the more polar substances will elute first and have a faster retention time than non-polar substances. In the study Gouvêa et al (2012) used HPLC as a determinant of the type of anthocyanin and its levels with samples of freeze-dried acai (*Euterpe oleraceae* Mart.). The results of this study obtained a retention time of 17.8 minutes for cyanidin-3-glucoside and 22. 2 minutes for cyanidin-3-rutinoside. Then the levels of cyanidin-3-glucoside and cyanidin-3-rutinoside were 35.29 ± 0.12 mg/100 g and 58.73 ± 0.22 mg/100 g, respectively.

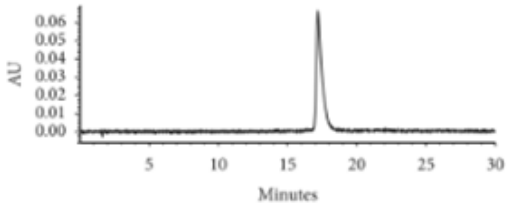

Figure 1 Chromatogram of cyanidin-3-glucoside

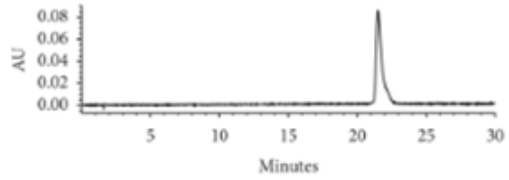

Figure 2 Chromatogram of cyanidin-3-rutinoside

4. CONCLUSION

Anthocyanins are secondary metabolites belonging to the flavonoid group which act as pigments or coloring agents in plants that give red, blue and purple colors to flowers, fruits and vegetables. Based on its structure, anthocyanin compounds have several pharmacological activities such as antioxidants, anti-carcinogenic, preventing impaired liver function, anti-hypertensive, and anti-diabetic. To obtain good anthocyanin compounds, specific sources and methods are needed, starting from the extraction process to its isolation. In this researchAnthocyanins were obtained by means of thin layer chromatography (TLC), UV-Vis spectrophotometer, thin layer chromatography-densitometry, and high performance liquid chromatography (HPLC).

REFERENCES

[1] S. Sutomo, N. Hasanah, A. Arnida, and A. Sriyono, "Standardisasi Simplisia dan Ekstrak Daun Matoa (Pometia pinnata J.R Forst & G. Forst) Asal Kalimantan Selatan," *J. Pharmascience*, vol. 8, no. 1, p. 101, 2021.

[2] R. Fitriyani, L. N. Lestario, and Y. Martono, "JENIS DAN KANDUNGAN ANTOSIANIN BUAH TOMI – TOMI," *J. Teknol. dan Ind. Pangan*, vol. 29, no. 2, pp. 137–144, 2018.

[3] M. R. Sahi, F. Fatimawali, and J. P. Siampa, "Ekstraksi dan Optimasi Antosianin Daun Gedi Merah (Abelmoschus manihot (L.) Medik.) Dengan Metode Spektrofotometri UV-VIS," *Pharmacon*, vol. 10, no. 1, pp. 668–675, 2021.

[4] F. Weber and L. R. Larsen, "Influence of fruit juice processing on anthocyanin stability," *Food Res. Int.*, vol. 100, pp. 354–365, 2017.

[5] P. Purwaniati, A. R. Arif, and A. Yuliantini, "ANALISIS KADAR ANTOSIANIN TOTAL PADA SEDIAAN BUNGA TELANG (Clitoria ternatea) DENGAN METODE pH DIFERENSIAL MENGGUNAKAN SPEKTROFOTOMETRI VISIBLE," *J. Farmagazine*, vol. 7, no. 1, p. 18, 2020.

[6] W. Arifuddin, "Aktivitas Antioksidan Senyawa Antosianin dari Ekstrak Etanol Ubi Jalar Ungu (Ipomoea batatas L)," *Celeb. Biodiversitas*, vol. 1, no. 2, pp. 26–29, 2018.

[7] R. Anggriani, N. Ain, and S. Adnan, "Identifikasi Fitokimia Dan Karakterisasi Antosianin Dari Sabut Kelapa Hijau (Cocos Nucifera L Var Varidis)," *J. Teknol. Pertan.*, vol. 18, no. 3, pp. 163–172, 2017.

[8] N. P. L. Laksmiani, N. P. E. Leliqia, N. N. T. Wiriyanti, I. A. Putu, C. Dewi, and I. M. A. G. Wirasuta, "IDENTIFIKASI DAN KARAKTERISASI ANTOSIANIN EKSTRAK ETANOL 70 % DALAM SUASANA ASAM DARI UBI JALAR UNGU (Ipomoea batatas L .) DENGAN KLT - SPEKTRODENSITOMETRI," *simodos unuid*, vol. 1, 2015.

[9] Y. Febriani, E. A. Ihsan, and S. Ardyati, "Analisis Fitokimia Dan Karakterisasi Senyawa

Antosianin Ubi Jalar Ungu (Ipomea Batatas) Sebagai Bahan Dasar Lulur Hasil Budidaya Daerah Jenggik Lombok," *Sint. J. Farm. Klin. dan Sains Bahan Alam*, vol. 1, no. 1, pp. 1–6, 2021.

[10] A. A. Arifin, S. Armiani, and H. Fitriani, "Isolasi Antosianin Kulit Terong Ungu (solanum melongena) Sebagai Biosensor Pendeteksi Kandungan Bahan Kimia Pada Makanan," *Biosci. J. Ilm. Biol.*, vol. 10, no. 1, pp. 361–382, 2022.

[11] A. Mahmudatussa'adah, D. Fardiaz, N. Andarwulan, and F. Kusnandar, "Karakteristik Warna dan Aktivitas Antioksidan Antosianin Ubi Jalar Ungu," *J. Teknol. dan Ind. Pangan*, vol. 25, no. 2, pp. 176–184, 2014.

[12] S. Wang *et al.*, "Identification of Anthocyanin Composition and Functional Analysis of an Anthocyanin Activator in Solanum nigrum Fruits," *Molecules*, vol. 22, no. 6, pp. 1–14, 2017.

[13] I. M. A. G. Wirasuta *et al.*, "IDENTIFIKASI ANTOSIANIN UBI UNGU (Ipomoea) dengan KLT-SPEKTROFOTODENSITOMETRI," *Semin. Nas. Sains dan Teknol.*, vol. 254, 2015.

[14] K. A. Adrianta, "IDENTIFIKASI SENYAWA ANTOSIANIN DAN METABOLIT SEKUNDER DARI EKSTRAK ETANOL BERAS KETAN HITAM (Oryza sativa L.) DALAM PEMANFAATANNYA SEBAGAI ALTERNATIF PENGOBATAN DEMAM BERDARAH DENGUE," *J. Ilm. Medicam.*, vol. 2, no. 1, pp. 17–22, 2016.

[15] M. D. Anggistia, H. Widiyandari, and K. Anam, "Identifikasi dan Kuantifikasi Antosianin dari Fraksi Bunga Rosela (Hibiscus Sabdariffa L) dan Pemanfaatannya sebagai Zat Warna Dye-Sensitized Solar Cell (DSSC)," *J. Kim. Sains dan Apl.*, vol. 19, no. 2, pp. 50–57, 2016.

[16] A. C. M. S. Gouvêa, M. C. P. de Araujo, D. F. Schulz, S. Pacheco, R. L. de O. Godoy, and L. M. C. Cabral, "Anthocyanins standards (cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside) isolation from freeze-dried açaí (Euterpe oleraceae Mart.) by HPLC," *Food Sci. Technol.*, vol. 32, no. 1, pp. 43–46, 2012.

[17] S. S. Aulia, I. Sopyan, and Muchtaridi, "Penetapan Kadar Simvastatin Menggunakan Kromatorafi Cair Kinerja Tinggi (KCKT) :Review," *Farmaka*, vol. 14, no. 4, pp. 70–78, 2016.