

Study of betacyanin level from cactus fruit (*Opuntia elatior* Mill) in Palu city using spectrophotometry UV-VIS

Yonelian Yuyun* and Jamaluddin

Department of Pharmacy, Faculty of Mathematic and Natural Science, Tadulako University, Palu, Indonesia

*Correspondence Info:

Yonelian Yuyun

Department of Pharmacy,

Faculty of Mathematic and Natural Science,

Tadulako University, Palu, Indonesia

Tel.: +6208112260512

E-mail: yoneli_redrose@yahoo.com

Abstract

A research has been done about comparative study of the levels of total anthocyanin fruit cactus (*Opuntia elatior* Mill) from Palu city using UV-Visible spectrophotometry in order to determine the levels of total betasianin cactus fruit that comes from the Mantikulore, East Palu and South Palu Subdistrict. Betacyanin pigment extraction using maceration method with a ratio of solvent: the sample (1: 1), then the separation using a centrifuge and filtered, the filtrate obtained was concentrated using a rotary evaporator at 35 °C to obtain a concentrated extract of ± 4 ml. The extraction is performed three times. The extract was tested qualitatively using reagent HCl 2 M and NaOH 2 M and for quantitative assay using UV-Vis spectrophotometry at wavelength of 538 nm. The results obtained in qualitative tests showed the three sampling sites containing betacyanin. On the quantitative test results betacyanin total levels from Mantikulore subdistrict of 0.022 mg/100 g, East Palu subdistrict of 0.021 mg/100 g and South Palu subdistrict of 0.021 mg /100 g.

Keywords: cactus fruit, *Opuntia elatior* Mill, betacyanin, spectrophotometry

1. Introduction

Palu city is an area located on the Sulawesi island, Indonesia, a region lying between the two continents, Asia and Australia. Sulawesi is a region with high levels of endemic species of flora and fauna. Central Sulawesi is the center of diversity in Sulawesi island. As a meeting point of three continental plates making Sulawesi as a unique and distinctive island which is the center of Wallace and unexplored with good use of its potential.[1]

One of the plants that grow in Palu particularly in barren area and has not been utilized to the fullest potential is cactus plant. Cactus is the name given to members of the family of flowering plants Cactaceae. Cactus can grow for a long time without water. Cactus commonly found in dry areas (desert). Generally, cactus comes from the barren plains such as South America and Mexico, which have low rainfall frequency indeterminate. More than 2000 species of cactus in the hemisphere. Genus of cactus are grouped into three types: Preskioidea, Opuntodeae and Cereoidea. Various types of cactus have used as a human food source for long time, one of which is *Opuntia*. Nowadays, *Opuntia* is also still used as animal feed, cosmetics, and pharmaceuticals.[2] Cactus fruit has been known containing natural pigment betacyanin.[3] Natural pigments of plant tissue include chlorophyll, carotenoids, flavonoids, anthocyanins, quinones, and betacyanin.[4]

As with other natural products in many plants betacyanin stored in the vacuole as glycosides.[5] Simple natural form of betacyanin is non-glycosylated betanidin or isobetanidin chromophores obtained from condensation

between cyclo-DOPA with betalamic acid. Other betacyanin forms can be obtained from isomerization on one or two free hydroxyl groups on the cyclo-DOPA. Glycosylation at the fifth position called betanyn.[6]

Betanin levels in plant can be influenced by several factors including temperature, humidity, rainfall, sunlight and air.[7] Ambient temperature, air and sunlight have a significant impact on the accumulation of secondary metabolites. *Opuntia elatior* Mill. known containing betacyanin. Betacyanin synthesis is affected by temperature and light, because the enzymes that affect the synthetic pathway of secondary metabolites is a light – sensitive enzymes.[8] The secondary metabolites contained in the cactus fruit which is useful as a coloring agent is betacyanin. Previous study showed that fruit cactus (*Opuntia elatior* Mill.) Rajkot, Gujarat, India, contains secondary metabolites betacyanin of 47.10 mg/100 ml.[3]

Based on the descriptive above, it was necessary to study the levels of total betacyanin from cactus fruit (*Opuntia elatior* Mill.) that grows wild in some places in the Palu region.

2. Material and Methods

2.1 Chemicals

Materials used in this study is the fruit of cactus (*Opuntia elatior* Mill.), Whatman paper No.1, ethanol p.a, hydrochloric acid 2 M, sodium hydroxide 2 M, citrate-phosphate buffer pH 5 and distilled water.

2.1 Sample Preparation

Ripe fruit is cleaned of thorns and dirt in the fruit and then washed with water, after a clean rind peeled, mashed fruit pulp in a blender until a cactus fruit juice.

2.2 Phase Extraction

Extraction is done by weighing 100 g of fruit juice that has been refined and incorporated into erlenmeyer, added ethanol: water (50:50) as much as 100 ml shaken for 15 minutes in cold conditions. The mixture was centrifuged (15000 rpm and 4°C) for 20 minutes, then filtered using Whatman paper No. 1, obtained filtrate was concentrated using a rotary evaporator vacuum at a temperature of 35°C to obtain extracts of ± 4 ml, then stored in a dark container prior to testing the extract. Stage extraction process above was repeated 3 times.[10]

2.3 Analysis of Samples

2.3.1 Qualitative Analysis

This qualitative test is used to ensure the content betasianin in cactus fruit extract. This test consists of multiple treatments, namely:

- Betacyanin extract was added with 2 M HCl and heated at a temperature of 1000°C for 5 minutes and observed discoloration is reduced or lost.
- Betacyanin extract was added with 2 M NaOH drop wise and the observed color changes to yellow.[11]

2.3.2 Quantitative Analysis

Extract weighed as much as 100 mg, reconstituted with the solvent citrate-phosphate buffer pH 5 to 50 ml volume, and then the absorbance is measured using a UV-Vis spectrophotometry, at a wavelength of 538 nm. Measurements were performed 3 times.

2.4 Data Analysis

Results of analysis betacyanin absorbance were measured using a UV-Visible spectrophotometry at a wavelength of 538 nm. Levels of total betacyanin calculated using equation:

$$\text{Betacyanin total (mg/L)} = \frac{A \times MW \times Df \times 1000}{\epsilon \times L}$$

Note:

A	=	absorbance
MW	=	molecular weight betacyanin (550 g/mol)
Df	=	dilution factor
ϵ	=	molar extinction coefficient
L	=	thick cuvette

$$\text{Betacyanin total (mg/kg)} = \frac{K \times L}{B}$$

K	:	sample concentration (mg / L)
V	:	final volume of the sample solution prepared (L)
B	:	sample weight (kg)

3. Results and discussion

Betacyanin is one of the natural red dyes that widely used in the food system. One of the plants that contain these pigments are contained in the cactus fruit.

Sampling sites are in Subdistrict of Mantikulore, Subdistrict of East Palu and Subdistrict of South Palu in Central Sulawesi, Indonesia. The basis of the selection of the appropriate sampling where the three sites is the habitat of cactus.

This research conducted in several steps, the first step was sampling by tweezers to pick the ripe fruit that marked red-violet colored fruits and weight about ± 20 -50 g. Sample preparation is done by dried sorting cactus fruit is cleaned of dirt and fine spines attached to the fruit. The next step is done by wet sorting, the fruit is washed with running water until clean, then the fruit peel, separated between flesh and skin, and flesh of the fruit blended for 5 minutes to reduce the sample size to increase the effectiveness of the process of extraction of the desired compound is interested because the surface area of material that is extracted to the extraction of materials will be even greater, thus the rate of dissolution of a material higher. Cactus fruit extract weighed as much as 100 g, then add about 100 ml of solvent. Preferred solvents that used in this experiment was ethanol: water (50:50). The selection of solvent is used based on characteristic of betacyanin is polar.[11] Other experiment showed that betacyanin have high solubility in water so that the extraction solvent is usually done with water or using a solvent mixture of water: ethanol (1: 1).[12] After the addition of the solvent, then the mixture is shaken for 15 minutes in cold temperatures is intended to dissolve and homogenize the mixture. Homogeneous mixture was centrifuged (15000 rpm and 40°C for 20 minutes) to separate the compounds of the mixture heavier the precipitation principle. Supernatant obtained was evaporated to obtain a thick extract using a rotary vacuum evaporator.

In the qualitative test used two reagents that HCl 2 M and NaOH 2 M. The purpose of this qualitative test to ensure betacyanin compounds contained in extracts of cactus fruit. Based on Table 1 using HCl 2 M seen that all samples contained betacyanin is characterized by discoloration after HCl 2 M is added and then heated at 100 °C for 5 minutes. This caused betacyanin compound is unstable at high temperatures and low pH. Previous research suggested that during the heating process betanin degraded by isomerization, decarboxylation, or termination (by heating or acid) produces a red color gradually decrease and eventually turn into brown color.[13] Reid *et al* (1980) showed the damage of betacyanin sharply occurred below pH 4 with HCl.¹⁴The results of using NaOH as reagent test was explained on table 2. All positive samples containing betacyanin extract characterized by a color change from red to yellow. These changes occur because under alkaline conditions cause hydrolysis of aldamin bonding.[4]

Quantitative test results by spectrophotometer present the levels of betacyanin are 0.0217 mg/100 g (Mantikulore Subdistrict), 0.0121 mg /100 g (East Palu Subdistrict) and 0.02118 mg /100 g (South Palu Subdistrict). These results indicate that the levels of total betacyanin obtained in cactus fruit is still small than bits (120 mg/100g fresh weight), amarantus (46-199 mg/100 g fresh weight), and the results of previous studies of cactus fruit (*Opuntia elatior* Mill) (47.10 mg/100 ml).[3,14,15] This is due to the possibility of the minerals contained in the soil where sampling is less thus affecting the levels of total betacyanin from cactus fruit.

Table 1: Result of Qualitative Analysis Using HCl 2 M

No	Sample	Colour of Sample	Result	Description
1	I a	Pink	Brown	Positive
2	I b	Pink	Brown	Positive
3	I c	Pink	Brown	Positive
4	II a	Pink	Brown	Positive
5	II b	Pink	Brown	Positive
6	II c	Pink	Brown	Positive
7	III a	Pink	Brown	Positive
8	III b	Pink	Brown	Positive
9	III c	Pink	Brown	Positive

Where, extract of cactus fruit from I) Mantikulore Subdistrict, II) East Palu Subdistrict, III) South Palu Subdistrict; a, b, c) Number of Replication

Table 2: Result of Qualitative Analysis Using HCl 2 M

No	Sample	Colour of Sample	Result	Description
1	I a	Pink	Yellow	Positive
2	I b	Pink	Yellow	Positive
3	I c	Pink	Yellow	Positive
4	II a	Pink	Yellow	Positive
5	II b	Pink	Yellow	Positive
6	II c	Pink	Yellow	Positive
7	III a	Pink	Yellow	Positive
8	III b	Pink	Yellow	Positive
9	III c	Pink	Yellow	Positive

Where, extract of cactus fruit from I) Mantikulore Subdistrict, II) East Palu Subdistrict, III) South Palu Subdistrict;
a,b,c) Number of Replication

Table 3: Result of Quantitative Analysis

No	Sample	Wet Weight (g)	A (λ 538 nm)	Conc. (mg/L)	Conc. Average (mg/L)	Conc. Average (mg/L)	Betacyanin Conc. (mg/100 g)
1	I a	100.019	0.5460 0.5505 0.5510	5.005 5.046 5.050	5.033	4.435	0.022
2	I b	100.017	0.4260 0.4250 0.4276	3.905 3.895 3.919	3.900		
3	I c	100.018	0.4779 0.4750 0.4760	4.380 4.354 4.363	4.365		
4	II a	100.007	0.4817 0.4835 0.4832	4.415 4.432 4.429	4.425	4.242	0.021
5	II b	100.005	0.4368 0.4389 0.4371	4.004 4.023 4.006	4.011		
6	II c	100.003	0.4681 0.4688 0.4674	4.290 4.297 4.284	4.290		
7	III a	100.015	0.4538 0.4553 0.4565	4.150 4.176 4.185	4.173	4.235	0.021
8	III b	100.011	0.4365 0.4355 0.4348	4.001 3.992 3.986	3.993		
9	III c	100.012	0.4953 0.4964 0.4938	4.540 4.550 4.526	4.539		

Where, extract of cactus fruit from I) Mantikulore Subdistrict, II) East Palu Subdistrict, III) South Palu Subdistrict;
a, b, c) Number of Replication

4. Conclusion

Based on the results obtained it can be concluded that the levels of total betasianin cactus fruit from Mantikulore Subdistrict 0.022 mg / 100 g, East Palu Subdistrict of 0.021 mg / 100 g and South Palu Subdistrict 0.021 mg / 100 g.

References

- [1] Pitopang R., Khaeruddin I., Tjoa A., Burhanuddin, F., I., 2008, Book of Types of Trees Lived In Sulawesi, UNTAD Press, Palu.
- [2] Anonim, 2010, <http://www.htysite.com/P-kaktus.htm> (Online 7 October 2013).
- [3] Chauhan, S.P., 2010, *Phytochemical and Pharmacological Screening of Fruit of Opuntia elatior Mill*, Thesis, Saurashtra University.
- [4] Schwartz, S.J, Von Elbe J.H. Identification of betanin degradation products, *Eur Food Res Technol.* 1983,
- [5] Grotewold E., *The Challenges of Moving Chemicals Within and Out of Cells: Insight Into the Transport of Plant Natural Products*, *Planta.* 2004.
- [6] Zyard, J.P., and Laurent, 2003, Betalains. In: Davies K (eds) *Plant Pigments and Their Manipulation*. Annu. *Plant Rev.* Oxford.
- [7] Rukmana, R., dan Yuniarsih Y.O., 1998, *Ornamental Plants: Cactus*, Penerbit Kanisius, Bandung.
- [8] Delgado, V.F., Jiménez A.R., Parades L.O., 2000, Natural Pigments: Carotenoids, Anthocyanins, and Betalains - Characteristics, Biosynthesis, Processing, and Stability, *Critical Reviews in Food Science and Nutrition*.
- [9] Naderi, N. *et al*, 2010, Betalain Extraction From *Hylocereus polyrhizus* for Natural Food Coloring Purpose. Departement of Food Technology, Faculty of Food Science and Technology, University Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.
- [10] Harborne, J.B., *Phytochemical methods: A Guide How to Analyze Plant*, ITB Publisher, Bandung. 1987,
- [11] Khuluk, A.D., *et al*, 2007, Extraction and Stability of Anthocyanin Leaves Blood (*Alternanthera dentata*) Comparative Study Air Solvent: Ethanol and Temperature Extraction. *Technology of Agriculture Journal*, Vol 8 No 3.
- [12] Eder, R., 1996. *Handbook of Food Analysis*, Vol. I. Marcel Dekker Inc. New York.
- [13] Huang, A.S., & Von elbe, J.H., *Kinetics of The Degradation and Regeneration of Betanine*. *Journal of Food Science.* 1985,
- [14] Anonim, 2009, *Merah Bit - (SEAFast) CENTER*, IPB, Bogor.
- [15] Cai, Y.Z., M. Sun, W. Schliemann & H. Corke, Chemical Stability And Colorant Properties of Betaxanthin Pigments from *Celosia argentea*, *J. Agric. Food Chem.* 2001.