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Research Article

CHARACTERIZATION OF BETACYANIN FROM DRAGON FRUIT FLESH AND ITS APPLICATION AS A NATURAL DYE FOR PHARMACEUTICAL PREPARATION

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ABSTRACT

The flesh of dragon fruit (*Hylocereus lemairel* (Hook.) Britton and Rose) contains betacyanin pigment. Betacyanin (6-O-3-hydroxy-3-methyl-glutaryl) -betanin) is red pigment that has many benefits in pharmaceutical preparation. The aim of this study was to characterize physicochemically, to measure the stability and to apply betacyanin as a natural dye. Extraction was carried out by water using Ultrasonic Assisted Extraction (UAE) method which is sonicated at 50 kHz for 30 minutes at 25°C. Extract was freeze dried for 48 hours. The dried extract was characterized and purified by using preparative TLC and physicochemically analysed using UV-Vis and FTIR spectrophotometers. Betacyanin stability against pH and temperature was tested and applied as a dye in tablet. The result of this study showed that betacyanin was detected with Rf value 0.6 which was equal to betacyanin standard Sigma Aldrich®. The maximum wavelength of betacyanin was obtained at 534 nm and the IR spectra showed similarity with betacyanin standard with the same functional groups between 4000 and 600 cm^{-1}. The stability study results were obtained stable at temperature below 40 °C and at pH 4. Betacyanin applications as natural dye of tablet have been successfully carried out with good colour stability exceeding 3 months of storage at room temperature.

Keywords: Betacyanin; Dragon Fruit; Natural Dye.

INTRODUCTION

Food colouring is widely used in the manufacturing process of pharmaceutical preparations. The purpose of this colouring is not only to increase the attractiveness of the product, but also to help patients distinguish between the drugs consumed and to help distinguish the dosage from the same drug, thereby reducing errors in drug used[1]. Various colouring agents, especially synthetic dyes have a negative effect on the human body because they are carcinogenic, and there are new rules in Germany, US, India and several other European countries which prohibit the use of several synthetic dyes, making fewer use of these dyes[2]. This led to the demand for natural dyes derived from natural increased, because of environmentally friendly, no side effects, non-toxic and has good antioxidant. One plant that contains natural dyes is dragon fruit (*Hylocereus lemairel* (Hook) Britton and Rose) or red pitaya; which is a family of Cactaceae[3]. Dragon fruit flesh is purple-red when ripe and has black seeds around it[4]. Dragon fruit flesh contains nutrients and minerals such as vitamin B_1, Vitamin B_2, Vitamin B_3 and Vitamin C, proteins, carbohydrates, fiber, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolic, polyphenols, carotene, phosphorus, iron, phytoalbumin and betacyanin[3]. One of the main components in dragon fruit is betacyanin which is a red pigment that has a potential as a natural dye[5]. Betacyanin (6'-O-3-hydroxy-3- methyl-glutaryl) -betanin) with N-heterocyclic is a chemical compound that has antioxidant activity against free radicals[6]. During this time betacyanin pigments are widely used as food colouring[7], so it is necessary to use betacyanin pigments as natural dyes in pharmaceutical preparations. Natural dyes are less stable against light, heat, and at certain pH values when compared to synthetic dyes[8]. This is a factor in the lack of use of natural dyes in pharmaceutical preparations. Natural dyes from different parts of plants can be extracted through various methods such as using water solvents, organic solvents and extraction using enzyme[9]. But from these methods, the right and good method for extracting red dyes in dragon fruit has not been obtained.

This study aims to extract betacyanin from dragon fruit flesh using an optimized ultrasonic extraction method to get betacyanin more stable and good physicochemical properties. Betacyanin was purified and characterized by using UV-Vis and FTIR spectrophotometers and its stability against pH and temperature was examined. The betacyanin was applied as a dye in the formulation of tablet and the results of this study are expected to be an alternative used of natural dyes for pharmaceutical preparations and can reduce environmental pollution problems because natural dyes are more eco-friendly and safe.

MATERIALS AND METHODS

Sample Collection and Preparation

Dragon fruit (*Hylocereus lemairel* (Hook) Britton and Rose) were purchased from local farmers in Padang West Sumatera Indonesia which were already in sterile packaging for trafficked. 5 kg dragon fruits were peeled and cleaned to separate the fruit flesh from the rind. The flesh of the dragon fruit were mashed and the flesh mass was used for the further analysis.

Chemicals and Reagents

Betacyanin used as standard material was purchased from Sigma Aldrich® and all the reagents used in the experiment were of

analytical grade and were supplied by Merck, Darmstadt, Germany.

Preparation of Betacyanin Extract

Betacyanin extraction process was carried out by using ultrasonic-assisted extraction technique. A total of 1000 g of dragon fruit flesh was homogenized with 500 ml of distilled water 2:1 (w/v). The mixture was placed in an ultrasonic bath and sonicated at 50 kHz for 30 minutes at 25ºC. Extract was separated and filtered using the Buchner funnel through Whatman No. 1 filter paper. The residue was extracted again with water 3 times. Solutions were mixed into a single solution and the colored solution was centrifuged at 6000 rpm for 15 minutes at room temperature and the supernatant was freeze dried using freeze dryer Alpha 1-2 LD plus® for 48 hours. The dried extract was stored at 4ºC to maintain the colour stability of the extract before use. Specific and non-specific characterization of dragon fruit extract was carried out based on the Ministry of Health Republic of Indonesia guidelines[10].

Chromatographic Procedure

Extract was purified on pre-coated TLC silica gel plates 60 F_{254} (20 cm × 10 cm) by means of band applicator CAMAG Linomat 5®sample applicator equipped with a 100 µL Hamilton® syringe. 1 gram of dried extract and standard betacyanin (Sigma Aldrich®) were dissolved with 10 mL solution of methanol : acetic acid (6:4) as mobile phase. 2 µL of sample and standard solutions were applied as a band of 4 mm wide, 0.3 mm high and 6 mm apart in the form of bands on TLC plates. The developing distance was 85 mm, measured from the lower edge of the plates. The plates were allowed to dry for 15 minutes for visualization of the spots using CAMAG® UV Cabinet dual wavelength, 254/366 nm. Migration distances were measured and retention factors (R_F) were calculated.

Physicochemical Characterization

Preparative TLC isolates were obtained by scraping the stationary phase where the sample stains on the plate, then dissolved with methanol as much as 10 mL and centrifuged. The supernatant was taken and analysed, whereas for standard betacyanin a concentration of 1000 µg/mL was made. Measurement of the maximum wavelength of the sample and standard was measured at 400-800 nm using a UV-Vis Spectrophotometer Shimadzu UV-1800®.

Sample and standard betacyanin functional groups were measured using an FTIR spectrometer Perkin Elmer®. Spectrums were measured at wave numbers 600-4000 cm^{-1}. This analysis will show a spectrum that describes functional groups of betacyanin.

Betacyanin Stability Study

Stability studies of betacyanin samples were carried out on temperatures (25 °C, 40 °C, 60 °C, 80 °C and 100 °C) and pH (2; 3; 4; 5; 6; 7; 8; 9; 10; 11 and 12) with addition 1% HCl and 1% NaOH within 30 minutes, then absorbance was measured using a UV-Vis Spectrophotometer Shimadzu UV-1800®.

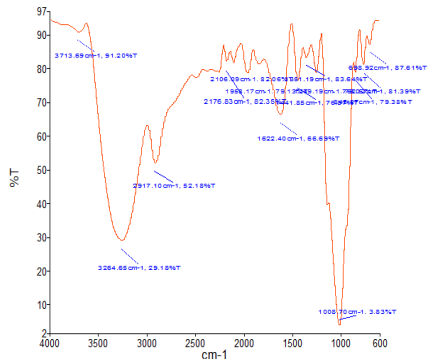
Application of Betacyanin Samples in The Formulation of Paracetamol Tablets

The dried extract was applied in making paracetamol tablets using direct press method with formula: Paracetamol 250 mg, 10% starch, Talcum 5%, betacyanin qs powder, Avicel®PH 102 added to a total weight of 300 mg, made for 10 tablets. Tablets were evaluated for the colour stability of the tablets in the range of storage 1, 2 and 3 months at room temperature.

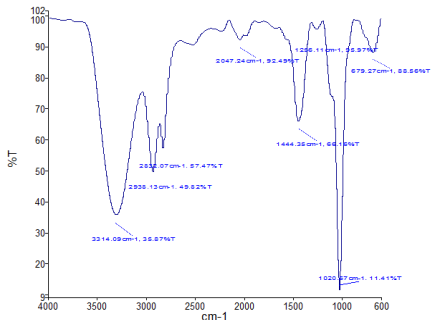
RESULTS AND DISCUSSION

Dragon fruit flesh contains pigment called betacyanin. Betacyanin is a red pigment that has many benefits, one of which is in pharmaceutical preparations as a natural dye in tablet printing. In this study, betacyanin was extracted using the Ultrasonic Assisted Extraction (UAE) method. This method uses ultrasonic which can cause a cavitation effect in order to break down the cell wall and betacyanin is released out easily thus maximizing extraction results[11]. The drying method used is freeze drying to remove the water solvent. This method aims to maintain sample quality because betacyanin is not stable to heating. The sample is frozen first to form solid phase, then dried using freeze dryer. The sublimation process occurs which forms a dry betacyanin extract. Betacyanin yield obtained from this method was 17, 6 %w/w.

The optimum conditions for analysis betacyanin by TLC was chosen experimentally by considering the effects of several factors such as the solution concentration, the ratio of solvents in the eluent and the type of TLC plates. The solvent system used was system methanol/acetic acid (6:4) which gave good resolution and good chromatogram with Rf value 0.6 which was equal to betacyanin standard (Sigma Aldrich®). Betacyanin was purified and isolates were analysed using UV-V is Spectrophotometer Shimadzu UV-1800® and FTIR spectrometer Perkin Elmer®. The results showed that both sample and standard have λ_{max} at 534 nm. Betacyanin absorbs light strongly at a wavelength of 532-538 nm[12].



Betacyanin (Sigma Aldrich®)



Betacyanin of Dragon Fruit Flesh

Figure 1: The FTIR spectra of betacyanin at wave numbers 600-4000 cm^{-1}

Betacyanin Spectrums were measured at wave numbers 600-4000 cm^{-1}. As shown in Figure 1, the FTIR spectra of betacyanin sample showed similarity with betacyanin standard, although there was a slight shift in the wave number still in the range. The peaks of 3714 cm^{-1} and 3320 cm^{-1} betacyanin standard and sample are O-H and N-H tensile vibrations. O-H and N-H bonds are at wave numbers between 3800-3200 cm^{-1}[13]. The peaks of 2917 cm^{-1} and 2939 cm^{-1} betacyanin standard and sample are C-H and CH_2 tensile vibrations. The peaks of 699 cm^{-1} and 672 cm^{-1} betacyanin standard and sample are bending vibrations of the C=CH group.

The stability of betacyanin pigments is affected by temperature and pH. It can be seen in the change in colour of the solution from red to yellow and absorbance reduction of betacyanin. The result showed that betacyanin stable at temperature below 40ºC and pH 4 as shown in Figure 2. At high temperatures and critical pH, hydrolysis reaction occurs in betacyaninon the N=C bond which causes the betacyanin changed to betalamic acid (yellow) and cyclo-Dopa 5-O-glycoside. While at lower pH, deglycolization occurs in betacyanin become betanidin. The bond between a betacyanin and a glycoside is an acetal bond that is easily broken by strong acids such as hydrochloric acid[14].

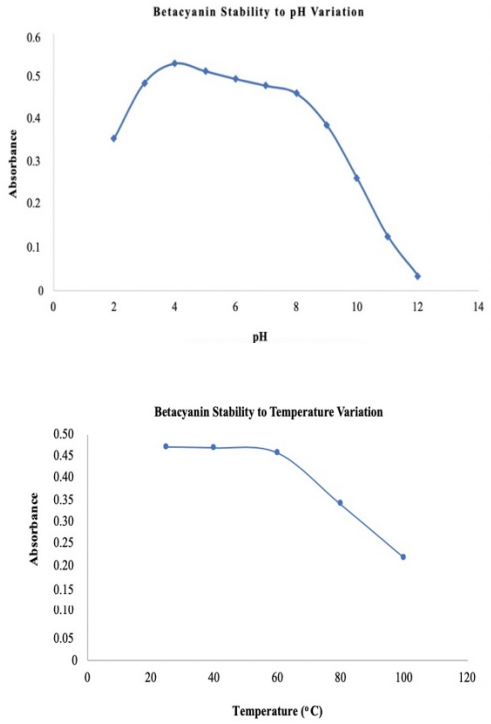


Figure 2: Betacyanin Stability to pH and Temperature

Betacyanin applications as natural dyes in tablet printing have been successfully carried out. The colour of the tablets produced is solid red and the tablets are stored and evaluated for 3 months. The results can be seen in Figure 3. For 3 months of storage, the tablet is still good and there are only a few colour changes. It can be concluded that betacyanin from dragon fruit flesh can be used as an alternative colouring agent in pharmaceutical products.

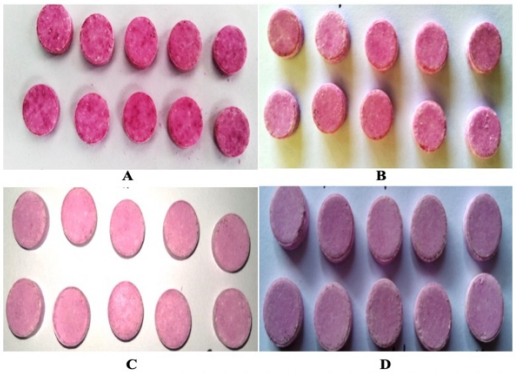


Figure 3: Tablet colour comparison for 3 months of storage, A (0 months), B (1 month), C (2 months), D (3 months)

CONCLUSION

Betacyanin of dragon fruit flesh has been successfully isolated and physio-chemically characterized. The extraction method used is simple, flexible, cost-effective and environmentally friendly. The results of this study showed the similarity between betacyanin sample and betacyanin Sigma Aldrich® and betacyanin sample can be used as an alternative colouring agent in pharmaceutical products. The colour stability of the tablets produced will be affected by storage. The colour will last longer if stored on betacyanin stability conditions at temperatures below 40 at pH 4.

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