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Red Dragon Peel (*Hylocereus polyrhizus*) as Antioxidant Source

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Abstract. Dragon fruit (*H. polyrhizus*) is a plant from South America which is easily found in tropical countries. The fruit is one of popular in Indonesia due to its unique shape, colours, and it is consumed as a beverage. However, the thickness of the peel from the fruit can cause problem in waste management. Therefore, in this study antioxidant activity from pigment and non-pigment extracts from the peel was determined. The pigment was extracted by using maceration with ethanol HCl while non-pigment extraction was carried out by using methanol followed by partition with hexane, dichloromethane, and ethyl acetate, respectively. The antioxidant activity was analyzed by using DPPH method. The results showed that the pigment extract exhibited high antioxidant activity with IC₅₀: 159.6 ppm while ethyl acetate extract showed weak activity and the hexane and DCM showed no antioxidant activity. Therefore, the pigment from this peel possess antioxidant activity and the further investigation of antioxidant activities are needed by using different methods and to determine the chemical structure which responsible in this activity.

INTRODUCTION

Pigments are chemical compounds that absorb light in the wavelength range of the visible region or it can be called as biochrome, which is defined as a specific chemical substance with a coloured molecule, produced by living organisms such as plants, fungi, human, animals.[1,2] In addition, the colourful of plants have become attention of man throughout history for art as well as for food colouring such as anthocyanins, betalains, carotenoids, and chlorophylls. [3,4]

Part of plants such as flowers, fruit, leaves, seeds, skin, stems and roots when extracted can produce dyes and the extracts have been used for different purposes such as textile or food industry.[5] One potential plant that can be used as a natural dye is dragon fruit (*H. cacti*) or by another name Pitaya. Published papers reported that dragon fruit has a potential as source to produce red pigment and it's called as betalains. Betalain is a water-soluble pigment that gives color to flowers and fruits. Betalain pigments are divided into two groups, namely betasianin which produces purplish red and betaxantin which produce yellow-orange colors. [6,7]

In the dragon fruit peel, it is also containing pigments which are usually only discarded as a food waste and have not been used optimally. This is very unfortunate because the fruit of the dragon fruit itself has several advantages. According to Wu *et. al.* (2006), the peel possessed polyphenol compounds with antioxidant activities. [8]. According to Rahmawati (2016), the peel contain betasianin, flavonoids, and phenol. [9] In addition, dragon fruit skin also contains vitamin C, vitamin E, vitamin A, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolic, carotene, and phytoalbumin which are thought to have antioxidant benefits and can also be potential for antimicrobial activities [10]. In this study, analysis of antioxidant activity was carried out on pigment extracts as well as non-pigments extracts.

EXPERIMENTAL DETAILS

EXTRACTION

The fresh peels were cleaned, cut into small pieces, blended and kept in refrigerator. For pigment extraction, one kg of the peels was extracted by using maceration with 80 % (v/v) ethanol and HCl 0.1 N for 12 hours. The mixture was sonicated for 25 minutes and then filtered. The extract was concentrated and obtained pigment extract. The extract was partitioned with *n*-hexane, dichloromethane, and ethyl acetate to obtain *n*-hexane, dichloromethane, ethyl acetate, and pigment extracts. Furthermore, the residue was extracted by using maceration with methanol for 24 hours to obtain non-pigment secondary metabolites. The mixture was filtered and concentrated to obtain methanol extract. In the same manner, the methanol extract was partitioned to obtain *n*-hexane, dichloromethane, ethyl acetate extracts.

ANTIOXIDANT ACTIVITY

Samples were prepared with a certain concentration in methanol. Approximately 100 μ L of the sample was transferred into row A of the microplate (plate consist of A-H rows, each row consists of 12 wells). Two-fold dilutions of the compound were added to the next row until the concentration was 31.25 μ g/mL. Five μ L Diphenylpicrylhydrazyl (DPPH) was added to each well of the sample. The microtiter plate was then vortexed and incubated for 30 min in the dark room.[11] Then, absorbance was measured, and the total percentage of radical scavenging activity was calculated based on the following formula:

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY ANALYSIS ON PIGMENT EXTRACT

In order to analyze the present of betalains, the HPLC analysis was performed and the methods was referred to Wybraniec *et al.* (2001). Reversed-phase HPLC was used with ODS column and he separation was performed isocratically using a mixture of 90% solvent A (0.5%aq.TFA) with 10% solvent B (acetonitrile) for 35 min, at a flow rate of 1 mL/min at 538 nm.[7]

RESULT AND DISCUSSION

In this research, the pigment from the dragon fruit peel was extracted by using ethanol 80% and 0.1 N HCl and obtained red pigment. The ethanol was used because from previous publication showed that this aqueous organic solvent was optimal solvent to extract betalains. In this extraction, the acid was added due to increasing the stability as well as to avoid oxidation by polyphenoloxidase. [3] In this research, after the pigment extraction, the residue was air-dried and then applied to maceration with methanol and followed by liquid-liquid extraction to obtain *n*-hexane, dichloromethane, and ethyl acetate extracts.

To each extract was then analyse for their antioxidant activity by using free radical (DPPH) scavenging activity method. This method is a popular in antioxidant assay and the method is simple in determination of antioxidant in plant extracts and foods.[12] The method can be characterized by the formation of stable radicals with electrons that can be delocalised and give a deep purple colour, and the density of this colour can be reduced if reacted with a compound that can donate hydrogen atoms.[13]In antioxidant activity analysis, each extract was tested in inhibition of free radicals with a concentration of two-fold dilution and then IC_{50} was obtained. The smaller the IC_{50} value of a compound or extract, the stronger the antioxidant ability. Analysis of antioxidant activity from several extracts from dragon fruit peel can be seen in Table 1.

TABLE 1. Antioxidant Activity from several extracts of Dragon fruit peel

Extract	IC ₅₀ (ppm)
Pigment	159.6
Ethyl acetate	648.9
Dicloromethane	>1000
<i>n</i> -Hexane	>1000

From the Table 1, pigment extract showed the highest antioxidant activity compared to ethyl acetate, dichloromethane, and *n*-hexane with IC₅₀ value 159,6 ppm. The antioxidant activity in pigment extracts might be due the present of betalains or phenolic compounds. Based on the previous research, it was stated that the betalains from beetroot extract possessed high antioxidant activity by inhibiting radical DPPH with IC₅₀ of 0.11 ppm.[14,15] The antioxidant activity of betalain compounds is related to the amount of hydroxy in phenols and imino groups and also sugars which are bound to betacyanins such as betanidine 6-O-β-glucoside. [16,17]

In order to investigate the present of betalains in the pigment extract, HPLC analyses was performed and the methods was described in Wybraniec *et al.* (2001).[7] From the HPLC chromatogram (Figure 1) showed that the present of betalain compounds. The HPLC pattern from the extract is similar which reported by Wybraniec *et al.* (2001). However, the antioxidant activity from individual compound has not been reported. Therefore, in this continuation of the research, the isolation of the compounds will be conducted and their antioxidant activities will analysed.

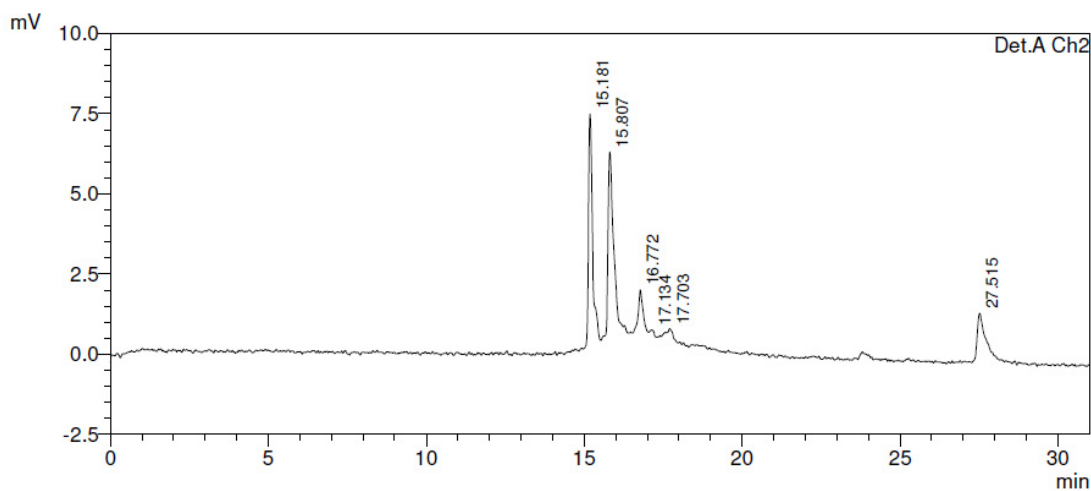


FIGURE 1. HPLC pattern of betacyanins from dragon fruit peel. λ : 538 nm

CONCLUSION

Antioxidant activity analysis of several extracts from dragon fruit peel showed that pigment extract showed good antioxidant activity by using DPPH radical scavenging with IC₅₀ value of 159.6 ppm.

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