Antibacterial Activity of Red Dragon Peel (Hylocereus polyrhizus) Pigment

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Abstract. Red dragon fruit (Hylocereus polyrhizus) is a species of plant that provides natural pigment which is betalain. Betalain is one of the pigments that gives a natural colour to flowers and fruits. Moreover, of betalain in red dragon fruit peel, there are bioactive compounds such as polyphenol and flavanoid with antioxidant and antibacteria activities. This research was objected to extract pigments and others secondary metabolites from red dragon fruit peel using ethanol, methanol, dichloromethane and ethyl acetate solvents, continued with determined potential antibacteria activity using Resazurin Microtiter Assay (REMA) method. The results of antibacterial activity showed inhibition percentage against test of positive Gram bacteria Streptococcus aureus ATCC 29213 with extract of n-hexane, ethyl acetate and pigment sequentially were 67.5, 67.0, 85.1, 83.1 % and Bacillus subtilis ATCC 11774 were 73.4, 98.6, 97.1, 100 % and negative Gram bacteria Escherichia coli ATCC 35218 were 70.0, 73.1, 74.2, 94, 9 % and Vibrio algynolyticus were 72.6, 73.1, 74.2, 94.9 % at final concentration of 20 μg/mL.

1. 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 (Hylocereus 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 betaxanthin 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] Whereas 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] From previous studies, the fruit possessed antimicrobial activities against pathogenic microbial with different assay.[11, 12] The use of antibiotics irrationally causes many pathogenic microbes to adapt to their environment and become resistant to synthetic antibiotic drugs. This has encouraged the development of novel antimicrobial substances derived from natural products.[13, 14] Therefore, in this study was conducted to evaluated the antibacterial activities of dragon fruit peel pigments against pathogenic bacteria.

2. Experimental

2.1. Extraction
The fresh peels were cleaned, cut into small pieces, blended and kept in refrigerator. One kg of the peels was extracted by using maceration with 80 % (v/v) methanol 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. The mixture was filtered and concentrated to obtained methanol extract. In the same manner, the methanol extract was partitioned to obtained n-hexane, dichloromethane, ethyl acetate extracts.

2.2. Antibacterial Activity
The antibacterial activities of the extracts were assayed against *E. coli* ATCC 35218, *V. algino*, *S. aureus* ATCC, and *B. subtilis* by microdilution method followed (Sarker et al. 2007). All the tested extract and positive controls (Amoxan®, Cefadroxil) were dissolved in DMSO to give final concentration of 20 µg. Briefly, precultures of the tested microorganisms were made by inoculating 25 mL of medium (NB medium for bacteria and WP medium for fungus) and incubated for 24 h at 37 °C for bacteria or 4 days at room temperature for fungus. Then the cell density was adjusted to 10^7 CFU/mL (Martins et al., 2011). Microbial suspension of 20 µL was distributed in each well containing extract, NB and resazurin. The plate was incubated at 37 °C for 24 h for the bacteria or 4 days for the fungus and the optical the density of each is measured by using microplate reader. The experiment was run in three replicates.[13, 15]

3. Results 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 obtained n-hexane, dichloromethane, and ethyl acetate extracts.

The antimicrobial activity against four pathogenic bacteria *E. coli*, *V. algino*, *S. aureus*, and *B. subtilis* by using microdilution method. This method was used due to it is having several advantages such as it can be used to analysed several different sample at once, requiring small amount of sample, time saving, more accuracy and high sensitivity. [16] In this assay, the resazurin was added as microbial growth indicator. The addition of resazurin in the assay can predict the presence of visual antimicrobial activity (qualitative) which is through colour change. A compound which has antimicrobial activity if the colour is changed from pink to blue, whereas it does not have activity or weak activity, the colour is stayed in pink. This resulted from the oxireductase enzyme in the microbe reduce blue resazurin into pinkish resofurin.[17] Furthermore, the antimicrobial activity can be
determined by measuring the optical density to give the inhibition growth. The antimicrobial activity from various extracts of the species in 20 µg was presented in Table 1.

**Table 1. The antimicrobial activity from various extracts of the species**

| Sample         | Growth Inhibition (%) |
|----------------|-----------------------|
|                | S. aureus | B. Subtilis | E. coli | V. alginoliticus |
| n-hexane       | 67,5      | 73,4        | 70,0    | 72,6             |
| DCM            | 67,0      | 98,6        | 69,3    | 73,1             |
| Ethyl acetate  | 85,1      | 97,1        | 71,5    | 74,2             |
| Pigment        | 83,1      | 100         | 68,0    | 94,9             |
| Amoxan®        | 90,0      | 98,4        | 67,9    | 100              |
| Cefadroxil     | 90,7      | 98,9        | 95,4    | 98,7             |

All extracts exhibited moderate to high inhibition activity toward Gram-positive bacteria. These results are in accordance with previous published paper showing that Gram-negative microorganisms are typically more resistant to antimicrobial agents than Gram-positive bacteria. This has long been explained by the presence of an outer membrane permeability barrier in Gram-negative bacteria, which limits the access of the antimicrobial agents to their targets in the bacterial cells.[18] The pigments showed high activity across the bacteria compared to the extracts and surprisingly the pigment exhibited the highest activity against *Vibrio alginoliticus* (pathogenic to human and animal such as fish). This finding is in line with a previous publication by Nurmahani et al. They reported that the peel of the fruit chloroform extracts from *H. polyrhizus* were found to exhibit good antibacterial activity where almost all the pathogens by using disc diffusion method and MIC.[11] In this study, we reported the antibacterial activities by using microdilution method and this is the first report of antibacterial activity from the peel by using the method.

### 4. Conclusions

The extracts responded differently toward different types of bacteria. The study showed that all the extracts exhibited antibacterial activity. It can be concluded that the pigment and the extracts from the peel can be classified as a good source of potent natural antibacterial agents and further investigation are needed.

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