Antifungal Activity of Red Dragon Peel (*Hylocereus polyrhizus*)

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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 give a natural color to flowers and fruits. Moreover, of betalain in red dragon fruit peel, there is a bioactive compound such as polyphenol and flavonoid with antioxidant and antifungal activities. This research was objected to extract pigments and other secondary metabolites from red dragon fruit peel using ethanol, methanol, dichloromethane, and ethyl acetate solvents, continued with determined potential activity against *Candida albicans* by using Resazurin Microtiter Assay (REMA) method. Furthermore, total phenolics compounds (TPC) and total flavonoid compounds (TFC) were determined from all the extracts. The results of antifungal activity showed various inhibition percentage against the fungal at 500 ppm. The ethyl acetate showed the highest activity among the extracts with 74.27% inhibition followed by pigment extracts (60.88%). Furthermore, from test TPC and TFC determination, the ethyl acetate possessed the highest amount with the value of 34.38 mg gallic acid equivalent/g fresh weight and 28.62 mg quercetin/g fresh weight, respectively. Therefore, to find the active compounds, multiple analysis is needed.

1. **Introduction**

Plants, when extracted, can produce dyes and the extracts have been used for different purposes such as the textile or food industry [1]. One potential plant that can be used as a natural dye is dragon fruit (*Hylocereus cacti*) or by another name Pitaya. Wybraniec and coworkers reported that dragon fruit has a potential as a source to produce red pigment and it's called betalains [2]. Betalain is a water-soluble pigment that gives color to flowers and fruits. Betalain pigments are divided into two groups, namely betacyanin which produces purplish red and betaxanthin which produce yellow-orange colors [2, 3].

In the dragon fruit peel, it is also containing pigments which are usually only discarded as 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 [4]. Whereas according to Rahmawati (2016), the peel contains betacyanin, flavonoids, and phenol. Also, dragon fruit skin contains some vitamins, 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 [5]. From previous studies, the fruit possessed antimicrobial activities against pathogenic microbial with different assay [6, 7]. Nowadays, there is growing interest in pharmaceutical industries to investigate plants as a source of active compounds with fewer side effects to human especially in combating infection diseases [8, 9].
Due to these facts, the exploration of new alternative medicines derived from plants is required. Flavonoids are one of phenolic group which has antimicrobial activity [10]. These compounds act to inhibit nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism [11]. Therefore, this study was conducted to evaluate the antifungal activities of dragon fruit peel extracts against pathogenic fungal as well as to determine their total phenolics and flavonoids compounds.

2. Experimental

2.1. Extraction
The fresh peels were cleaned, cut into small pieces, blended and kept in a refrigerator. 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. 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. Antifungal Activity
The antifungal activities of the extracts were assayed against C. albicans by the microdilution method followed by Sarker et al. 2007 [12, 13].

2.3. Total Phenolics and Flavonoids Compounds
Total phenolic compounds were determined according to Hendra et al. [9] and the results were expressed as mg gallic acid equivalents/g fresh weight (DW). Total Flavonoid Assay The total flavonoid compounds in each extract was determined according to Hendra et al. [9] and expressed as mg quercetin equivalents/g fresh weight.

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. [14] 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 antifungal activity against Candida albicans by using microdilution method. In this assay, the resazurin was added as a microbial growth indicator. The addition of resazurin in the assay can calculate existence of visual antimicrobial activity (qualitative) which is through color change. A sample or extracts which have antimicrobial activity if the color is changed from pink to blue, whereas it does not have an activity or weak activity, the color stays in pink. This resulted from the oxyreductase enzyme in the microbe reduce blue resazurin into pinkish resofurin. [15] 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 500 ppm was presented in Table 1.

All extracts exhibited moderate to low inhibition activity toward the fungal. The ethyl acetate showed the highest activity among the extracts followed by the pigment extract, but these activities are lower than the positive control (Table. 1). Based on our knowledge, there is no report regarding antifungal activity from the peel of these fruits. Furthermore, the table showed that the extracts exhibited various amounts of TPC and TFC. The ethyl acetate showed a high amount of TPC and TFC with a value of 34.38 mg gallic acid equivalent/ g fresh weight and 28.62 mg quercetin/ g fresh weight, respectively.
Table 1. The antimicrobial activity from various extracts of the species in 500 ppm.

| Extracts   | % Inhibition a | Total Phenolic compounds (TPC) b | Total Flavonoids compounds (TFC) c |
|------------|----------------|---------------------------------|-----------------------------------|
| Pigment    | 60.88          | 29.07                           | 25.00                             |
| n-Hexane   | 44.95          | 16.29                           | 14.27                             |
| DCM        | 47.23          | 27.54                           | 25.95                             |
| Ethyl acetate | 74.27          | 34.38                           | 28.62                             |
| Ketoconazole | 90.97          | -                               | -                                 |

a. at concentration 500 ppm; b mg gallic acid eq./g fresh weight; c mg quercetin eq./ g fresh weight.

This study showed that the extract with high antifungal activity showed high TPC and TFC. This might be there is a correlation between phenolic and flavonoid compounds with antifungal activity. Previous reports showed that some medicinal plants contain phenolic and flavonoid compounds had antimicrobial activities [16–18]. Rodriguez Vaquero et al. (2007) investigated properties of some phenolic in different wine as antibacterial gallic, caffeic, vanillic acid, rutin, and quercetin) of different wine against pathogen bacteria and the result showed that they could inhibit the growth of E. coli. Further, these activities related to the concentration of phenolic compounds present in the wine [19]. The presence of flavonoid compounds in the ethyl acetate extracts suggests antifungal activities, for example, kaempferol and quercetin showed antibacterial activity against Bacillus sp. and other human pathogenic bacteria. [17, 20–22]. Therefore, to find the bioactive compounds from the extracts, an ongoing study is conducted by using chromatography and spectroscopy techniques.

4. Conclusions

The extracts responded differently toward antifungal and TPC and TFC. It can conclude that the pigment and the extracts from the peel can be classified as a moderate source of potent natural antifungal agents and it might be abundant of phenolic and flavonoids compounds. Therefore, further investigation is needed.

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