Fibroblast Viability Test Toward Red Dragon Fruit 
(*Hylocereus polyrhizus*) Peel Ethanolic Extract

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**Abstract**

The nutritional content of red dragon (*Hylocereus polyrhizus*) fruit is not only limited to the flesh, the peel contains polyphenols and antioxidant activity along with the antiproliferative effects that are higher than the flesh. Plant bioactive constituents is believed to have various benefits with minimum side effects but yet not standardized. Phytochemical and viability test are needed to analyze the toxic concentration and 50% inhibition concentration (IC50) from *Hylocereus polyrhizus* peel 70% ethanolic crude extract.

**Materials and methods:** Qualitative phytochemical test was determined using modified Farnsworth method. Fibroblast cells (3T3BALB/C) given seven different extract (500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 μg / mL) concentrations. Analysis using colorimetric MTS assay with triplicate. The value of formatzan absorbance indicated the viability of the cells.

**Results:** Flavonoids, saponin, phenols, tannins, triterpenoids, terpenoids, and alkaloids were presence. Statistical data analysis obtained using ANOVA where each group has significantly different effect (p < 0.05) and IC50 value was obtained 1576.867 μg / mL, higher than the concentration group that was planned before.

**Conclusion:** RDFP 70% ethanol extract proven to be not toxic, particularly at a concentration below 250 μg / mL. Meanwhile, the IC50 higher than the 100% concentration.

**INTRODUCTION**

Herbal medicines are mainly used to promote healing by accelerate blood clotting and fight infections. 1 The actual side effects of herbal medicines are not yet known because it is not standardized. Some primary consideration of herbal medicines is still used today are because its plant crude extract are widely available with different nutritional content yet economically cheaper compared to the results of purification of specific compounds. 2 The use of crude extracts which contain several elements of different active substances is believed to work synergistically so that the impact is greater than the effect of each bioactive constituent and its toxicity will also decrease. Therefore phytoconstituents from various plants in the form of crude extract need to be further identified and screened so it is standardized with the aim of healing management, in which red dragon fruit peel (RDFP) is one of the plants with the potential to be extracted as a crude extract which later can be processed into herbal medicines. 1

The nutritional content of red dragon fruit is not only limited to the flesh, the RDFP which comprises only one-third of the fruit is often thrown away in the process, especially in the food and beverage production industry. 1,4 But it turns out that RDFP contains polyphenols and antioxidant activity along with the antiproliferative effects on B16F10 melanoma cancer cells that are higher than the flesh. 3 Although it can be said to be a ‘leftover’ product, RDFP contain vitamin C and antibacterial effect on *Salmonella typhi* while its extract of chloroform has an antibacterial effect on Gram-positive and Gram-negative which is higher than dragon fruit peel species *Hylocereus undatus* (Haw.) Britton & Rose (Cactaceae). 3-6

The main compounds of *H. polyrhizus* peel are chlorogenic acid, gallic acid, and quercetin. 5 Quercetin, is one of the flavonoid groups and well absorbed by the body, is known to inhibit mast cell degranulation, basophil histamine release and formation from other inflammatory mediators, which in histopathological analysis of male Wistar groups treated with quercetin, fewer inflammatory cells, an increase in microvessel density, more proliferation of fibroblasts, more regular collagen deposition and epithelialization were found. 7,8 Disodium cromoglycate (anti-allergic drug) has a structure and function similar to quercetin effective in treating recurrent aphthous stomatitis. 8 RCT studies in the management of minor aphthous ulcers using topical quercetin, resulting in a total recovery in 35% of cases in 2-4 days and 90% of cases in 4-7 days compared to patients receiving topical benzydamine hydrochloride mouthwash. 9 Therefore quercetin can be used to reduce the frequency of recurrences by eliminating mild symptoms. 8 Chlorogenic acids is considered a fairly economical therapeutic agent since it can be found abundant in common plants. The antioxidant activity of chlorogenic acids can contribute to the success of wound healing by increasing the effect of collagen production and capillary density, antioxidant and free radical scavenger effects on oxidative parameters, and anti-inflammatory effects on MMPs in wound tissue. Study revealed that gallic acid a viable wound healing agent which in normal and hyperglycemic conditions, gallic acid has powerful antioxidants that directly increase the expression of antioxidant genes and also accelerate the migration of keratinocyte and fibroblast cells which leads to activation of growth factors responsible for wound healing, such as focal...
Viability test

The data seen in Figure, there was a decrease in the number of viable cells with increasing concentrations ranging from the lowest concentration of 7.81 µg / mL to the highest concentration of 500 µg / mL. Based on ISO 10993-5, the cell viability classification, concentrations of 250, 125, 62.5, 31.25, 15.63 and 7.81 µg / mL are nontoxic. In this study, the IC₅₀ was 1576.867µg / mL, higher than the concentration group that was planned before.

DISCUSSION

The result of phytochemical test obtained are slightly different from the studies conducted previously in which research the RDFP extract did not contain alkaloid compounds but contained saponins, whereas, in this study, RDFP extract did not contain saponins and terpenoids. These differences in results can be influenced by several factors related to discrepancy in edaphology and climate because the secondary metabolites of plants can differ depending on the season or location of plants (diversity in nutrients and soil conditions) and bioactive compounds synthesis is also depending on time so the sampling time could be one of the determining factors. Flavonoid content in RDFP extract can be used as an anti-inflammatory therapy because flavonoids are known to accelerate proliferation process by shortening the time of inflammation. Flavonoids have a function to stall bleeding by increasing the number of platelets, so that when there is bleeding, platelets will rupture and produce thromboplastin or thrombokinase enzymes that will work and activate prothrombin which helps by Ca²⁺ and vitamin K contained in blood plasma can activate thrombin which will eventually change fibrinogen molecules to form fibrin monomers. Triterpenoids are known to work as antiviral, antimicrobial, anti-inflammatory, antitumoral, and become immunomodulatory compounds. Some medicinal plants in traditional medication with pharmacological effects that play a role in fighting the disease of the immune system such as allergies and hypersensitivity (especially types I and IV), have triterpenoids, although further research needs to be done on the specifications of the triterpenoid type and the appropriate dosage in this crude extract study.

The content of phenols, alkaloids, and tannins can act as an antibacterial. Alkaloids will disrupt the peptidoglycan of the bacterial cell, so that the cell wall layer is not fully formed which will eventually cause death in these cells, whereas tannins will shrink cell walls or cell membranes that can disrupt cell permeability so that bacterial cells cannot carry out living (growth is obstructed or even dead) The presence of anthocyanins on the RDFP can increase tannin content, so in addition to its antibacterial properties according to Iranian Traditional Medicine (ITM), tannins are used in RAS treatment because they have astrincent effects. Gallic acid, which is one of the groups of tannins found in RDFP, has a migration effect on fibroblasts and is a potential agent for treating damage to wound healing, both acute and chronic
skin disorders. Treatment with gallic acid does not lead to cytotoxicity in normal mouse fibroblast and endothelial cells, besides there is visible potential for anti-apoptotic effects on normal human lymphocytes. Regardless of its dosage, other factors that play a role in viability include the duration and mechanism of exogenous agents against cells. Cells exposed to exogenous agents can react with different results. An exogen agent can be metabolized without any effects that can be observed in certain cell lines, but the opposite can also occur. Exogenous agents that are cytotoxic can affect various cell functions through a variety of different mechanisms, such as prevention of protein synthesis, destruction of cell membranes, inhibition of elongation of polydeoxynucleotide, irreversible binding to receptors, and other enzymatic activities can make cells experience necrosis or apoptosis. Necrosis occurs when cells experience hypothermia or are exposed to conditions that are very different from their physiological conditions so that the cell membrane becomes damaged. Necrosis interposes with the cell's ability to maintain homeostasis, which is essential for ATP production, enzyme activity, and the influx of water and extracellular ions so that intracellular organelles of cells such as mitochondria, can swell and rupture (cell lysis). As a result of the disruption of the plasma membrane, cytoplasmic contents, including the lysosome enzyme, will be released into the extracellular fluid which can be used to measure the level of necrosis. In this study, agents that have the potential to play a role in cell death are (including) alkaloids. Due to their toxicity, plants alkaloids efficient in protecting against predators by causing changes in the central nervous system, impaired protein function after consumption and metabolism. Alkaloids also has bitter taste as defense. But again in general, toxicity effect can be dangerous and beneficial depend on the specific dose, time of exposure, and individual characteristics such as developmental stage, sensitivity, site of action, and depending on the pharmacological context. As a tropical plant of the family of Cactaceae, the red dragon fruit has low toxicity index of alkaloid. Therefore, further research is needed to identify the amount of content and type of alkaloid or purification to remove toxic substances in the RDPF extract.

Hence to ascertain the effective dose of this peel extract as RAS therapy, a further viability test can be conducted to determine its ED50, LD50, MIC, etc. as appropriate and to know the exact percentage of phytochemical composition and nutritional content of the peel extracts, other phytochemical tests or methods can be carried out in other ways or using different ethanol concentrations or solvents.

CONCLUSIONS
RDPF 70% ethanol extract proven to be not toxic, particularly at a concentration below 250µg/mL. Meanwhile, the IC₅₀ higher than the 100% concentration.

ACKNOWLEDGEMENT
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REFERENCES
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Tables and Figures

Table 1. Phytochemical Test Result

<table>
<thead>
<tr>
<th>Phytochemical Components</th>
<th>Qualitative Results</th>
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<tbody>
<tr>
<td>Flavonoid</td>
<td>(+)</td>
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<tr>
<td>Saponin</td>
<td>(+)</td>
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<tr>
<td>Phenol</td>
<td>(+)</td>
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<tr>
<td>Tanin</td>
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<tr>
<td>Triterpenoid</td>
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<td>Steroid</td>
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<tr>
<td>Terpenoid</td>
<td>(-)</td>
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<tr>
<td>Alkaloid</td>
<td>(+)</td>
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Note: +/− indicates the existence of substances in the extract
There was a decrease in the number of viable cells with increasing concentrations ranging from the lowest concentration of 7.81 µg / mL to the highest concentration of 500 µg / mL.

Figure 1. Relationship between concentration of RDFP extract and 3T3BALB/C viability.