"ANTHOCYANIN ACTIVITY OF RED DRAGON FRUIT (*Hylocereus* polyrhizus) PEEL AS IMMUNOSTIMULAN IN THE COVID-19 PANDEMIC ERA"

SKRIPSI

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PROGRAM STUDI FARMASI

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Diajukan Kepada : Fakultas Kedokteran dan Ilmu Kesehatan Universitas Islam Negeri Maulana Malik Ibrahim Malang Untuk Memenuhi Salah Satu Persyaratan dalam Memperoleh Gelar Sarjana Farmasi (S. Farm)

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Batu, 1 November 2023





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ΜΟΤΤΟ

There is no success without effort and prayer "Tidak ada kesuksesan tanpa usaha dan doa"

نِّبَايُّهَا الَّذِيْنَ أُمَنُوا اصْبِرُوًا وَصَابِرُوًا وَرَابِطُوًا وَاتَّقُوا اللهَ لَعَلَّكُمْ تُقْلِحُوْنَ

"Wahai orang-orang yang beriman! Bersabarlah kamu dan kuatkanlah kesabaranmu dan tetaplah bersiap-siaga (di perbatasan negerimu) dan bertakwalah kepada Allah agar kamu beruntung" (Q.S. Ali Imran: 200)

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ABSTRACT

Background: Coronavirus disease (Covid-19) are generally self-limiting diseases that rely on immune power. Certain food nutrients can be essential in maintaining health and boosting the immune system. One of the nutrients that can maintain and boost the immune system is a flavonoid, which is found in the peel of the red dragon fruit (Hylocereus polyrhizus). The red dragon fruit peel is rich in polyphenols and anthocyanins-flavonoid, which are sources of antioxidants. Dragon fruit peel waste is developed and processed into innovative food in the form of "bakpao" as a source of nutrition and adds to the public's attractiveness, which is then labelled the product "Dragon Pao". Purpose: To help people choose food innovations that can help them increase their immunity in the Covid-19 pandemic era. Methods: This study uses a laboratory study with a qualitative approach followed by several tests of substances in the bakpao product from red dragon fruit peel. The intended tests are organoleptic, hedonic, and anthocyanin tests. The total number of panellists used for the hedonic test was 20 people in the Malang area. Panellists were randomly selected and came from various age groups. The hedonic test was carried out using a google form through a questionnaire. Result: The results of the organoleptic test obtained a soft texture, a distinctive smell of meatballs, a pink colour, and a sweet taste. Hedonic test results obtained 80-90% of respondents' answers are in the options like and really like. Then in the anthocyanin test, the dragon fruit peel was positive for anthocyanin. Conclusion: Dragon Pao" have the potential to be a nutraceutical product as an immunostimulant in the Covid-19 pandemic era that all people like. Consumption of "Dragon Pao" is an option to increase the body's immunity as an effort to prevent exposure to Covid-19.

Keywords : covid-19, immune system, nutraceutical product, dragon pao

CHAPTER I

INTRODUCTION

The Coronavirus Disease (Covid-19) pandemic has become a global health problem, especially in Indonesia. Diseases caused by viruses, such as Covid-19, are generally self-limiting diseases that rely on immune power. Many things can support the immune system, one of which is food. Certain nutrients in food can be essential in maintaining health and boosting the immune system, one of which is a flavonoid (Devagaran and Diantini in Putra *et al.*, 2020). Flavonoids can be found inseveral foods, fruits, and vegetables. Even flavonoids can be found in the waste from the fruit. One of the fruit waste that contains flavonoids isred dragon fruit peel waste.

Red dragon fruit peel (*Hylocereus polyrhizus*) is a waste commonly found in the daily environment. This is because dragon fruit is one of the food commodities the community favours and is used in various food products. Red dragon fruit reaches 737.5 tons. This consumption pattern can cause the waste population to increase, which has a solid potential to damage the environment. Therefore, the utilization of dragon fruit peel waste is necessary in addition to overcoming the waste problem. This is because dragon fruit peel has many health benefits (Yulianto, 2020).

The peel of the red dragon fruit weighs 30-35% of the whole weight of the fruit (Utami *et al.*, 2020). The fruit's peel contains vitamin C, E, and A, alkaloids, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolic,

carotenoids and phytoalbumin (Putri *et al.*, 2015). The advantages of red dragon fruit peel are that it is rich in polyphenols and anthocyanins, which are also flavonoid compounds as a source of antioxidants. The antioxidant activity of the red dragon fruit peel is greater than the flesh (Nizori *et al.*, 2020). Research stated that at 1 mg/ml, dragon fruit peel inhibited $83.48 \pm 1.02\%$ of free radicals, while dragon fruit flesh was only able to inhibit free radicals of $27.48 \pm 5.03\%$ (Nurliyana *et al.*, 2010). In addition, red dragon fruit peel is also rich in nutrients, such as 8.98% protein, 2.60% fat, and 25.56% fibre (Rochmawati, 2019).

The nutritional content of red dragon fruit peel needs an innovation through a food product as an effort to process and utilize red dragon fruit peel waste. In this study, the innovation of dragon fruit peel into a much-needed food product, especially in this case, the product will become a food product that is favourable for the community, namely bakpao. Bakpao is a typical Chinese food that is well known by the public and has a delicious taste, light texture and is liked by everyone (Trenggonowati *et al.*, 2019). Bakpao innovation from the main ingredient of red dragon fruit peel will become an attractive food product and is in demand by the public so that it is expected to have an effect on increasing body immunity for the community during the pandemic and reduce the population of dragon fruit peel waste. This study aims to examine the anthocyanin content in dragon fruit peel and utilize dragon fruit peel in bakpao food products that are efficacious as immunostimulants. This research can be an alternative and healthy food to face the era of the Covid-19 pandemic for the wider community, increase public knowledge, and utilize waste in the environment into products with high selling power.

CHAPTER II

RESEARCH METHODS

This study uses a laboratory research design. In this study, researchers used a qualitative approach. The independent variables used in this study were red dragon fruit peel and bakpao products. In contrast, the dependent variable is anthocyanin, HCl reagent, and 2M NaOH. This research was conducted at the researcher's house and the Chemistry, Pharmacy Laboratory, Faculty of Medicine and Health Sciences, Maulana Malik Ibrahim University, Malang, on 27th January to 20th February 2022.

The process of making the product begins with cleaning the peel of the red dragon fruit with clean water and removing the scales. Then the dragon fruit peel is boiled and blended until smooth. The blender results are divided into the dough for the bakpao peel and jam. Making jam is by mixing smooth dragon fruit peel, water, sugar, and vanilla, then cooking in a pan until the jam thickens. As for the process of making the bakpao, mix the fine red dragon fruit peel, wheat flour, water, butter, egg yolk, yeast, and baking powder. They were stirred by hand until smooth and then allowed to stand for 30 minutes. After that, the dough for thebakpao peel is shaped into spheres whose size is adjusted to taste and filled with jam. Then the bakpao are allowed to stand for another 20 minutes. After that, the bakpao is steamed for 15 minutes. The ingredients for making bakpao are dragon fruit peel 300g, flour 300g, water 150g, butter 25g, egg yolk two items, yeast (saf instant) 1

tsp, and baking powder 1 tsp. The formulation of red dragon fruit peel jam is dragon fruit peel 150g, sugar 100g, water 100g, and vanilla 1 tsp.

The ingredients for making bakpao are red dragon fruit peel, wheat flour, water, butter, egg yolk, instant yeast (saf instant), and baking powder. As for the stuffing, jam is used, which is also made from dragon fruit peel. The ingredients include red dragon fruit peel, granulated sugar, vanilla powder, and water. The ingredients are purchased at the mini-market except for the red dragon fruit peel, which comes from the consumed red dragon fruit. For anthocyanin test materials, the reagents are HCl, 2M NaOH, and aqua dest. Then, tools for making bakpao include knives, blenders, stoves, basins, frying pans, spatulas, and paper buns. The tools used in the anthocyanin test were test tubes, tube racks, dropper pipettes, tube clamps, measuring cups, stirring rods, beakers, tripods, and Bunsen. An organoleptic test is a test that uses the human senses as the primary tool in measuring the acceptance of a product (Ningrum et al., 2017). The senses used in this test include the senses of sight, touch, smell, and taste. Panellists are some persons who give organoleptic tests. They were asked to explain the organoleptic (texture, smell, colour, and taste) of the red dragon fruit peel product, namely bakpao. The Hedonic test is an analyzing sensory organoleptic technique used to determine the magnitude of the difference in quality between several products that match the assessment or score against specific properties of a product and to determine the level of preference of a product (Tarwendah, 2017). The hedonic test measures the level of preference for a product. This preference is known as the hedonic scale. Panellists were asked to indicate their preference level for each

sample by selecting the appropriate category (Ningrum *et al.*, 2017). The panellists used were untrained panellists from 20 people in the Malang area. Panellists were randomly selected and came from various age groups. The hedonic test was carried outusing a google form through a questionnaire.Parameters tested were texture, colour, aroma, taste, and total acceptance of the product. The hedonic test scores of the panellists started from 1 (dislike very much), 2 (disliked), 3 (liked quite a bit), 4 (liked), and 5 (liked very much).

The qualitative analysis aims to identify the presence of anthocyanins in samples of dragon fruit peel and red dragon fruit peel bakpao products. In the first stage, the sample was heated with 2M HCl for 2 minutes using a temperature of 100 °C, and then the colour of the sample was observed. If the red colour in the sample does not change (constant), then it indicates a positive anthocyanin result. In the second stage, the sample was mixed by adding 2M NaOH dropwise. When the red colour turns green, it also shows positive anthocyanins (Herfayati *et al.*, 2020). This research was conducted by considering research ethics, such as confidentiality and voluntary panellists. In the selection of panellists, there was no coercion, and respondents' personal data was not shared, but only the results of the hedonic test were shared.

CHAPTER III

RESULT

Organoleptic Test

An organoleptic test is used to examine the physical appearance of the raw material for red dragon fruit peel and bakpao products, including texture, smell, colour, and taste. The organoleptic results are in the tables below. Table 1 shows the results of the organoleptic test of red dragon fruit peel, and table 2 shows of organoleptic test of bakpao.

Hedonic Test

The hedonic test checks the panellists' preferences regarding bakpao products. The population in this hedonic test are men and women living in the Malang area with an age range ranging from children (8 years) to old adults (52 years), totalling 20 panellists. The percentage of male panellists is 30%, and the percentage of female panellists is 70%. Data on the number of panellists by age and gender are in the diagram 1 and 2.

Parameter	Information
Texture	Thick
Smell	No smell
Colour	Purplish red
Flavour	Bitter

Table 1. Results of Organoleptic Test of Red Dragon Fruit Peel

- ····	Table 2.	Results of	Organoleptic	Test of Bakpao
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Parameter	Information
Texture	Gentle
Smell	Typical buns
Colour	Pink
Flavour	Sweet



Figure 1. Female Panellists Age Data



Parameters tested in the hedonic test include texture, colour, smell, taste, and overall assessment of the bakpao. Parameters were assessed using a score of 1-5 (dislike very much to like very much) with a panel of 20 people of various ages through the google form media. The results of the hedonic test are in the table 3.

Table 5. Results of Hedoline Test						
Question	1	2	3	4	5	
Do you like the texture	0	0	0	2	18	
of the "Dragon Pao	(0%)	(0%)	(0%)	(10%)	(0%)	
Product"						
Do you like the colour	0	0	1	1	18	
of the "Dragon Pao	(0%)	(0%)	(5%)	(5%)	(90%)	
Product"				. ,		
Do you like the smell	0	0	2	3	15	
of the "Dragon Pao	(0%)	(0%)	(10%)	(15%)	(75%)	
Product"	Ì,				. ,	
Do you like the taste	0	0	0	7	13	
of the "Dragon Pao	(0%)	(0%)	(0%)	(0%)	(0%)	
Product"						
How do you rate the	0	0	0	3	17	
"Dragon Pao"	(0%)	(0%)	(0%)	(15%)	(85%)	
C	. /	~ /		` '	```	

Table 3. Results of Hedonic Test

Anthocyanin Test

An anthocyanin test was conducted to determine the secondary metabolite compounds of anthocyanin in red dragon fruit peel. The anthocyanin test was carried out using a phytochemical screening method. The samples tested were red dragon fruit peel and bakpao products. The anthocyanin test results are in Figure 3 below.



Figure 3. (a) Anthocyanin test (NaOH of red dragon fruit peel; (b) Anthocyanin test (NaOH) of Dragon Pao; (c) Anthocyanin (HCl) of red dragon fruit peel; (d) Anthocyanin (HCl) of Dragon Pao

CHAPTER IV

DISCUSSION

Based on the test results for filling out question number 1 in the questionnaire, two panellists chose option number 4, which means like, and 18 panellists chose option number 5, which means really like the texture of the "Dragon Pao" product. The results of filling out the number 2 in the questionnaire, one panellist chose option number 3, which means neutral, one panellist chose option number 4, which means like, and 18 panellists chose option number 5, which means really like the colour of the "Dragon Pao" product. The results of filling out question number 5, which means really like the colour of the "Dragon Pao" product. The results of filling out question number 3 in the questionnaire show two neutral panellists, three panellists like, and 15 panellists who really like the smell of the "Dragon Pao" product. The results of filling out the number 4 questionnaire show seven panellists who like and 13 panellists who really like the taste of the product "Dragon Pao".

The processed product from the peel of the red dragon fruit "Dragon Pao" has good organoleptic (texture, colour, smell, and taste) so that various age groups can enjoy it, whether they come from young ages to old ones. Based on the results of filling out questionnaire number 5 regarding the overall assessment of the product, three panellists liked it, and 17 panellists really liked the "Dragon Pao" product. This result proves that the processed product of the red dragon fruit peel "Dragon Pao" has received a positive response from the wider community and has great potential to be developed as a food innovation that is beneficial to health, especially as an immunostimulant during the Covid-19 pandemic. Based on the results of the anthocyanin test using the phytochemical screening method on the red dragon fruit peel obtained from the waste, it was positive that it contained anthocyanins. This positive result is indicated by a change in the colour of samples a and b from purple to light green and a change in sample b. from pink to green on adding a 2M NaOH reagent. Meanwhile, in the heating reaction and the addition of HCl (c and d), positive results were indicated by a change in the colour of sample c from purple to red and sample d from pink to red. However, there was a difference in the colour of the positive results in samples of red dragon fruit peel and the bakpao.

Bakpao products have a lighter cheerful colour than the red dragon fruit peel sample. Reduced levels of anthocyanins cause this during the process of making bakpao products. Although the content of anthocyanin compounds in the peel of red dragon fruit is quite large, processing treatments such as heating can result in changes in the concentration and colour of anthocyanins in processed products (Mahmudatussa'adah *et al.*, 2015). Anthocyanins are natural dyes belonging to the flavonoid group with three carbon atoms bonded by an oxygen atom to link two benzene aromatic rings (C6H6) in the main structure. As a bioactive compound, the arrangement of conjugated double bonds in the anthocyanin structure makes anthocyanins function as natural antioxidant compounds in humans. Anthocyanins can scavenge various types of reactive oxygenderived free radicals such as hydroxyl (OH*), peroxyl (ROO*), and single oxygen (O2*) (Siti Azima *et al.*, 2014).

Based on the previous research conducted by Putri *et al.* (2015), red dragon fruit peel has antioxidant activity with an IC50 value of 73.2772 ppm and a total

anthocyanin content of 58.070 ± 0.0001 mg/L. Fauziah *et al.* (2021) also found that antioxidant compounds are said to be very strong if the inhibition concentration 50% (IC50) value is 200ppm. This result shows that the peel of the red dragon fruit has intense antioxidant activity in counteracting free radicals to prevent various diseases, including Covid-19 effectively.

In patients affected by Covid-19, cell damage occurs due to increased oxidative stress, leading to organ failure. ARDS is the main cause of oxidative stress in Covid-19 patients because it causes free radicals and sticks to increase. Therefore, the provision of antioxidants has an essential role in the case of Covid-19 (Abdulkadir et al., 2021). Antioxidants play a role in protecting the body from free radical attacks. Giving certain antioxidants can increase immunity and protect the body from bacterial and viral infections (Rusnelly & Rotua, 2012). Anthocyanins have high antiviral, antifungal, and antibacterial activity (Saira & Kamran, 2017). Anthocyanins as immunostimulants work by stimulating lymphocytes produce interleukin (IL)-2 interferon to and (INF)-y. Immunostimulants are substances that can improve system function and activity immune. General mechanism of immunostimulants namely correcting the imbalance of the immune system by increasing immunity, both specific and nonspecific (Batarawidjaja & Rengganis, 2012). In general, cells involved in the immune system are T cells and B cells produced by the thymus and bone marrow behind. The cell development process can be stimulated with an immunostimulant (Sukmayadi et al., 2014). Anthocyanins or flavonoids can also modulate various immune functions, including increasing PBMC responsiveness, IL-4 cytokine

secretion, and NK cell lytic activity (Aliyah *et al.*, 2021). Several flavonoids, such as anthocyanins, are thought to have biological activity in inhibiting several coronavirus proteins, preventing lung inflammation, and cytokine storms which are severe consequences of SARS-CoV-2 infection. So alternative immunostimulants are needed deriving from plants that are expected to have side effects and toxicity are relatively small in the body that can stimulate the growth of the body's defence cells in the immune system (Sukmayadi in Listiani & Susilawati, 2019). Because it has many benefits, the flavonoid anthocyanin compounds contained in the "Dragon Pao" product have the potential to be used to improve the body's immune system during the Covid-19 pandemic. Based on the research that has been done, it can increase public knowledge about the benefits of dragon fruit peel to increase immunity so that later people can utilize and manage red dragon fruit peel waste into healthy and nutritious food products, both for their consumption and produced with high selling power.

CHAPTER V

CONCLUSION

The laboratory test show that the "Dragon Pao" product is positive for anthocyanins. Anthocyanin, which is one of the natural dyes of flavonoids, indicates that the product contains flavonoids and has the potential to be used to improve the immune system during the Covid-19 pandemic. Moreover, the hedonic test results also show that 80-90% of the answers indicate the choice of like and realy like with the product. Thus, "Dragon Pao" has the potential to be a nutraceutical product as an immunostimulant in the Covid-19 pandemic era that all people like. Based on the research that has been done, people should always maintain their health so they do not get infected with the Covid-19 virus by maintaining the immune system through a clean and healthy lifestyle and consuming foods that contain lots of anthocyanin compound as immunostimulants.

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