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Comparison of Antioxidant Activity Test of Red Dragon Fruit Extract 70% Ethanol Solvent and 96% Ethanol

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Abstract*:* Super Red Dragon Fruit is known to have various compounds with high antioxidant activity, which are important in preventing or assisting therapies to reduce free radicals in the body. Different types of solvents, including ethanol, can affect the antioxidant levels in dragon fruit compounds. Therefore, it is important to determine the level of ethanol solvent that provides the highest antioxidant activity. This study aims to evaluate the antioxidant activity in red dragon fruit extract using 70% and 96% ethanol solvents and determine whether there is a difference in activity between the two. Using the true experimental method, antioxidant activity in the fruit and skin of red super dragon fruit extracted using two different solvent levels was compared. Based on the shapiro-wilk test which showed a p value of 0.410, it was found that there was no significant difference between the two solvents in terms of antioxidant activity. This result indicates that the antioxidant activity in red dragon fruit extracts with 70% and 96% ethanol solvents has no significant difference.

Keywords: Antioxidant activity, ethanol solvent, red dragon fruit, compounds.

Introduction

In the modern era with the development of science and technology, changes in people's lifestyles have an adverse impact on health. Habits such as unbalanced food consumption, lack of exercise, lack of rest, smoking, and consuming alcohol are some examples of unhealthy lifestyles. In addition, deteriorating environmental conditions due to air pollution from motor vehicles and factory fumes also worsen health. This pollution contributes to the reduced production of the body's protective compounds against free radicals produced by air pollution, radiation, harmful chemicals, and other sources (Umayah., Amrun., 2007; Andarina., Djauhari., 2017; Wahjuni., 2015; Young and Woodside., 2014).

An unhealthy lifestyle is one of the causes of degenerative diseases that are often caused by free radicals. Free radicals are atoms or molecules with one or more unpaired electrons, which can damage the structure and function of cells by taking electrons from other molecules. This process can damage lipids, proteins, and DNA in the body, increasing

oxidative stress, which is a condition where the amount of free radicals exceeds the body's capacity to neutralize them. This oxidative stress can cause cellular damage and contribute to various diseases and aging. An example of free radicals is Reactive Oxygen Species (ROS), small molecules that can rapidly damage cell structures. Excessive production of ROS can lead to oxidative stress that damages important macromolecules in the body. To prevent the increase of free radicals that can cause degenerative diseases, antioxidants are needed that can neutralize, reduce, and inhibit the formation of new free radicals by donating electrons so as to stop damage in the body (Arnanda *et al*., 2019; Susilawati *et al*., 2022; Wedayani *et al.*, 2024; Amin *et al.*,2015; Sari., 2015).

Antioxidants are compounds that capture free radicals. The mechanism of action is done by donating one electron from the antioxidant compound to unstable free radicals. This process neutralizes the free radicals so that they no longer interfere with the body's metabolism. Based on its source, antioxidants are divided into two types, namely enzymatic antioxidants (such as glutathione peroxidase, superoxide dismutase, and catalase) and non-enzymatic antioxidants (including tocopherols, carotenoids, quinones, flavonoid polyphenols, vitamin C, vitamin E, and β-carotene) (Sari, A.N., 2015; Putri, M.A., 2018; Hani & Milanda., 2016; Prawitasari, D.S., 2019).

A plant can have an antioxidant effect if it contains compounds that can counteract free radicals, such as anthocyanins found in dragon fruit. The antioxidant activity of dragon fruit skin is higher than the antioxidant activity of the pulp, so it can be developed as a source of natural antioxidants. This is because the exogenous antioxidant properties of dragon fruit skin can be used as an inhibitor of oxidative damage in the body (Rakhmadhan & Helda, 2016; Winahyu *et al*., 2019; Aryanta., 2022; Mastuti., 2016; Yanty&Siska., 2017).

Research related to the antioxidant activity of red dragon fruit extract with ethanol solvent has been conducted by Pujiastuti & El'Zeba (2021) in a study entitled "Comparison of Total Flavonoid Levels in 70% and 96% Ethanol Extracts of Red Dragon Fruit Peels by Spectrophotometry". The purpose of this study was to measure total flavonoid levels and determine whether there are significant differences between total flavonoid levels in 70% and 96% ethanol extracts of red dragon fruit skin (Hylocereus polyrhizus) using the UV-Vis Spectrophotometric method. In this study, phytochemical screening and measurement of total flavonoid levels with UV-Vis Spectrophotometer were carried out on 70% and 96% ethanol extracts of red dragon fruit skin. The results showed that the type of solvent had a significant effect on the extraction yield. This difference in yield can be influenced by the extraction method, extraction duration, simplisia size, and type of solvent used (Hakim, A.R., Saputri, R. 2020). Based on the results of the Mann-Whitney Test using SPSS, the value of Asymp. Sig. 2-tailed is 0.046, which is smaller than 0.05. Therefore, it can be concluded that there is a significant difference between total flavonoid levels in 70% and 96% ethanol extracts of red dragon fruit skin (Hylocereus polyrhizus).

This study will review the comparison of antioxidant activity in red dragon fruit extract using 70% ethanol solvent and 96% ethanol solvent. This is done because there is no similar study that tests antioxidant activity as a whole, not only flavonoid levels, and also tests extracts from the skin and flesh of red dragon fruit. The results of this study are expected to provide comparative data on the antioxidant activity of red dragon fruit extract with 70% ethanol solvent and 96% ethanol solvent. The benefits of this research are expected to provide information on antioxidant activity in red dragon fruit extract with 70% ethanol solvent and antioxidant activity in red dragon fruit extract with 96% solvent.

Materials and Methods

The sample size in this study was carried out with reference to research conducted by Wahdaningsih (2022) as much as 1kg of dragon fruit peel used to obtain thick extract results. Then in this study I used 1 whole Red Dragon Fruit whose skin and pulp were mashed and dissolved using 70% ethanol solvent and 96% ethanol solvent until a thick extract was obtained. Measurement of absorbance value was done using UV-Vis spectrophotometry method. The calculation results were then statistically tested

using IBM SPSS Statistic 25 software.

This research instrument includes various tools and materials needed in the extraction process and antioxidant activity analysis of red dragon fruit extract. The tools used consisted of a blender machine to crush the sample, a glass jar as a temporary storage container, and a rotary evaporator that serves to evaporate the ethanol solvent in the extraction process, so that a thick extract is obtained. A knife is also needed to cut the red dragon fruit, while the mori cloth and black cloth are used for the filtering process and protection of samples from excessive light exposure, which can affect the stability of antioxidant compounds. Scales were used to measure the weight of the sample, and stirring rods served to mix the solution well. The UV-Vis spectrophotometer is an important instrument in this study because it is used to measure the antioxidant activity in red dragon fruit extract.

Results and Discussion

Univariate Analysis

Univariate analysis was conducted on the variables of antioxidant activity of red dragon fruit. The results of univariate analysis can be seen in Table 1.

Table 1 Results of univariate analysis of antioxidant activity of red dragon fruit with 70% ethanol solvent and 96% ethanol solvent

		Statistic
Antioxidant Activity of Red Dragon Fruit	Mean	2,10
	Median	2,00
	Variance	1,878
	Std. Deviation	1,370
	Minimum	0
	Maksimum	4
	Range	4

Bivariate Analysis

The results of bivariate analysis showed a significance of p> 0.05, indicating that the results of the normality test were normally distributed or significant. The results of the data normality test with Saphiro-Wilk antioxidant activity of red dragon fruit can be seen in Table 2.

Table 2 The results of the data normality test using the Shapiro-Wilk test showed a p value of more than 0.05 (0.410).

	Saphiro - Wilk		
Antioxidant Activity of Red Dragon Fruit	Statistic	df	Sig.
	0,926	10	0,410

Parametric Independent Samples Test results obtained Significance p < 0.05 which indicates that the test results are not normally distributed or there is no difference between antioxidant activity with 70% ethanol solvent and antioxidant activity with 96% ethanol solvent. The results of the Independent Sample parametric test can be seen in Table 3.

Table 3 Independent Samples Test parametric test

		T-Test for Equal Means		**95% Confidence Interval of Difference**	
		Sig. (2 - tailed)	**Mean Difference**	**lower**	**Upper**
Antioxidant Activity of Red Dragon Fruit	Equal Variances Assumed	0,833	-0,200	-2,313	1,913
	Equal Variances Not Assumed	0,833	-0,200	-2,327	1,927

Based on the results of the study, there is no difference in antioxidant activity of red dragon fruit extract using 70% and 96% ethanol solvent which can be seen in Table 4.

Table 4 Overview of antioxidant activity contained in red dragon fruit extract dissolved with 70% and 96% ethanol

Antioxidant Activity	Betanin	Flavonoid	Tiamin	Niasin	Pyridoksin
70%	+++	++++	+	+	++
96%	+++	++++	-	+	++

Discussion

Comparison of Research Results With

Previous Research

Based on the results of my research, there was no difference in antioxidant activity in red dragon fruit extract using 70% and 96% ethanol solvents. This is due to the high polarity of ethanol and the use of cold extraction method, which produces relatively the same antioxidant compounds. However, the results of this study contradict previous research entitled "Comparison of Total Flavonoid Content of 70% and 96% Ethanol Extracts of Red Dragon Fruit Skin (Hylocereus polyrhizus) with Spectrophotometry." The study showed a significant difference in total flavonoid levels between 70% and 96% ethanol extracts. This study used maceration method to extract the red dragon fruit skin that has been dried into simplisia, with the analysis of total flavonoid levels carried out using UV-Vis Spectrophotometry at a maximum wavelength of 444 nm. The results showed that 96% ethanol extract produced higher total flavonoid levels, namely 108.184 ± 0.0224 mgQE/g extract (10.82 ± 0.02%), compared to 70% ethanol extract which only amounted to 88.695 ± 0.0922 mgQE/g extract (8.87 ± 0.01%). This difference could be due to different approaches in the research objectives and the compounds analyzed, where flavonoids are semi-polar and thus more extracted with 96% ethanol, while antioxidant activity involves more diverse compounds with polarity properties that may be similar in both ethanol concentrations.

Effect of Solvent on Antioxidant Activity

Research by Harimurti *et al.* (2021) compared the antioxidant activity between temulawak extract (*Curcuma xanthorrhiza* Roxb) and super red dragon fruit extract (*Hylocereus polyrhizus*). In the study, it was found that curcumin compounds in temulawak and betanin in dragon fruit have high bioactive value. The results showed that the use of oil and water solvents resulted in higher IC50 values in super red dragon fruit containing betanin compared to the IC50 in temulawak extract containing curcumin. In addition, the percentage of antioxidant content in 2 grams of temulawak is 32.3%, while in 2 grams of red dragon fruit is only 0.15%, although there are differences in antioxidant potential based on the IC50 value produced. This study supports the finding that different solvents of 70% and 96% ethanol do not affect the antioxidant potential or the content of compounds in red dragon fruit. This result is also in line with research using cold extraction method on red dragon fruit extract.

In a study conducted by Putri, Nastiti and Hidayah in 2023 there was an effect of 70% ethanol and methanol solvents on antioxidant compounds in soursop leaf extract. In this study it was found that the results of qualitative detection tests of compounds useful as antioxidants produced in 70% ethanol and methanol extracts. The One Way ANOVA test results state that different types of solvents will have a significant effect on the levels of antioxidant compounds in soursop leaves, ethanol solvents are better for maintaining the concentration of antioxidant compounds compared to the use of methanol compounds (Putri., *et al*, 2023). So it is in line with the research conducted that ethanol solvent is better than methanol.

Ethanol Concentration and Extraction Time

Research comparing ethanol concentration and extraction time was conducted by Puspaningtyas, Putra, and Suhendra in 2021 the ethanol concentration used for solvents was 75%, 85% and 95%. Data analyzed by analysis of variance showed that concentration and concentration time had a significant and significant effect on antioxidant capacity. The higher the concentration of ethanol used, the less time it takes to produce good antioxidant capacity (Puspitaningtyas., *et al*, 2021).

Solvent Concentration and Antioxidant Activity

Research related to solvent differences conducted by Yunita and Khodijah in 2020 stated that higher concentrations have higher antioxidant activity compared to solvents with lower concentrations. This research was conducted to see the effect of ethanol solvent concentration during maceration on the levels of compounds contained in the extract. After obtaining tamarind leaves, the tamarind leaves will be dried and then extracted by multistage maceration method using ethanol solvent. The ethanol solvents used were 70% and 96%, then determined the levels of compounds with validated UV-Vis spectrophotometry. Level

testing is done by looking at the absorbance value obtained in the equation. The obtained levels were analyzed using the independent t test (Yunita and Khodijah, 2020).

Comparison of Solvent Type to IC50

The 95% ethanol solvent has the best antioxidant activity (IC50 value). The following are the results of research conducted by Noviyanti, Salingkat and Syamsiar (2019). The research conducted was to determine the effect of solvent type on dragon fruit extract by looking at the highest or best compounds and IC50. The difference with the research conducted is the results in the research conducted there is no difference between the two solvents used. According to research by Noviyanti and friends in 2019 the compounds contained in red dragon fruit extract have a polarity that is close to the polarity of 95% ethanol solvent so that the extract obtained is higher than acetone solvent. Betanin is a polar compound that can be extracted with a solvent that is also polar, namely ethanol (Noviyanty, Salingkat and Syamsiar, 2019).

Study on Dragon Fruit Peel

In the same study conducted by Pujiastuti and El'Zeba (2021) comparing the levels of antioxidant compounds in the administration of 70% and 96% ethanol extracts but only in dragon fruit peels in the study stated that there were differences between the two solvents 70% and 96% ethanol. In this study, phytochemical screening was carried out, namely: Wilstatter test, 10% NaOH test, and Bate-Smith test. The weight of red dragon fruit used is 25 mg using 70% and 96% ethanol extracts. In this study, the water used should not be excessive, which is less than 10%. Because it can affect the quality of ingredients that have a water base. Water content of less than 10% is expected to have optimum stability of materials such as red dragon fruit. The difference in solvent type has a significant effect on the extraction results. In research using gedi leaves and it is said that 96% ethanol solvent is the best solvent to use (Pujiastuti and El'Zeba, 2021).

Research Limitations and Recommendations

The difference in antioxidant activity in red dragon fruit extract using 70% and 96% ethanol solvents can be used as a reference for future research. However, this study has limitations, such as the use of mori cloth for extract filtration which can be replaced with a mesh sieve for better results, and the focus of the study is only on one type of red dragon fruit, whereas there are various varieties with varying antioxidant activity. Therefore, it is necessary to conduct further research on the comparison of antioxidant activity of dragon fruit extracts by considering factors that can affect the results of antioxidant activity.

Conclusion

This study shows that there is no significant difference in the antioxidant activity of red dragon fruit extract using 70% and 96% ethanol solvents. Both solvents produced extracts with relatively similar antioxidant ability. This result indicates that different ethanol concentrations do not have a major influence on the effectiveness of extracting antioxidant compounds from red dragon fruit.

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