Antioxidant Activity of Red Rice (Oryza Nivara. L) Through the DPPH (2,2-Diphenyl-1pilkrilhidrazil) Method

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Keywords: Red Rice, Oryza Nivara, Antioxidant Activity, DPPH.

Abstract: Free radicals are one of the causes of various degenerative disease that can be overcome with the use of antioxidant. One antioxidant source is a plant that contains high polyphenol and flavonoids. Red rice (*Oryza nivara* L.) is known to be rich in anthocianin pigment, polyphenol, flavonoids, protein, and vitamins. Due to these contents, red rice was developed to be tea, as a drink consumed for the treatment of some degenerative diseases. Antioxidant activity tests were conducted on red rice tea harvested and processed from Apuan, Baturiti, Tabanan, Bali. The antioxidant activity test utilized the DPPH method by observing colour changes after DPPH incubation. If all DPPH electrons paired with electrons from red rice samples, the sample colour changed from dark purple to bright yellow. The absorbed sample was then measured with a Uv-Vis-spectrophotometer in 515.6 nm. Infused red rice tea had an IC level of 50 for 3,635.72 ppm with the regression equation $y = 13.328x - 1.5431$ and $r^2 = 0.9834$. It can be concluded that infused red rice tea had weak antioxidant activity.

1 INTRODUCTION

Degenerative diseases are the diseases with the highest mortality in Southeast Asia. The increase in deaths due to degenerative diseases is also predicted to increase from year to year (World Health Organization (WHO), 2020). One of the causes of these diseases is free radicals.

Free radicals are molecules or compounds that can stand alone containing one or more unpaired and highly reactive electrons that tend to react with other molecules to achieve stability (Yuslianti, 2018). The high reactivity of these free radicals will then initiate a chain reaction in one formation. The result of such reactions will give rise to abnormal compounds and initiate a chain reaction that can damage important cells in the body. Free radicals can be overcome through the use of anthoxydants (Yuslianti, 2018).

Antioxidants have a very important role for the human body's health because their function can inhibit and neutralize oxidation reactions involving free radicals. Based on the aforementioned source, antioxidants can be divided into synthetic antioxidants and natural antioxidants. Synthetic antioxidants are chemically synthesized compounds. Meanwhile, natural antioxidants are antioxidant compounds that are naturally present in the body as a normal body defence mechanism or come from external intake.

One of the antioxidant compound sources is a plant with high polyphenol and flavonoid content. Brown rice (*Oryza nivara.* L) is one of the carbohydrate sources that have been frequently consumed. Brown rice is rich in anthocyanin pigments, phytochemicals, proteins and vitamins (Pengkumsri *et al.,* 2015). Another advantage of brown rice is the presence of components that are suspected to be antioxidants, and can play a role in warding off free radicals in the body. The total phenolic content in brown rice ranges from 200-700 mg EAG / 100g of ingredients, depending on the variety used (Sompong *et al.*, 2011). Polyphenols present in brown rice are compounds from the flavonoid group, such as flavones, flavones-3-ols, flavonone, flavan-3-ols and anthocyanidin.

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Puspitasari, L., Dira, M. and Dari, N.
Antioxidant Activity of Red Rice (Oryza Nivara. L) Through the DPPH (2,2-Diphenyl-1pilkrilhidrazil) Method.
DOI: 10.5220/0011937600003576
In *Proceedings of the 2nd Bali Biennial International Conference on Health Sciences (Bali BICHS 2022)*, pages 5-7
ISBN: 978-989-758-625-5

Anthocyanin pigments themselves are known to act as antioxidants, antimicrobials, antivirals, anti-inflammatories, photoreceptors, as well as antiallergenics (Pietta, 2000). In brown rice, there are a number of carotenoid, tocopherol and tocotrienol compounds that can also act as antioxidants (Shao *et al*., 2013).

Various ingredients are contained in brown rice, allowing for carbohydrate sources to be developed in the form of brown rice tea. Brown rice tea itself is made through a series of simple processes, and it has been widely consumed for the treatment of various degenerative diseases such as diabetes and cholesterol. Until now, scientific testing of the antioxidant content contained in brown rice tea has never been done in Indonesia. Hence, it is necessary to test brown rice tea's antioxidants through DPPH testing to allow further testing of brown rice tea as an antidiabetic.

2 METHOD

This is an invitro study with an experimental type of research. The research was carried out at the Integrated Service Laboratory, Faculty of Agricultural Technology, Udayana University in December 2020-March 2021. The sample used was brown rice harvested from the Apuan Baturiti Village, Bali.

2.1 Red Rice Tea

Brown rice was processed into tea through a series of processes that began with the washing process, and continued with the roasting process until it was ready to be brewed into brown rice tea. The tea was manufactured by adding 1200 ml of aquadest to 100 grams of brown rice tea; it was then heated to a boil and filtered.

2.2 Antioxidant Activity Test

DPPH compounds in quantities of 15.8 mg were weighed and dissolved into up to 100 ml of methanol p.a, and a concentration of DPPH solution of 0.4 mM was obtained. The solution was covered with aluminium foil. As much as 1 ml of the solution was mixed with 4 ml of the test extract, fraction, and quercetin (standard) for differences in concentration. The solution was vortexed for 30 minutes and read with UV-Visible spectrophotometry at a wavelength of 515.6 nm.

3 RESULT

Based on the results of the antioxidant activity test, the brown rice tea infusion's antioxidant activity in a spectrophotometric manner are shown in the following table:

Table 1: Antioxidant activity of brown rice tea infusion.

Concentration (mg/mL)	Equation (y = bx + a)	IC (PPM)
0.00	y = 13.328x – 1.5431 R^2 = 0.9834	3,635.72
2.09		
4.18		
6.28		

The brown rice tea infusion's IC50 value was obtained from the linear regression equation calculations in Table 1 above, with the regression equation being y = 13.328x – 1.5431 and r^2 = 0.9834. The y coefficient in this equation is as IC50, while the x coefficient in this equation is the concentration of squeezed water, where the value of x is the concentration needed to be able to reduce 50% of the DPPH radical's activity. The brown rice tea's IC50 value based on the calculation results obtained was 3,635.72 ppm. The following is the curve of the brown rice tea infusion concentration's relationship with the inhibition percent in Figure 1 below:

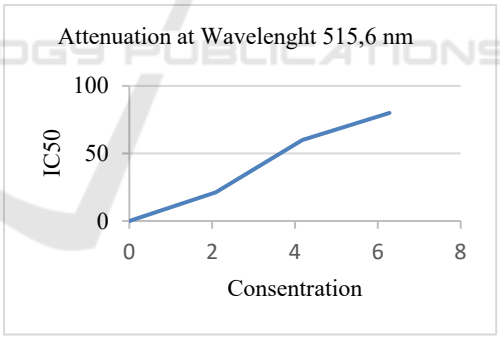

Figure 1: Linear Regression Curve of Brown Rice Tea Infusion.

The DPPH method was used to measure antioxidant activity. The antioxidant activity test method with DPPH (2,2-diphenyl-1-picrilhydrazyl) was chosen because this method is a simple, easy, fast, and sensitive method, and requires only a small sample for evaluation of natural ingredient compounds' antioxidant activity. Antioxidant activity was tested using UV-Vis spectrophotometry. The optimal wavelength of DPPH is between 515-517 nm (Julizan *et al.*, 2019). In this study, brown rice tea

infusion absorption was measured at a wavelength of 515.6 nm.

This antioxidant activity test method's principle is the quantitative measurement of antioxidant activity by measuring the captured DPPH radicals with a compound that has antioxidant activity using UV-Vis spectrophotometry. Thus, the free radical suppression activity can be identified, which is expressed by the IC50 (Inhibitory Concentration) value. The IC50 value is defined as the magnitude of the test compound's concentration that can dampen free radicals by as much as 50%. The smaller the IC50 value, the higher the free radical suppression activity (Molyneux, 2004).

The brown rice tea's IC50 value based on the calculation results was 3,635.72 ppm. This indicated that the brown rice tea infusion had weak antioxidant activity. If the IC50 value ranges from 150-200 ppm, the antioxidant properties possessed are considered weak, but still have the potential to be antioxidant substances (Molyneux, 2004). The relatively weak antioxidant activity in brown rice tea infusions is likely due to the use of water solvents that can only dissolve polar compounds. Boiling brown rice tea at high temperatures may also result in a decrease in its antioxidant activity.

4 CONCLUSION

Based on the results of the research that has been carried out, the following conclusion can be drawn: brown rice tea (*Oryza Nivara*. L) has weak antioxidant activity.

ACKNOWLEDGEMENTS

The authors would like to thank ITEKES BALI for providing research grants to allow this study to run well and smoothly. The authors also express their gratitude to ITEKES Bali for the support and all forms of facilities provided.

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