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Antioxidant Activity of Flavonoid Glycoside Extract of *Solanum Betaceum* on the Kidney of Wistar Rats

Ida Ayu Raka Astiti Asih[1*], Wiwik Susanah Rita[1], Wayan Suirta[1], Ahmad Fudholi[2,3]

[1] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Udayana University, Denpasar, Bali 80114, Indonesia
[2] Solar Energy Research Institute, Universiti Kebangsaan Malaysia, Bangi, Selangor 43600, Malaysia
[3] Research Centre for Electrical Power and Mechatronics, National Research and Innovation Agency (BRIN), Bandung 40135, Indonesia

Corresponding Author Email: astiti_asih@unud.ac.id

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ABSTRACT

Flavonoid glycosides are a type of secondary metabolite that is one of the active chemicals in plants. The antioxidant efficacy of solanum betaceum flavonoid glycosides extract on malondialdehyde levels and superoxide dismutase activity in Wistar rat kidneys with maximum physical activity is the focus of this investigation. The DPPH technique was used to conduct an in vitro activity test. The in vivo test includes four treatment groups: control, stress, ethanol extract, and flavonoid glycoside extract group. Swimming almost one and a half hours every day for five days and being fed and drinking ad libitum is the treatment for stress conditions. Ethanol and flavonoids glycosides extract were given at doses of 50 mg/kg/BW/day respectively. In vitro test result with DPPH technique glycoside flavonoid extract was categorized as a strong antioxidant with an IC50 of 69.89 ppm. The intake of ethanol extract and flavonoid glycoside extract at a dose of 50 mg / Kg BW significantly lower MDA levels ($p < 0.05$), and can prevent oxidative stress through SOD in Wistar rat kidneys.

1. INTRODUCTION

Excessive physical activity is one of the factors that can trigger oxidative stress. Oxidative stress can reduce the work of superoxide dismutase, which acts as an endogenous antioxidant. As a result, it indirectly affects the fat peroxidation reaction, resulting in the breakdown of fatty acid chains into various toxic compounds, such as malondialdehyde (MDA). However, it can be controlled by exogenous antioxidants such as flavonoid glycosides to help work superoxide dismutase in preventing cell damage.

Flavonoid glycosides are one of the active ingredients of plants included in the group of secondary metabolites. The antioxidant activity of flavonoid glycosides depends on their molecular structure, especially the prenyl $(CH_3)_2C = CH\text{-}CH_2$- and hydroxyl group substituents [1]. Flavonoid glycoside prenyl groups are developed to prevent or treat diseases associated with free radicals [2]. Free radicals are molecules that lose electrons, becoming unstable and taking electrons from other molecules or cells. Heart attacks, cancer, cataracts, and decreased kidney function are often associated with free radicals. A Kidney has a significant role in maintaining a healthy body because it is one of the vital organs in the body. It regulates fluid balance in the body, regulates the blood's salt concentration, acid-base balance in the blood, and excretion of waste materials such as urea and waste other nitrogen in the blood [3] or reduces chronic disease because free radicals need antioxidants [4].

Solanum betaceum is a fruit containing nutrients and vitamins vital for human health, such as flavonoid glycosides, carotenoids, vitamins (A, B6, C, E). It also contains minerals, fiber, iron, and potassium [5]. *Solanum* is widely cultivated in Bali, especially Kintamani and Bedugul. Solanum fruit is a very nutritious fruit; however, not many people know, so it is necessary to explore the benefits of this fruit for the health of our bodies. One of them is an antioxidant activity that has been scientifically proven. The *n*-butanol fraction of solanum betaceum peels displayed an excellent antioxidant activity with an Inhibitory Concentration 50 (IC_{50}) value of 68.14 mg/L and total phenol of 3.37 mg /g eq gallic acid, according to a previous study [6]. Jeane [7] added that giving Wistar rats the *n*-butanol flavonoid glycosides extract of Solanum boosted SOD activity of Wistar rats by 22.79% higher compared to the intake of ethanol extract. The three flavonoid glycosides group of flavonol substituted with glucos in C-3 atoms are potent antioxidants [8]. Based on this, we will study the antioxidant activity of flavonoid glycosides extract of Solanum fruit in vitro by DPPH method and in vivo on kidney tissue.

2. MATERIALS AND METHODS

2.1 Research material

The materials used include: *solanum*, 70% ethanol, diethyl ether, n-hexane, chloroform, *n*-butanol, SOD Kit (Biovision, K335-100), 10% ammonia, ketamine anesthetic, TCA, TBA solution, BHT, male wistar rats, standard rat feed and distilled water.

2.2 Sample preparation

Fresh solanum betaceum fruit is washed clean and peeled. A total of 1 kg of solanum betaceum is then cut into small pieces and mashed with a blender. Samples of flavonoid glycoside extract were obtained by maceration, precipitation, and partitioning methods.

2.3 DPPH radical scavenging activity

Using the DPPH method, the radical scavenging activity of flavonoid glycoside extract was investigated, following the procedure carried out by Shimada [9].

2.4 Animals testing and sampling process

The sampling process of this study was tuned to the method conducted by Wresdiyati [10]. The animal testing was carried out using 28 male Wistar strain rats weighing roughly 250 g. After two weeks of acclimating to their new habitat, they were divided into four treatment groups, consisting of 7 rats per group. Then, to induce in rats oxidative stress, they were made for swimming until almost drowning/day (90 minutes), given ad libitum feed, ethanol extract, water, and n butanol extract (flavonoid glycosides) 2 mL at a dose of 50 mg /Kg/ BW/day. After the treatment according to the design (Table 1), they were then taken as samples and dissected to obtain their kidney tissues. Then, the TBARS technique was used to evaluate SOD activity and MDA levels in rat kidney samples. All animal experiments were completed after being agreed upon by the ethical clearance (No:53/UN.14.2.9/PT.01.04 /2020).

2.5 Analysis SOD activity and fat peroxidation levels of rat kidney

The Superoxide Dismutase Kit was used to measure SOD activity in rat kidneys using a colorimetric technique (Biovision, K335-100). The Singh et al technique was used to prepare kidney samples [11]. Furthermore, the MDA levels were measured following the method conducted by Capeyron [12] and Suarsana [13] with several modifications.

Table 1. The treatment group of rats and type of treatment given

Group	Treatment		
	Ethanol extract	Flavonoid Glycoside extract	Stress (5 days)*
C	-	-	-
S	-	-	+
EES	+	-	+
GFS	-	+	+

Note:
+: treated;
C: Control;
-: without treatment;
EE: Ethanol extract;
S: stress;
*: maximum physical activity;
GF: flavonoid glycoside extract.

3. RESULTS

3.1 In vitro antioxidant activity

One of the free radicals commonly used for testing the preliminary radical scavenging activity of plant extracts is diphenyl picrylhydrazyll (DPPH) [14], because it is a straightforward and dependable method for determining radical scavenging activity [15]. Table 2 shows the radical-scavenging activity of flavonoid glycoside extract.

Table 2. Test results IC_{50} of flavonoid glycoside extract

Concentration (ppm)	Absorbance	% Inhibition	Linear Regression
0	0.677	0.00	y = 0.693x + 1.562
10	0.630	7.08	
25	0.529	21.78	
50	0.420	37.85	R^2 = 0.995
75	0.318	53.15	
100	0.205	69.90	IC_{50} = 69.89 mg/L

Note:
y = % inhibition, x = concentration.

3.2 In vivo antioxidant activities

In the experimental study, a randomized post-test only control group design was used. The data from the observations were then evaluated using SPSS software (version 23.0). The average variable, the variance homogeneity of each variable, and normal distribution of each group can be found in Table 3, Figure 1, and Figure 2.

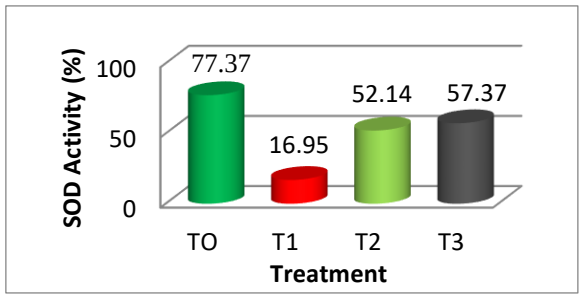

Figure 1. SOD activity (%) in the kidney of Wistar rat

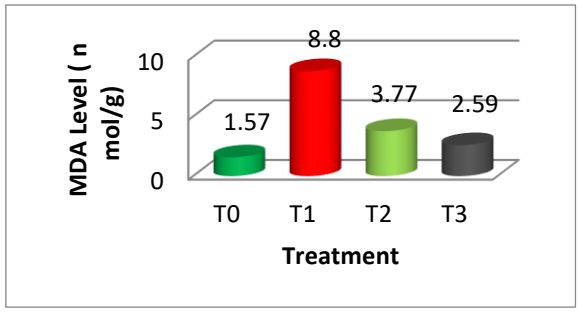

Figure 2. MDA levels (nmol/g) in the kidneys of Wistar rats

Table 4 shows the findings of the comparative examination of SOD activity following treatment among groups.

Table 3. Average, normality and homogeneity of variants

	T0	T1	T2	T3	p*
SOD (%)	77.37± 1.35	16.95± 1.06	52.14± 1.30	57.37± 1.07	0.788
p**	0.558	0.136	0.679	0.980	
MDA(nmol/g)	1.35± 0.06	8.80± 0.06	3.77± 0.09	2.59± 0.04	0.314
p **	0.642	0.880	0.878	0.128	

Note:
T0 = Control group (without treatment);
T1 =Stress group;
T2 =ethanol extract group;
T3 =flavonoid glycosides extract group;
* = homogeneous data (p> 0.05);
** = normally distributed data (p> 0.05).

Table 4. Results of statistical analysis of SOD enzyme activity

Group	n	Average enzyme activity SOD (%) ± SD
Control (T0)	6	77.37 ± 1.35 ^{b,c,d}
Treatment 1 (T1)	6	16.95 ± 1.06^{a,c,d}
Treatment 2 (T2)	6	52.14 ± 1.07^{a,b,d}
Treatment 3 (T3)	6	57.37 ± 1.26^{a,b,c}

Note:
n = number of test animals, SD = standard deviation;
a = The significant difference with T0;
b = The significant difference with treatment group 1(T1);
c = The significant difference with treatment group 2 (T2);
d = The significant difference with treatment group 3 (T3).

4. DISCUSSION

The flavonoid glycosides present in the n-butanol extract of the *solanum betaceum* Cav have an IC_{50} value of 69.89 mg/L, based on the in vitro studies shown in Table 2. Antioxidants with IC_{50} values ranging from 50-100 mg/L are vigorous. As a result, the solanum n-butanol extract shows high antioxidant activity. It is consistent with Asih [6], who found an IC_{50} value of 68.14 mg/L in *solanum n*-butanol extract peels.

The average SOD among groups was used to analyze the impact on each treatment group. In the one-way Anova test, the p-value of the average SOD activity was 0.0001. This number indicates that the four treatments administered to the rat statistically showed a significant difference ($p<0.05$). According to an analysis of superoxide dismutase activity in rat kidneys, the treatment group (T1) had the lowest superoxide dismutase activity of 16.95%. It is due to stress treatment in which antioxidants in flavonoid glycosides n-butanol extract of solanum betaceum are not administered. Figure 1 shows the SOD activity of each treatment group.

The control group (T0) had the highest SOD activity, 77.37%. Treatment group 3 (T3) had a superoxide dismutase activity of 57.37%. This group demonstrated more vigorous superoxide dismutase activity than the groups that received treatment 1 (T1) and 2 (T2). Overall, the experimental animals' medication had a considerably different effect. Table 3 summarizes the findings of a comparative examination of SOD activity following treatment among groups. The body's need for oxygen is not met when engaging in strenuous physical exercise, resulting in hypoxemia. Xanthine dehydrogenase (XD) converts to xanthine oxidase (XO), which produces reactive oxygen species (ROS) in the form of $\bullet O_2^-$ [16]. The greater the number of free radicals produced, the better. The control group (T0) had the highest SOD activity, 76.28%. It occurs as the control group received no stress treatment, making no free radical in the body. Under normal circumstances, the body's endogenous antioxidant defense system, superoxide dismutase, is still effective. With no treatment, the high superoxide dismutase activity in the control group supports this.

Treatment group 3 (P3) had a superoxide activity of 57.37%. This group demonstrated more vigorous superoxide dismutase activity than the groups that received treatment 1 (T1) and 2 (T2). The flavonoid glycoside molecules in the extract are most likely to blame for this. Flavonoid glycosides from n-butanol extract are more effective than ethanol extract at increasing SOD enzyme activity. According to Nilesh [17], the Beta vulgaris L. leaf n-butanol extract intake boosted the rat livers' SOD enzyme activity compared to the ethanol extract intake. It is most likely owing to the fact that are still molecules in the ethanol extract that are hostile to the active chemical, limiting its action.

Flavonoids assist superoxide dismutase's activity in the body with the neutralization of free radicals. They are reducing chemicals preventing oxidation reactions in a variety of ways. With the ability to transfer H^+ ions (HAT) to free radical compounds, they can be antioxidants. The mechanism of flavonoid compounds suspected is to be present in *n*-butanol extracts reducing free radicals is similar to the mechanism of quercetin compounds reducing superoxide radicals. Flavonoids have a direct and indirect method of action in boosting the activity of SOD enzymes. The presence of hydroxyl groups allows flavonoids to trap free radicals by providing hydrogen atoms. Directly, flavonoids trap free radicals by giving hydrogen atoms, contributing to the generation of reactive oxygen species (ROS) [18]. Flavonoids can indirectly boost the expression of endogenous antioxidant genes through various ways, activating nuclear factor erythroid 2 of factors 2 (Nrf2) that increases SOD enzyme production [19].

Based on the statistical findings, stress treatment harms experimental animals, notably higher levels of free radicals in the body, as proven by decreased superoxide dismutase activity. The group of rats given treatment 1 and treatment 3 (flavonoid glycoside extract) had the highest growth in SOD activity, with a rise of 238.46%. Overall, the experimental animals' medication had a considerably different effect.

Lipid peroxidation, mainly in unsaturated fatty acids, frequently generates malondialdehyde (MDA) as an end product through oxidation by free radicals.

Oxidative damage mainly caused by unsaturated fatty acids has long employed malondialdehyde as its indicator [20]. The technique to gauge MDA levels uses the reaction between MDA and thiobarbiturate (TBA), which results in the formation of a TBA-MDA complex which creates red color detected using a spectrophotometer UV-Vis at a total length of 532 nm. The treatments administered to the rats resulted in a substantially distinct effect ($p <0.05$), according to a One-Way ANOVA analysis of the mean of MDA levels indicated in Table 5. MDA research revealed that treatment group 1 (T1) had the highest MDA content of 8.8 nmol/g, compared to the other treatment groups. The increased levels of MDA in treatment group 1 were produced by oxidative stress in the

animals. Stress by swimming till almost drowning causes oxidative damage in experimental animals. The body's antioxidant defense system and free radical generation become unbalanced due to excessive physical activity. Oxygen consumption increases 20-fold throughout the body during maximal physical activity, but oxygen consumption in muscle fibers increases 100-fold. Hypoxia, or a lack of oxygen in the body, can result from this. As the amount of oxygen consumed rises, the amount of oxygen produced rises.

Table 5. The results of Duncan's different test analyses of MDA levels

Group	n	MDA Level ±SD
Control (T0)	6	1.35 ± 0.06^{a}
Treatment 1 (T1)	6	8.8 ± 1.06^{b}
Treatment 2 (T2)	6	3.77 ± 0.09^{c}
Treatment 3 (T3)	6	2.59 ± 0.04^{d}

Note: Different notations in the column show significantly different values ($p < 0.05$).

Giving physical exercise to swim produces an increase in free radicals in the body. MDA is produced when free radicals induce oxidative damage and lipid peroxidation in the cell membrane.

Lipids (LH) make up the cell membranes in the body, which are typically polyunsaturated fatty acids. When oxidants and unsaturated fatty acids (LH) combine to produce free fat carbon radicals (L•), the peroxidation reaction begins at the initiation phase. Peroxyl radicals (LOO•) are formed when free fat combines with oxygen. Lipid hydroperoxide (LOOH), a cytotoxic compound, and free fatty acid (L•) are generated, resulting in a chain when the peroxyl radical and other unsaturated fatty acids combine again. The reaction will cease in the termination phase when free fat is generated in the initiation phase, or other radicals formed at the propagation phase react back to other radicals to form non-radical products [21] in Arsana [22]. Fat endoperoxide is also produced, which decomposes to malondialdehyde [13]. As a result, the elevated MDA levels in treatment group 1 point to many free radicals forming under oxidative stress.

The MDA content in the control group (T0) was the lowest, at 1.36 nmol/g protein. Compared to the other treatments, the control therapy produced the least MDA. It suggests that the number of free radicals generated in the body is relatively minimal without maximum physical activity, resulting in deficient MDA levels. In addition, MDA levels in treatment group 2 were 3.77 nmo/g, while MDA levels in treatment group 3 were 2.76 nmo/g. It is most likely due to the flavonoid glycoside components. As a result, flavonoid glycoside molecules can bind to free radicals and react with them.

Boligon [23] found that an *n*-butanol extract of *T. catharinensis* leaves reduces MDA levels in Wistar rats more effectively than an ethanolic extract. It is because the polyphenol and flavonoid in a gram of *n*-butanol fraction is higher than that of ethanol extract, which is indicated by an IC_{50} value of the *n*-butanol fraction.

5. CONCLUSIONS

According to the findings, the flavonoid glycoside extract of *solanum* is a potent antioxidant with an IC50 = 69.89 mg/L, and at a dose of 50mg/kg, BW induced increased SOD activity and lower MDA levels in the renal tissue of Wistar rats that were not administered flavonoid glycoside extract.

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AUTHOR CONTRIBUTIONS

Ida Ayu Raka Astiti Asih, Wiwik Susanah Rita, and Wayan Suirta contributed to the research design, the analysis of the results, and the writing of the manuscripts. Ahmad Fudholi contributed to editing, reviewing, and improving the first draft of the manuscript.

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