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## Dewandaru fruit extracts (*Eugenia uniflora L*) reduces oxidative stress and increases Superoxide Dismutase level in excessive activity-induced rats



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### ABSTRACT

**Background:** Oxidative stress occurs when free radicals levels are higher than antioxidants levels. This can trigger cell damage which leads to various diseases such as Alzheimer, atherosclerosis, and degenerative diseases such as heart disease, cancer, and premature aging. Oxidative stress can be caused by excessive physical activities. Dewandaru fruit (*Eugenia uniflora L*), which contains high levels of antioxidants such as phenol, flavonoid, and anthocyanin, can prevent excessive oxidation caused by maximum physical activities. This research aims to test the activity of Dewandaru fruit extract in reducing oxidative levels.

**Methods:** This study used purely experimental planning of post-test only control group design. Twenty-four rats were divided into four groups; control group, P1, P2, and P3. All groups were given maximal physical exercise. The P1, P2, and P3 received 50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW of n-butanol extract of Dewandaru

fruit respectively. Measurement of Malondialdehyde (MDA), F2-isoprostane, 8-OHdG, and superoxide dismutase (SOD) with ELISA was used to determine the level of oxidative stress.

**Result:** The levels of MDA, F2-isoprostane, and 8-OHdG were higher in control group when compared to the treatment group; a higher dose of the extract resulted in lower MDA, F2-isoprostane, and 8OHdG levels ( $p = 0.001$ ). While the level of SOD was lower in the control group when compared to the treatment group; a higher dose of the extract resulted in a lower SOD level ( $p = 0.001$ ).

**Conclusion:** N-butanol extracts of Dewandaru fruit at a dose of 50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW significantly reduce MDA, Isoprostane, 8-OHdG and increase SOD in rats given maximum physical activities.

**Keywords:** Dewandaru fruit extract, MDA, F2-Isoprostane, 8-OHdG, SOD.

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### INTRODUCTION

Free radicals can originate from outside the body, or it can also be formed inside the body as a product of an integral part of physiological processes such as the oxidative phosphorylation in mitochondria. The main source of reactive oxygen species (ROS) inside the body is oxidative phosphorylation. During the physical activity, ROS is formed as a side product of the oxidative phosphorylation reaction to form adenosine triphosphate (ATP) through electron transport chain reaction in the mitochondria. Excessive physical activities beyond the limit of fatigue can form free radicals that can lead to oxidative stress.<sup>1</sup>

Oxidative stress occurs when the free radicals levels are higher than the antioxidants levels. This condition can trigger various cell damages, which will lead to degenerative diseases such as heart disease, cancer, premature aging, and also other various diseases such as Alzheimer and atherosclerosis.<sup>2</sup>

The oxidative damage level of cells/tissues due to free radicals can be determined by measuring malondialdehyde (MDA) level in the blood and

pentane level in the breath; both are indicators of lipid peroxidation.<sup>3</sup> The MDA is a reactive aldehyde compound, and it is one of many reactive electrophilic compounds that causes toxic stress in cells and covalent reaction that changes the protein shape, resulted in a final product known as lipoxidation products.<sup>4</sup> Another oxidative stress marker is F<sub>2</sub>-isoprostane, which can be measured in the urine sample of overtraining subject.<sup>5</sup>

The reaction of oxidation involving these free radicals can damage the surrounding normal cell membrane and also the DNA composition, which might lead to mutation. Mutation of the DNA can cause several degenerative diseases such as cancer, heart disease, cataracts, and premature aging. Oxidation of guanosine, one of the bases that form DNA, will form the 8-OHdG compound. Thus it can be used as a marker that shows DNA damage caused by excessive free radicals.<sup>9</sup>

The body produced enzymes and special molecules to eliminate free radicals (endogenous antioxidant). Such compounds may be obtained from external sources such as vitamin C, vitamin E, and

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beta-carotene (exogenous antioxidants). Exogenous antioxidants work by cutting off the chain of the oxidation reaction of free radicals or by trapping it. It also prevents the oxidative stress.<sup>6</sup> In oxidative stress condition caused by excessive activities, the body requires a considerable amount of nutrients that contain antioxidants.

One of the fruits that contain antioxidants is Dewandaru fruit (*Eugenia uniflora* L). Dewandaru fruit extracts reduce lipid peroxidation reaction. Flavonoid contained in Dewandaru fruit has strong antioxidant activity.<sup>7</sup> The content of flavonoids, phenols, and anthocyanins contained in Dewandaru fruit has antihyperglycemic, antidyslipidemic and antioxidant effects in rats fed a high-glucose compound diet.<sup>8</sup>

This study aims to test Dewandaru fruit extracts in reducing oxidative stress in excessive physical activity-induced rats. The oxidative stress was measured from MDA, 8-OHdG, Isoprostane, and SOD level.

## MATERIALS AND METHODS

As many as 500 grams dewandaru fruits (*Eugenia uniflora* L) was macerated with 2000 mL n-butanol in a sealed container and kept away from the sunlight. The filtrate was then evaporated by using rotary evaporator at a temperature of 60°C. The thick extract obtained was then evaporated in an oven at a temperature of 50°C until the dry extract was obtained.

This study used purely experimental planning of post-test only control group design. Twenty-four Wistar rats (*Rattus norvegicus*) were divided into four groups consist of six rats each. The rats went through adaptation process for seven days. The first group was set as control; the rats were given excessive activities and CMC-Na 0.5%. The second group (P1) was given excessive activities, CMC-Na 0.5%, and 50 mg/kgBW dewandaru extract. The third group (P2) was given excessive activities, CMC-Na 0.5%, and 100 mg/kgBW dewandaru extract. The

fourth group (P3) was given excessive activities, CMC-Na 0.5%, and 200 mg/kgBW dewandaru extract. The treatment was given for 14 days. For sampling collection, the rats were anesthetized (with ketamine + xylazine, 0.1 ml, i.m), and then 1 ml of their blood was collected to be observed.

All data were analyzed by using SPSS software version 20. One way ANOVA test was used to analyze the statistical significance.

## THE RESULTS

The mean level of MDA, F<sub>2</sub>-Isoprostane, 8 OHdG and SOD from all the groups can be seen in Table 1 below. The differences of oxidative stress marker levels among the group were statistically significant, as showed by *p*-value <0.05 from the one way ANOVA test in all variables.

## DISCUSSION

This study found that dosage of 200 mg/kgBW of N-butanol Dewandaru fruit extract has the most potent ability in reducing MDA. The high contents of phenol, flavonoid and terpenoid compounds can work significantly to decrease the level of MDA. Bioactivity of secondary metabolites in the form of phenol (flavonoid) in n-butanol extracts can prevent and neutralize ROS reaction, which resulted from body metabolism, with polyunsaturated fatty acids (PUFA). This lipid peroxidation, which is suspected to be inhibited, can produce a product called MDA. Phenol, chlorophyll, and terpenoid compounds contain a lot of -OH, >C=C<, and >C=O groups. These groups can donate their electrons or one of their hydrogen molecules to ROS, therefore forming a less reactive free radical. This reaction can reduce lipid peroxidation reaction between ROS and PUFA, hence decreasing the MDA level.

Natural flavonoids can protect phospholipid PUFA membrane by donating or giving one of its Hydrogen (H+) ions to the radical lipid peroxyl

**Table 1** Mean level of MDA, isoprostane, 8-OHdG, and SOD of each group

Marker	Group				<i>p</i> value
	Control	P1	P2	P3	
MDA (µmol/L)	11.99 ± 1.46	9.07 ± 0.66	5.39 ± 0.92	3.69 ± 0.78	0.0001
F <sub>2</sub> -Isoprostane (ng/mL)	5.39 ± 0.28	4.37 ± 0.19	2.88 ± 0.63	2.02 ± 0.67	0.0001
8-OHdG (ng/mL)	3.44 ± 0.38	2.83 ± 0.17	2.30 ± 0.25	1.69 ± 0.29	0.0001
SOD (U/L)	8.37 ± 1.27	13.38 ± 0.90	19.21 ± 0.91	23.93 ± 0.60	0.0001

MDA: malondialdehyde, SOD: superoxide dismutase  
 K(-) : control, without extract ( CMC-Na only)  
 P1 : was given 50 mg/kgBW dewandaru extract  
 P2 : was given 100 mg/kgBW dewandaru extract  
 P3 : was given 200 mg/kgBW dewandaru extract

( $\text{LOO}^*$ ), producing further radical reactions as follows:<sup>10</sup>



The result of this study is supported by several previous studies regarding the dewandaru fruit extract and the antioxidants compound. The flavonoids, tannins, and anthocyanins found in Dewandaru fruit reduces lipid peroxidation and improve SOD in rats given a high glucose diet. Flavonoid compounds in *Adiantum capillus-veneris* L. have a high antioxidant capacity and reduce the level of MDA. The content of flavonoids in lotus leaves can reduce oxidative stress by lowering SOD activities in rats given swimming exercise.<sup>12</sup> Flavonoids in *Allium* can rapidly reduce MDA level.<sup>13</sup> Flavonoids in *Hypericum perforatum* L. extracts can decrease MDA level in rats with high cholesterol.<sup>14</sup>

This study used  $\text{F}_2$ -Isoprostane as a biomarker of oxidative stress because currently,  $\text{F}_2$ -Isoprostane is the best biomarker for oxidative stress status and lipid peroxidation in vivo.<sup>15,16</sup> The measurement of  $\text{F}_2$ -Isoprostane as oxidative stress marker has several advantages compared with other methods.  $\text{F}_2$ -Isoprostane is a specific, chemically stable, product of peroxidation, it is formed in vivo, and can be detected in tissues and liquid. It increases substantially in animal models with oxidant injury, and it is not affected by the amount of fat in the diet, and most importantly it is sensitive to the dose of antioxidants.<sup>16</sup>

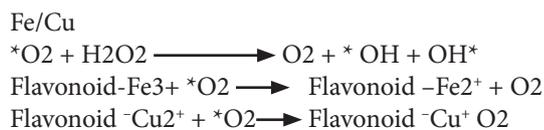
The effect of n-butanol extract which contains several compounds as antioxidants can lower the level of  $\text{F}_2$ -Isoprostane in the urine of Wistar rats. This effect is due to the flavonoid, polyphenol, and carotene compounds, which is a lipophilic, chain-breaking antioxidant, which plays a role in protecting cell membranes by preventing lipid peroxidation. Flavonoids, beta-carotenes, and vitamin C are efficacious as antioxidants, which protect LDL cholesterol from oxidation process, due to non-cyclooxygenase oxidative modification of arachidonic acid that produces  $\text{F}_2$ -Isoprostane.<sup>17,18,19</sup>

The result of this study shows that isoprostane level was increased in control group after the rats received physical exercises. Administration of n-butanol dewandaru fruit extract at a dose of 100 mg/kgBW resulted in lower isoprostane level. At a dose of 200 mg/kgBW, isoprostane level was decreased to a mean of 2.02 ng/mL. This finding was statistically significant ( $p = 0.001$ ). It is thought that the phenol compound (flavonoid) in the

extract were able to neutralize free radicals (ROS) formed during the training.

The content of Flavonoids in n-butanol extracts of Dewandaru fruit can inhibit the occurrence of DNA damage resulted by  $\text{HO}^*$  reaction with nitrogenous bases of DNA and stimulate the formation of enzymatic antioxidants such as SOD, catalase, and GPx. Flavonoids can protect our bodies from further reactions of ROS and reactive nitrogen species (RNS) by catching ROS, blocking propaganda reactions, and stimulating the formation of endogenous antioxidants such as GPx, SOD and catalase as well as lowering MDA level because of the absence of fat peroxidation (PUFA) and decreasing level of 8-OHdG due to  $\text{HO}^*$ , which normally enters and reacts into DNA, being neutralized by Flavonoid.<sup>20</sup>

Flavonoids can function as a chelating agent of Cu and Fe metals, which function as catalysts in Fenton reaction. This reaction also includes a reaction that turns hydrogen peroxide into  $^*\text{OH}$ . This chelating process will decrease the catalytic activity of Cu and Fe metals reducing the formation of  $^*\text{OH}$  radicals and automatically decreasing the process of DNA damage and fat peroxidation (PUFA), through the following reactions:<sup>11,21,22</sup>



N-butanol extract of Dewandaru fruit at a dose of 200 mg/kgBW has the highest ability in reducing the level of 8-OHdG. This effect happens due to the highest quantity of phenol, flavonoid and terpenoid compounds and works synergistically in reducing the 8-OHdG level.

These results were supported by several studies such as 8-OHdG found to be decreased significantly in a group of pregnant women who were given a moderate exercise compared to the other group without moderate exercise. Exercise with moderate category (submaximal exercise) affects neuroendocrine functions to stimulate the synthesis of the hormone, resulting in raising body temperature, increasing production of prolactin hormone and decreased production of growth hormone.<sup>23</sup>

Administration of Dewandaru d-butanol extract at a dose of 50 mg / kgBW has been significantly ( $p < 0.05$ ) increasing the SOD activity. This effect is thought to be caused by the quantity of phenol compound (flavonoid) in this dosage can reduce or neutralize ROS. Meanwhile, at a dose of 200 mg / kgBW, the SOD activity has the highest increase ( $p < 0.05$ ). The results of this study supported several studies such as flavonoid compounds, and

anthocyanins contained in Dewandaru (*Eugenia uniflora* L.) may increase SOD, catalase and glutathione levels and reduce lipid peroxidation in hyperglycemic streptozotocin-induced rats.<sup>24</sup>

## CONCLUSION

The extracts of Dewandaru fruit n-butanol dose 50 mg/kgBB, 100 mg/kgBB and 200 mg/kgBB can significantly reduce MDA content, F<sub>2</sub>-Isoprostane, 8 OHdG and increase SOD (p < 0.05) in wistar rats given maximum physical activities.

## REFERENCES

- Pham-Hu LAP, He H, Pham-Huy C. Free Radicals, Antioxidants in Disease and Health. *Int J Biomed Sci.* 2008;4:89-96.
- Murray RK, Granner DK, Rodwell VW. *Biokimia Harper.* 27<sup>th</sup> ed. Jakarta; Penerbit Buku Kedokteran EGC: 2012.
- Clarkson PM, Thomson HS. Antioxidants: What role do they play in physical activity and health? *Am J Clin Nutr.* 2000;729 (Suppl):637-646
- Davoine C, Farmer EE. Reactive Electrophile Species, *Current Opinion in Plant Biology.* 10(4):380-386.
- Margonis I, Fatouros AB, Athanasios Z, Jamurtas BC, Nikolaidis MG, Papassotiropoulos I, Taxildaris K. Oxidative stress biomarkers responses to physical overtraining Implication for diagnosis. *Journal Free Radical Biologi and Medicine.* 2007;43(6):901-10.
- Winarsi H. Antioksidan Alami dan Radikal Bebas Potensi dan Aplikasi nya dalam Kesehatan. Yogyakarta; Penerbit Canisius: 2007.
- Celli GB, Pereira-Netto AB. Comparative analysis of total phenolic content, antioxidant activity, and flavonoids profile of fruits from two varieties of Brazilian cherry (*Eugenia uniflora* L.) throughout the fruit developmental stages. *Food Research International Journal.* 2011;44(8):2442-2451.
- Cardoso de Souza J, Oliveira PS, Bona NP, Vasconcellos FA, Jucimara B, Vizzotto M. Antioxidant, antihyperglycemic, and antidyslipidemic effects of Brazilian-native fruits extracts in animal model of insuline resistance. *Journal of Redox Report Communication in Free Radical Research.* 2017. pp. 1-6.
- Jena NR. DNA damage by reactive species : Mechanisms, mutation and repair. *J. Biosci.* 2012;37(3):503-17.
- Hamid AA, Aiyelaagbe OO, Usman LA, Ameen OM, Lawal A. Antioxidants: Its medicinal and pharmacological applications. *African Journal of Pure and Applied Chemistry.* 2010;4(8):142-151.
- Akhlaghi M, Brian B. Mechanisms of flavonoid protection against myocardial ischemia-reperfusion injury. *Journal of Molecular and Cellular Cardiology.* 2009;46:309-17.
- Xu HC, Wang MY. Effect of flavonoid from Lotes (*Nelumbo nuficera Gacrti*) leaf on biochemical parameters related to oxidative stress induced by exhauctive swimming exercise of mice. *Biomedical Research.* 2014;25(1):1-5.
- Nausheen QN, Ali SA, Subur K. Cardioprotective and Antioxidant activity of Onion (*Allium cepa*)Leaves Extract in Doxorubicin Induced Cardiotoxicity in Rats. *Annals of Experimental Biology.* 2014;2(2):37-42.
- Zou Y, Lu Y, Wei D. Hypocholesterolemic Effects of a Flavonoid-rich extract of Hypericum perforatum L. in Rats Fed a Cholesterol-Rich Diet. *J. Agric. Food Chem.* 2005;53:2462-66.
- Morrow JD, Zackert WE, Van der Ende DS, Reich EE, Terry ES, Cox B, et al. Quantification of isoprostanes as indicators of oxidant stress in vivo. In Cadenas E, Packer L (eds), *Handbook of Antioxidants.* ed 2. New York;Dekker:2002.
- Montuschi P, Barnes JP, Roberts II LJ. Isoprostanes: Markers and Mediators of Oxidative Stress. *FASEB J.* 2004;18:1791-1800.
- Patrono C. Isoprostanes : Potential Markers of oxidant Stress in Atherothrombotic Disease. *Thromb. Vasc. Biol.* 1997;17:2309-2315.
- McMichael, M. 2004. Ischemia-Reperfusion Injury: Assessment and Treatment, part II. *J. Vet. Emerg.Crit. Care,* 14: 242-252
- Nalsen C, Vessby B, Berglund L, Uusitupa M, Hermansen K, Riccardi G, et al. Dietary (n-3) Fatty Acids Reduce Plasma F2-Isoprostane but Not Prostaglandin F2a in Healthy Humans. *Journal of Nutrition.* 2006;136:1222-1228.
- Cooke MS, Mark DE. 8-oxo-deoxyguanosin : Reduce, reuse, recycle. *PNAS.* 2007;124(134):1353-1536.
- Valko M, Mario I, Milan M, Christopher JR, Joshua T. Role of oxygen radicals in DNA damage and cancer incidence. *Molecular and Cellular Biochemistry.* 2004;266:36-37.
- Chevion S, Molan DS, Heled Y. Plasma antioxidant status and cell injury after severe physical exercise. *PNAS.* 2003;100(9):5119-23.
- Pathise OS, Victor CC, Natalia PB, Mayara SP, Juliane SC. *Eugenia uniflora* (red type) standardized extrac: a potent pharmacological metabolic syndrome damage management. *Biomedicine & Pharmacotherapy.* 2017;92(5):935-941.



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