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DOI: [10.31965/infokes.Vol21Iss3.1144](https://doi.org/10.31965/infokes.Vol21Iss3.1144)Journal homepage: <http://jurnal.poltekkeskupang.ac.id/index.php/infokes>**RESEARCH****Open Access****The Effect of Dayak Onion Extract (*Eleutherine palmifolia* (L.) Merr) on Swimming Time and Oxidative Stress Levels in Mice with the Forced Swimming Test Model****Aulia Ramadhani<sup>1a\*</sup>, Muchsin Doewes<sup>2b</sup>, Shanti Listyawati<sup>3c</sup>**<sup>1</sup> Department of Clinical Nutrition, Postgraduate Program, Universitas Sebelas Maret, Surakarta City, Central Java, Indonesia<sup>2</sup> Department of Pharmacology, Internal Medicine Subspecialist, Universitas Sebelas Maret, Surakarta City, Central Java, Indonesia<sup>3</sup> Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta City, Central Java, Indonesia<sup>a</sup> Email address: [ramadhani94.ar@gmail.com](mailto:ramadhani94.ar@gmail.com)<sup>b</sup> Email address: [mdowes2000@yahoo.com](mailto:mdowes2000@yahoo.com)<sup>c</sup> Email address: [shantilistyawati@staff.uns.ac.id](mailto:shantilistyawati@staff.uns.ac.id)

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**Abstract**

Excessive physical exercise can increase the occurrence of oxidative stress, which is characterized by cell damage caused by free radicals. Antioxidants found in Dayak Onion such as flavonoids, saponins, tannins, and vitamin C can be used as alternative ingredients to neutralize free radicals. The purpose of this study was to determine the effect of dose and duration of administration of Dayak onion extract on malondialdehyde (MDA) levels in a rat model of the forced gum test, extraction of Dayak onions using 96% ethanol. The type of research is Randomized Pre and Post Test Control Group Design. Male Sprague Dawley rats aged 2 months (weighing 150-200g) were divided into 5 groups: K- (control group, rats not given Dayak Onion extract but underwent forced swimming test), K+ (rats given xanthine and forced swimming test), P1 (rats given Dayak Onion extract at a dose of 50 mg/200 rats body weight/day), P2 (rats given Dayak Onion extract at a dose of 100 mg/200 rats body weight/day), P3 (rats given Dayak Onion extract at a dose of 200 mg/200 rats body weight/day). Dayak Onion extract was administered for 21 days with for 21 days with forced swimming test treatment, and changes in MDA levels were observed. The results showed that the administration of Dayak onion extract at a dose of 50 mg/200 rats/day, 100 mg/200 rats/day, and 200 mg/200 rats/day had an effect on preventing the increase in MDA, but at a dose of 200 mg/200 rats mice/day is the optimal dose. The conclusion is administration of Dayak Onion (*Eleutherine Palmifolia* (L.) Merr) significantly decreased MDA levels in male Sprague Dawley rats with forced swimming test model.

**Keywords:** Dayak Onion, Antioxidant, MDA, Forced Swimming Test.**\*Corresponding Author:**

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## 1. INTRODUCTION

Humans spend a large portion of their waking hours being physically active, around 85-90% (Dewi et al., 2016). The physical activity as any movement that involves the skeletal muscles and requires energy expenditure (Westerterp, 2013). With the increasing demands of modern life, including the need to work harder to maintain one's livelihood, physical activity in the work setting continues to rise (Mattioli et al., 2020). Unfortunately, due to poor time management, people often engage in physical activity that surpasses their body's endurance, leading to potential negative impacts on their health (Asih et al., 2018).

As part of the body's metabolism, free radicals and reactive oxygen species (ROS) are routinely produced by cells (Andrés et al., 2021). If the production of free radicals exceeds the body's antioxidant defenses, it can lead to oxidative stress. One of the contributing factors to oxidative stress is excessive physical activity (Kruk & Duchnik, 2014). Various studies have reported that acute aerobic physical activity, particularly at high intensities can lead to increased oxidative stress (Pingitore et al., 2015). The mechanisms behind this include the impact of increased oxygen consumption during exercise, which is 10 to 15 times higher than at rest, leading to increased pro-oxidants and relatively inadequate antioxidants compared to pro-oxidants (Arazi et al., 2021). According to Krzeszowiak et al., (2014) and Pittaluga et al., (2015), excessive physical activity can increase the occurrence of oxidative stress and malondialdehyde (MDA) levels in the blood (Krzeszowiak et al., 2014; Pittaluga et al., 2015). MDA is a useful indicator of oxidative stress, as higher levels of MDA indicate higher levels of oxidative stress (Singh et al., 2014). When free radicals cause lipid peroxidation in cell membranes under oxidative stress conditions, lipid production occurs, as noted by (Morales and Munné-Bosch, 2019). Since cell membranes function as enzymes or receptors, oxidative stress in cell membranes can lead to cellular dysfunction and cell death (Sari, 2018)

Base on the information provided, Malondialdehyde (MDA) is a byproduct of the homolytic decomposition of lipid peroxidation in cell membranes. This process generates more free radicals, which can cause metabolic and cellular disorders by damaging the cell membrane and causing lipid peroxidation. The effects of lipid peroxidation include altered membrane fluidity, permeability, and function (Riyana, Mudigdo & Wasita, et al., 2019). The production of MDA and other lipid peroxides increases during high-intensity exercise compared to low-intensity exercise, as evidenced by an increase in lipid hydroperoxides. This suggest that exercise-induced oxidative stress can contribute to cellular damage through the production of free radicals and lipid peroxidation (Souza-Silva et al., 2016). Overall, MDA and lipid peroxidation are indicators of oxidative stress and cellular damage. Strategies to reduce oxidative stress, such as antioxidant supplementation of exercise training, may help mitigate the negative effects of free radicals and lipid peroxidation on cellular function and health (Riyana et al., 2022).

Prevent oxidative stress, antioxidants can be administered through natural food sources of supplements. Natural food sources of antioxidants include Vitamin A, vitamin C, vitamin e, saponins, and flavonoids. Indonesia, which is a tropical region, has various plants that have the potential to help improve health, one of which is *Eleutherine palmifolia*, also know as "Bawang Dayak" (Hendrawan et al., 2020). This plant is commonly found on the island of Borneo and has been traditionally used by local people as a medicinal plant. The part of the plant that can be used is the bulb. This plant is empirically known to have anti-inflammatory and anti-cancer properties. Phytochemical testing conducted by Wigati and Rahardian, (2018) revealed that Dayak Onion contains flavonoids, saponins, tannins, alkaloids, quinones, provitamins, and various other essential minerals that are useful as central nervous system stimulants, antioxidants, anti-inflammatories, and blood circulatory enhancers (Wigati & Rahardian,

2018). Ginsenosides, derivatives of saponin compounds, are useful as tonics that can increase resistance to stress, fatigue, and various other diseases (Chen et al., 2016).

The results of the Dayak Onion extract test showed an inhibitory or antioxidant activity on free radicals using the DPPH method. The IC<sub>50</sub> value of the ethanol extract of Dayak Onion bulbs reached 52.38 ppm (Yuswi, et al., 2017). The antioxidant activity in Dayak Onion is able to prevent the oxidation of body cells by oxygen, such as hydrogen peroxide, superoxide, hydroxyl radicals, and other free radicals (Muti'ah et al., 2020). However, no research has been conducted on the effect of using Dayak Onions to prevent oxidative stress in experimental animals that perform physical activity using the forced swimming test method.

The purpose of this study is to examine the impact of Dayak Onion extract on the levels of MDA in male Sprague Dawley rats by utilizing the forced swimming test model. There has been limited investigation conducted on the influence of Dayak Onion extract on MDA levels as a marker for oxidative stress, particularly using the forced swimming test model in rats. Therefore, additional research is required to gain a more comprehensive understanding of this subject.

## 2. RESEARCH METHOD

This study used a Randomized Pre and Post Test Control Group Design, with an experimental population of male white rats (Sprague Dawley) aged 2 months with a body weight of 150-200g. The research was conducted for 21 days during March 2023 at the Laboratory of the Center for Food and Nutrition Research, Gadjah Mada University. Five groups of experimental animals were installed, namely K- (control group without Onion Dayak extract but given FST), K+ (control group given xanthine and FST), P1 (given Onion Dayak extract at a dose of 50 mg/200 rat body weight/day), P2 (given Dayak onion extract at a dose of 100 mg/200 rats/day), and P3 (given Dayak onion extract at a dose of 200 mg/200 rats/day). Dayak onions are crushed (chopped) and dried in an oven at  $\pm 40^{\circ}\text{C}$  for 8 hours. The dried simplicia was then crushed and sieved using mesh no. 40. As much as 10 kg of Dayak onion bulbs were obtained from the Palangkaraya area. Dayak onions were macerated using 96% ethanol with a ratio of 1:5 for 3 days; every 24 hours the stirring process is carried out. The maserate obtained was then filtered and evaporated using a temperature of  $50^{\circ}\text{C}$  to obtain a thick extract of Dayak onion. The extraction results were in the form of thick extract of Dayak onion as a test material which was stored in a bottle at refrigerator temperature ( $\pm 4^{\circ}\text{C}$ ) and protected from sunlight to prevent nutrient damage. Mice were acclimatized for 7 days, after the condition was completed, blood samples were taken to measure MDA levels before treatment. Dayak onion extraction using mice was measured swimming in a water bath measuring 90cm x 45cm and 35cm deep, with a water temperature of  $25^{\circ} \pm 1^{\circ}\text{C}$ , one hour after administration of shallot extract on day 21 (Li et al., 2022). Mice were considered exhausted if they showed no movement to the surface for 10 seconds, with their heads under the air, tails stretched, bodies stretched, and all four legs immobile (Abbasi-Maleki et al., 2020). After the force swim test, another blood sample was taken to determine MDA levels (posttest).

The data gathered in this study were reported as the mean standard deviation. Before performing any statistical analysis, tests were conducted to assess the homogeneity and normality of the data. The Paired T-Test was utilized to analyze the data, and Post-Hoc LSD was employed to perform further statistical analysis. Statistical significance was determined using a p-value of less than 0,05. Ethical certificate number: 47/UN27.06.11/KEP/EC/2023.

### 3. RESULTS AND DISCUSSION

**Tabel 1.** Test of Onion Dayak Determination.

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Ordo	Liliales
Famil	Iridaceae
Sub-Familia	Papilionoideae
Genus	Eleutherine
Spesies	Eleutherine Palmifolia (L.) Merr
Sinonim	Eleutherine bulbosa (Mil.) Urb., Eleutherine americana (Aubl.) Merr. Ex K.Heyne, Sisyrinchium palmifolium (Klatt) Baker.
Region Name	Dayak Onion

A determination test on the Dayak onion (*eleutherine palmifolia* (L.) Merr) was carried out to determine the authenticity of the Dayak onion species. Dayak onions themselves have many types so the determination test avoids ingredients being mixed with other ingredients so that they have fewer benefits. The determination test on Dayak onions was carried out using visual observation techniques. This method is used to observe the external characteristics of plants which may indicate sex or plant determination. For example, in some plants, differences in size, shape, color, or patterns in reproductive organs such as flowers or other structures can make a difference (Malendes & Bunyamin, 2017). By carrying out a determination test, it can be guaranteed that the Dayak onions used contain active compounds that are beneficial to health. In addition, the determination test can also help avoid consuming products whose authenticity and quality are not guaranteed. Therefore, the determination test is very important to ensure the safety and efficacy of Dayak onions as natural ingredients used in medicine or health supplements.

Dayak onion determination test description of the determination test analysis. A total of 10 kg of Dayak onion bulbs were obtained from the Palangkaraya area. A total of 50 grams of the material was determined at the Plant Systematics Laboratory, Faculty of Biology, Gadjah Mada University, Yogyakarta. The results of the determination show that the onion bulbs used have the Latin name *eleutherine palmifolia* (L.) Merr, with the regional name Bawang Dayak.

**Table 2.** Testing the content of Dayak Onion.

Type of Analysis	Analysis Methods	Analysis Results
Antioksidan IC-50	Dpph-Spectrophotometry	196,36
Vitamin C	Spectrophotometry	2182,15 mg/100g

Table 2 presents the content of Dayak Onion extract obtained from Buntok District, Palangkaraya City, Central Kalimantan Province. The extraction process was carried out at the Laboratory of the Center for Food and Nutrition Research, Gadjah Mada University. The starting material consists of 10 kg of Dayak onions which are sliced and dried in an oven at 60°C until the moisture content reaches  $\leq 10\%$ . The dry material was then ground and sieved using mesh no. 40. Furthermore, the Dayak onions were macerated in 96% ethanol solvent with a ratio of 1:7 for 3 days, with stirring every 24 hours. The resulting maceration was then filtered and concentrated using a rotary evaporator at 50°C to obtain concentrated Dayak onion extract.

Antioxidant yield was obtained in Dayak onion extract of 196.36 ppm. Using 96% ethanol as a supplier, referring to previous research (Yuswi et al., 2017), in order to get maximum antioxidant results.

**Table 3.** The effect of Dayak Onion extract on the MDA level of Sprague Dawley White rats using the forced Swimming Test on day 1 to day 28.

Group	MDA Levels (nmol/ml)			<i>P</i> <sup>b</sup>
	Day-1	Day-21	Δ MDA Days 1 - 21	
K-	1,03±0.09	10,16±0,29	9,13±0,35	0,000*
K+	0,97±0,15	2,20±0,30	1,23±0,16	0,000*
P1	0,88±0,15	5,34±0,53	4,45±0,58	0,000*
P2	0,98±0,16	3,53±0,42	2,55±0,34	0,000*
P3	0,95±0,12	2,68±0,35	1,73±0,31	0,000*

Note:

K- : Test animals group not given Dayak Onion extract but still given FST

K+ : Test animals group given xanthine and FST

P1 : Test animals group given Dayak Onion extract dose of 50 mg/ 200 g/BW and FST

P2 : Test animals group given Dayak Onion extract dose of 100 mg/ 200 g/BW and FST

P3 : Test animals group given Dayak Onion extract dose of 200 mg/ 200 g/BW and FST

Table 3 presents the impact of Dayak Onion extract on MDA levels in male Sprague Dawley white rats using the FST from day 1 to day 28. MDA level measurement was conducted after acclimation to establish a baseline for normal MDA levels in rats that have not yet experienced oxidative stress or are still in good health. The findings revealed that high-intensity physical activity could cause an increase in oxidative stress, which was indicated by an increase in MDA levels. All treatment groups, including the control groups, demonstrated an increase in MDA levels. The K- group showed a sharp increase in MDA levels of 9,13 nmol/ml. the K+ group, which was given xanthine, showed an increase in MDA levels of 1,23 nmol/ml, while the P3 group had a similar increase in MDA levels as the K+ group, which was 1,73 nmol/mL. overall, the administration of Dayak Onion extract with P1 (extract dose of 50 mg/200 rats body weight/day), P2 (extract dose of 100 mg/200 rats body weight/day), and P3 (extract dose of 200 mg/200 rats body weight/day) treatments significantly prevented an increase in MDA levels ( $p < 0,001$ ).

**Table 4.** Post-Hoc LSD Analysis of the Effect of Dayak Onion Extract on Swimming Test Levels of Sprague Dawley White Rats using Forced Swimming Test.

Groups	Treatment	<i>P</i>
K-	K+	0,000*
	P1	0,000*
	P2	0,000*
	P3	0,000*
K+	P1	0,000*
	P2	0,000*
	P3	0,0367
P1	P2	0,000*
	P3	0,000*
P2	P3	0,000*

Note:

K- : Test animals group not given Dayak Onion extract but still given FST

K+ : Test animals group given xanthine and FST

P1 : Test animals group given Dayak Onion extract dose of 50 mg/ 200 rats body weight/day

P2 : Test animals group given Dayak Onion extract dose of 100 mg/ 200 rats body weight/day

P3 : Test animals group given Dayak Onion extract dose of 200 mg/ 200 rats body weight/day



Table 4, it can be observed that all groups, P1, P2, and P3, exhibit significant values. Hence, it can be deduced that the bawang dayak extract can influence the endurance and swimming duration in the forced swimming test rat model. Vigorous physical activities such as swimming can lead to an upsurge in the generation of free radicals within the body. These unstable molecules, known as free radicals, are capable of causing oxidative stress and cellular damage. This is why the role of antioxidants becomes pivotal, as they function to safeguard the body's cells against harm stemming from free radicals. When the test subjects engage in swimming, their bodily metabolism escalates to produce the requisite energy (Gomes et al., 2012). Consequently, there is an increase in the production of free radicals as a byproduct of this metabolic process. Uncontrolled generation of free radicals may result in oxidative stress, which in turn damages the body's cells and contributes to fatigue as well as the risk of injuries (Abbasi-Maleki et al., 2020).

Antioxidants act as a protective shield for the body against free radicals. They bind with these radicals and put a halt to potentially hazardous chain reactions that could harm the cells (Aversa et al., 2016). By mitigating oxidative stress, antioxidants play a role in maintaining cellular equilibrium and supporting recovery following intense physical activities (Brancaccio et al., 2020). However, it's imperative to note the intricacies of the relationship between antioxidants and physical exertion. Certain studies have suggested that the intake of antioxidant supplements could have adverse effects on the body's adaptation to physical exercises (Higgins et al., 2020). This is because the body requires a certain level of mild oxidative stress to adapt and reinforce its natural antioxidant defenses. Consequently, it is advisable to acquire antioxidants from natural food sources like fruits, vegetables, legumes, and grains (Poletta et al., 2016).

**Table 5.** The effect of Dayak Onion extract on the MDA level of Sprague Dawley White rats using the forced Swimming Test on day 1 to day 21.

Groups	Treatment	P
K-	P+	0,000*
	P1	0,000*
	P2	0,000*
	P3	0,000*
K+	P1	0,000*
	P2	0,000*
	P3	0,051
P1	P2	0,000*
	P3	0,000*
P2	P3	0,002*

Note:

K- : Test animals group not given Dayak Onion extract but still given FST

K+ : Test animals group given xanthine and FST

P1 : Test animals group given Dayak Onion extract dose of 50 mg/ 200 rats body weight/day

P2 : Test animals group given Dayak Onion extract dose of 100 mg/ 200 rats body weight/day

P3 : Test animals group given Dayak Onion extract dose of 200 mg/ 200 rats body weight/day

Table 5 presents the impact of Dayak Onion extract on MDA levels in male Sprague Dawley white rats using the FST from day 1 to day 21. MDA level measurement was conducted after acclimation to establish a baseline for normal MDA levels in rats that have not yet experienced oxidative stress or are still in good health. The findings revealed that high-intensity physical activity could cause an increase in oxidative stress, which was indicated by an increase in MDA levels. All treatment groups, including the control groups, demonstrated an increase

in MDA levels. The K- group showed a sharp increase in MDA levels of 9,13 nmol/ml. the K+ group, which was given xanthine, showed an increase in MDA levels of 1,23 nmol/ml, while the P3 group had a similar increase in MDA levels as the K+ group, which was 1,73 nmol/mL. Overall, the administration of Dayak Onion extract with P1 (extract dose of 50 mg/200 rats body weight/day), P2 (extract dose of 100 mg/200 rats body weight/day), and P3 (extract dose of 200 mg/200 rats body weight/day) treatments significantly prevented an increase in MDA levels ( $p < 0,000$ ). The advantage of using the forced swimming test method is that it is easy and effective to make the rat model exhausted, and can cause an increase in oxidative stress in experimental animals (Qi et al., 2014).

Post-Hoc LSD in table 4 of the effect Dayak onion extract on MDA levels in male Sprague Dawley white rats in the FST model showed significant results for almost all of the test samples. The test results for groups P1, P2, and P3 showed a significant difference in blood MDA levels in male Sprague Dawley rats among the treatment groups. However, different results were shown by the K+ and P5 groups, indicating that the p-value was greater than 0,05. This suggests that there is no significant difference in blood MDA levels in male Sprague Dawley rats between the FST + Xanthine and FST + Dayak Onion extract 200mg/200rats body weight/day treatment groups on day 28. It can be concluded that Dayak Onion extract has therapeutic effects equivalent to those of xanthine drugs. Therefore, Dayak Onion can be used as an adjuvant to enhance the optimization of the effects obtained. Xanthine drugs work by inhibiting the enzyme xanthine oxidase, which can convert xanthine into uric acid, which can accumulate and cause fatigue (Rachmania et al., 2021).

The antioxidant process affects MDA (malondialdehyde) by protecting cells from oxidative damage induced by free radicals. MDA is a product of lipid oxidation which is formed when free radicals damage fat molecules in cell membranes. Free radicals are molecules that are unstable and have an excess of a single electron. They can damage cells and cause oxidative stress in the body. Oxidative stress occurs when the production of free radicals exceeds the body's ability to cope with and neutralize them using endogenous (produced by the body) or exogenous (obtained from food and supplements) antioxidants. Antioxidants act as the body's defense against free radicals by stopping the chain reactions produced by free radicals. They stop free radicals by donating electrons to free radical molecules, thereby preventing them from damaging healthy cells. In terms of MDA, antioxidants can reduce MDA formation by protecting lipids in cell membranes from oxidative damage. MDA has toxic properties and can damage DNA, proteins, and cell membranes. Excessive accumulation of MDA can contribute to various diseases, including cardiovascular, neurodegenerative, and cancer (Wigati & Rahardian, 2018). By consuming antioxidant-rich foods or using antioxidant supplements, it can help the body slow down the formation of MDA and reduce the risk of cell damage caused by oxidative stress. Some examples of foods rich in antioxidants include berries, dark green vegetables, nuts, seeds, and spices. In the study of the effect of Dayak onion extract on blood MDA in Sprague Dawley male white rats, the FST model showed significant results for all test samples. The test results in groups P1, P2, P3 showed significant differences in blood MDA levels in male Sprague Dawley rats between groups for each treatment given ( $p < 0,05$ ). The antioxidant compound of Dayak onion extract can reduce blood MDA levels in FST model SD male rats. This decrease was influenced by the antioxidant content of Dayak onions which can inhibit free radicals by suppressing the process of lipid peroxidation. Phytochemical compounds in Dayak onions such as flavonoids, tannins, saponins, alkaloids and other compounds can physiologically improve circulation in the central nervous system or blood circulation in the peripheral nerves. Flavonoid compounds can suppress free radicals and stabilize ROS because they will be oxidized by radical compounds resulting in more stable and less reactive radicals (Panche et al., 2016). Tannins have a cooling effect and can coat the

underlying tissue, so that nerve cells are protected from harmful external stimuli (Qi et al., 2014).

Vitamin C can efficiently prevent the formation of superoxide, hydrogen peroxide, hydroxyl radicals, peroxy radicals, and oxygen radicals (Zuraida et al., 2015). Vitamin C is more effective in inhibiting lipid peroxidation by peroxy radicals than other plasma components such as -tocopherol. Vitamin C can prevent membrane peroxidation by increasing tocopherol activity and preventing cell damage due to free radicals. Antioxidants can help protect muscle cells from the oxidative damage that occurs during intense exercise, by protecting the integrity of muscle cells. Antioxidants can help prevent the damage that DOMS can cause. By reducing inflammation and cell damage, antioxidants reduce the intensity and duration of DOMS. This study also showed that the forced swimming test model increased blood serum MDA levels in Sprague Dawley male white rats and administration of Dayak onion extract could reduce serum MDA levels for 21 days.

#### 4. CONCLUSION

The current study was the first of its kind to examine the impact of Dayak Onion extract on malondialdehyde (MDA) levels in male Sprague Dawley rats utilizing the forced swimming test model. Results showed that the administration of Dayak Onion extract significantly decreased serum MDA levels, indicating a suppression of oxidative stress conditions. The data also revealed that the P3 treatment group, which received a dose of 200 mg/200 rats body weight/day, was the most effective treatment, showing better outcomes than the other treatment groups.

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