# THE EFFECT OF *Hibiscus sabdarifa* L. CALYX ETHANOL EXTRACT ON INCREASING GLUTATHION PEROXIDASE (GPX) ACTIVITY AND DECREASING MALONDIALDEHYDE (MDA) AGAINST 7,12-DIMETHYLBENZ-{A} ANTRASENA (DMBA) INDUCED IN RATS

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**ABSTRACT**

**Background:** The existence of free radicals in the body can lead to cellular damage, tissue, and genetic (mutation). To prevent the adverse effects of free radicals needed antioxidants. body has enzin glutathione peroxidase, is a coenzyme that serves to protect cells from oxygen radical attack. Roselle is one of the plants that can be used as a source of antioxidants.

**Objective**: The objective of this study was to determine the effect of ethanol extract of petals Roselle on the enzyme activity of GPx and activity on levels of malondiaIdehyde (MDA), in mice DMBA-induced strain SD.

**Method**: Strain male rats of Sprague Dawley (SD) were divided into 5 groups. Group I and II is baseline. Group III-V is ethanol extract of roselle calyx 10, 50, 100 mg / kg. Data were analyzed with Kolmogorof Smirnov test, leven test, ANOVA and LSD tests,

**Result**: The results showed significant differences compared to the activity of GPx DMBA control ($p < 0.05$). An increase in GPx activity in roselle extract, a dose of 10 mg / kg (11.1125 ± 0.62136), a dose of 50 mg / kg (6.31 ± 1.15779), a dose of 100 mg / kg (29.96 ± 1.15536) compared of DMBA (8.7 ± 0.12884) ($p < 0.05$). Antioksigen effects were also seen in roselle extract ability to reduce levels of MDA. Decreased levels of MDA in roselle extract giving a dose of 10 mg / kg (7.86 ± 0.2692), a dose of 50 mg / kg (2.065 ± 0.21947), a dose of 100 mg / kg (1,935 ± 0.39102) compared of DMBA (9.3475 ± 0.22202) ($p < 0.05$).

**Keywords** : Antioxidants, Roselle, glutathione peroxidase (GPx) , malondialdehyde (MDA ), DMBA

## INTRODUCTION

The existence of free radicals in the body can lead to cellular damage , tissue damage , and genetic ( mutation ). To prevent the adverse effects of free radicals, antioxidants is needed. Antioxidants are substances that can resist the influence of the dangers of free radicals that are formed as a result of oxidative metabolism, which is the result of chemical reactions and metabolic processes that occur in the body .

Roselle is one of the plants that can be used as a source of antioxidants . Results of research conducted by Suwandi( 2012) concluded that the roselle calyx extract dose of 250 mg / kg lowered malondialdehyde which is the end product of lipid peroxidation (lipid

peroxidation is oxidative stress which arise due to the triggering of free radicals) of 28.0 % in rats fed with waste of oil cooking and roselle calyx extract dose of 500 mg / kg lowered malondialdehyde by 50.2 %

Primary antioxidant enzymes include superoxide dismutase (SOD), glutathione peroksiase (GPx), and catalase. GPx and catalase enzyme works by dismutase catalyzes the reaction of superoxide anion radicals into H2O2 (Winarsi, 2007)**.** MDA is a product of the unsaturated fatty acid oxidation by free radicals. In addition, MDA is also a component of cell metabolites produced by free radicals. High concentration of MDA indicate oxidation processes in the cell membrane. High antioxidant status is usually followed by a decrease in the levels of MDA.

Purpose of this study was to determine the effect of ethanol extract of petals Roselle on the enzyme activity of gpxand activity on levels of malondialdehyde (MDA), in mice DMBA-induced strain SD.

## METHOD

### Material Preparation

Materials used in this study is roselle calyx from Malang, East Java;Strain male rats of Sprague Dawley (SD) from UPHP, GadjahMadaUniversity (UGM)

### Extraction of Roselle calyx

Powder of roselle calyx (Hibiscus sabdariffa L.) were extracted with 70% ethanol (1: 5) by using the method of maceration. Macerate were collected and evaporated with a rotary vacuum evaporator at 60 ° C and 100 rpm, then concentrated on water bath with a temperature of 60-70 ° C to obtain a thick extract. Ethanol extract of Roselle calyx is weighed and the yield is measured (Anonymous, 2004).

### Extract Standardization

Qualitative analysis was performed with silica gel TLC 254 with the mobile phase (toluene: acetone: formic acid) (6: 6: 1). Then the assay of flavonoids with quersetin standard which is method by Chang et al (2002).

### Animal treatment

Rats were located in a fairly light room, ample ventilation and maintained humidity. Rats fed and watered ad libitum. Rats were adapted in a cage for 1 week prior to the treatment. Each rat was given DMBA dissolved in corn oil at a concentration of 1% b/v which to be given by intra gastric past the sonde (chrome, 2013).

The male of Sprague Dawley (SD) rats with age of 4 weeks were divided into 5 groups (each group consist of 5 rats). Group I was the control group baseline which was fed with a standard level, group II is a group with a given standard feed and DMBA 75 mg / kg, Group III, IV and V is the group treated with addition of ethanol extract dose variation calyx 10, 50 and 100 mg / kgBB / day for 7 days. Then on the eighth day, they were given DMBA 75 mg /kg single dose intragastric. A week after DMBA addition, rats were fasted for 16 hours, then sacrificed and blood drawn past the eye (plexus orbitalis) to measure the levels of MDA, then the liver tissue of rats each were taken for measurement of enzyme activity GPx. Data were analyzed using ANOVA and LSD.

Blood samples were centrifuged at 4° C temperature at 10,000 rpm speed. The top layer (serum) and then pipetted into the container for inspection GPx enzyme activity test and MDA level test.

## RESULTS AND DISCUSSION

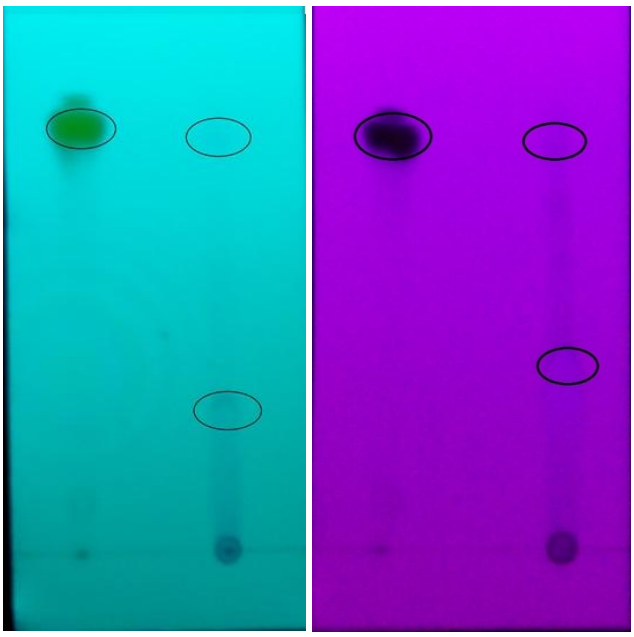
### Extraction and standardization of active substance

Rosella flowers freshly cleaned and dried aerated in the open air. Once dried it was made into powder then with sieved by sieve No. 20/50. A total of 1.5 kg of powder rosella were then macerated by using ethanol 70%. Extraction results are as much as 615.45 grams of 70% ethanol extract of rosella flower with a yield of 41.03%. The characteristic of extract were performed in Table I.

**Table 1. Extract Characteristics**

| **ORGANOLEPTIK** | |
|---|---|
| **Colour** | Red |
| **Smelling** | Typical |
| **Flavor** | Sour |
| **Extract form** | Thick |

Standardization of the active agent of ethanol extract of rosella was done by preparative TLC method which were read in the UV 366 and UV 254, then determine the total flavonoid content by using quersetin standard



A B

**Figure 1. TLC profile of rosella extract detected with , A. UV 254, B UV 366**

The TLC profile with quercetin standard showed that rosella extract contain a quercetin, but quercetin was not a major content. The major content of rosella extract has Rf of 0.38 lower than quercetin with Rf of 0.87.

The total flavonoids were assayed with quercetin standard using spectrophotometric method with AlCl3. The standard curve of quercetin was found with equation of y = 0.003 x + 0.179 and $R^2$ = 0.964. The total flavonoids content of rosella extract were perfomed on Table 2.

**Table 2. Total Flavonoid content of rosella extract**

| Sample Replication | Abs | X (µg/mL) | **Flavonoid total** (g/100 g extract) | X̄ ± SD |
|---|---|---|---|---|
| 1 | 0,336 | 11,219 | 0,37 | |
| 2 | 0,310 | 10,612 | 0,35 | 0,363±0,012 |
| 3 | 0,331 | 11,105 | 0,37 | |

**The MDA level**

The MDA level of rosella extract treted rats were showed on Table 3.

**Table 3. The results of MDA**

| GROUP | MDA level |
|---|---|
| **Baseline** | 1.295 ± 0.05686 |
| **DMBA** | 9.3475 ± 0.22202 |
| **Rosela 10 mg/KgBB** | 7.86 ± 0.2692 |
| **Rosela 50 mg/KgBB** | 2.065 ± 0.21947 |
| **Rosela 100 mg/KgBB** | 1.935 ± 0.39102 |

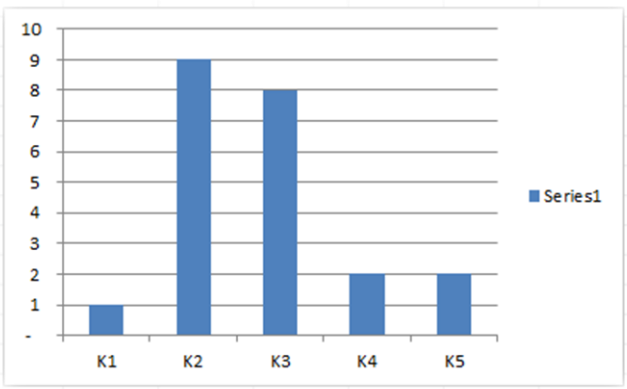



**Figure 2. The MDA level of rosella extract treated rats.**

It shows that 70% ethanol extract of rosella able to reduce levels of MDA. Based on the results of the Kolmogorov-Smirnov test for normality and Levene's test of homogeneity indicates that the data content of MDA, normally distributed and homogeneous. This is shown on the Sig > 0.05. Results of one-way ANOVA statistical analysis showed the Sig

<0.05, meaning that there is a significant difference between the concentration of MDA in each test group. The results of multiple comparison test (Tukey) showed differences significant (Sig <0.05) between group I and group II, III, IV, and V, group II with group III, IV, and V, group III and group III and group IV and V. However, there is no significant difference (Sig> 0.05) between the groups IV and group V.

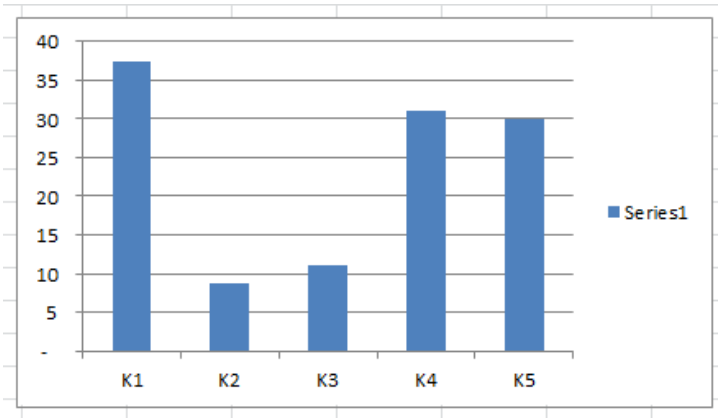
Roselle extracts is contain antocyanin that reported to have antioxidants effect, The decreasing of MDA level could cause by antocyanin content on rosella extract. The treatment of DMBA cause free radicals. Free radical would cause oxidation stress. That triggers the process of peroksidasi of the lipids and increase the MDA level. After treatment with rosella extract the MDA level could decrease.

**The GPx enzyme activity**

The Average levels of GPx activity obtained from each experimental group were showed on Table 4.

**Table 4. Average values of GPx enzyme activity**

| GROUP | RESULTS |
|---|---|
| **Baseline** | 37.4475 ± 0.27072 |
| **DMBA** | 8.7 ± 0.12884 |
| **Rosela 10 mg/KgBB** | 11.1125 ± 0.62136 |
| **Rosela 50 mg/KgBB** | 31.06 ± 1.15779 |
| **Rosela 100 mg/KgBB** | 29.96 ± 1.15536 |




**Figuer 3.The average values of GPx enzyme activity after treatment with rosella extract**

Based on the test results of GPx levels in each test group can be seen that in group 4 and 5 with doses of 50 and 100 mg extract / kg bw obtained percentage of GPx levels can reduce levels of MDA, although not nearly normal. It shows that 70% ethanol extract of roselle flowers can increase levels of GPx. Based on the results of the Kolmogorov-Smirnov test for normality and Levene's test of homogeneity indicates that the data content of Gpx were normally distributed and homogeneous. This is indicated by the Sig> 0.05. Results of one-way ANOVA statistical analysis showed the Sig <0.05, meaning that there is a significant difference between the concentration levels of GPx in each test group. The results of multiple comparison test (Tukey) showed a significant difference

(Sig <0.05) between group I and group II, III, IV, and V, group II, group III, III and IV, group III to group V and group IV with group V. However, there is no difference (Sig> 0.05) was significant between group IV with group V.

Roselle extract will increase the levels of antioxidants in the body, thus increasing the activity GPx

## CONCLUSION

The roselle calyx ethanol extract at doses of 10, 50 and 100 mg / kgBW can decrease levels of MDA and increasing GPx activity in rats blood.

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## CONFLICT OF INTEREST

The authors state that there is no conflict of interest

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