

## The Effect of Roselle (*Hibiscus sabdariffa*) Extract on Malondialdehyde Level in Rat Liver

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### ABSTRACT

This study aimed to determine the efficacy of roselle flower extract in reducing the Malondialdehyde (MDA) level in rats after induction of 20% ethanol. This experimental study used a post-test design on 24 male white rats, Wistar strain which were grouped into six type of treatment. The K1 group was given daily aquadest only, K2 was given 20% ethanol. K3 was given 20% ethanol and vitamin C, K4 was given 20% ethanol and 250 mg/kgBW/day roselle flower extracts, K5 was given 20% ethanol and 500 mg/kgBW/day roselle flower extract, K6 was given 20% ethanol and 750 mg/kgBW/day roselle flower extract. Each group received treatment for 30 days. At the time of termination, the rat's liver was collected and the liver's MDA level was examined. One Way Anova test and the Post Hoc Tukey test were used for data analysis. There was a decrease in MDA levels ( $3.1578 \pm 0.37$  ng/ml) in K4 compared to K2 as well as to K5 and K6 with higher extract concentrations. Thus, despite its benefit as antioxidant, excess of flavonoid compounds undergoing oxidation will produce a metabolite compound that can damage the endogenous antioxidant. Hence, 250 mg/kgBW/day roselle flower extract given daily can reduce MDA levels in mice induced with 20% alcohol.

**Keywords:** antioxidant, ethanol, malondialdehyde, roselle

### INTRODUCTION

Alcoholic beverages have become inseparable part of human civilization. Many traditional drinks in Indonesia such as *tuak*, *sopi*, *arak*, and *badeg* are consumed by local communities as part of their local tradition (Riskiyani *et al.* 2015). According to Riskesdas (MoHRI 2018), 4.6% of the population consumed alcoholic drinks and the highest prevalence was found in East Nusa Tenggara (NTT) province, which is 17.7%.

The type of alcoholic drinks accessed were traditional alcoholic drinks (38.7%), beer with (29.5%), wine (21.6%) and whiskey (3.8%) respectively. In Indonesia, there are many types of alcohol with various ethanol levels. According to Presidential Regulation of the Republic of Indonesia no 74 year 2013, alcohol beverages are divided based on the ethanol levels: Group A with 1%–5% ethanol level, group B with 5%–20% ethanol level, and group C with 20%–55% ethanol level.

Excessive alcohol consumption can cause various health problems both in the short

and long term. These include central nervous system disorders, cardiovascular disorders and digestive disorders as well as psychological disorders that can cause changes and distortions of an individual's behavior and minds so that it can endanger the individual and others (Tritama 2015).

In metabolism process, alcohol is converted to acetaldehyde. The acetaldehyde formed is oxidized in the liver. Alcohol metabolism in the liver cells (hepatocytes) cause an increase of free radicals that react with poly unsaturated fatty acids leading to lipid peroxidation which produce Malondialdehyde (MDA). This triggers oxidative stress in the liver tissue (Zakhari 2006; Ayala *et al.* 2014)

Oxidative stress in the body caused by free radicals due to Lipid peroxidation reactions can be neutralized by endogenous or exogenous antioxidants. Many Indonesian natural ingredients contain antioxidants with various types of active ingredients and can be obtained at affordable prices (Werdhasari 2014). One of such plants is roselle (*Hibiscus sabdariffa* L.).

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roselle cultivation can be found in the low and highlands. According to research by Hidayah (2011) the highest antioxidant activity came from the roselle found in the lowlands.

Roselle petals is often used in food processing, it contains anthocyanin which gives color to the roselle petals and is believed to have antioxidant activity that can neutralize free radicals (Nurnasari & Khuluq 2017). Anthocyanins belong to flavonoids group which can act as antioxidant. The conjugated double bond system in anthocyanin helps in capturing free radicals (Ulialbab *et al.* 2015). In addition to anthocyanins, roselle petals also contain beta-carotene, vitamin C, thiamine, riboflavin, niacin and flavonoids (Mardiah *et al.* 2009).

Ulialbab *et al.* (2015) reported that roselle extract has the ability to reduce levels of malondialdehyde. Dianasari and Fajrin (2015) was using roselle extracts of 250 mg/kgBW/day, 500 mg/kgBW/day, and 750 mg/kgBW/day doses in diabetic mice, the results obtained at doses of 500 mg/kgBW/day and 750 mg/kgBW/day had antidiabetic activity. Further, Zuraida *et al.* (2015) reported that roselle extract can reduce MDA levels and increases catalase enzyme levels in rats exposed to Carbon Tetrachloride, and Pramita (2014) reported that roselle extract can reduce MDA levels in rat eyes and can increase SOD levels in rat eyes that have been exposed to 300 rad gamma radiation. Therefore, the researchers were interested in investigating the efficacy of roselle extract at doses of 250 mg/kgBW/day, 500 mg/kgBW/day, and 750 mg/kgBW/day levels on malondialdehyde level in ethanol induced rats' liver.

## METHODS

### Design, location, and time

The research was held in Pharmacology Laboratorium Medicine Faculty of Padjajaran University from July to December 2019. The study was conducted based on ethical treatment agreement from Ethics Commission, UPN Veteran Jakarta. This experimental study used a post test-only control group design. The study was conducted at the Pharmacology Laboratorium with treatments to one or more experimental groups. The results were then compared with the control group.

### Material and tools

The subjects of this study were 24 male white rats (*Rattus norvegicus*) Wistar strain obtained from the Pharmacology and Therapy Laboratory of the Faculty of Medicine, Padjajaran University, Bandung. The mice were aged 2–3 month old with a body weight of 250–300 grams. Mice which were ill before treatment and had any anatomic abnormalities were excluded from the study.

The tools used in this study were: spectrophotometer, centrifugator, micropipette, 1.5 ml and 2 ml microtube, filter paper, and sample tubes. The material used in this study were roselle flower extract (*Hibiscus sabdariffa* L.), ethanol 20% solution which were fed to the rats everyday for 30 days. The type of extraction used was multilevel maceration with water solvents. The extract dosage used were 250 mg/kgBW/day, 500 mg/kgBW/day, 750 mg/kgBW/day. The doses was chosen according to Zuraida *et al.* (2015) where the study found that 250 mg/kgBW and 500 mg/kgBW of roselle extract were able to reduce MDA level in rats' blood induced with carbon tetrachloride. Meanwhile the dose of 750 mg/kgBW in this research used to determine the lethal dose. Moreover, other antioxidant used in this study were vitamin C at a dose of 1.8 mg/day (Christijanti *et al.* 2011).

### Procedures

#### Extraction

Roselle flower extract was made from dried roselle petals. Twenty five grams of roselle petals macerated with water in 250 ml Erlenmeyer and covered with aluminum foil. Then, stored in the refrigerator for at least 24 hours. After 24 hours, it was filtered (Oktiarni *et al.* 2016). Examination of the characteristics of the extract identified the chemical content of the roselle flower extract included alkaloids, saponins, phenolics, flavonoids, triterpenoids, and glycosides.

#### Intervention

The treatment was divided into six groups, four rats were assigned per group: in the negative control group/ normal (K1) rats were given standard feed and aquades; in the positive control group I (K2) rats were given standard feed and 20% ethanol as much as 2 ml/day; in the positive control group II (K3) rats were given standard feed, 20% ethanol as much

as 2 ml/day, and vitamin C dose 1.8 mg/day; in the treatment group rats given 20% and roselle flower extract with different dosages namely in K4 rats were given roselle flower extract 250 mg/kgBW/day, in K5 rats were given roselle flower extract 500 mg/kgBW/day, and in K6 rats were given roselle flower extract 750 mg/kgBW/day. In groups K2, K3, K4, K5, and K6 rats were given ethanol 20% every day for 30 days. Each the roselle extract and vitamin C was given one hour after the administration of ethanol 20% orally. The rats were terminated on the last day after the 30 days of intervention. The mice were anesthetized before being terminated. The anesthetic substance used was ketamine injected intraperitoneally with a dosage of 75–100 mg/kgBW. The administration of ketamine was done in an early stage of euthanasia process because it causes quick unconsciousness about three to five seconds after injection (AVMA 2013).

#### Measurement of malondialdehyde

The liver were weighed as much as 10 mg then crushed until smooth and put in the appendorf tube; 100 cc distilled water was then added. Homogenate was added 100 µl of 100% Trichloroacetic Acid (TCA) solution, thiobarbiturate Na 1%, and HCl 1 N 250 µl. The mixture was centrifuged until homogeneous and incubated for 20 minutes at 100°C. After that, centrifuged at 3500 rpm for 10 minutes. The results were read on a spectrophotometer with a wavelength of 532 nm. The results of the spectrophotometer were converted into MDA standard curves (Fitria *et al.* 2015).

The standard curve was made to find out the relationship between the concentration of the solution and the absorbance value so that the concentration of the sample can be measured. Tetraethoxypropane (TEP) solution was used to create the MDA standard curve. MDA standard curves were made by reacting TEP in various concentrations with 0.67% thio-barbituric acid (TBA). The standard Tetraethoxypropane (TEP) solution used was 10.0; 20.0; 40.0; 60.0; 80.0; 100.0 and 120.0 µl; distilled water was added until each volume reached 250 µl. After that, 1.25 ml of Trichloroacetic acid (TCA) 20% and 0.5 ml of Thiobarbituric Acid (TBA) 0.67% were added and solution was shaken until homogeneous. All samples were heated for 30 minutes at 100°C and cooled immediately (Agustini 2017).

#### Phytochemical tests

Phytochemical tests was done to determine the content of the active composition in roselle petals extract. Roselle extract was dissolved in each special solvent to determine the content in the extract. The tests carried out included alkaloid tests, flavonoid tests, tests saponin, tannin test, and triterpenoid test. For alkaloid test, the steps included roselle extract of 0.1 g reacted with 10 ml of chloroform and a few drops of ammonia. The chloroform fraction was separated and acidified with a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> and divided into 3 test tubes, then Dragendorf reagents, Meyer reagents, and Wagner reagents were added.

In the flavonoid test, roselle extract was added with 0.1 mg and 0.4 magnesium tablets ml of amyl alcohol (a mixture of 37% hydrochloric acid and 95% ethanol by volume) and 4 ml of alcohol then the mixture was shaken. The steps for saponin test detected by foam testing in hot water. Extract was heated and then the foam was taken. Stable foam during 10 minutes and did not disappear with the addition of 1 drop of HCl 2%. The steps of tanin test, extract as much as 1 g added with 10 ml of distilled water and then bring to a boil. After cooling the filtrate was added with FeCl<sub>3</sub> 1%. And the last step were the triterpenoid and steroid test. Extract as much as 1 g was dissolved with 25 ml of hot ethanol 50°C, then filtered into porcelain dish and dried. The residue was dissolved with ether and transferred into a test tube, then 3 drops of anhydrous acetic acid and 1 concentrated H<sub>2</sub>SO<sub>4</sub> were added. Extracts with purple or red color indicated the presence triterpenoids and green or blue extracts indicated steroids (Syafitri *et al.* 2014).

#### Data analysis

Data were analyzed using the One way ANOVA test with a significance level set at 0.05 to determine whether there were differences in MDA levels in all groups. Post-Hoc test was conducted following the ANOVA test to determine the differences in each group. Data were analyzed using SPSS 21 software.

## RESULTS AND DISCUSSION

#### Phytochemical test

The qualitative phytochemical test results of roselle flower extract revealed that the

extract used in the study contained antioxidant compounds as shown in Table 1. The qualitative phytochemical test showed that roselle flower extract contained flavonoid, saponin, phenolic, triterpenoid, glycoside, and alkaloids and roselle flower did not contain tannin and steroid. Previous research has reported that roselle flowers contains chemical properties including anthocyanin, beta-carotene, vitamin C, thiamine, riboflavin, flavonoids and niacin (Aisiyah *et al.* 2017). Anthocyanin, a flavonoid, is a natural antioxidant and is the red pigment of roselle flowers (Aisiyah *et al.* 2017). The long conjugated double bond system in anthocyanins is thought to capture free radicals by breaking down the propagation chain of free radicals, where all hydroxyl groups (OH) in ring B can contribute or act as electron or hydrogen donors so that cleaning or interception of free radicals occurs (Priska *et al.* 2018). In addition to anthocyanins as antioxidants, other phytochemical compounds contained in roselle flower such as saponin, alkaloid, triterpenoids, glycoside, and phenolics also have function as antioxidants and can be used as antibacterials (Nurnasari & Khuluq 2017). However, we did not find Tannin and Steroid in the roselle extract. The tannin content may decrease with increasing extraction time. This is because all the compounds contained, especially tannins, will be extracted and mixed with solvents (Sukardi *et al.* 2007). Other study also found that roselle flower petals, whether they are fresh or extracted with ethanol did not contain any steroid compounds (Hayati *et al.* 2012).

### Malondialdehyde measurement

Data were analyzed using one way ANOVA test. The results obtained a significance value of 0.001. This means the extract given has an effect on liver malondialdehyde level induced with 20% ethanol.

Table 2 indicates data from the average liver MDA level in each group assessed from the rats' liver tissue taken after 30 days of treatments. The differences in the mean MDA levels for each group, ranged from 2.4 ng/ml to 3.73 ng/ml. Data analysis using One Way Anova showed that there was a significant difference in malondialdehyde levels between groups ( $p=0.001$ ;  $\alpha=0.05$ ). The lowest average MDA level was found in K3 (2.4334±0.17), the group treated with vitamin C. This is because vitamin C acts as potent antioxidant

Table 1. Phytochemical test

Phytochemical Test	Results
Flavonoid	+
Saponin	+
Tannin	-
Phenolic	+
Triterpenoid	+
Steroid	-
Glycoside	+
Alkaloid	+

by acting as an electron donor to ward off free radicals (Lieberman & Peet 2018). This group is the reference to determine the efficacy of roselle extract compared to a well-known or standardize antioxidant which is the vitamin C. The average MDA level in K4, given roselle extract of 250 mg/kgBW/day is 3.1578±0.37 and it showed a statistically non-significant difference with the K3. This supports the fact that roselle extract of 250 mg/kgBW/day can work as good as vitamin C. This is in line with research by Zuraida *et al.* (2015) which showed that roselle extract at a dose of 250 mg/kgBW/day in rats exposed to carbon tetrachloride was able to reduce MDA levels. This might due to the combination of anthocyanin or flavonoid compounds contained in roselle flowers. The flavonoid compounds in the roselle flower are able to ward off free radicals through

Table 2. Differences of average levels of malondialdehyde

Group	Treatment	MDA	
		Level±SD (ng/ml)	P*
K 1	Negative control	3.5668±0.48 <sup>b</sup>	0.001
K 2	Positive control 1	3.6830±0.32 <sup>b</sup>	
K 3	Positive control 2 (Vitamin C)	2.4334±0.17 <sup>a</sup>	
K 4	Roselle extract 250 mg/kgBW/day	3.1578±0.37 <sup>a</sup>	
K 5	Roselle extract 500 mg/kgBW/day	3.6053±0.54 <sup>b</sup>	
K 6	Roselle extract 750 mg/kgBW/day	3.7306±0.28 <sup>b</sup>	

One way ANOVA; a and b: Post Hoc Tukey with significant difference; MDA: Malondialdehyde

the process of radical scavenging by giving one hydrogen atom from the group to react with free radicals (Zuraida *et al.* 2015). As shown in Harun *et al.* (2017) that administration of tempeh which contains flavonoids to rats can reduce the level of malondialdehyde.

The highest average MDA is at K6 with an average of  $3.7306 \pm 0.28$ , which is the group treated with roselle extract at a dose of 750 mg/kg/BW. It also shows that the average K5 and K6 are higher than K4, which means that each additional dose can cause an increase from the average MDA. It means that dose of 500 mg/kgBW/day and 750 mg/kgBW/day was less effective in neutralizing free radical. According to Lemmens *et al.* (2014), flavonoid compounds undergoing oxidation will produce a metabolite compound that can damage the endogenous antioxidant glutathione (GSH). The effect of the damage to endogenous antioxidants is thought to cause a decrease in intracellular antioxidant action which causes the Reactive Oxygen Species (ROS) formed to react with hepatocyte cell molecules so that the lipid peroxidation process still occurs and a high MDA is instead produced (Lemmens *et al.* 2014).

In addition, there is no significant difference between K5 and K6 with K1 and K2. This shows that the K1 and K2 rats were already under stress, the stress increased the formation of free radicals. The formation of Reactive Oxygen Species (ROS) or active free radicals cause excessive lipid peroxidation reactions between ROS and polyunsaturated fatty acids contained in the hepatocyte cell membrane wall. This causing damage to the structure and function of the hepatocyte cells. The final product of the lipid peroxidation process is an increase in the levels of malondialdehyde (MDA) (Ayala *et al.* 2014).

## CONCLUSION

The qualitative phytochemical test showed that roselle flower extract contained flavonoid, saponin, phenolic, triterpenoid, glycoside, and alkaloids as anti oxidants but did not contain tannin and steroid. The administration of roselle flower extract 250 mg/kgBW/day for 30 days to the rats' liver malondialdehyde level induced by 20% ethanol was comparable to the administration of Vit C 1.8 mg/day as the standard antioxidant. While higher dosage of roselle flower extract 500 mg/kgBW/day and 750 mg/kgBW/day did

The effect of roselle on malondialdehyde levels

not show comparable efficacy in reducing the rats' liver MDA levels.

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## AUTHOR DISCLOSURES

The authors have no conflict of interest.

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