Pharmaciana
Vol.8, No.2, Nov 2018, Page. 257-266
ISSN: 2088 4559; e-ISSN: 2477 0256
DOI: 10.12928/pharmaciana.v8i2.8738

Effect of the ethanolic extract of red roselle calyx (*Hibiscus sabdariffa* L.) on hematocrit, platelets, and erythrocytes in healthy volunteers

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Submitted: 29-01-2018 ***Reviewed: 08-03-2018*** ***Accepted: 21-11-2018***

ABSTRACT

Roselle calyx (*Hibiscus sabdariffa L*) is widely used as an antioxidant, antihepatotoxic, diuretic, anti-cholesterol, and immunostimulant. Roselle contains anthocyanin and quercetin that have antioxidant activity. This study aimed to determine the effects of the ethanolic extract of roselle calyx on hematocrit, platelets, and erythrocytes in healthy volunteers during the 30-day administration and 15 days after it ceased. This study employed pre- and post-treatment design on 21 healthy volunteers, comprising 11 males and 10 females who fulfilled the inclusion criteria and filled out the informed consent form willingly. Volunteers were provided with roselle capsules for 30 days with a dosage of 500 mg a day taken 10-15 minutes after dinner. The hematologic examination was performed on Day 0, 31, and 45, including the measurement of hematocrit, platelet, and erythrocyte levels using a hematology analyzer. The obtained data were analyzed in SPSS with paired t-test and Wilcoxon test. The results showed that the hematocrit, platelets, and erythrocytes of all male and female volunteers on Day 0, 31, and 45 were within the normal range. The comparison analysis affirmed that there was no significant difference between these three parameters from Day 0 to 31, Day 31 to 45, and Day 0 to 45 ($p>0.05$). As a conclusion, the administration of the ethanolic extract of roselle calyx with a dosage of one 500mg capsule per day for 30 days did not affect the hematocrit, platelets, and erythrocytes in healthy volunteers. There was also no delayed effect on these three hematological parameters 15 days after the administration stopped.

Keywords: *Hibiscus sabdariffa*, hematology, healthy volunteers, capsule, safety

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INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is empirically efficacious as an antiseptic, diuretic, stamina booster, immunostimulant, sedative, antihypertensive, anti-cholesterol, and antibacterial (Kusumastuti, 2014). According to Morton (1999), every 100 g of roselle calyx contains 1.145 g protein, 2.61 g fat, 12 g fiber, 1,263 g calcium, 273.2 mg phosphor, 8.98 mg iron, 0.029 mg carotene, 0.117 mg thiamine, 0.227 mg riboflavin, 3.765 mg niacin, and 244.4 mg vitamin C. The ascorbic acid (vitamin C) and flavonoid (flavonols and anthocyanin pigments) content make this plant a natural antioxidant that can counteract various free radicals and affect the immune system (immunostimulant) (Mardiyah *et al.*, 2009). Iron and vitamin C contained in roselle calyx are also believed to affect the production of erythrocytes in the spinal cord. Anthocyanin and vitamin C in this plant function as antioxidants by scavenging free radicals and highly reactive molecules (Xu *et al.*, 2011). Chu and Chen (2006) claim anthocyanin compounds as a stimulant to the production of erythropoietin, which determines the formation of red blood cells. According to Sembiring *et al.* (2009), fresh extract of roselle leaves can increase the number of red blood cells of anemic male rat proportionally. Munawaroh (2009) affirms that the administration of roselle calyx extract affects the level of erythrocytes in anemic white rats. Moreover, Hidayati *et al.* (2014) suggest that the control group of rats receiving roselle tea with different dosages for 30 days has normal erythrocytes and leukocytes, whereas the treatment group tends to have higher erythrocytes and lower leukocytes.

Blood is a unique connective tissue because it only has a liquid matrix. It acts as a carrier of compounds throughout the body. It consists of cells and plasma. The blood matrix is blood plasma amounting to 52%-62% of the total blood volume in the body. Blood cells are produced from stem cells in the red bone marrow (hemopoietic tissue) (Chalik, 2016). Hematological panel examinations include, for instance, hematocrit, platelets, and erythrocytes. A decrease in erythrocyte can occur in anemic condition (Kemenkes, 2011). Hematocrit levels are closely related to erythrocyte; when hematocrit is below normal, it may be caused by erythrocyte damage. When the production of erythrocyte decreases, it reduces blood formation and, consequently, the hematocrit levels (Virden *et al.*, 2007). Decreased hematocrit is also an indicator of anemia, leukemia, cirrhosis, loss of blood, and hyperthyroidism, e.g., a decrease of 30% indicates moderate to severe anemia (Kemenkes, 2011). Platelet deficiency likely leads to slow blood clotting and excessive bleeding or, in other words, a wound does not stop bleeding. Thrombocytopenia can cause homeostatic disorders, manifested in gum bleeding, hematemesis, and melena (Shepherd, 2007).

According to Suwandi (2012), the lethal dose 50 (LD_{50}) of the ethanolic extract of roselle in rats is ≥15 g/kg BW (classified as practically non-toxic, LD_{50}> 5-15 g) (BPOM RI, 2014). Meanwhile, Sari *et al.* (2016) claim that the LD_{50} of the ethanolic extract of roselle in Sprague Dawley rats is 850.90 mg/kg BW (classified as mild toxic, LD_{50}> 500-5000 mg) (BPOM RI, 2014).

Wulandari (2010) confirms a significant difference in the histological profile between the groups of Wistar rat kidneys that are administered with different dosages of roselle, i.e., 40, 60, and 90 mg/day, orally for 30 days. Decreased kidney function is closely related to erythropoietin deficiency, which causes a fall in glomerular function. Patients with chronic kidney disease have a risk of blood loss due to platelet dysfunction. Blood loss may lead to iron deficiency (National Kidney Foundation, 2012). Another research on test animals asserts that roselle has therapeutic potential as immunostimulants (optimal dose of 50 mg/kg BW) without toxic effects (tolerated dose) (Nurkhasanah, 2015); hence, it is expected to provide favorable results when consumed by humans.

Based on the description above, the preclinical test results prove that the ethanolic extract of roselle fulfills the requirements to proceed to the clinical test phase. Phase I, which involves testing on healthy volunteers, aims to identify the tolerability and safety of the studied preparations (BPOM RI, 2015). Therefore, in this study, roselle was prepared into 500-mg capsules according to the conversion of the dose in the test rats (SD) to the one in humans. Phase I was conducted to identify the effects of the ethanolic extract of roselle on hematocrit, platelets, and erythrocytes in healthy volunteers with a dosage of 500 mg/day for 30 days.

RESEARCH METHOD

Tools and Materials

Capsules containing the powdered ethanolic extract of red roselle calyx were obtained from PT Natura (*Product code:* 5055, *Batch*: RH162703). The hematology analyzer was ABX micros 60®.

Research Procedure

Ethical clearance submission

This research began with the submission of ethical clearance request to Muhammadiyah University, Yogyakarta. The research permit was granted after the issuance of a certificate of ethics No. 255/EP-FKIK-UMY/IV/2017.

Volunteer recruitment

The recruitment process of healthy volunteers in this study used short message service (SMS) broadcast and a face-to-face encounter. The SMS broadcast contained information about a request for participation in the clinical test research. One short message was sent to one cell phone number and, then, to other numbers joined in a particular group. This broadcasted text was then forwarded to every cell phone number in other groups (Harioso *et al.*, 2009). All willing volunteers who fulfilled the inclusion criteria were involved in the study, and the recruitment stopped when the required number of volunteers was met.

Informed consent

Volunteers were first provided with oral and written explanations regarding the research procedure and their role in the clinical test. After every volunteer expressed their willingness to participate and understood the research description, as well as their rights and obligations as volunteers, they were asked to fill out and sign the informed consent form.

Volunteer health examination

After giving consent to participating in the research, the volunteers were subjected to a clinical examination to ensure the state of their health. The examination was performed by doctors with medical licenses who issued a health certificate describing the results of the examination.

Dosage selection

The dosage of the capsule containing the ethanolic extract of roselle calyx was determined according to the research conducted by Nurkhasanah (2015), which suggests that at a dose of 50 and 100 mg/kg BW, the ethanolic extract of this flower has the potential as an immunomodulatory agent in rats. To obtain the desired results, this research conversed the dose given to the test rats to the optimal dose in humans (Laurence and Bacharach, 1964) and, therefore, applied the dosage of 500 mg per day.

The provision of the capsules of the ethanolic extract of roselle

A total of 30 capsules containing 500-mg roselle extract powder were given to all healthy volunteers. Each capsule was taken once a day at night shortly after dinner for 30 days.

Blood sampling

Blood samples were taken as much as 5 ml using a syringe and inserted into a tube containing EDTA (ethylenediaminetetraacetate), and then centrifuged at 3500 rpm for 5 minutes. Afterward, the clear yellow supernatant (serum) was separated from the underlying solid residue. If the serum was not processed directly, it was stored in a freezer at -20˚C for preservation.

Hematocrit, thrombocytes, and erythrocytes examination

The nurses took the blood samples as much as 5 ml and collected them in vacutainer tubes containing anticoagulants (EDTA). Then, the samples were analyzed for their hematological properties (hematocrit, thrombocyte, and erythrocyte) by health analysts using the hematology analyzer ABX micros 60®.

Data Analysis

The research data were analyzed statistically in SPSS v.23 to identify any differences between the results of the pre- and post-treatment in volunteers by comparing the data on Day 0 with Day 31, and Day 31 with Day 45. The hematological parameters tested in this study were hematocrit,

thrombocyte, and erythrocyte levels. The data analysis began with the Shapiro-Wilk test of normality to determine the data distribution. If these data had a normal distribution, then they were later subjected to a paired sample t-test; otherwise, the analysis continued with the Wilcoxon test at 95% confidence level (Dahlan, 2014).

RESULTS AND DISCUSSION

The physical examination results of healthy volunteers

This study is a Phase I clinical trial attempting to determine the effects of capsules of red roselle extract on hematocrit, platelet, and erythrocyte levels in healthy volunteers for 30 days. The capsules were obtained from PT Natura Indonesia. The physical examination of the volunteers, performed by doctors with a medical license, aimed to assess the health of volunteers before, during, and after the study. All healthy volunteers had to first fill out an informed consent form as proof of their willingness to participate in the study without any elements of coercion from the researcher. Ethical clearance is a basis for the feasibility of test and analysis, particularly in clinical trials. This research has met the ethical feasibility requirements according to the Ethics Committee of the Faculty of Medicine and Health Sciences, Muhammadiyah University, Yogyakarta and been granted a certificate of ethics No. 255/EP-FKIK-UMY/IV/2017.

The physical examination of the volunteers was conducted at Hidayatullah Hospital in Yogyakarta on Day 0, 31, and 45. On Day 0, the volunteers had not taken the roselle capsule yet. Day 31 was the day when the volunteers have stopped consuming the capsules (end of treatment), and Day 45 was 15 days after the end of the capsule administration. The physical examination measured vital signs, namely blood pressure (BP), body temperature (t), pulse (RR), and respiratory rate (HR). Every day during the 30-day treatment, the volunteers were interviewed to assess any clinical and psychological side effects and, at the same time, provided with education so that they did not forget to take the roselle capsule. The results of the physical examination of the volunteers are presented in Table I.

The results showed that the systolic and diastolic blood pressure, HR, RR, and body temperature during the 30-day treatment and 15 days after the consumption of roselle capsules stopped were within the normal range. Taking roselle capsules for 30 days did not affect the blood pressures of healthy male and female volunteers ($p>0.05$), and after the consumption stopped for 15 days (Day 30-45), no effects on blood pressure were found ($po>0.05$).

Blood pressure represents the strength required by blood to flow in the blood vessels and carry nutrients throughout the organs of the body. It is influenced by various factors, such as age, physical activity, and changes in position (Manembu *et al.*, 2015). It is measured in millimeters of mercury (mmHg) and recorded as two different values, namely systolic blood pressure and diastolic blood pressure. Systolic blood pressure occurs when the ventricle contracts and secretes blood to the arteries, while diastolic blood pressure occurs when the ventricle relaxes and is filled with blood from the atrium (Amiruddin *et al.*, 2015). The normal value of blood pressure in adults is <120/80 mmHg (JNC VII, 2004). The results showed that the blood pressures of the volunteers were within the normal range, indicating that the consumption of capsules containing the ethanolic extract of red roselle for 30 days is relatively safe for healthy people because it does not affect systolic and diastolic blood pressure. Red roselle contains polyphenols that play a role in maintaining blood pressure by reducing renin-angiotensin (RAS) activity and increasing vasodilators in bradykinin (Debon *et al*., 2015). The most important flavonoid content in this flower is anthocyanin. Anthocyanin is a water-soluble plant pigment. It is only found in plants with bright colors on their edible flowers, leaves, and fruits. It is a type of flavonoid compound. In hypertension management, flavonoids are useful for inhibiting ACE (Angiotensin-Converting Enzyme) and, consequently, preventing the conversion of angiotensin I to angiotensin II, which triggers the increase of sympathetic nervous system activity, vasoconstriction of vascular smooth muscle, and water and sodium retention. Flavonoids prevent the formation of angiotensin II (Kusumastuti, 2014).

Table I. The physical examination results of the healthy male and female volunteers

Parameters	Mean ± SD			*P*-values			Normal Range
	Day 0	Day 31	Day 45	d1	d2	d3	
Male							
Systolic (mmHg)	117 ± 9.31	117 ± 9.31	117 ± 9.31	1.000	1.000	1.000	≤ 120
Diastolic (mmHg)	78.18 ± 5.60	78.63 ± 5.04	76.36 ± 7.10	0.317	0.180	0.414	≤ 80
HR (x/minute)	78.18 ± 2.60	77.45 ± 6.99	78.00 ± 2.36	0.248	0. 190	0.317	60-100
RR (x/minute)	18.36 ± 0.80	17.93 ± 1.96	18.54 ± 0.93	0.671	0.667	0.317	16-20
Temperature (˚C)	36.60 ± 0.26	36.15 ± 0.45	36.66 ± 0.37	0.005	0.005	0.357	36.1-37.2
Female							
Systolic (mmHg)	113.50 ± 8.83	113.50 ± 8.83	112.50 ± 7.16	1.000	0.581	0.619	≤ 120
Diastolic (mmHg)	73.00 ± 6.75	73.00 ± 6.75	72.60 ± 6.75	1.000	0.854	0.854	≤ 80
HR (x/minute)	76.90 ± 5.62	80.40 ± 7.16	76.90 ± 5.62	0.527	0.527	1.000	60-100
RR (x/minute)	18.20 ± 1.13	17.20 ± 1.75	18.20 ± 1.13	0.182	0.182	0.383	16-20
Temperature (˚C)	36.6 ± 0.42	36.16 ± 0.18	36.63 ± 0.44	0.15	0.15	0.180	36.1-37.2

Note:
Day 0 : before treatment (the consumption of capsules containing the ethanolic extract of roselle calyx)
Day 31 : the end of treatment
Day 45 : 15 days after the treatment ceased (no consumption)
Normal systolic and diastolic levels (JNC VII, 2004); Pulse (AHA,2015); RR (Agrawal and Whorwell, 2008); Temperature (NHS, 2016)
d1: the comparison between parameters on Day 1 and Day 31; d2: the comparison between Day 31 and Day 45; d3: the comparison between Day 0 and Day 45

Table I shows that there is no significant difference between the vital signs during and after the consumption of the capsules ($p > 0.05$). Pulse was included in the examination to assess cardiovascular function. According to the American Heart Association (AHA) (2015), the normal pulse range is 60-100 times per minute. Berman *et al.* (2009) explain that the pulse of a healthy individual is the reflection of the heart rate, meaning that the number of pulses is equal to the number of heart's ventricular contractions. Pulse rate lowers (bradycardia) at bedtime and rises (tachycardia) due to air temperature, emotion, body activity, and size. Heat pressure can increase blood circulation because it induces a situation where blood must carry oxygen to the muscles and bring heat from the body to the surface of the skin, resulting in increased pulse frequency (AHA, 2015; Kumalasari, 2017).

There was no significant difference during the consumption of the capsules for 30 days ($p>0.05$). Fifteen days after the administration ceased, no significant difference was identified as well ($p > 0.05$). The respiratory rate of the healthy volunteers was in the normal range, meaning that the capsule of the ethanolic extract of roselle does not affect the respiratory rate and is, therefore, relatively safe for consumption. The normal respiratory rate in adults is 12-20 times per minute. Breathing becomes faster after physical exercise and lowers when at rest (Agrawal and Whorwell, 2008). Doctors measured respiratory rate by observing the volunteer's chest or abdomen during respiration (inhalation and exhalation) in one minute.

On the contrary, the consumption of the capsules for 30 days reduced the body temperature in male volunteers ($p <0.05$). However, the same effect was not detected in female volunteers ($p > 0.05$). Accordingly, after stopping the consumption for 15 days, the body temperature of male volunteers

increased ($p < 0.05$), while the effect on body temperature of female volunteers remained insignificant ($p > 0.05$). Although the body temperature of the male volunteers statistically showed a significant difference, it was still within the normal range. The same case applied to the female volunteers. As a conclusion, the consumption of capsules containing the ethanolic extract of roselle does not affect the body temperature of a healthy individual and is, therefore, relatively safe.

The effects of red roselle capsule on hematocrit, thrombocyte, and erythrocyte

The hematological examination carried out in this study included the parameters of hematocrit, platelets, and erythrocytes in blood samples. Hayati (2013) employes this examination to determine the effects of consuming capsules that contain the ethanolic extract of *Eurycoma longifolia* roots on the hematology systems. Santoso *et al.* (2006) suggest that hematological examination can identify the safety and toxicity of drugs on a healthy subject

The hematological data were analyzed at a 95% confidence level. This research employed the Shapiro-Wilk test of normality because the number of samples was less than 50. If the data were normally distributed, then they were later subjected to the paired t-test; otherwise, the analysis continued with the Wilcoxon test. The average levels of hematocrit, platelet, and erythrocyte in healthy male and female volunteers are presented in Table II.

The statistical analysis results of the administration of the roselle extract capsules for 30 days showed no significant difference ($p > 0.05$) in the hematocrit, platelet, and erythrocyte counts of healthy male and female volunteers. Aiming to determine any delayed effects of roselle extract capsules, the volunteers were asked to stop consuming these capsules for 15 days, starting from Day 31 to Day 45. This research performed the examination on Day 45 according to the International Conference on Harmonization (ICH) (2010), which recommends a further evaluation two weeks after the administration of drug treatment to assess any delayed toxicity and recovery. On Day 45, there was no significant difference in hematocrit, platelet, and erythrocyte counts in both male and female volunteers ($p > 0.05$). After consuming roselle capsules for 30 days and then stopping this treatment for 15 days, the levels of hematocrit, platelet, and erythrocyte of the volunteers were within the normal range. Therefore, based on these blood profile, the red roselle capsules are relatively safe.

Table II. The average hematocrit, thrombocyte, and erythrocyte counts in healthy male and female volunteers before, during, and after consuming the ethanolic extract of roselle

Parameters	Mean ± SD			*P*-value			Normal Range
	Day 0	Day 31	Day 45	d1	d2	d3	
Male							
Hematocrit (%)	43.50± 4.73	43.23±4.12	44.61±3.78	0.563	0.026	0.109	40.7-50.3
Thrombocyte ($10^3/\mu m$)	260.54 ± 48.66	258.81 ± 43.28	275.81 ± 41.32	0.777	0.163	0.235	150–300
Erythrocyte (million cells/ul)	4.72 ± 0.29	4.73 ± 1.38	4.87 ± 0.19	1.00	0.33	0.050	4.7-6.1
Female							
Hematocrit (%)	36.15 ± 2.19	36.79 ± 4.52	36.11 ± 5.04	0.139	0.168	0.155	36.1-44.3
Thrombocyte ($10^3/\mu m$)	275.40 ± 70.61	276.20 ± 90.82	283.00 ± 83.42	0.646	0.959	0.646	150–300
Erythrocyte (million cells/ul)	4.20 ± 0.14	4.21 ± 0.10	4.20 ± 0.14	0.943	0.886	0.948	4.2-5.4

Notes:
Day 0 : before treatment (the consumption of capsules containing the ethanolic extract of roselle calyx)
Day 31 : the end of treatment
Day 45 : 15 days after the treatment ceased (no consumption)
d1 : the comparison between parameters on Day 1 and Day 31
d2 : the comparison between Day 31 and Day 45
d3 : the comparison between Day 0 and Day 45

Table II shows that the average hematocrit levels in male volunteers decrease after consuming the red roselle capsules, while in female volunteers, these numbers increase. Hematocrit level is the concentration of erythrocytes in 100 mL of a person's blood sample (expressed in %). It rises when the number of blood cells increases or the volume of blood plasma decreases. On the contrary, it decreases (hemodilution) due to a decline in the cell number or an increase in blood plasma level, as in anemic condition (Virden *et al.*, 2007). Decreased hematocrit level by 30% indicates moderate to severe anemia. Hematocrit level can drop below normal due to erythrocyte damage. The lysis of the erythrocyte membrane releases hemoglobin into the plasma, reducing the number of red blood cells and the hemoglobin levels in them. Consequently, the cells in the body experience oxygen deficiency. Continuous damage on erythrocyte membrane can lead to anemia and reduce blood hematocrit levels (Ahumibe and Braide, 2009). In this study, the decline in hematocrit level after consuming the roselle capsules for 30 days was still within the normal range. In other words, roselle is relatively safe for consumption.

Platelets help the process of blood clotting and maintain vascular integrity. The results showed that the average platelet count in male and female volunteers was in the normal range, i.e., 150–300 10^3/μm. Platelet deficiency can cause bleeding or decelerate blood clotting. In other words, it will lead to prolonged bleeding if a wound occurs. The risk of bleeding increases if the thrombocyte level is <60,000. Furthermore, if it drops to <20,000, it can cause severe bleeding; a continuous drop in platelet count to <5,000 will cause brain hemorrhage (Tisnadjaja, 2006). The preclinical tests conducted by Santi (2004) on the activity of the standardized ethanolic extract of guava leaves as a cure for dengue fever (immunological aspects) in rats have found that the leaf extract can increase the number of megakaryocytes in the bone marrow and, subsequently, raise the platelet count in the blood. In this study, roselle capsules were proven to be relatively safe to consume because the platelet count was persistently within the normal range.

The average erythrocyte levels in male and female volunteers increased after consuming the capsules of red roselle calyx for 30 days. Such an increase is closely related to the state of bone marrow. Patria *et al.* (2013) state that erythrocyte level increases after the administration of a solution containing a combination of microminerals (Cu, Fe, Zn, Co) and vitamins (A, B1, B12, C) twice the normal dose in drinking water. The role of vitamin C in bone marrow accelerates the absorption and transport of Fe minerals from the small intestinal mucosa to bone marrow; these minerals are then used to form hemoglobin. Anthocyanin is a flavonoid compound that can stimulate the production of erythropoietin that plays a role in red blood cells (erythrocytes) formation (Chu and Chan, 2006). Flavonoids are active compounds of polyphenols that act as antioxidants, which can increase erythropoiesis in bone marrow and exhibit immunostimulant effects (Sundaryono 2011). Vitamin C is needed to increase Fe absorption. According to Sembiring *et al.* (2009), the high content of vitamin C and Fe in roselle can increase the number of erythrocytes in the blood of anemic white rats. Vitamin C and Fe are factors that influence the formation of red blood cells.

The comparison between the hematocrit, platelet, and erythrocyte counts in healthy male and female volunteers on Day 0 and Day 45 showed no significant differences ($p > 0.05$).

The monitoring results of the side effects of the red roselle capsules

The side effects of the red roselle capsules might exhibit some symptoms in volunteers. Therefore, this research monitored their condition during the 30-day treatment. The monitoring

continued 15 days after the treatment stopped to identify whether the side effects could occur in healthy individuals. The monitoring was performed by interviewing the volunteers via SMS.

The clinical symptoms found during the consumption of the capsules included diuresis or frequent urination in 6 healthy volunteers (28.57%) and dyspepsia in 4 healthy volunteers. These side effects were considered mild because they appeared at a low frequency, i.e., 2-3 times in 30 days. After exhibiting the symptoms of dyspepsia, the volunteers were recommended to consume the roselle capsules immediately after eating. Meanwhile, the volunteers who experienced frequent urination were provided with education regarding the possible diuresis effect that might occur after consuming the capsules.

The dyspepsia felt by the volunteers can be explained by the presence of organic acid and ascorbic acid in the forms of hydroxy citric acid and hibiscus acid in the ethanolic extract of roselle calyx (Da-Costa-Rocha *et al.*, 2014). Volunteers with a history of dyspepsia could feel stomach discomfort. Meanwhile, the case of mild diuresis in healthy volunteers is in line with Kusumastuti (2014), which describes that the anthocyanin, gossypetin, and hibiscin content found in roselle promote diuresis that can lower blood pressure. Furthermore, Yulinah and Wahyuningsih (2015) explain that quercetin affects the endothelium of the blood vessels and releases nitric oxide, increasing the vasorelaxation of the kidneys that induces diuresis.

CONCLUSION

The administration of capsules containing the ethanolic extract of red roselle calyx for 30 days does not affect the hematological parameters, i.e., hematocrit, thrombocyte, and erythrocyte count, in healthy male and female volunteers ($p > 0.05$). The same case applies to the following 15 days after the consumption stops ($p > 0.05$).

ACKNOWLEDGMENT

This study is supported by the research grant provided by the LPP of Universitas Ahmad Dahlan, Yogyakarta for the Study Program in 2017.

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