The Utilization of Rosella (Hibiscus Sabdariffa Linn.) Calyx Extract to Increase Hemoglobin and Hematocrit of Female Adolescents Anemia

(Pemanfaatan Ekstrak Kelopak Bunga Rosella (Hibiscus Sabdariffa Linn.) untuk Meningkatkan Kadar Hemoglobin dan Hematokrit Remaja Putri Penderita Anemia)

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Abstract: This research aims to prove the provision benefits of Rosella (Hibiscus sabdariffa Linn.) calyx extract to increase hemoglobin and hematocrit levels in female adolescents anemia. This research is a true experiment with a randomized pre-test and post-test control group design. Totals of samples were 40 female adolescents with anemia according to the inclusion and exclusion criteria divided by randomization sampling technique, consisting of two groups intervention groups and control groups. Data analysis used paired t-test statistical test to determine the difference between pretest and post-test each group and an independent t-test to determine the difference between the different groups. The average difference in hemoglobin and hematocrit levels in the intervention group was higher than in the control group. The average difference in hemoglobin levels in the intervention group was 1.8 g/dL in and the control group was 0.9 g/dL, (p < 0.05), The difference in the average hematocrit level in the intervention group was 5.6% and the control group was 2.7% (p < 0.05). The researchers also found significant statistics differences between hemoglobin and hematocrit levels of female adolescents from intervention and control groups s with a p-value < 0.05. The combination of Rosella (Hibiscus sabdariffa Linn.) calyx extract and Fe²⁺ tablet was effective to increasing hemoglobin and hematocrit levels in female adolescents with anemia.

Keywords: rosella calyx, hemoglobin, hematocrit, anemia

Abstrak: Tujuan dari penelitian adalah membuktikan manfaat pemberian ekstrak kelopak bunga Rosella (Hibiscus sabdariffa Linn.) terhadap peningkatan kadar hemoglobin, hematokrit dan eritrosit remaja putri penderita anemia. Penelitian ini merupakan true eksperimen dengan randomized pre-test and post-test control group design. Jumlah sampel 40 remaja putri anemia sesuai kriteria inklusi dan eksklusi dibagi dengan teknik sampling acak, terdiri dari dua kelompok yaitu kelompok intervensi dan kontrol. Analisis data dengan uji statistik paired t-test dan independent t-test. Hasil penelitian menunjukkan selisih rata-rata kadar hemoglobin dan hematokrit kelompok intervensi lebih tinggi dibandingkan kelompok kontrol. Selisih rata-rata kadar hemoglobin kelompok intervensi 1,8 gr/dL dan kelompok kontrol 0,9 gr/dL (p<0,05), selisih rata-rata kadar hematokrit kelompok intervensi 5,6% dan kelompok kontrol 2,7% (p<0,05). Serta terdapat perbedaan yang bermakna secara statistik pada kadar hemoglobin dan hematokrit remaja putri anemia antara kelompok intervensi dan kelompok kontrol p-value < 0,05. Pemberian ekstrak kelopak bunga Rosella (Hibiscus Sabdariffa Linn.) dan tablet Fe²⁺ efektif meningkatkan kadar hemoglobin dan hematokrit pada remaja putri penderita anemia.

Kata kunci: kelopak bunga rosella, anemia, hemoglobin, hematokrit
INTRODUCTION

ANEMIA is a decrease in red blood cells that causes impaired hemoglobin function and the need for oxygen to all peripheral tissues is not met in an adequate amount\(^{(1)}\). World Health Organization (WHO) found the prevalence of anemia in 53.7\% of all adolescent girls in developing countries\(^{(2)}\). Anemia rate in Southeast Asia of female adolescents ranges from 25-40\% with mild and severe levels of anemia\(^{(3)}\). According to Basic Health Research, 32\% of women aged 15-24 years have anemia\(^{(4)}\). Women of reproductive age, especially adolescent girls and pregnant women, are the most prone to anemia\(^{(5)}\).

Government prog that focus on adolescent girls by giving supplementation of blood-added tablets once a week and 10 tablets during menstruation for junior high and high school students are still not effective, due to several factors that affect iron absorption\(^{(6)}\). Non-pharmacological therapy is needed as a companion to the blood-added tablet supplement prog. Administration of vitamin C tablets and Fe tablets increased hemoglobin levels lower than the use of herbal ingredients\(^{(7)}\). The side effects caused by the combination of Fe\(^{2+}\) tablets with pharmacological will be greater than in combination with herbal plants. Herbal therapy has been widely used after the government issued a Regulation of the Minister of Health concerning Sanitification of Herbal Medicine in 2010\(^{(8)}\). One of the plants that contain vitamin C and iron, is Rosella (Hibiscus sabdariff a Linn.)\(^{(9)}\).

Rosella is known for its function for health\(^{(10)}\). In 100 g of rosella calyx (Hibiscus sabdariff a Linn.) contains 8.98 mg of iron and 244.4 mg of vitamin C\(^{(11)}\). Rosella calyx also contain ten times the vitamin C content of star fruit, nine times that of citrus oranges, seven times that of black grapes as well as vitamin C and iron minerals in Rosella calyx is higher than katuk (Sauropus androgynus), cassava (Manihot esculenta), spinach (Amaranthus janjeticus)\(^{(11,12)}\). Rosella calyx in the form of extract have a higher content because the extraction process can obtain active substances in Rosella calyx. Therefore, this research aims to prove the administration of Rosella (Hibiscus sabdariff a Linn.) calyx extract to improve hemoglobin and hematocrit of female adolescents with anemia.

MATERIAL AND METHOD

MATERIAL. Red rosella (Hibiscus sabdariff a Linn.) calyx obtained from PT. Temu Kencono, Gunung Pati, Semarang, Indonesia, ethanol 70\%.

Tools. The tools used in the extraction are blender, wind evaporator, digital scale, bucher funnel, food rehydrator, spray dryer.

METHOD. Research Design. This research is a quantitative used by true experimental research design with pretest and posttest control group design. Anemic adolescent girls were divided into two groups. The intervention group was given Rosella (Hibiscus Sabdariff a Linn.) calyx ethanolic extract and Fe\(^{2+}\) tablets, the control group was given Fe\(^{2+}\) tablets and a placebo. The division of groups in this study was done randomly.

Extraction. In this study rosella calyx were extracted by the maceration method. The extraction process was carried out at the Integrated Laboratory of Diponegoro University, Semarang assisted by laboratory personnel. The dried rosella calyx is made into powder by blender. The extraction method used is UAE (Ultrasond-Assisted Extraction) maceration using 96\% ethanol as solvent. The macerate was filtered to separate the pulp and using a bucher funnel, then evaporated in Rotavapor at a temperature of 30-40 °C to obtained viscous extract. Then the crystallization process was carried out, the semi-solid rosella calyx extract was dried with a food dehydrator to become powder and ready to be encapsulated.

Extract Dose. The dose in preclinical study was obtained based on a Sembiring study, administration of rosera flower extract at a dose of 42 mg/20kgBW in mice was effective in increasing red blood cells\(^{(11)}\). When converted into a human, the obtained dose is 279 mg/60 kgBW. This is dose conversion calculation:

\[
\text{Abs. dose for mice } 20 \text{ g} = 42 \text{ mg/BW x 0.02 kg} = 0.84 \text{ mg}
\]

\[
\text{Human dose} = \text{Abs. Dose x mouse-human conversion} = 0.84 \text{ mg x 387.9} = 325.8 \text{ mg/70 kgBW}
\]

Dosage per kg body weight

\[
= 325.8 \text{ mg/70 kgBW} = 4.65 \text{ mg/kgBW}
\]

The average weight of school children is 50-60 kg, then the required dose

\[
= 4.65 \text{ x 60 kgBW} = 279 \text{ mg/60 kgBW}
\]

Ethical Clearence. The researcher has obtained a research permit from the Health Research Ethics Committee of Regional Public Hospital (RSUD) Dr.Moewardi, Surakarta, Faculty of Medicine, Universitas Sebelas Maret on March 18, 2021, numbered:333 /III /HREC/2021. Before conducting the research, the researcher provided information and
asked for informed consent to obtain the willingness of the research subject.

**Clinical Study.** Researchers brought two boxes containing Rosella calyx extract and a placebo, the two boxes were divided into code A and code B whose contents were unknown to the research respondents (Single Blind). In this study, Code A was the intervention group that was given Rosella calyx extract 279 mg/60 kgBW and 60 mg Fe tablets and Code B was the control group who was given 60 mg Fe tablets and a placebo. Data analysis used paired t-test statistical test to determine the difference between pretest and posttest in each group and independent t-test to determine the difference between the different groups.

**Analysis of Hemoglobin and Hematocrit Level.**

**Table 1. The distribution of the subjects’ characteristics based on age and IMT**

| Characteristics | Intervention Group | Control Group | P value |
|-----------------|--------------------|---------------|---------|
|                 | (n=20)             | (n=20)        |         |
| Age             | Mean ± SD          | Mean ± SD     |         |
| IMT             | 21.8 ± 1.23        | 21.62 ± 1.06  | 0.734*  |

*Independent t-test, †Mann-Whitney*

The hemoglobin and hematocrit levels of adolescent girls were measured used hematology analyzer which was carried out at the IBL laboratory and the Srondol Health Center Laboratory.

**RESULT AND DISCUSSION**

**Subjects Characteristic.** The subjects’ characteristics in this study were done randomly. Their characteristics shown in Table 1. They were divided into two groups, the intervention group received Rosella calyx extract and control group received a placebo. The tablet shows that both subject characteristics groups have similar characteristics of age. Most intervention and control group subjects are aged 16 years old. Data on subjects characteristics based on IMT values in the intervention group and control group are included in the normal category. The IMT score of intervention group is 21.8 while the control group is 21.6. The independent t-test of the subjects characteristics based on the IMT show p > 0.05 for intervention and control groups. It indicates no differences IMT of intervention and control groups.

The Mann-Whitney test for the subjects characteristics, in terms of age, of intervention and control groups obtains a p-value > 0.05. It indicates no differences of age of intervention and control groups.

The normality test ensures the data normality.
to examine the hypotheses before the bivariate test. The researchers used the Shapiro-Wilk normality test because the sample was lesser than 50 members. The normality test shown in Table 2 result with Shapiro-Wilk on hemoglobin and hematocrit levels for both groups obtains \( p > 0.05 \). It indicates the data distribution is normal.

**The Level of Hemoglobin.** The hemoglobin level shown in Table 3 of the intervention group increases higher than the control group. The hemoglobin level after the administration of Rosella calyx extract and Fe tablet increases into 1.8 gr/dL, higher than the control group with Fe and placebo administrations, 0.9 gr/dL. The paired t-test of hemoglobin in the intervention group obtains a \( p \)-value < 0.05, indicating significant differences of before and after the intervention with 279 mg Rosella calyx extract and Fe 60 mg tablet for 14 days. The paired t-test of hemoglobin in the control group obtains a \( p \)-value < 0.05, indicating significant differences of before and after the intervention with Fe 60 mg tablet and placebo for 14 days. The independent t-test obtains \( p \)-value < 0.05 for the hemoglobin level of both groups. It indicates significant differences between both groups.

The consumption of Rosella calyx extract, *Hibiscus sabdariffa* Linn, for 14 days could increase the hemoglobin level of female adolescents with anemia in Riyadlus Sholihin and Darut Taqwa Islamic Dormitory Schools, Semarang. The hemoglobin increases due to the vitamin C content. The vitamin C in Rosella Calyx is nine times higher than orange, seven times higher than star fruits, and three times higher than black grapes. The iron content of the calyx is also higher than katuk leaves, cassava leaves, and spinach leaves \(^{(11,12)}\). From the content test in the Integrated Laboratory, Diponegoro University, the researchers found 100 g Rosella calyx extract has 13.59 mg iron, 336.3 mg vitamin C, and 1160 mg flavonoid. The contents are higher than dried Rosella Calyx that have 8.98 mg iron and 244.4 mg vitamin C \(^{(11)}\). The extraction process of the Calyx can enrich the vitamin C and iron contents. It allows the production of bioactive compounds. The extracted Rosella Calyx in the form of capsules facilitates the consumption and long storing procedure because of the pH and temperature durability. In this research, 279 mg extract has 0.038 mg iron and 9.40 mg vitamin C. It can increase the hemoglobin level 1.8 gr/dL, with a percentage of 16.4%. Nurjannah also found 200 mg long bean leave extract for female adolescents for 14 days had 0.026 mg iron. It could increase the hemoglobin level 1.45 gr/dL with a percentage of 14%, effect size value 0.7, and \( p \)-value 0.021 \(^{(14)}\).

The iron content and vitamin C are correlated factors to produce erythrocyte and hemoglobin. Vitamin C in Rosella calyx reduces the ferrous iron (Fe\(^{3+}\)) into ferrous (Fe\(^{2+}\)). Thus, it will be easily absorbed by small intestines. It could also freed the iron when it is needed by the body, by hindering the production of hemosiderin that is difficult to mobilize. Vitamin C can increase four or five times the absorption of iron in the form of non heme. It is also useful to remove the iron from the transferrins in the plasma into liver ferritin. Then, most blood transferrins bring the iron into bone marrow and other limbs. The iron inside the bone marrow is useful to produce hemoglobin \(^{(15)}\). Thus, the administration of vitamin C with iron tablets for the absorption process and iron releasing process from the transferrin into the body tissues \(^{(16)}\).

The researchers compared the previous studies with the current study with the same ingredient and research period. However, they have different doses. The previous study used Rosella calyx extract 279 mg/60kgBW in 14 days for female adolescents. It was effective to increase the hemoglobin. The average of hemoglobin level after the investigation was 12.60 gr/dL. It indicated that the respondents did not suffer

### Table 2. The normality test results of hemoglobin and hematocrit of both groups

| Variables | Intervention Group | Control group |
|-----------|-------------------|---------------|
|          | (n=20)            | (n=20)        |
|          | Mean±SD           | p-value       | Mean±SD           | p-value |
| Hemoglobin |                   |               |                   |         |
| (gr/dL)   |                   |               |                   |         |
| Pretest   | 10.8 ± 0.59       | 0.053         | 11.05 ± 0.58      | 0.086   |
| Posttest  | 12.6 ± 0.60       | 0.334         | 12.04 ± 0.68      | 0.178   |
| \( \Delta \) | 1.78 ± 0.51       | 0.561         | 0.99 ± 0.41       | 0.417   |
| Hematocrit |                   |               |                   |         |
| (%)       |                   |               |                   |         |
| Pretest   | 32.65 ± 1.46      | 0.428         | 32.85 ± 1.42      | 0.116   |
| Posttest  | 38.20 ± 1.93      | 0.556         | 35.55 ± 1.90      | 0.688   |
| \( \Delta \) | 5.55 ± 1.95       | 0.052         | 2.70 ± 1.12       | 0.117   |

*shapiro-wilk*
anemia. The increase rate of 1.8 gr/dL and the effect size, 1.68, showed a strong effect. Thus, when the results were converted into 10 days, the averages were 12.13 gr/dL with increasing rate 1.3 gr/dL. Nisa’s study, on the other hand, administered 115.2 mg Rosella calyx extract in 10 days for pregnant women with anemia. The result obtained an average of 10.4 gr/dL that indicated the respondents still suffered anemia. The increasing rate, 1.09 gr/dL and the effect size, 0.11, indicated a weak influence (17).

Mizwati administered dried Rosella tea with 200 mg dose for pregnant women with anemia within 14 days. The result obtained an average score of 12.44 gr/dL, indicating suffering no anemia. The increasing rate was 1.11 gr/dL and the effect size was 0.40 (weak) (18).

Current research has a higher increasing rate and effect size than Nisa and Mizawati’s studies. It was because the contents of iron and vitamin C were higher due to the added doses. Thus, the contents of vitamin C and iron were higher than Mizawati and Nisa’s studies. In Mizawati’s study, the brewing process was with hot water that damaged the vitamin C of the Calyx.

The Level of Hematocrit. The hematocrit level of the intervention group increases higher than the control group. After the administration of the extract and Fe tablet, the intervention group’s hematocrit level increases 5.6%, higher than the administration of Fe and placebo for the control group, 2.7%. The paired t-test of hematocrit level in the intervention group obtains a p-value < 0.05, indicating significant differences of before and after the intervention with 279 mg Rosella calyx extract and Fe 60 mg tablet for 14 days. The independent t-test obtains p-value < 0.05 for the hematocrit level of both groups. It indicates significant differences between both groups.

Hematocrit refers to the percentage of erythrocyte as the benchmark to assess the lowering period of erythrocyte besides the hemoglobin level (19). During anemia conditions, the hematocrit (hemodilusi) decreases due to lower erythrocyte. In this case, the sizes of erythrocytes in body influence the hematocrit level. The size of erythrocyte is an important factor to assess the hematocrit because it influences the blood viscosity. Hematocrit will be higher if the blood viscosity is high. The increasing rate is in line with the increasing hemoglobin rate and the total of erythrocyte. Rosella calyx extract is an alternative to meet the iron as a source of non-heme iron. In this research, after the intervention, the hematocrit level of the female adolescent was 38.20%. Thus, it could be concluded the category was normal physiologically healthy. It proved that the increasing rate of hemoglobin leads to higher erythrocyte content.

This current research is in line with Nurjannah. The administration of long bean leave extract for female adolescents with anemia for 14 days, 200 mg dose, everyday, could increase the hematocrit level with a percentage of 3.61%, p-value = 0.019, and increasing rate 2.3%, and effect size 0.74 (moderate) (14). In this current research, the dose was different to the previous studies, 279mg/60kg BW Rosella calyx extract in 14 days for female adolescents. It was effective to improve the hematocrit level. The hematocrit level average after the intervention was 38.20%, indicating normal hematocrit with increasing

| Variables      | Intervention Group | Control group | p-value |
|----------------|--------------------|---------------|---------|
|                | (n=20)             | (n=20)        |         |
| Hemoglobin level |                   |               |         |
| Pretest        | 10.86 ± 0.59       | 11.04 ± 0.58  | 0.314b  |
| Posttest       | 12.64 ± 0.59       | 12.05 ± 0.68  | 0.005b  |
| p-value        | 0.001a             | 0.001a        |         |
| Difference     | 1.78 ± 0.51        | 0.99±0.41     | 0.001b  |
| Hematocrit level |                   |               |         |
| Pretest        | 32.65 ± 1.46       | 32.85 ± 1.42  | 0.664b  |
| Posttest       | 38.20 ± 1.94       | 35.55 ± 1.90  | 0.001b  |
| p-value        | 0.001a             | 0.001a        |         |
| Difference     | 5.55 ± 1.95        | 2.70 ± 1.12   | 0.001b  |

*aPaired t-test, *Independent t-test
rate 5.55% and effect size 1.79 (strong effect). The current research is different with Nurjannah’s research with long bean leave administration with 200 mg dose in 14 days for female adolescents with anemia. The average hematocrit level after the intervention was 38.60%, with increasing rate 3.61% and effect size 0.74 (moderate). This current research has a higher increasing rate and effect size. It was caused due to the different iron and vitamin C levels or other factors triggering hematocrit level.

CONCLUSION

The administration of Rosella calyx extraction (Hibiscus sabdariffa Linn.) 279 mg and Fe 60 mg tablet in 14 days significantly increase hemoglobin and hematocrit levels of female adolescents with anemia. It was higher than the administrations with Fe 60 mg and placebo for the control group. After the study the average hemoglobin and hematocrit levels in the intervention group increased and teenage girls were in good health. The researchers hope future researchers could develop the study by adding research time to check the menstrual period and control other influential factors, such as stress, wormy, and physical activity.

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