

## THE EFFECT OF ROSELLA FLOWER EXTRACT (*HIBISCUS SABDARIFFA*. LINN) INCREASE ON ERYTHROCYTE LEVELS IN ANEMIC TEENAGERS

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### ABSTRACT

**Objective:** The purpose of the study was to prove whether the rosella flower capsule has benefits to improve the level of erythrocytes for anemic teenagers.

**Methods:** The type of research was a true experiment with randomized pretest and posttest control group design. The sample number of 40 anemic teenagers with inclusion criteria. There were 20 girls as an intervention group given Rosella flower l extract of 279 mg and Fe 60 mg tablets and 20 teenagers as control groups given Fe 60 mg tablets and placebos. The intervention was done for 14 d. Data were analyzed with t-test statistics.

**Results:** The results showed an average difference in the erythrocyte number of intervention groups was higher than the control group (0.77 million/mm<sup>3</sup> and 0.35 million/mm<sup>3</sup>, respectively). There was a statistical difference in the erythrocyte number of anemic teenagers between the intervention and control groups with a p-value= 0.001.

**Conclusion:** Consuming extract of *rosella* flower capsule (*Hibiscus sabdariffa* Linn) increases the erythrocytes level of an anemic teenager.

**Keywords:** Rosella flower capsule, Erythrocyte, Anemia, Teenager

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### INTRODUCTION

Anemia is a nutritional problem suffered by women, especially pregnant women and teenagers. Almost 30% of women suffered from anemia in the world. The number of anemic young adult women at 15-24 y old was 32%. There were 4.8 million from 21 million of the teenager who experienced lack of red blood cell [1, 2]. The prevalence of anemia (50%) in Indonesia is mainly caused by iron deficit [3].

Anemia in young women is caused due to bleeding during menstruation, so the iron loss is about 0.4-0.5 mg/day. Iron needs increase due to rapid growth (growth spurt), experiencing chronic diseases and infectious diseases, and poor diet [4].

Giving tablets blood-boosting supplements experienced several obstacles in overcoming anemia, one caused by food intake that did not meet nutritional adequacy (RDA) numbers [5]. Food intake, especially the intake of iron and vitamin C that is not as needed, will decrease the effectiveness of giving blood-boosting tablets. As many as 73.3% of adolescents experience anemia due to low levels of vitamin C and iron intake [6].

Rosella flower petals have a high iron and vitamin C content. Iron and vitamin C content in 100 grams of Rosella flower petals, namely Iron of 8.98 mg and vitamin C of 244.4 mg [7]. The content of Rosella flower petals in the form of extracts is higher, namely iron 13.59 mg and vitamin C 336.3 mg per 100 gr of rosella petal extract. Rosella flower petals have a higher iron and vitamin C content than the iron content in moringa leaves 6 mg/100gram, long bean leaves 6.2 mg/100 gr, red spinach 7 mg/100gr, and vitamin C content is higher compared to guava leaves 32.41 mg/100gr, tomatoes 40 mg/100 grams and papaya fruit 78 mg/100 gr.

Bioactive compounds in rosella flower petals are obtained through the extraction process. The Rosella flower contained bioactive compounds flavonoids and anthocyanins in the phytochemical screening test. Most of these compounds are detected in the leaves of sorghum caudatum to treat anemia [8]. Anthocyanin might stimulate the production of erythropoietin. This influences the formation of red blood cells (erythrocytes) [9, 10]. Giving Rosella flower petal extract doses of 279 mg/60 kg body weight converted from squeak to human doses is expected to reduce anemia. This

study was done by Sembiring's who gave 48 mg/20 kgBB and increased red blood cells of mice [7].

Therefore, this study aimed to prove does the consumption of Rosella petal extract (*Hibiscus sabdariffa* Linn) at a dose of 279 mg and Fe 60 mg tablets for 14 d was more effective at increasing the erythrocyte count than the control group who given Fe 60 mg tablets and placebo of anemic teenagers.

### MATERIALS AND METHODS

#### Research design

This type of this study was quantitative with a true experimental research design with randomized pretest and posttest control group design. The study sample included 40 anemic teenagers divided into two groups: the intervention and control groups. This sample was a randomization process taking 40 envelopes bearing code A 20 and code B 20. All envelopes are simplified randomized. Each subject that meets the research criteria gets one envelope selected by the subject and opened by the enumerator (single-blind).

Code A was an intervention group in this study, and Code B was a control group. The intervention group was given rosella flower petal extract at 279 mg and a 60 mg Fe tablet for 14 d. The control group was given Fe 60 mg tablets and a placebo for 14 d. Hematology analyzer tools examined in IBL Laboratory and Srdol Health Center Laboratory analyzed the erythrocytes count.

#### Ethical approval

This research has been approved by the Health Research Ethics Committee of Dr. Moewardi Hospital, Faculty of Medicine, Universitas Sebelas Maret on March 18<sup>th</sup>, 2021, number: 333/III/HREC/2021. Researchers used informed consent to be the subject of the study. The study used a parametric paired t-test in each group, an independent t-test in a different group.

#### Extraction of rosella petals

The extraction process of rosella petals involves some stages. Rosella flower petals are dry, blended into a simplisia form. The extraction method used was Ultrasound-Assisted Extraction (UAE)

maceration, which was simplisia amounting to 1 kg in maceration using 96% ethanol to be submerged using ultrasonic assistance of 48 kHz+/-30". Then filtration was carried out so that it was separated between the dregs and the maserat. The result of the maceration of rosella flower petals filtered using a Bucher funnel was then evaporated with a wind evaporator at a temperature of 30-40 degrees to remove the solvent content of ethanol [11].

### Rosella flower capsule preparation

Rosella flower petal extract (*Hibiscus sabdariffa* Linn) was semi-solid and deep dark red or thick extract. Dryer until obtained a thick section. After the process of crystallization or compaction, a viscous extract was added with simplisia residue with the amount adjusted to the needs. It is mixed until more homogeneous and dried with a food dehydrator until it becomes a powder.

## RESULTS

### The extraction

The results of rosella flower extract have become powder, ready for encapsulation. Rosella flower petal extract was put into a capsule at 279 mg/60 kg per body weight. Rosella flower petal extract that has

been done encapsulation and placebo. Rosella flower petal extract capsules that have been packaged.

### The study samples

The samples involved in the study were 15-to 19-year-old girls living in boarding school. The sample count was 20 in the intervention group and 20 teenagers in the control group. The inclusion criteria are adolescent girls who are mildly anemic, with hemoglobin levels (8-11.9 gr/dl). Intervention is given to adolescent girls after a menstruation span of 10 d, starting from the first day of the last period: young women who are not taking drugs and a normal menstrual cycle. The characteristic sample of this research was shown in table 1.

Data on the subject's characteristics above was based on daily food intake. Both menstrual length characteristics in the intervention group and the control group were in the normal category. The average length of menstrual time for the intervention and control group was 7 and 6 d, respectively. Protein consumption in both groups was in the normal category. The intake of vitamin C and Iron in the intervention group was less than in the control group. The t-test of the characteristics data based on the length of time, protein intake, vitamin C, and iron of both groups indicate no differences with a p-value>0.05.

**Table 1: The distribution of the subjects' characteristics based on length of menstrual time, protein, vitamin C, and iron**

Characteristics	Intervention group		Control group		P-value
	n	mean±SD	n	mean±SD	
Length of menstrual time	20	7±0.51	20	6±0.59	0.395 <sup>b</sup>
Protein	20	50.2±2.99	20	53.0±2.41	0.131 <sup>a</sup>
Vitamin C	20	66.7±6.88	20	71.9±7.64	0.637 <sup>b</sup>
Iron	20	7.4±0.99	20	8.3±0.98	0.674 <sup>a</sup>

Independent t-test<sup>a</sup>, Mann-Whitney<sup>b</sup>

**Table 2: The normality test results of erythrocyte level of both groups**

Variables		Intervention group			Control group		
		n	mean±SD	p-value*	n	mean±SD	p-value*
Erythrocyte (μ)	Pretest	20	3.40±0.14	0.264	20	3.38±0.13	0.075
	Posttest	20	4.18±0.27	0.604	20	3.73±0.20	0.303
	Δ		0.77±0.23	0.739		0.35±0.17	0.147

Shapiro-wilk\*

The normality test result with *Shapiro-Wilk* on erythrocyte levels for both groups obtains p>0.05. It indicates the data distribution is normal.

### The level of erythrocyte

The intervention group's erythrocyte level (table 3) increases higher than that of the control group. After administering rosella petal extract and Fe tablet, the erythrocyte level increases to 0.77 million/mm<sup>3</sup>, higher than the control group with Fe and placebo administrations, 0.35 million/mm<sup>3</sup>.

The paired t-test of erythrocyte in the intervention and control group obtains a p-value=0.001. This indicates significant differences

between before and after the intervention with 279 mg Rosella petal extract and Fe 60 mg tablet and the control group with Fe 60 mg tablet and placebo for 14 d.

The *independent t-test* obtains a significant difference with p-value=0.001 for the erythrocyte levels of both groups. The mean differences increase the level of erythrocyt in the intervention group more than in the control group (0.77±0.23 and 0.35±0.17, respectively). It indicates that consuming 279 mg Rosella petal extract capsule per day and Fe 60 mg per day was effective in increasing the erythrocyte levels than the administration of Fe 60 mg tablet and placebo.

**Table 3: The level changes of erythrocyte levels before and after intervention**

Variables		Intervention group		Control group		p-value
		n	mean±SD	n	mean±SD	
Erythrocyte level (Million/mm <sup>3</sup> )	Pretest	20	3.40±0.14	20	3.38±0.14	0.570 <sup>b</sup>
	Posttest	20	4.17±0.27	20	3.73±0.21	0.001 <sup>b</sup>
	p-value		0.001 <sup>a</sup>		0.001 <sup>a</sup>	
	Difference		0.77±0.23		0.35±0.17	0.001 <sup>b</sup>

Paired t-test<sup>a</sup>, Independent t-test<sup>b</sup>

## DISCUSSION

Rosella flower (*Hibiscus sabdariffa* Linn) is a medicinal plant whose petals are used to treat anemia in Benin. There are bioactive

compounds in the phytochemical screening test in rosella flowers, such as flavonoids and anthocyanins [11]. Most of these compounds are also detected in the leaves of sorghum caudatum used to treat anemia. Most combinations are available [8]. The most abundant

flavonoid content in the Rosella plant is gossypetin, hibisten and sabbdaretin. Several other metabolite compounds are also found in the Rosella plant that can help increase the number of erythrocytes [9, 10]. Anthocyanin compounds can stimulate erythropoietin production, affecting red blood cells or erythrocytes.

The most common extraction method used to obtain anthocyanins in mangosteen peels is maceration. Maceration generally runs slowly and produces low yields. Therefore, faster extraction methods are needed, one of which is ultrasonic-assisted extraction (UAE). Ultrasonic waves are sound waves above human hearing ( $\geq 20$  kHz). UAE is categorized into two, namely UAE sonication directly and indirectly. UAE direct sonication has not been used on rosella flower extract. The UAE method can increase yield. The use of 70% ethanol compounds in the maceration process in rosella petals produces the highest and highest antioxidant substance activity compared to water and citric acid. In this case, the polarization of rosella anthocyanin compounds is close to the 70% ethanol polarization as well as with the polarization of the 70% ethanol solvent mixture plus water with a ratio of 50:44. Phenolic compounds as secondary metabolites in plants have the potential to be antioxidant substances [14].

Previous research that has been conducted by Sembiring giving rosella extract on *Mus musculus* L anemia with three dose variants, namely doses of 14 mg, doses of 28 mg, and 42 mg for 14 d, found that a dose of 42 mg more effectively increases the number of erythrocytes compared to doses of 14 mg and 28 mg. The average increase in erythrocyte count at a dose of 42 mg by 20.23 cells/mm<sup>3</sup> compared to an amount of 0.28 mg of 15.87 cells/mm<sup>3</sup> and a dose of 14 mg by 14.50 cells/mm<sup>3</sup> with statistical test results in the treatment and control group obtained a  $p < 0.05$ , thus showing that Rosella extract could increase the number of blood cells mice anemia along with an increase in the concentration of extracts in the treatment group. In this study conducted the conversion of doses from mice to humans with an effective dose conducted by Sembiring, which is a dose of 42 mg/20 kg weight; after converting the dose from mice to humans obtained a dose of rosella petal extract of 279 mg [7].

Ghislain's research showed that the feeding of Rosella flower petals was significant to hematological parameters with a  $p$ -value of  $< 0.05$ . Increased red blood cell levels indicate that rosella flower petal drinks contain phytochemical compounds that stimulate the formation of erythropoietin in the erythropoiesis process [12].

Previous research conducted by Nurjannah, giving long bean leaf extract in adolescent girls anemia for 14 d dose of 200 mg increased the amount of erythrocytes by 0.53 million/mm<sup>3</sup>, the percentage increase was 13.9%, the value of  $p = 0.013$  and the effect size is weak at 0.25 [13].

Based on Sari's research, consuming rosella flower petal extract capsule to female rats Sprague Dawley strain orally doses 850.90 mg/kg weight does not cause symptoms of mortality, and no toxic effect is delayed during observation of 14 d [14].

The administration of red Rosella ethanol extract at a dose of 500 mg for 30 d showed an increase in erythrocytes. There was no toxic effect after daily monitoring for 30 d while taking rosella petal ethanol extract capsules, and continued with 15 d of tightening after not consuming Rosella flower extract in healthy volunteers [15].

In this research, the female adolescents did not experience the side effects during consumption. In both groups, they consumed Rosella extract in the evening. Moreover, the teenagers might have meals beforehand. It was important due to the high vitamin C content. Thus, Rosella is relatively safe to consume. Another study stated that rosella petals extract gel had significant protein, vitamin C, beta carotene, and calcium value. This study supported that the rosella petals gel added at the time of dental implant insertion, the result obtained after examination of bone tissue formation at days 14, 28, and 56 levels of calcium increased compared with the control group that did not use the addition of rosella petal extract gel [19]. This study supports the administration of capsule rosella extract to stimulate the formation of erythrocytes in the bone marrow.

The current research and the previous studies, with the same ingredient and research period except for the dose, 279 mg/60 kg

weight in 14 d for female adolescents with anemia, effectively increased erythrocyte levels. The average erythrocyte level average, 4.18 million/mm<sup>3</sup>, showed that the healthy respondents with normal erythrocyte level had a 0.77 million/mm<sup>3</sup> difference with a percentage of 22.6% and effect size 2.0, strong effect. Another study stated that tea of rosella leaf might decrease anemic for pregnant women. The consumption of rosella extract combined with Fe tablet showed a significant increase in hemoglobin levels compared with the consumption of Fe table tal. one. In this study, the dose of rosella flower extract given to the respondent was 115.2 mg/kg/day/day for ten days [20].

This research is different from Nurjannah because the long bean leaves extract administration, 200 mg, in 14 d for female adolescents with anemia, obtained an average of 4.34 million/mm<sup>3</sup>, increasing rate of 0.53 million/mm<sup>3</sup>, increasing rate 13.9%, and effect size 0.25 (weak effect) [13]. This current research has a higher increasing rate and effect size than the previous study because of the different vitamin C and iron contents. It might also be caused by other factors that could trigger erythrocyte numbers. The use of Rosella petal extract was more effective in increasing the erythrocyte than the brewing method with hot water.

## CONCLUSION

Consuming a capsule of rosella flower petal extract (*Hibiscus sabdariffa*. Linn) at a dose of 279 mg and Fe 60 mg tablets for 14 d was more effective at increasing the erythrocyte count of adolescent girls with anemia than the control group who given Fe 60 mg tablets and placebo. Rosella flower petal extract capsule (*Hibiscus sabdariffa*. Linn) can be a companion herbal therapy program giving Fe tablets to reduce the anemia status of teenagers.

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## AUTHORS CONTRIBUTIONS

All the authors have contributed equally to this lovely study.

## CONFLICT OF INTERESTS

All the authors declare to have no conflict of interest in this study.

## REFERENCES

1. Kemenkes RI, Kesehatan P, Pertama Drg Rudy Kurniawan, M Kes Yudianto, SKM, M, Si Boga Hardhana S Si, MM Tanti Siswanti, SKM MK, editor. Kementerian Kesehatan Republik Indonesia. Indonesia. 2018. p. 556.
2. Vinet L, Zhedanov A. A "missing" family of classical orthogonal polynomials. *J Phys A Math Theor: Math Theor*. 2011;44(8). doi: 10.1088/1751-8113/44/8/085201; 085201PMID 085201.
3. Bappenas. Pembangunan gizi di indonesia; Kajian sektor kesehatan. 1st ed. Jakarta: Kementerian PPN/Bappenas; 2019.
4. Pola Konsumsi MH. (Faktor Inhibitor dan Enhancer Fe) Terhadap Status anemia Remaja Putri. *J Kesehat Masy*. 2015;11:80.
5. Sari P, Azizah DI, Gumilang L, Judistiani RTD, Mandiri A, Asupan Zat Besi, Asam Folat. Dan vitamin C pada remaja putri di daerah jatinangor. *J Kesehat Vokasional*. 2020;4:169.
6. Akib A, Sumarni S. Kebiasaan makan remaja putri yang berhubungan dengan Anemia: Kajian positive deviance. *Amerta Nutr*. 2017;1(2):105-16. doi: 10.20473/amnt.v1i2.6232.
7. Sembiring A, Tanjung M, Sabri E, Rosela PES. (Hibiscus sabdariffa L.) terhadap jumlah eritrosit dan kadar hemoglobin mencit jantan (*Mus musculus* L.) anemia strain ddw melalui induksi natrium nitrit (Nano2). *Saintia Biol*. 2013;1:60-5.
8. Agbangan PDC, Tachon C, Bonin H, Chrostowka A, Fouquet E, Sohounhloue DCK. Phytochemical study of a tinctorial plant of

- Benin traditional pharmacopoeia: The red sorghum (*Sorghum caudatum*) of Benin. *Sci Study Res Chem Chem Eng Biotechnol Food Ind.* 2012;13:121-35.
9. Da-Costa Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. Hibiscus sabdariffa L.-a phytochemical and pharmacological review. *Food Chem.* 2014;165:424-43. doi: 10.1016/j.foodchem.2014.05.002, PMID 25038696.
  10. Trie Oktaviani SMF. Review: aktivitas farmakologik ekstrak rosella (*Hibiscus sabdariffa* L.). *Farmaka.* 2018;16:345-51.
  11. Sari F, Nurkhasanah MSB. Acute toxicity test of rosella (*Hibiscus sabdariffa* L.) calyx ethanolic extract on sprague dawley rats. *Tradit J.* 2016;21:12-8.
  12. Obouayeba AP, Diarrassouba M, Soumahin EF, Kouakou H. Phytochemical analysis, purification and identification of hibiscus anthocyanins. *J Pharm Chem Biol Sci.* 2015;3:156-68.
  13. Oktaviani T, Megantara S. Review: aktivitas farmakologi ekstrak rosella (*Hibiscus sabdariffa* L.). *Farmaka.* 2018;16:345-51.
  14. Choiriyah NA. Ekstraksi senyawa antosianin dan fenolik rosella ungu dengan variasi pelarut. *DNJ J* 2017;1:16;1(1). doi: 10.21111/dnj.v1i1.1017.
  15. Ghislain MT. Proximate and mineral composition, the protein quality of hibiscus sabdariffa L. (Roselle) seeds cultivated in two agro-ecological areas in cameroon. *Int J Nutr Food Sci.* 2014;3(4):251. doi: 10.11648/j.ijnfs.20140304.14.
  16. Nurjanah FW, Hadisaputro S, Fatmasari D. Long bean leaf extract for improving haematological status of the female adolescent with anemia that gets Fe supplementation. *J Kesehat Masy Kemas.* 2020 Jul;16(1):81-7. doi: 10.15294/kemas.v16i1.23203.
  17. Sari F, Nurkhasanah BMS. Uji toksisitas akut ekstrak etanol Kelopak rosella (*Hibiscus sabdariffa* L.) Pada tikus sprague dawley. *Tradit J.* 2016;21:12-8.
  18. Setianingsih S, Nurani LH, Rohman A. Effect of the ethanolic extract of red roselle calyx (*Hibiscus sabdariffa* L.) on hematocrit, platelets, and erythrocytes in healthy volunteers. *Pharmaciana.* 2018;8(2):266. doi: 10.12928/pharmaciana.v8i2.8738.
  19. Dharma Utama M, Amiruddin M, Arpa S, Ambo Asse R, Ikhriaeni MM. The content of compound and bioactive substance of the rosella petals extract gel to accelerate the remodeling process of the alveolar bone. *Int J Appl Pharm.* 2019;11:16-8.
  20. Nisa R, Soejoenoes A, Wahyuni S. Effect of roselle (*Hibiscus sabdariffa*) on changes in hemoglobin levels in pregnant women with anemia taking iron supplement. *Belitung Nurs J.* 2017;3(6):771-7. doi: 10.33546/bnj.305.