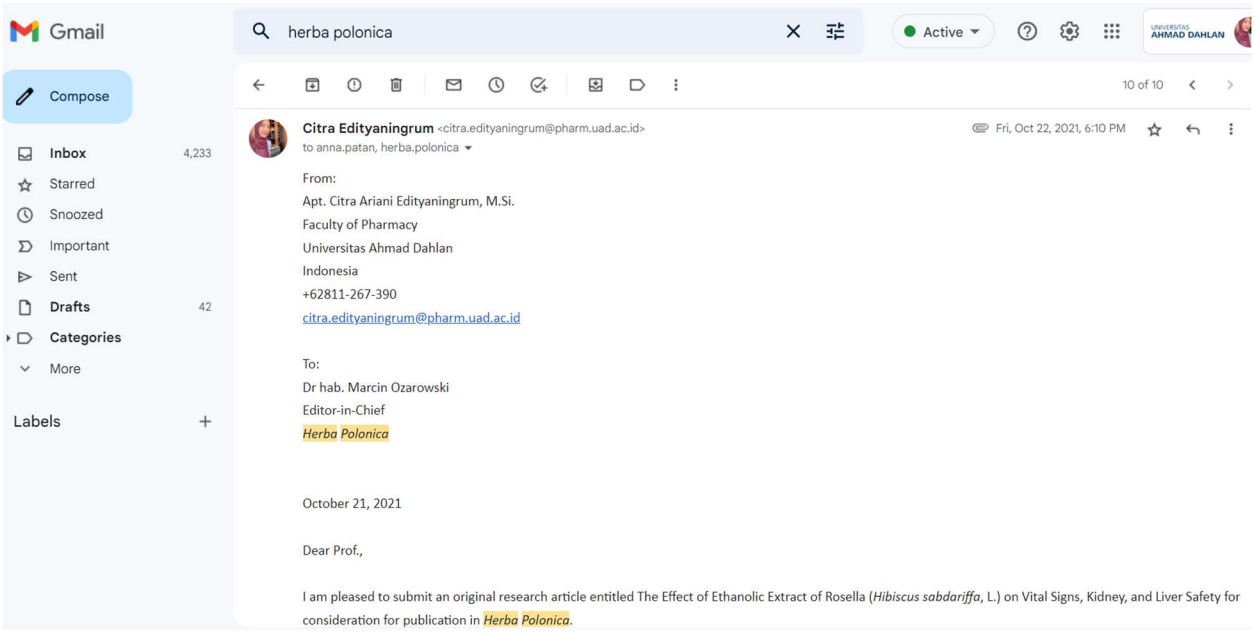
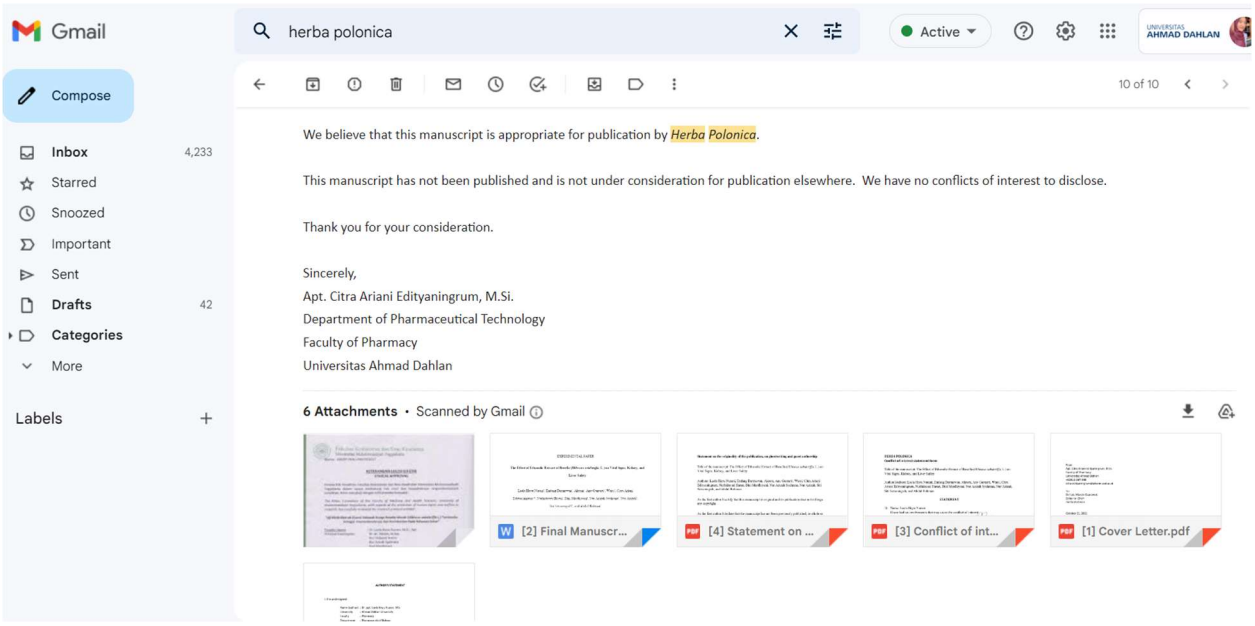
Bukti submit di Jurnal Herba Polonica, artikel berjudul “The Effect of Ethanolic Extract of Rosella (*Hibiscus sabdariffa*, L.) on Vital Signs, Kidney, and Liver Safety”





Artikel

EXPERIMENTAL PAPER

The Effect of Ethanolic Extract of Rosella (*Hibiscus sabdariffa,* L.) on Vital Signs, Kidney, and Liver Safety

Laela Hayu Nurani[1], Endang Darmawan[1], Akrom[1], Any Guntarti[1], Warsi[1], Citra Ariani Edityaningrum[1]*, Nurhidayati Harun[2], Dini Mardhiyani[3], Nur Azizah Syahrana[4], Nur Azizah[5], Siti Setianingsih[6], and Abdul Rohman[7]

[1]Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta 55164, Indonesia.

[2]Diploma of Pharmacy, STIKes Muhammadiyah Ciamis, Ciamis, West Java

[3]Department of Pharmacy, Faculty of Pharmacy and Health Sciences, Universitas Abdurrab, Pekanbaru, Indonesia

[4]Department of Pharmacy, Faculty of Medicine and Health Sciences, UIN Alauddin Makassar, Indonesia

[5]Faculty of Pharmacy, STIKes Muhammadiyah Kuningan, Kuningan, West Java 45552, Indonesia

[6]Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang, Central Java 50232, Indonesia

[7]Centre of Excellence Institute for Halal Industry & Systems (IHIS), Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia.

**corresponding author: citra.edityaningrum@pharm.uad.ac.id*

Summary

Introduction: Rosella (*Hibiscus sabdariffa,* L) extract is often used as immunostimulant because it contains flavonoids, especially anthocyanin and quercetin with antioxidant activities.

Objective: This study aimed to determine the safety of the rosella extract consumed in the form of capsules on the vital signs, hematologic parameters as well as kidney and liver function.

Methode: This research was conducted using clinical trial pre and post-test design in healthy participants. There were twenty-one participants including twenty-one healthy participants (52 % male, age ranged 8-45 years old) consuming rosella capsules for thirty days, with the dose of 500 mg extract daily. Leukocytes, lymphocytes, blood urea nitrogen (BUN), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and vital signs were consecutively evaluated on days 0, 31, and 45, respectively. The Wilcoxon and paired sample t-test were used to compare the parameters among the evaluated times.

Result: The result showed that no significant difference for all parameters among the three time points ($p > 0.05$).

Conclusion: These findings suggested that the administration of ethanolic extract of rosella is potential safe and does not negatively affect the vital signs, hemoglobin, leukocytes, lymphocytes, BUN, SGOT, and SGPT.

Key words: leukocytes, lymphocytes, BUN, SGOT, SGPT, *Hibiscus sabdariffa*

INTRODUCTION

Rosella (*Hibiscus sabdariffa,* L) contains antioxidant compounds capable of enhancing the immune system by maintaining cell damage due to its ability to absorb excessive ultraviolet light, therefore, it protects the body cells from free radical changes [1]. The antioxidant compounds include metabolite of phenolics and flavonoids [2] such as hibiscitrin (hibiscetin-3-glucoside), quercetin, gossytrin, sabdaritrin, gossypitrin, gossypetin glucosides, and luteolin [3], fatty acid [4], alkaloids, steroids, triterpenoids, saponins, tannins, anthocyanins, and B-carotene [5] [6]. The antioxidant activity of rosella are mainly due to red pigment of anthocyanin capable of increasing the scavenging activity of free radicals [7]. Anthocyanins also stimulated the production of erythropoietin, which promotes the formation of red blood cells [8]. Thus, it affects the number of leukocytes and lymphocytes in the immune system [9].

Antioxidants are needed to maintain the immune system in the human body. Any deficiencies in the antioxidant defense system make the human body more susceptible to oxidative stress [10] due to imbalance between reactive oxygen species (ROS) and endogenous antioxidant. Induction of ROS can damage lymphocytes in healthy humans. In the immune system, leukocytes play important roles in producing, transporting, and distributing antibodies, as part of the immune response to antigens [11]. Immune reactions to microorganism attacks, foreign macro-molecules, and cancer cells are initiated by lymphocytes that are the granular form of leucocytes. Lymphocytes specifically recognize and respond to foreign antigens and are also serve as a mediator of humoral and cellular immunity [12].

The preclinical study revealed that rosella provided therapeutic potential of immune-stimulator at an optimal dose of 50 mg/kg BW [12] herefore, its use in humans are feasible. Clinical

parameters that can be used in describing the tolerated dosage is a hematologic, kidney, and liver changes which is a phase 1 clinical test parameter [13].

The ethanol extract of rosella petals has a lethal dose 50% (LD_{50}) toxicity value of 850.90 mg/kgWB in Sprague Dawley mice in the category of ring toxicity and has no effect on the enzyme activity of Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), and Alkaline Phosphatase (ALP) [14]. These enzymes are specific indicators for determining liver cell damage. Kidney function can also be a parameter in determining the safety of medicinal plants [15]. The examination of blood urea nitrogen (BUN) is one of the markers of renal function, which can be used to evaluate kidney function, although the elevated BUN levels do not actually reflect renal function [16]. Hemoglobin (Hb) is part of the hematology that serves as the oxygen-carrying substance present in the erythrocytes [17]. Increased or high Hb levels are generally associated with increased amounts of erythrocytes produced in bone marrow with raw materials of iron (Fe), amino acids, vitamin B12, folic acid, B2, B6, vitamin C, vitamin E, minerals (cobalt and copper), and the hormone erythropoietin. A decrease in Hb level may indicate a hematological disorder of anemia [18].

This research aimed to assess the safety of the rosella extract consumed in the form of capsules on the vital signs, hematologic parameters as well as kidney and liver function.

MATERIAL AND METHODS

Place and time of research

This research was conducted at a private clinic in Yogyakarta. Yogyakarta on April 3 to May 17, 2017, for 45 days. The research was conducted after securing an ethical clearance from Muhammadiyah University of Yogyakarta (255 / EP-FKIK-UMY / IV / 2017).

Research Instrument

Instrument used for measuring lymphocytes is flow-cytometry, Hb and leukocyte count were hematology analyzer, autoanalyzer photometer SGOT-SGPT examination, and BUN examination using spectrophotometer instrument with Modiff-Berthelot method.

Health Checkup and Preparation

Medical examination

This research was conducted by using pre and post treatments research design involving 21 volunteers consisting of 11 male and 10 females selected based on inclusion and exclusion criteria. The inclusion criteria included doctor's certificate confirming a health status, age 18-45 years, BMI 18-30 kg/m^2, non-smokers, and signed an informed consent. Health criteria included vital signs, blood pressure body temperature, heart rate (HR) and respiration rate (RR). The medical examination results were stated in a health certificate supported by clinical laboratory examination including routine haematology, liver function, kidney function, history of disease, and physical examination. Exclusion criteria included pregnant women, consuming other drug during research duration, and using other herbs during the study.

Administration of rosella extract capsule

Rosella extract capsule was given at a dose of 500 mg/day (Product code: 5055C, Batch: RH162703). The capsules given to volunteers for 30 days. Haemoglobin, leukocytes, lymphocytes, BUN, SGOT, SGPT and vital sign examined on day 0 (before treatment), 30th day (after 30 days consuming rosella capsule) and 45 days (15 days after not consuming rosella capsule).

Preparation of blood sampling

Blood samples were taken from the median cubital vein or brachial vein using 5 cc syringe for 4 cc blood. The sample was then stored in a centrifugal micro tube containing EDTA as a coagulant. The separation of the plasma was carried out by centrifugation at a rate of 3,500 rpm for 2 hours so that the transparent yellow liquid (serum). If the serum was not used immediately, then the liquid was stored in a freezer with a temperature of -200°C.

Examination of hemoglobin, leukocytes, lymphocytes, BUN, SGOT and SGPT.

Hemoglobin and leukocytes

Blood samples on the EDTA tube were then analyzed using an automated hematology analyzer.

Lymphocytes

The results of blood sampling of volunteers on the EDTA tube were stored into the falcon tube as much as 50 µL. The tube was added with each 5 µL tritest CD3APC / CD4PerCP / CD25PE reagent. It was then homogenized using vortex for 1 minute. It was then incubated for 15 minutes

with temperature 20-25 °C in a dark room. The 450 µL of solution of lytic agent then was added, followed by an incubation for 15 minutes with temperature 20-25 °C in the dark room. After the incubation period was completed, an analysis was done using FACS flow cytometer

Blood Ureum Nitrogen (BUN)

The levels of BUN were determined by the Modiff-Berthelot method. The volunteer's blood sampling was fed into a tube using a microphone. A-0.5 mL color reagent was added to the bottle labeled: unknown, control, standard, blank. A-10 uL was added to the sample to the appropriate bottle. A-0.5 mL of enzyme reagent was added to all bottles. It then was mixed well and incubated at 37 °C for 5 minutes. A-2.0 mL base reagent was added. The mixture was then stirred and incubated at 37°C for 5 min. The wavelength was set at 630 nm and the zero of the spectrophotometer with blank. The concentration of unknown samples was read directly using standard (25 mg/dL).

Serum Glutamic Oxaloacetic Transaminase (SGOT) – Serum Glutamic Pyruvate Transaminase (SGPT)

A total of 1000 µL of reagents were introduced into the test tube. The reagents used were ready-to-use reagents consisting of SGOT and SGPT reagents. Furthermore, 100 µL serum was added and shaken for 1 min in a water heater at 37ºC. Spectrophotometer readings were performed at a 340 nm wavelength with a conversion factor of 1745 to obtain SGOT and SGPT levels. SGOT and SGPT levels were expressed in units of units/liter (U/L).

Data analysis

The data were presented as the mean ± SD using SPSS 23 statistics. The normality test was carried out using Shapiro-Wilk. The normally distributed data were further analyzed using paired t-test while the non-normally distributed data were analyzed using a Wilcoxon test at a 95% confidence level.

RESULT AND DISCUSSION

In this study, Hb, Leucocytes, lymphocytes, BUN, SGOT, and SGPT levels in male and female volunteers were found to be different in mean levels at day 0, 30 and 45. Clinically, however, the levels were still within the normal limits (**Table 1** and **Table 2**).

Table 1. Results of Hemoglobin, Leukocytes, Lymphocytes, BUN, SGOT, and SGPT on Healthy Volunteers (n = 21)

Parameters	Mean ± SD			Normal value
	Day 0	Day 31	Day 45	
Male				
Hemoglobin (g/dL)	14.29±1.59	14.49±1.64	14.77±1.57	13-18
Leukocytes (10^3u/L)	7.31±1.96	6.52±1.34	7.10±0.87	4-11
Limphocytes (%)	37.92±10.2	32.84±6.71	32.38±7.47	15-45
BUN (mg/dL)	21.81±5.79	19.90±5.41	23.90±5.37	10-50
SGOT(U/L)	23.69±4.45	21.18±4.02	20.45±3.64	10-36

SGPT(U/L)	13.81±5.87	12.72±4.22	12.54±3.93	9-43
Female				
Hemoglobin (g/Dl	12.01±1.06	12.39±1.54	12. 03±1.70	12-16
Leukocytes (10^3u/L)	6.70±1.24	6.29±1.88	6.86±2.19	4-11
Limphocytes (%)	32.25±9.61	31.32±1.17	28.48±5.59	15-45
BUN (mg/dL)	21.44±5.81	22.89±8.31	21.00±6.82	10-50
SGOT(U/L)	19.80±5.39	19.80±5.39	19.80±5.39	10-36
SGPT(U/L)	10.10±4.70	11.20±4.51	11.00±3.49	9-43

Note:

BUN: Blood Ureum Nitrogen

SGOT: Serum Glutamic Oxaloacetic Transaminase

SGPT: Serum Glutamic Pyruvate Transaminase

Table 2. Results of Paired t-Tests for Changes of Hemoglobin, Leucocyte, Lymphocyte, BUN, SGOT and SGPT on Healthy Volunteers (n = 21)

Examination	**p-value (Male)**			**p-value (Female)**		
	d1	**d2**	**d3**	**d1**	**d2**	**d3**
Hemoglobin	0.266	0.113	0.066	0.460	0.570	0.414
Leucocyte	0.130	0.090	0.624	0.420	0.230	0.755
Lymphocyte	0.131	0.776	0.047*	0.818	0.332	0.049*
BUN	0.448	0.061	0.285	0.321	0.428	0.486

SGOT	0.161	0.531	0.070	1.000	0.848	0.725
SGPT	0.545	0.913	0.165	0.733	0.933	0.634

Note:

Paired t-test:

d1 = day 0 and 31

d2 = day 31 and 45

d3 = day 0 and 45

Table 3. Results of Vital Check-ups on Healthy Volunteers (n = 21)

Examination	**Normal value**	**Value (n=21)**		**p-value**
		Day 30	**Day 45**	
Systole (mmHg)	≤ 120	115.95±8.45	115.00±6.32	0.43
Diastole (mmHg)	≤ 80	75.90±6.44	74.57±7.80	0.43
Pulse (x/menit)	70-80	78.86±7.05	77.48±4.16	0.27
Respiratory Rate (RR) (x/menit)	16-20	17.71±1.82	18.38±1.02	0.15
Temperature (ºC)	36.1-37.2	36.16±0.35	36.65±0.40	0.01

Note:

Test wilcoxon ($p < 0.05$)

Day 30: after administration of ethanol extract capsule of rosella petals

Day 45: after 15 days do not consume ethanol extract capsule of rosella petals

Effect of ethanolic extract capsule of rosella on vital signs

Table 3 showed the results of healthy vocabulary check-ups after 30 days of taking rosella capsules and after not consuming rosella capsules for 15 days. It showed that there was no significant difference in volunteer vital signs. Vital signs of the 31st day of volunteers were within the normal range. In this study, there was no significant difference after rosella capsule and after not being given rosella capsule on systolic blood pressure value, diastolic blood pressure, pulse, respiration ($p > 0.05$). Previous research shows that rosella plays a role in maintaining blood pressure in healthy volunteers, by decreasing the activity of renin-angiotensin (RAS) and the active vasodilator metabolite of bradykinin (des-Arg(9)-bradykinin). Consume rosella tea effectively lower blood pressure in patients with mild hypertension. There was no significant difference in the body temperature ($P < 0.05$). Literature reviews suggest that flavonoid contained in rosella has the ability to inhibit cyclooxygenase reactions which can affect the biosynthesis of prostaglandins, a mediator of febrile formation.

Effect of ethanolic extract capsule of rosella on hemoglobin, leucocytes and lymphocytes

The comparison of hemoglobin, leukocyte, and lymphocyte levels in male and female volunteers on day 0 and day 31 in **Table 2** showed that there is no significant difference ($p > 0.05$). This indicates that ethanolic extract of rosella at the given doses does not affect the hematology of healthy human blood during and after administration. **Table 1** revealed the increased levels of hemoglobin but within normal limits. The anthocyanin present in rosella can stimulate the production of erythropoietin thus affecting the formation of red blood cells. The erythrocytes are then synthesized to produce hemoglobin [19]. The result showed that iron mineral levels as well as vitamins from rosella also help increase the number of Hb volunteers. Vitamin C is needed to

increase iron absorption. While Fe binds to hemoglobin to carry oxygen throughout the body. High vitamin C and Fe content, in rosella can increase the number of erythrocytes and Hb levels in white blood of anemia mice. Vitamin C and Fe are essential sources in the body. Iron (Fe) and vitamin C are factors associated with the formation of red blood cells and hemoglobin in the blood [20].

The average of leukocyte, lymphocytes from day 0 and 31 on men and women were decreased but were still within normal limits. This was possibly because of the content of anthocyanin and vitamin C from rosella which acts as an antioxidant in the body's defense. The role of anthocyanin and vitamin C as an antioxidant was binding free radicals and molecules that *are* highly reactive. High consumption of antioxidants could increase the body's immune system against foreign objects or antigens [21]. Leukocytes play a role in the body's natural immunity as phagocytes, antigens, and antibodies [22] (Campbell et al., 2010). The number of leukocytes plays an important role in improving the immune system and also protects the body cells against pathogens [23]. Anthocyanin also plays a role in inhibiting the proliferation of lymphocytes. This proliferation of lymphocytes is associated with a process of hematopoiesis in the mechanism of apoptosis. The process of apoptosis has an important role in maintaining the correct number of hematopoietic progenitors for erythrocytes as well as various types of leukocytes such as lymphocytes one of them. Each cell has a different lifetime. Lymphocytes have a life span of one day (neutrophil) and some are up to 20-30 years old for some T cells [24]. The circulation of lymphocytes in the blood increases, if the immune system increases compared to the state of illness, where the number of lymphocytes becomes indicator of immune response [25]. The comparison of hemoglobin, leukocyte, and lymphocyte levels on 31^{st} and 45^{th} days of both men and women had no significant different ($p > 0.05$). It indicated that delay of effect did not occur.

The mean of male hemoglobin from day 31 to day 45 had increased, while in women, it decreased but was still in normal limit.

The comparison of hemoglobin and leukocyte levels on days 0 and 45 days was not significant ($p > 0.05$), while the percentage of lymphocyte revealed the significant difference ($p < 0.05$). However, the lymphocyte percentage during the study was within the normal range of 15-45% [26].

The effect of ethanolic extract of rosella on BUN

Table 2 and Table 3 revealed that BUN levels of male and female volunteers in the day 1 had no significant difference compared to those of day 31 ($p > 0.05$). However, there was a significant difference in the average BUN level from each measurement day. A decrease in BUN levels from day 0 and 31, however these values are within normal levels.

On the day 31 and 45 after not being given by extract capsule, there was no significant difference ($p > 0.05$). The use of rosella extract on male volunteers decreased the amount of BUN and increased again on the day 45 (15 days after did not consume the rosella). In female volunteers, the number of BUN levels remained at the same levels either on day 30 or day 45. The increased levels of BUN in women was in line with those reported by Ukoha et al. [27], indicating that the increased BUN that is due to an integrity distortion of renal cytoarchitecture in the administration of rosella flower water extract for 21 days. It is because rosella has a diuretic effect, caused by the compound of quercetin and anthocyanin [28]. BUN levels are also influenced by the amount of protein intake in the body. The status of hydration is also a factor affecting the amount of BUN in the blood [3].

SGOT and SGPT

The comparison of SGOT and SGPT levels was shown in Table 2. There is no significant difference ($p > 0.05$) on day 0 and day 31 of male and female volunteers. The average of SGOT and SGPT on days 0 and 31 decreased in male but did not in women. Clinically, the average levels of days 0 and 30 were still within normal limits.

The comparison of the 30th and 45th days of SGOT and SGPT levels of male and female shows no significantly different ($p > 0.05$). The mean of SGOT in male decreased while in women remained the same level. While SGPT levels decreased in men and women. This decrease in value is in line with previous studies showing that ethanol extract of red rosella flower petals has a hepatoprotector activity by decreasing the activity of SGOT and SGPT [14].

CONCLUSION

The administration of rosella extract capsule for 30 days did not affect the vital sign, hemoglobin value, leucocytes, lymphocytes, BUN, SGOT, and SGPT ($p > 0.05$). In addition, the ethanolic extract of Rosella does not negatively affect the vital signs, hemoglobin, leukocytes, lymphocytes, BUN, SGOT, and SGPT, thus, are safe for consumption.

ACKNOWLEDGEMENT

The leading research grant of study program from Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPP) Universitas Ahmad Dahlan (UAD) in 2016 and 2017 PUPS-049/LPP-UAD/III/2016; PHB-212/LPP-UAD/III/2016; PIPP-001/SP3/LPP-UAD/IV/2017.

REFERENCES

[1] I. Da-Costa-Rocha, B. Bonnlaender, H. Sievers, I. Pischel, and M. Heinrich, "Hibiscus sabdariffa L.–A phytochemical and pharmacological review," *Food chemistry,* vol. 165, pp. 424-443, 2014.

[2] A. Formagio *et al.*, "Phenolic compounds of Hibiscus sabdariffa and influence of organic residues on its antioxidant and antitumoral properties," *Brazilian Journal of Biology,* vol. 75, pp. 69-76, 2015.

[3] T. Villani, H. R. Juliani, J. E. Simon, and Q.-L. Wu, "Hibiscus sabdariffa: Phytochemistry, quality control, and health properties," in *African Natural Plant Products Volume II: Discoveries and Challenges in Chemistry, Health, and Nutrition*: ACS Publications, 2013, pp. 209-230.

[4] O. Kosakowska, Z. Weglarz, M. Sidky, J. Przybyl, and A. Geszprych, "Sterols and fatty acids in the seeds of roselle [Hibiscus sabdariffa L.] cultivated in Egypt," *Herba polonica,* vol. 1, no. 51, 2005.

[5] L. Opletal *et al.*, "Preparation and validated analysis of anthocyanin concentrate from the calyces of Hibiscus sabdariffa," *Natural product communications,* vol. 12, no. 1, p. 1934578X1701200112, 2017.

[6] I. S. M. Purbowati and A. Maksum, "The antioxidant activity of Roselle (Hibiscus sabdariffa Linii) phenolic compounds in different variations microwave-assisted extraction time and power," in *IOP Conference Series: Earth and Environmental Science*, 2019, vol. 406, no. 1: IOP Publishing, p. 012005.

[7] G. I. Peredo Pozos *et al.*, "Antioxidant capacity and antigenotoxic effect of Hibiscus sabdariffa L. extracts obtained with ultrasound-assisted extraction process," *Applied Sciences,* vol. 10, no. 2, p. 560, 2020.

[8] P. E. Aba, P. E. Joshua, and C. Oli, "In vivo Studies on the Possible Haematological Changes in Rats Administered Hibiscus sabdariffa Aqueous Extract," *International Blood Research & Reviews,* pp. 1-8, 2016.

[9] M. Huse, "Mechanical forces in the immune system," *Nature Reviews Immunology,* vol. 17, no. 11, pp. 679-690, 2017.

[10] M. Schieber and N. S. Chandel, "ROS function in redox signaling and oxidative stress," *Current biology,* vol. 24, no. 10, pp. R453-R462, 2014.

[11] Y. Yang, A. V. Bazhin, J. Werner, and S. Karakhanova, "Reactive oxygen species in the immune system," *International reviews of immunology,* vol. 32, no. 3, pp. 249-270, 2013.

[12] M. Kruijsen-Jaarsma, D. Révész, M. B. Bierings, L. M. Buffart, and T. Takken, "Effects of exercise on immune function in patients with cancer: a systematic review," *Exercise immunology review,* vol. 19, 2013.

[13] Q.-N. Zhu, D. Zhang, T. Jin, Q. Wu, J. Liu, and Y.-F. Lu, "Rutaecarpine effects on expression of hepatic phase-1, phase-2 metabolism and transporter genes as a basis of herb–drug interactions," *Journal of ethnopharmacology,* vol. 147, no. 1, pp. 215-219, 2013.

[14] L. H. N. Nurkhasanah and Z. R. Hakim, "Effect of rosella (Hibiscus sabdariffa L) extract on glutathione-S-transferase activity in rats," *Tropical Journal of Pharmaceutical Research,* vol. 16, no. 10, pp. 2411-2416, 2017.

[15] J. P. Dzoyem, V. Kuete, and J. N. Eloff, "Biochemical parameters in toxicological studies in Africa: significance, principle of methods, data interpretation, and use in plant

screenings," in *Toxicological survey of African medicinal plants*: Elsevier, 2014, pp. 659-715.

[16] L. Llauger, J. Jacob, and O. Miro, "Renal function and acute heart failure outcome," *Medicina Clínica (English Edition),* vol. 151, no. 7, pp. 281-290, 2018.

[17] B. F. Rodak, E. M. Keohane, and G. A. Fritsma, *Hematology-E-Book: Clinical Principles and Applications*. Elsevier Health Sciences, 2013.

[18] B. Fattizzo *et al.*, "Efficacy of recombinant erythropoietin in autoimmune hemolytic anemia: a multicenter international study," *haematologica,* vol. 106, no. 2, p. 622, 2021.

[19] E. Dzierzak and S. Philipsen, "Erythropoiesis: development and differentiation," *Cold Spring Harbor perspectives in medicine,* vol. 3, no. 4, p. a011601, 2013.

[20] R. a. Nisa, "Effect Of Roselle (Hibiscus Sabdariffa) On Changes In Hemoglobin Levels In Pregnant Women With Anemia Taking Iron Supplement," ed: ISSN, 2017.

[21] R. Nova, A. S. Hoemardani, and M. Louisa, "Potential of herbal medicines in cancer therapy," *The Indonesia Journal of Cancer Control,* vol. 1, no. 1, pp. 32-42, 2021.

[22] S. Gordon, "Phagocytosis: an immunobiologic process," *Immunity,* vol. 44, no. 3, pp. 463-475, 2016.

[23] S. M. Flint *et al.*, "Leucocyte subset-specific type 1 interferon signatures in SLE and other immune-mediated diseases," *RMD open,* vol. 2, no. 1, p. e000183, 2016.

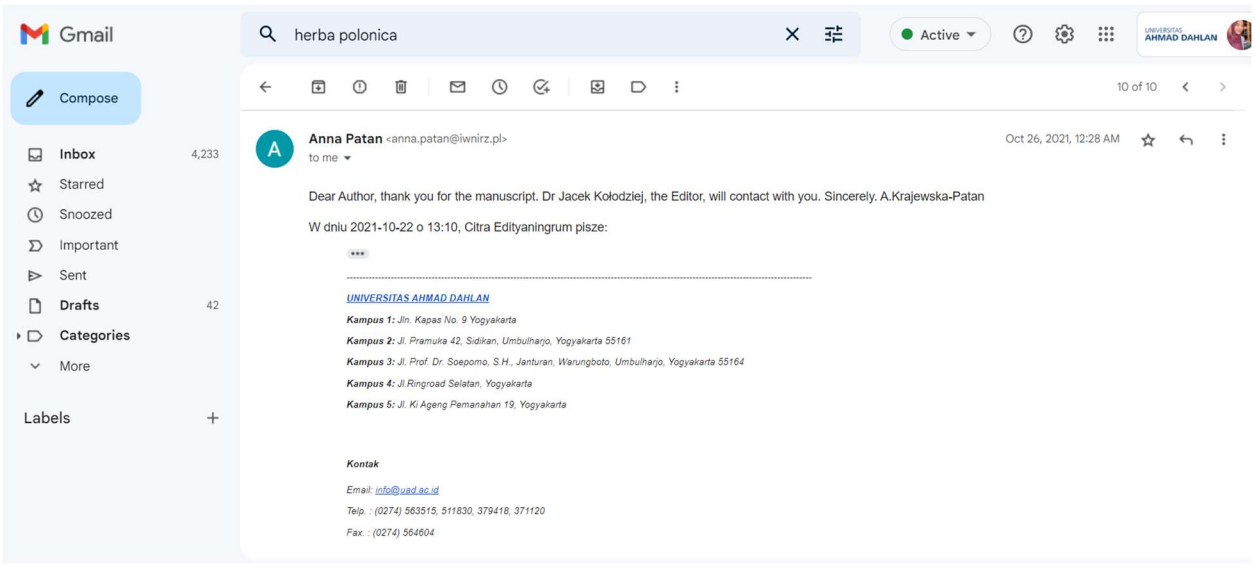
[24] I. A. M. Groh *et al.*, "Long-Term Consumption of Anthocyanin-Rich Fruit Juice: Impact on Gut Microbiota and Antioxidant Markers in Lymphocytes of Healthy Males," *Antioxidants,* vol. 10, no. 1, p. 27, 2021.

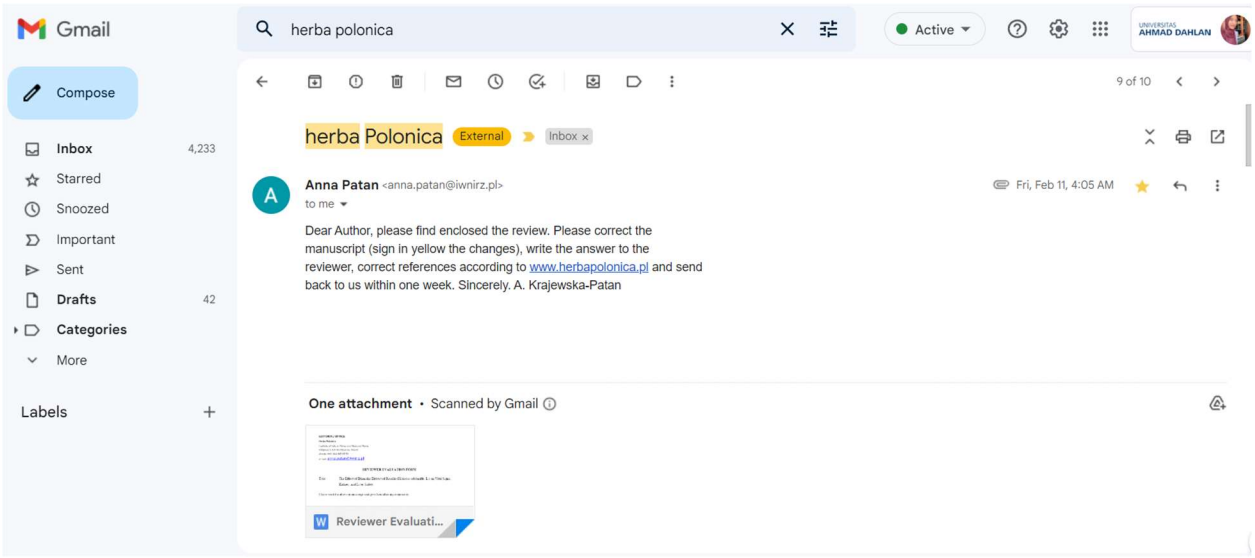
[25] L. Madaro and M. Bouché, "From innate to adaptive immune response in muscular dystrophies and skeletal muscle regeneration: the role of lymphocytes," *BioMed research international,* vol. 2014, 2014.

[26] A. Al-Mawali, A. D. Pinto, R. Al Busaidi, and I. Al-Zakwani, "Lymphocyte subsets: reference ranges in an age-and gender-balanced population of Omani healthy adults," *Cytometry Part A,* vol. 83, no. 8, pp. 739-744, 2013.

[27] U. Ukoha, S. Mbagwu, G. Ndukwe, and C. Obiagboso, "Histological and biochemical evaluation of the kidney following chronic consumption of Hibiscus sabdariffa," *Advances in Biology,* vol. 2015, 2015.

[28] G. Riaz and R. Chopra, "A review on phytochemistry and therapeutic uses of Hibiscus sabdariffa L," *Biomedicine & Pharmacotherapy,* vol. 102, pp. 575-586, 2018.


Gmail
herba polonica
Active
Compose
Inbox 4,233
Starred
Snoozed
Important
Sent
Drafts 42
Categories
More
Labels
10 of 10
Anna Patan <anna.patan@iwnirz.pl>
to me
Oct 26, 2021, 12:28 AM
Dear Author, thank you for the manuscript. Dr Jacek Kołodziej, the Editor, will contact with you. Sincerely. A.Krajewska-Patan
W dniu 2021-10-22 o 13:10, Citra Erityaningrum pisze:
UNIVERSITAS AHMAD DAHLAN
Kampus 1: Jln. Kapas No. 9 Yogyakarta
Kampus 2: Jl. Pramuka 42, Sidikan, Umbulharjo, Yogyakarta 55161
Kampus 3: Jl. Prof. Dr. Soepomo, S.H., Janturan, Warungboto, Umbulharjo, Yogyakarta 55164
Kampus 4: Jl.Ringroad Selatan, Yogyakarta
Kampus 5: Jl. Ki Ageng Pemanahan 19, Yogyakarta
Kontak
Email: info@uad.ac.id
Telp. : (0274) 563515, 511830, 379418, 371120
Fax. : (0274) 564604


Gmail
herba polonica
Active
Compose
Inbox 4,233
Starred
Snoozed
Important
Sent
Drafts 42
Categories
More
Labels
9 of 10
herba Polonica
External
Inbox ×
Anna Patan <anna.patan@iwnirz.pl>
to me
Fri, Feb 11, 4:05 AM
Dear Author, please find enclosed the review. Please correct the manuscript (sign in yellow the changes), write the answer to the reviewer, correct references according to www.herbapolonica.pl and send back to us within one week. Sincerely. A. Krajewska-Patan
One attachment • Scanned by Gmail
Reviewer Evaluati...

Komentar Reviewer

EDITORIAL OFFICE

Herba Polonica

Institute of Natural Fibres and Medicinal Plants
Kolejowa 2, 62-064 Plewiska, Poland
phone: +48 (61) 665 95 50
e-mail: anna.patan@iwnirz.pl

REVIEWER EVALUATION FORM

Title: The Effect of Ethanolic Extract of Rosella (Hibiscus sabdariffa, L.) on Vital Signs, Kidney, and Liver Safety

I have read the above manuscript and give hereafter my comments:

The manuscript by …no information …is focusing on the safety of the rosella extract consumed in the form of capsules on the vital signs, hematologic parameters as well as kidney and liver function.

1º/ **consistency with the journal profile**

non-consistency with the journal profile

2º/ a manuscript is based on extensive study with proper controls

a manuscript is not based on extensive study with proper controls

3º/ **the information concerning the identification and documentation of plants is included**

the information concerning the identification and documentation of plants is not included

4º/ **the information concerning medicinal use of the plant is clarified**

the information concerning medicinal use of the plant is not clarified

5º/ the approach to the subject is innovative

the approach to the subject is not innovative

6º/ **in the article based on animal or clinical studies written approvals of Ethics/Bioethics Committee is cited**

in the article based on animal or clinical studies written approvals of Ethics/Bioethics Committee is not cited

7º/ **the article is written carefully**

the article is written carelessly

8º/ Language of the paper:

excellent □

good □

need corrections □

9º/ **the article is written according to the 'Instructions to Authors'**

the article is not written according to the 'Instructions to Authors'

These following concerns need to be addressed:

I. Major errors:

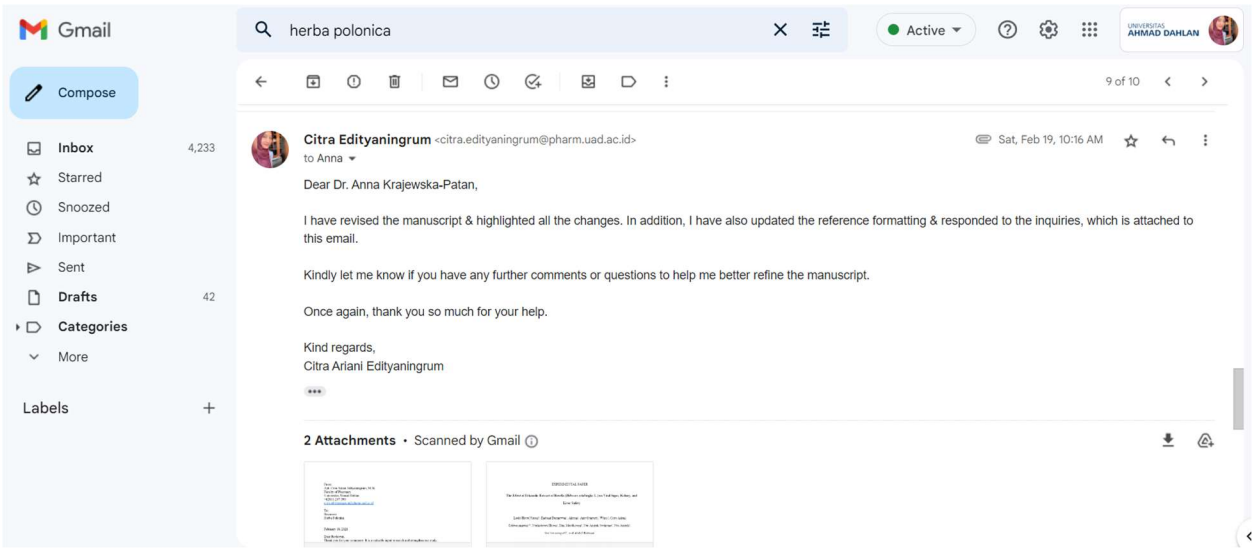
1. Ethical clearance means ethical approval? It should be clarified.
2. The authors gave a range for normal values of many parameters, but there are no sources about it.
3. Why ANOVA test is not used for the statistical comparison?
4. The Authors wrote that the rosella extract is consumed in the form of capsules. In the article we have no information about the brand name of the capsule.
5. In the article we have no information about validation of the used methods.
6. The authors write: "The levels of BUN were determined by the Modiff-Berthelot method" but we have no source to the method.
7. What statistical programme was used for the calculations?
8. It should be completed in the article, if the methodology of the experiment is compatible with international regulations concerning the experiments on humans.

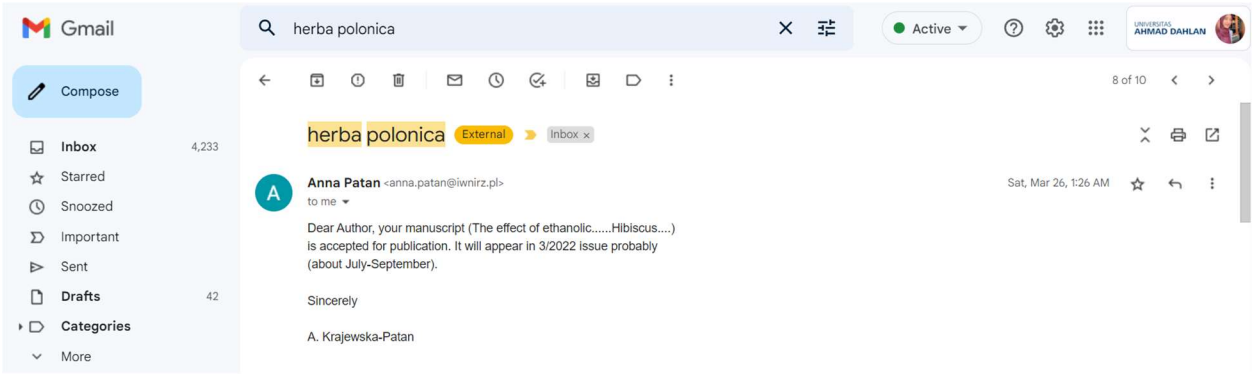
II. Minor errors:

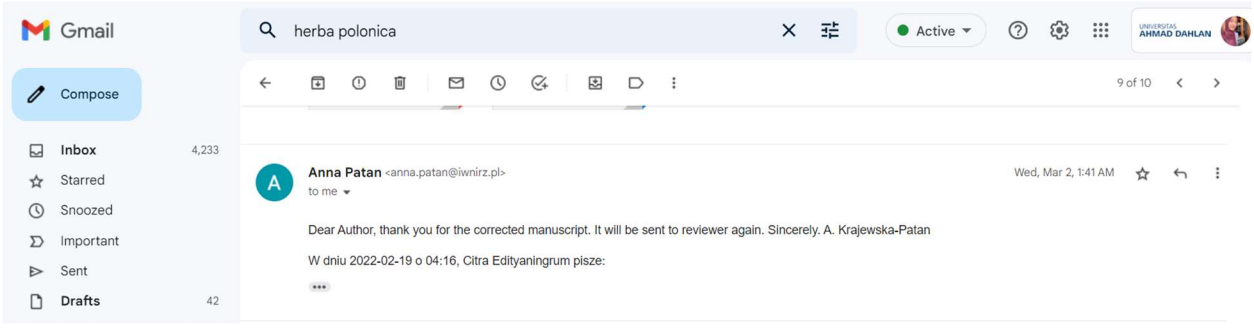
1. ……..
2. ……..

Concluding,

- I recommend this paper for publication in *Herba Polonica* after strong revision and inserting the above-mentioned changes in the manuscript □
- I agree to evaluate the revised body text of the paper □
- I do not recommend this paper for publication in *Herba Polonica* □
- I recommend this paper for publication in *Herba Polonica* after minor revisions □
- I recommend this paper for publication in *Herba Polonica* without any changes □


Gmail
herba polonica
Active
Compose
Inbox 4,233
Starred
Snoozed
Important
Sent
Drafts 42
Categories
More
Labels
9 of 10
Citra Edityaningrum <citra.edityaningrum@pharm.uad.ac.id>
to Anna
Sat, Feb 19, 10:16 AM
Dear Dr. Anna Krajewska-Patan,
I have revised the manuscript & highlighted all the changes. In addition, I have also updated the reference formatting & responded to the inquiries, which is attached to this email.
Kindly let me know if you have any further comments or questions to help me better refine the manuscript.
Once again, thank you so much for your help.
Kind regards,
Citra Ariani Edityaningrum
2 Attachments • Scanned by Gmail


Gmail
herba polonica
Active
Compose
Inbox 4,233
Starred
Snoozed
Important
Sent
Drafts 42
Categories
More
8 of 10
herba polonica
External
Inbox
Anna Patan <anna.patan@iwnirz.pl>
to me
Sat, Mar 26, 1:26 AM
Dear Author, your manuscript (The effect of ethanolic.....Hibiscus....) is accepted for publication. It will appear in 3/2022 issue probably (about July-September).
Sincerely
A. Krajewska-Patan


Gmail
herba polonica
Active
Compose
Inbox 4,233
Starred
Snoozed
Important
Sent
Drafts 42
9 of 10
Anna Patan <anna.patan@iwnirz.pl>
to me
Wed, Mar 2, 1:41 AM
Dear Author, thank you for the corrected manuscript. It will be sent to reviewer again. Sincerely. A. Krajewska-Patan
W dniu 2022-02-19 o 04:16, Citra Edityaningrum pisze:

Gmail

herba polonica

Active

Compose

Inbox 4,233
Starred
Snoozed
Important
Sent
Drafts 42
Categories
More

Labels

herba polonica urgent

External Inbox ×

Anna Patan
to me

Mon, Oct 10, 10:52 PM

Dear Author, I am waiting for the ORCID numbers of all authors. And see the galley proof once again. The order of the articles cited was wrong. I changed it. Please check if it is correct now. URGENT!

Sincerely

A. Krajewska-Patan

One attachment • Scanned by Gmail

Herba_3-2022_a

Gmail

herba polonica

Active

Compose

Inbox 4,233
Starred
Snoozed
Important
Sent
Drafts 42
Categories
More

Labels

1 of 10

Citra Edityaningrum <citra.edityaningrum@pharm.uad.ac.id>
to Anna

Wed, Oct 12, 1:12 PM

Dear Anna Pattan,

The article is OK.
The following is the authors' Orchid ID:

LAELA HAYU NURANI: https://orcid.org/0000-0003-3327-0624
ENDANG DARMAWAN: https://orcid.org/0000-0001-6143-4126
AKROM: https://orcid.org/0000-0002-1792-722X
ANY GUNTARTI: https://orcid.org/0000-0001-5428-0261
WARSI: https://orcid.org/0000-0001-5210-4416
CITRA ARIANI EDITYANINGRUM: https://orcid.org/0000-0002-4113-6233
NURHIDAYATI HARUN: https://orcid.org/0000-0003-3454-9459
DINI MARDHIYANI: https://orcid.org/0000-0003-0823-7259
NUR AZIZAH SYAHRANA: https://orcid.org/0000-0003-3364-0998
NUR AZIZAH: https://orcid.org/0000-0003-2562-0866
SITI SETIANINGSIH https://orcid.org/0000-0001-7532-6783
ABDUL ROHMAN: https://orcid.org/0000-0002-1141-7093

Thank you very much for your help.

Anna Patan
to me

Wed, Oct 12, 4:58 PM

Polish > English Translate message

Turn off for: Polish

thank you

W dniu 2022-10-12 o 08:12, Citra Edityaningrum pisze:

•••

UNIVERSITAS AHMAD DAHLAN

Kampus 1: *Jln. Kapas No. 9 Yogyakarta*

Kampus 2: *Jl. Pramuka 42, Sidikan, Umbulharjo, Yogyakarta 55161*

Kampus 3: *Jl. Prof. Dr. Soepomo, S.H., Janturan, Warungboto, Umbulharjo, Yogyakarta 55164*

Kampus 4: *Jl.Ringroad Selatan, Yogyakarta*

Kampus 5: *Jl. Ki Ageng Pemanahan 19, Yogyakarta*

Kontak

Email: info@uad.ac.id

Telp. : (0274) 563515, 511830, 379418, 371120

sciendo

herba polonica
International journal edited by the Institute of Natural Fibres and Medicinal Plants
National Research Institute
Vol. 68 No. 3 2022

Received: 2022-02-04
Accepted: 2022-04-20
Available online: 2022-09-30

DOI: 10.2478/hepo-2022-0018

EXPERIMENTAL PAPER

Effect of ethanolic extract of rosella (*Hibiscus sabdariffa* L.) on vital signs, kidney, and liver safety (*ahead of print*)

LAELA HAYU NURANI[1], ENDANG DARMAWAN[1], AKROM[1], ANY GUNTARTI[1], WARSI[1], CITRA ARIANI EDITYANINGRUM[1]*, NURHIDAYATI HARUN[2], DINI MARDHIYANI[3], NUR AZIZAH SYAHRANA[4], NUR AZIZAH[5], SITI SETIANINGSIH[6], ABDUL ROHMAN[7]

[1]Faculty of Pharmacy
Universitas Ahmad Dahlan
Yogyakarta 55164, Indonesia

[2]Diploma of Pharmacy
STIKes Muhammadiyah Ciamis
Ciamis, West Java

[3]Department of Pharmacy, Faculty of Pharmacy and Health Sciences
Universitas Abdurrab
Pekanbaru, Indonesia

[4]Department of Pharmacy, UIN Alauddin Makassar
Indonesia

[5]STIKES Muhammadiyah Kuningan
Kuningan, West Java 45552, Indonesia

[6]Faculty of Pharmacy, Universitas Wahid Hasyim
Semarang, Central Java 50232, Indonesia

[7]Centre of Excellence Institute for Halal Industry & Systems (IHIS)
Universitas Gadjah Mada
Yogyakarta, 55281, Indonesia

*corresponding author: email: citra.edityaningrum@pharm.uad.ac.id

Summary

Introduction: Rosella (*Hibiscus sabdariffa* L) extract is often used as immune-stimulant because it contains flavonoids, especially anthocyanin and quercetin with antioxidant activities.

Objective: This study aimed to determine the safety of the rosella extract consumed in the form of capsules on the vital signs, haematologic parameters as well as kidney and liver function.

Methods: This research was conducted using clinical trial pre- and post-test design in healthy participants. There were 21 healthy participants (52 % male, age ranged 8–45) consuming rosella capsules for thirty days, in a dose of 500 mg extract daily. Leukocytes, lymphocytes, blood urea nitrogen (BUN), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and vital signs were consecutively evaluated on days 0, 31, and 45, respectively. The Wilcoxon and paired sample t-test were used to compare the parameters among the evaluated times.

Result: The result showed that no significant difference for all parameters among the three time points (p>0.05).

Conclusion: These findings suggested that the administration of ethanolic extract of rosella is potential safe and does not negatively affect the vital signs, haemoglobin, leukocytes, lymphocytes, BUN, SGOT, and SGPT.

Key words: ***leukocytes, lymphocytes, BUN, SGOT, SGPT, Hibiscus sabdariffa***

Słowa kluczowe: ***leukocyty, limfocyty, BUN, SGOT, SGPT, Hibiscus sabdariffa***

INTRODUCTION

Rosella (*Hibiscus sabdariffa* L) contains antioxidant compounds capable of enhancing the immune system by maintaining cell damage due to its ability to absorb excessive ultraviolet light, therefore, it protects body cells from free radical changes [1]. The antioxidant compounds include metabolite of phenolics and flavonoids [2] such as hibiscitrin (hibiscetin-3-glucoside), quercetin, gossytrin, sabdaritrin, gossypitrin, gossypetin glucosides, and luteolin [3], fatty acid [4], alkaloids, steroids, triterpenoids, saponins, tannins, anthocyanins, and β-carotene [5, 6]. The antioxidant activity of rosella are mainly due to red pigment of anthocyanin, increasing the scavenging activity of free radicals [7]. Anthocyanins also stimulated the production of erythropoietin, which promotes the formation of red blood cells [8]. Thus, it affects the number of leukocytes and lymphocytes in the immune system [9].

Antioxidants maintain the immune system in human body. Every deficiency in the antioxidant defence system make the human body more susceptible to oxidative stress [10] due to imbalance between reactive oxygen species (ROS) and endogenous antioxidant. Induction of ROS can damage lymphocytes in healthy humans. In the immune system, leukocytes play important role in producing, transporting, and distributing antibodies, as a part of the immune response to antigens [11]. Immune reactions to microorganism attacks, foreign macromolecules, and cancer cells are initiated by lymphocytes that are the granular form of leucocytes. Lymphocytes specifically recognize and respond to foreign antigens and also serve as a mediator of humoral and cellular immunity [12].

The preclinical study revealed that rosella provided therapeutic potential of immune-stimulator at an optimal dose of 50 mg/kg BW [12]. Therefore, its use in humans is feasible. Clinical parameters that can be used in describing the tolerated dosage is haematologic, kidney, and liver changes which is a phase 1 clinical test parameter [13].

The ethanol extract of rosella petals has a lethal dose 50% (LD_{50}) toxicity value of 850.90 mg/kgWB in Sprague Dawley mice in the category of ring toxicity and has no effect on the enzyme activity of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) [14]. These enzymes are specific indicators for determining liver cell damage. Kidney function can also be a parameter in determining the safety of medicinal plants [15]. The examination of blood urea nitrogen (BUN) is one of the markers of renal function, which can be used to evaluate kidney function, although, the elevated BUN levels do not actually reflect renal function [16]. Haemoglobin (Hb) serves as the oxygen-carrying substance present in the erythrocytes [17]. Increased or high Hb levels are generally associated with increased amounts of erythrocytes produced in bone marrow with raw materials of iron (Fe), amino acids, vitamin B_{12}, folic acid, B_2, B_6, vitamin C, vitamin E, minerals (cobalt and copper), and the hormone erythropoietin. A decrease in Hb level may indicate a haematological disorder of anaemia [18].

This research aimed to assess the safety of the rosella extract consumed in the form of capsules on

the vital signs, hematologic parameters as well as kidney and liver function.

MATERIAL AND METHODS

Materials

The sample used in this study was obtained from Natura (Food and Nutratecal Company; Jakarta, Indonesia) with brand name of rosella PE® with product code: 5055C and batch number: RH 162703.

Place and time of research

This research was conducted at a private clinic in Yogyakarta, Indonesia from April 3 to May 17, 2017, for 45 days. The ethical clearance, or ethical approval, regarding the clinical study was obtained from Muhammadiyah University of Yogyakarta (approval number: 255/EP-FKIK-UMY/IV/2017).

Research instrument

Instrument used for measuring lymphocytes is flowcytometry, Hb and leukocyte count were haematological analyser, autoanalyser photometer SGOT-SGPT examination, and BUN examination using spectrophotometer with Modiff-Berthelot method.

Health checkup and preparation

Medical examination

This research was conducted by using pre- and post-treatments research design involving 21 volunteers consisting of 11 males and 10 females selected based on inclusion and exclusion criteria [19, 20]. The inclusion criteria included doctor's certificate confirming a health status, age 18–45, BMI 18–30, non-smokers, and signed consent. Health criteria included vital signs, blood pressure, body temperature, heart rate (HR) and respiration rate (RR). The medical examination results were stated in a health certificate supported by clinical laboratory examination including routine haematology, liver function, kidney function, history of disease, and physical examination. Exclusion criteria included pregnant women, consuming other drug during research duration, and using other herbs during the study.

Administration of rosella extract capsule

Rosella extract capsule was given at a dose of 500 mg/day (Product code: 5055C, Batch: RH162703). The capsules were administered to volunteers for 30 days. Haemoglobin, leukocytes, lymphocytes, BUN, SGOT, SGPT and vital sign were examined on day 0 (before treatment), 30th day (after 30 days of consuming rosella capsule) and 45th day (15 days after not consuming rosella capsule) [19].

Preparation of blood sampling

Blood samples were taken from the median cubital vein or brachial vein using 5 cc syringe for 4 cc blood. Then, the sample was stored in a centrifugal micro tube containing EDTA as a coagulant. The separation of plasma was carried out by centrifugation at a rate of 3,500 rpm for 2 hours so that the transparent yellow liquid (serum) occurred. If the serum was not used immediately, then the liquid was stored in a freezer in a temperature of –200°C [21, 22].

Examination of haemoglobin, leukocytes, lymphocytes, BUN, SGOT and SGPT

Haemoglobin and leukocytes

Blood samples on the EDTA tube were analysed using an automated haematology analyser [23].

Lymphocytes

The results of blood samples on the EDTA tube were stored in the falcon tube as much as 50 μl. The tube was added with each 5 μl tritest CD3APC/CD-4PerCP/CD25PE reagent. It was then homogenized using vortex for 1 minute. It was then incubated for 15 minutes with temperature 20-25°C in a dark room. Then, 450 μl of solution of lytic agent was added, followed by an incubation for 15 minutes in a temperature of 20–25°C in the dark room. After the incubation period was completed, an analysis was done using FACS flow cytometer [22].

Blood urea nitrogen (BUN)

The levels of BUN were determined by the Modiff-Berthelot method. The volunteer's blood sampling was fed into a tube using a microphone. A-0.5 ml colour reagent was added to the bottle labelled: unknown,

control, standard, blank. A-10 μl was added to the sample into appropriate bottle. A-0.5 ml of enzyme reagent was added to all bottles. Then, it was mixed well and incubated at 37°C for 5 minutes. A-2.0 ml base reagent was added. The mixture was then stirred and incubated at 37°C for 5 min. The wavelength was set at 630 nm and the zero of the spectrophotometer with blank. The concentration of unknown samples was read directly using standard (25 mg/dl) [24].

Serum glutamic oxaloacetic transaminase (SGOT) – serum glutamic pyruvate transaminase (SGPT)

A total of 1000 μl of reagents were introduced into the test tube. The reagents used were ready-to-use reagents consisting of SGOT and SGPT reagents. Furthermore, 100 μl serum was added and shaken for 1 min in a water heater at 37°C. Spectrophotometer readings were performed at a 340 nm wavelength with a conversion factor of 1745 to obtain SGOT and SGPT levels. SGOT and SGPT levels were expressed in units/litre (U/L) [25].

Data analysis

The data were presented as mean ±SD. The normality test was carried out using Shapiro-Wilk. If data are normally distributed, the means of pre- and post-treatments were subjected to paired t-test, while for the non-normally distributed data, two medians were analysed using a Wilcoxon test at a 95% confidence level. All statistical test were carried out using IBM SPSS 23 statistics.

RESULT AND DISCUSSION

In this study, Hb, Leucocytes, lymphocytes, BUN, SGOT, and SGPT levels in male and female volunteers were found to be different in mean levels at day 0, 30 and 45. Clinically, however, the levels were still within the normal limits (tab. 1, tab. 2).

Effect of ethanolic extract capsule of rosella on vital signs

Table 3 showed the results of healthy volunteers' check-ups after 30 days of administration of rosella capsules and after not consuming rosella capsules for 15 days. It showed that there was no significant difference in vital signs. Vital signs of the 31st day were within the normal range. In this study, there was no significant difference after the administration of rosella capsule and after not being given it on systolic blood pressure value, diastolic blood

Table 1.

Results of haemoglobin, leukocytes, lymphocytes, BUN, SGOT, and SGPT on healthy volunteers (n=21)

Parameters	Mean ±SD			Normal value [26]
	Day 0	Day 31	Day 45	
		Male		
Haemoglobin [g/dl]	14.29±1.59	14.49±1.64	14.77±1.57	13–18
Leukocytes [10^3u/l]	7.31±1.96	6.52±1.34	7.10±0.87	4–11
Limphocytes [%]	37.92±10.2	32.84±6.71	32.38±7.47	15–45
BUN [mg/dL]	21.81±5.79	19.90±5.41	23.90±5.37	10–50
SGOT [U/L]	23.69±4.45	21.18±4.02	20.45±3.64	10–36
SGPT [U/L]	13.81±5.87	12.72±4.22	12.54±3.93	9–43
		Female		
Haemoglobin [g/dl]	12.01±1.06	12.39±1.54	12. 03±1.70	12–16
Leukocytes [10^3u/l]	6.70±1.24	6.29±1.88	6.86±2.19	4–11
Limphocytes [%]	32.25±9.61	31.32±1.17	28.48±5.59	15–45
BUN [mg/dl]	21.44±5.81	22.89±8.31	21.00±6.82	10–50
SGOT [U/L]	19.80±5.39	19.80±5.39	19.80±5.39	10–36
SGPT [U/L]	10.10±4.70	11.20±4.51	11.00±3.49	9–43

Note: BUN: blood ureum nitrogen; SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic pyruvate transaminase

Table 2.

Results of paired t-tests for changes of haemoglobin, leucocyte, lymphocyte, BUN, SGOT and SGPT on healthy volunteers (n=21)

Examination	*p*-value (male)			*p*-value (female)		
	d1	d2	d3	d1	d2	d3
Haemoglobin	0.266	0.113	0.066	0.460	0.570	0.414
Leucocyte	0.130	0.090	0.624	0.420	0.230	0.755
Lymphocyte	0.131	0.776	0.047*	0.818	0.332	0.049*
BUN	0.448	0.061	0.285	0.321	0.428	0.486
SGOT	0.161	0.531	0.070	1.000	0.848	0.725
SGPT	0.545	0.913	0.165	0.733	0.933	0.634

Note: Paired t-test: d1 = day 0 and 31; d2 = day 31 and 45; d3 = day 0 and 45

Table 3.

Results of vital check-ups on healthy volunteers (n=21)

Examination	Normal value	Value (n=21)		*p*-value
		Day 30	Day 45	
Systolic [mmHg]	≤20	115.95±8.45	115.00±6.32	0.43
Diastolic [mmHg]	≤80	75.90±6.44	74.57±7.80	0.43
Pulse [x/menit]	70–80	78.86±7.05	77.48±4.16	0.27
Respiratory rate (RR) [x/menit]	16–20	17.71±1.82	18.38±1.02	0.15
Temperature [°C]	36.1–37.2	36.16±0.35	36.65±0.40	0.01

Note: Wilcoxon test ($p<0.05$); Day 30: after administration of ethanol extract capsule of rosella petals; Day 45: after 15 days do not consume ethanol extract capsule of rosella petals

pressure, pulse, respiration ($p>0.05$). Previous research showed that rosella plays a role in maintaining blood pressure in healthy volunteers by decreasing the activity of renin-angiotensin (RAS) and the active vasodilator metabolite of bradykinin (des-Arg(9)-bradykinin). Rosella tea effectively lower blood pressure in patients with mild hypertension. There was no significant difference in the body temperature ($p<0.05$). Literature reviews suggest that flavonoid contained in rosella has the ability to inhibit cyclooxygenase reactions which can affect the biosynthesis of prostaglandins, a mediator of febrile formation.

Effect of ethanolic extract capsule of rosella on haemoglobin, leucocytes and lymphocytes

The comparison of haemoglobin, leukocyte, and lymphocyte levels in male and female volunteers on day 0 and day 31 in table 2 showed that there is no significant difference ($p>0.05$). This indicates that ethanolic extract of rosella at given doses does not affect the haematology of healthy human blood during and after administration. Table 1 revealed the increased levels of haemoglobin but within normal limits. The anthocyanin present in rosella can stimulate the production of erythropoietin thus affecting the formation of red blood cells. The erythrocytes are then synthesized to produce haemoglobin [27]. The result showed that iron mineral levels as well as vitamins from rosella also help increase the number of Hb volunteers. Vitamin C is needed to increase iron absorption. While Fe binds to haemoglobin to carry oxygen throughout the body. High vitamin C and Fe content, in rosella can increase the number of erythrocytes and Hb levels in white blood of anaemic mice. Vitamin C and Fe are essential sources in the body. Iron (Fe) and vitamin C are factors associated with the formation of red blood cells and hemoglobin in the blood [28].

The average number of leukocytes, lymphocytes from day 0 and day 31 on males and females were decreased but were still within normal limits. This was possibly because of the content of anthocyanin and vitamin C from rosella which acts as an

antioxidant The role of anthocyanin and vitamin C as an antioxidant was binding free radicals and molecules that are highly reactive. High consumption of antioxidants could increase immune system against foreign objects or antigens [29]. Leukocytes play a role in natural immunity as phagocytes, antigens, and antibodies [30]. The number of leukocytes plays an important role in improving the immune system and also protects the body cells against pathogens [31]. Anthocyanin also plays a role in inhibiting the proliferation of lymphocytes. This proliferation of lymphocytes is associated with a process of haematopoiesis in the mechanism of apoptosis. The process of apoptosis plays an important role in maintaining the correct number of haematopoietic progenitors for erythrocytes as well as various types of leukocytes, such as lymphocytes. Each cell has a different lifetime. Lymphocytes have a life span of one day (neutrophil) and some are up to 20–30 years old for some T cells [32]. The circulation of lymphocytes in the blood increases, if the immune system increases compared to the state of illness, where the number of lymphocytes becomes indicator of immune response [33]. The comparison of haemoglobin, leukocyte, and lymphocyte levels on 31st and 45th days of both men and women had no significant different (p>0.05). It indicated that delay of effect did not occur. The mean of male haemoglobin from day 31 to day 45 had increased, while in women, it decreased but was still in normal limit.

The comparison of haemoglobin and leukocyte levels on days 0 and 45 days was not significant (p>0.05), while the percentage of lymphocyte revealed the significant difference (p<0.05). However, the lymphocyte percentage during the study was within the normal range of 15-45% [34].

The effect of ethanolic extract of rosella on BUN

Table 2 and Table 3 revealed that BUN levels of male and female volunteers in the day 1 had no significant difference compared to those of day 31 (p>0.05). However, there was a significant difference in the average BUN level from each measurement day. A decrease in BUN levels from day 0 and 31, however these values are within normal levels.

On the day 31 and 45 after not being given extract capsule, there was no significant difference (p>0.05). The use of rosella extract on male volunteers decreased the amount of BUN and increased again on the day 45 (15 days after did not consume the rosella). In female volunteers, the number of BUN levels remained at the same levels either on day 30 or day 45. The increased levels of BUN in women was in line with those reported by Ukoha *et al.* [35], indicating that the increased BUN is due to an integrity distortion of renal cytoarchitecture in the administration of rosella flower water extract for 21 days. It is because rosella has a diuretic effect, caused by quercetin and anthocyanin [36]. BUN levels are also influenced by the amount of protein intake in the body. The status of hydration is also a factor affecting the amount of BUN in blood [3].

SGOT and SGPT

The comparison of SGOT and SGPT levels was shown in table 2. There is no significant difference (p>0.05) between day 0 and day 31 in male and female volunteers. The average of SGOT and SGPT on days 0 and 31 decreased in males but did not in females. Clinically, the average levels in days 0 and 30 were still within normal limits.

The comparison of SGOT and SGPT levels in day 30 and day 45 of male and female shows no significant difference (p>0.05). The mean of SGOT in males decreased while in females remained at the same level, while SGPT levels decreased both in females and males. This decrease in value is in line with previous studies showing that ethanol extract of red rosella flower petals has a hepatoprotective activity by decreasing the activity of SGOT and SGPT [14].

CONCLUSION

The administration of rosella extract capsule for 30 days did not affect the vital sign, haemoglobin value, leucocytes, lymphocytes, BUN, SGOT, and SGPT (p>0.05). In addition, the ethanolic extract of rosella does not negatively affect the vital signs, haemoglobin, leukocytes, lymphocytes, BUN, SGOT, and SGPT, thus, its consumption is safe.

ACKNOWLEDGEMENT

The leading research grant of study program from Lembaga Penelitian dan Pengabdian kepada Masyarakat (LPP) Universitas Ahmad Dahlan (UAD) in 2016 and 2017 PUPS-049/LPP-UAD/III/2016; PHB-212/LPP-UAD/III/2016; PIPP-001/SP3/LPP-UAD/IV/2017.

Conflict of interest: Authors declare no conflict of interest.

REFERENCES

1. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. *Hibiscus sabdariffa* L. – A phytochemical and pharmacological review. Food chem 2014; 165:424-443. doi: https://dx.doi.org/10.1016/j.foodchem.2014.05.002

2. Formagio AS, Ramos DD, Vieira MC, Ramalho SR, Silva MM, Zárate NA, et al. Phenolic compounds of *Hibiscus sabdariffa* and influence of organic residues on its antioxidant and antitumoral properties. Braz J Biol 2015; 75(1):69-76. doi: https://dx.doi.org/10.1590/1519-6984.07413

3. Villani T, Juliani HR, Simon JE, Wu QL. *Hibiscus sabdariffa*: Phytochemistry, quality control, and health properties. In: African Natural Plant Products Volume II: Discoveries and Challenges in Chemistry, Health, and Nutrition. ACS Publications, 2013:209-230.

4. Kosakowska O, Weglarz Z, Sidky M, Przybyl JL, Geszprych A. Sterols and fatty acids in the seeds of roselle [*Hibiscus sabdariffa* L.] cultivated in Egypt. Herba Pol 2005; 51(1):18-23.

5. Opletala L, Chocholousova-Havlikova L, Siatka T, Cahliková L, Locarek M, Ali BH, et al. Preparation and validated analysis of anthocyanin concentrate from the calyces of *Hibiscus sabdariffa*. Nat Prod Commun 2017; 12(1):43-45.

6. Purbowati ISM, Maksum A. The antioxidant activity of roselle (*Hibiscus sabdariffa Linii*) phenolic compounds in different variations microwave-assisted extraction time and power. IOP Conference Series: Earth and Environmental Science 2019; 406. http://doi.org/10.1088/1755-1315/406/1/012005

7. Pozos GIP, Lopez MAR, Natera JFZ, Moya CA, Ramirez LB, Silva MR, et al. Antioxidant capacity and antigenotoxic effect of *Hibiscus sabdariffa* L. extracts obtained with ultrasound-assisted extraction process. Appl Sci 2020; 10(2):560. doi: https://dx.doi.org/10.3390/app10020560

8. Aba PE, Joshua P, Oli C. *In vivo* Studies on the possible haematological changes in rats administered *Hibiscus sabdariffa* aqueous extract. International Blood Research & Reviews 2016; 51(1): 1-8. doi: https://dx.doi.org/10.9734/IBRR/2016/23170

9. Huse M. Mechanical forces in the immune system. Nat Rev Immunol 2017; 17(11):679-690. doi: https://dx.doi.org/10.1038/nri.2017.74

10. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol 2014; 24(10):R453-462. doi: https://dx.doi.org/10.1016/j.cub.2014.03.034

11. Yang Y, Bazhin AV, Werner J, Karakhanova S. Reactive oxygen species in the immune system. Int Rev Immunol 2013; 32(3):249-270. doi: https://dx.doi.org/10.3109/08830185.2012.755176

12. Kruijsen-Jaarsma M, Révész D, Bierings MB, Buffart LM, Takken T. Effects of exercise on immune function in patients with cancer: a systematic review. Exerc Immunol Rev 2013; 19:120-143.

13. Zhu QN, Zhang D, Jin T, Wu Q, Liu J, Lu YF. Rutaecarpine effects on expression of hepatic phase-1, phase-2 metabolism and transporter genes as a basis of herb-drug interactions. J Ethnopharmacol 2013; 147(1):215-259. doi: https://dx.doi.org/10.1016/j.jep.2013.03.005

14. Nurkhasanah, Nurani LH, Hakim ZR, Effect of rosella (*Hibiscus sabdariffa* L.) extract on glutathione-S-transferase activity in rats. Trop J Pharm Res 2017; 16(10):2411-2416. http://dx.doi.org/10.4314/tjpr.v16i10.14

15. Dzoyem JP, Kuete V, Eloff JN. Biochemical parameters in toxicological studies in Africa: significance, principle of methods, data interpretation, and use in plant screenings. In: Toxicological survey of African medicinal plants. Elsevier, 2014:659-715. doi: https://dx.doi.org/10.1016/B978-0-12-800018-2.00023-6

16. Llauger L, Jacob J, Miró Ò. Renal function and acute heart failure outcome. Med Clin (Barc) 2018; 151(7):281-290. doi: https://dx.doi.org/10.1016/j.medcli.2018.05.010

17. Rodak BF, Keohane EM, Fritsma GA, Hematology: Clinical Principles and Applications. Elsevier Health Sciences, 2013; 152.

18. Fattizzo B, Michel M, Zaninoni A, Giannotta J, Guillet S, Frederiksen H et al. Efficacy of recombinant erythropoietin in autoimmune hemolytic

anemia: a multicenter international study. Haematologica 2021; 106(2):622-625. doi: https://dx.doi.org/10.3324/haematol.2020.250522

19. Harun N, Darmawan E, Nurani LH. Effect of calyx capsule-ethanol extract *Hibiscus sabdariffa* L. on renal function of healthy volunteers. IOP Conference Series: Materials Science and Engineering 2017; 259(1):1-6. doi: https://dx.doi.org/10.1088/1757-899X/259/1/012019

20. Kitagawa H, Munekage M, Ichikawa K, Fukudome I, Munekage E, Takezaki Y, et al. Pharmacokinetics of active components of Yokukansan, a traditional Japanese herbal medicine after a single oral administration to healthy Japanese volunteers: a cross-over, randomized study. PLOS ONE 2015; 10(7):1-14. https://doi.org/10.1371/journal.pone.0131165

21. Jager WD, Bourcier K, Rijkers GT, Prakken BJ, Margolis-Seyfert V. Prerequisites for cytokines measurements in clinical trials with multiplex immunoassays. BMC Immunol 2009; 10(52):1. doi: https://dx.doi.org/10.1186/1471-2172-10-52

22. Kong eng-Ming, Zhao L, Wang L, Chen L, Hu L, Fu X, et al. Ensuring sample quality for blood biomarker studies in clinical trials: a multicenter international study for plasma and serum sample preparation. Transl Lung Cancer 2017; 6(6):625-634. doi: https://dx.doi.org/10.21037/tlcr.2017.09.13

23. Tazoho GM, Gouado I, Ndomou M, Bonsi MT, Wamba YM, Agbor EE. Clinical, Hematological and biochemical health benefit effects of *Hibiscus sabdariffa* Lin dried calyces beverage in human. Food and Nutrition Sciences 2016; 7:383-395. http://dx.doi.org/10.4236/fns.2016.75040

24. Bzura J, Koncki R. A mechanized urease activity assay. Enzyme Microb Technol 2019; 123:1-7. doi: https://dx.doi.org/10.1016/j.enzmictec.2019.01.001

25. Rani J, Raju. Destimation of serum glutamic oxaloacetic transaminase, serum glutamic-pyruvic transaminase, gamma-glutamyl transferase and cholesterol levels in prolonged (30 years) daily consumption coffe in people. International Journal of Research Medical Sciences 2016; 4(5):1564-1573. doi: https://dx.doi.org/10.18203/2320-6012.IJRMS20161228

26. The Ministry of Health. Buku Pedoman Interpretasi Data Klinik (Guideline book on interpretation og clinical data). The Ministry of Health, 2011:8-71.

27. Dzierzak E, Philipsen S. Erythropoiesis: development and differentiation. Cold Spring Harbor M 2013; 3(4):1-17. doi: https://dx.doi.org/10.1101/cshperspect.a011601

28. 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 Nursing Journal 2017; 3(6):771-777.

29. Nova R, Hoemardani ASD, Louisa M. Potential of herbal medicines in cancer therapy. The Indonesia Journal of Cancer Control 2021, 1(1):32-42. doi: https://dx.doi.org/10.52830/inajcc.v1i1.44

30. Gordon S. Phagocytosis: An Immunobiologic Process. Immunity 2016; 44(3):463-475. doi: https://dx.doi.org/10.1016/j.immuni.2016.02.026

31. Flint SM, Jovanovic V, Teo BW, Mak A, Thumboo J, McKinney EF, et al. Leucocyte subset-specific type 1 interferon signatures in SLE and other immune-mediated diseases. RMD Open. 2016; 16;2(1):e000183. doi: https://dx.doi.org/10.1136/rmdopen-2015-000183

32. Groh IAM, Riva A, Braun D, Sutherland HG, Williams O, Bakuradze T, et al. Long-term consumption of anthocyanin-rich fruit juice: impact on gut microbiota and antioxidant markers in lymphocytes of healthy males. Antioxidants (Basel) 2020; 10(1):27. doi: https://dx.doi.org/10.3390/antiox10010027

33. Madaro L, Bouché M. From innate to adaptive immune response in muscular dystrophies and skeletal muscle regeneration: the role of lymphocytes. Biomed Res Int 2014; 2014:438675. doi: https://dx.doi.org/10.1155/2014/438675

34. Al-Mawali A, Pinto AD, Al Busaidi R, Al-Zakwani I. Lymphocyte subsets: reference ranges in an age- and gender-balanced population of Omani healthy adults. Cytometry A 2013; 83(8):739-44. doi: https://dx.doi.org/10.1002/cyto.a.22322

35. Ukoha UU, Mbagwu SI, Ndukwe GU, Obiagboso C. Histological and biochemical evalua-

tion of the kidney following chronic consumption of *Hibiscus sabdariffa*. Advances in Biology 2015; Article ID 486510:1-5. https://doi.org/10.1155/2015/486510

36. Riaz G, Chopra R. A review on phytochemistry and therapeutic uses of *Hibiscus sabdariffa* L. Biomed Pharmacother 2018; 102:575-586. https://doi/org/10.1016/j.biopha.2018.03.023