

Beetroot Extracts as Haematopoietic Agents on Rats

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ABSTRACT

Introduction: Beetroot (*Beta vulgaris* L.) contains flavonoid compounds that play a role in the haematopoietic process. It is known that methanol extract of beetroot has benefits in the process of haematopoiesis in normal white rats. **Aims:** To evaluate the beetroot extracts as hematopoietic agents on male rats. **Methods:** Beetroot dried powder was divided into two parts. One part was macerated separately with dichloromethane and 70% ethanol, while the other part was added with citric acid and washed with water to remove alkaloids and then extracted with 70% ethanol. The study used 24 rats which were divided into four groups. Each group consisted of 6 rats, namely the normal group, dichloromethane extract group, ethanolic extract group, and free alkaloids-ethanolic extract group. Each extract was given at a dose of 200 mg.Kg⁻¹ for 21 days. Analyzed blood parameters are erythrocytes, haemoglobin, MCV, MCH, MCHC, leukocytes, and platelets. The data obtained consisted of the number of cells analyzed using one-way ANOVA then obtained by the Tukey test. **Results:** This study showed a significant increase in the number of erythrocytes, haemoglobin, MCV, MCH, MCHC, leukocytes, and platelets in rats that were given each extract compared to the normal group ($p < 0.05$). The ethanolic extract of beetroot increased erythrocytes, haemoglobin, MCV, MCH, MCHC, leukocytes, and platelets by 41.49%, 24.95%, 14.92%, 33.54%, 27.19%, 59.40%, and 35.37%, respectively. **Conclusions:** The ethanolic extract of beetroot has the potential as a good natural haematopoietic agent.

Key words: Beetroots, Extract, Haematopoietic

INTRODUCTION

Haematopoiesis is the process of forming blood cells such as erythrocytes, leukocytes, and platelets. Haematopoiesis is an excellent model for studying the molecular mechanisms of cell control (Rieger and Schroeder, 2012). If hematopoiesis is disturbed, it can cause various problems related to blood component diseases, one of which is anaemia. Natural ingredients that are efficacious as antianaemia can be used to overcome the problem of anaemia, one of which is beetroot (*Beta vulgaris* L.) (Jaiswal *et al.*, 2014; (Hikmawanti *et al.*, 2021). Beetroot is widely used as a detoxifying agent for the liver, lowering blood pressure, cholesterol, and inflammation, overcoming menstrual problems, increasing stamina, and so on (Neha *et al.*, 2018).

Beetroot contains vitamins, carbohydrates (6.99%), protein (1.35%), fat, and oils (0.3%) which are useful for health. It also contains minerals such as Iron (Fe), Sodium (Na), Zinc (Zn), Calcium (Ca), Potassium (K), Magnesium (Mg), and Phosphorus (P). Red beetroot contains vitamin A, C, E, K, and B. Beetroot contains secondary metabolite compounds such as tannins (6.055 mg/100g), alkaloids (128.90mg/100g), flavonoids (6.417mg/100g), glycosides (0.652 mg/100g), and saponins (3.780 mg/100g) (Odoh and Okoro, 2013).

The methanol extract of beetroot has benefits in the process of haematopoiesis in normal white rats. This extract significantly increases the levels of haemoglobin, erythrocytes, platelets, Mean Corpuscular Volume (MCV),

Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), and Packed Cell Volume (PCV) at a dose of 400 mg.Kg⁻¹ BW compared to normal control (Indhumathi and Kannikaparameswari, 2012). The 96% ethanol extract of beetroot at a dose of 200 mg.Kg⁻¹ effectively increased the levels of haemoglobin and erythrocytes in white rats induced by phenylhydrazine (Jaiswal *et al.*, 2014).

Flavonoids have a protective effect against erythrocytes in anaemic conditions (Mazhar *et al.*, 2017). Tannins can form complexes with protein, starch, and digestive enzymes. However, this matter causes a decrease in the nutritional value of the food. Thus, tannins are often considered undesirable nutrients. Tannins could cause protein disruption in the body that can cause decreased absorption of iron in the body, which will then affect the levels of haemoglobin (Chung *et al.*, 1998; Pratiwi and Widari 2018); Benouadah *et al.*, (2016) reported that alkaloids could reduce levels of erythrocytes, haemoglobin, and hematocrit by interfering with the process of erythropoiesis and destruction of blood cells.

Obtaining metabolites in plants during the extraction process is influenced by the extraction procedure itself. In this research, the extraction procedure was modified by selecting solvents with different polarities. Nevertheless, these solvents are still able to attract flavonoids and iron as compounds that are thought to play a role in the process of hematopoiesis. The difference in chemical content in each extract produced different haematopoietic activities. Thus, through this research, it is known that beetroot extract has the most potential as a natural haematopoietic agent in normal rats.

MATERIALS AND METHODS

Collection of plant material

Fresh Beetroots were obtained from a farm in Lembang, West Java, Indonesia. The plants were harvested at the age of 2-3 months. The plant was authenticated in Herbarium Bogoriense, Biology Research Center, Indonesian Institute of Sciences, Cibinong, Indonesia.

Preparation of the extracts

The plant material was air-dried at room temperature. The dried beetroots were ground to powder. Afterward, the dried powder of beetroot was divided into two parts. The first part, namely a beetroot powder (820.0g) was extracted

separately using 70% ethanol or dichloromethane for 24h in a macerator to obtain filtrates. The filtrates are then referred to as ethanolic extract of Beetroots (EEBR) and dichloromethane extract of Beetroots (DEBR), respectively. Whereas, the second part was alkaloids free-beetroot powder. The procedure was performed as in Widiyanti *et al.*, (2016) with modifications. This powder (820.0 g) was prepared by adding a citric acid solution so that the alkaloids were converted into water-soluble salts and washed with water. The residue was then extracted using 70% ethanol for 24 hours in a macerator. The filtrate is then referred to as alkaloids free-ethanolic extract of Beetroots (AF-EEBR). The residue was re-macerated two times. Then, each filtrate was evaporated using a vacuum rotary evaporator N-1200 BS Series (EYELA, Shanghai, China) at 50°C.

Physicochemical evaluation

Physicochemical characteristics of each extract, such as organoleptic, percentage of extraction yield, total-ash content, and loss on drying were assessed according to the Indonesian Herb Pharmacopoeia (Ministry of Health Republic of Indonesia, 2008) and WHO guidelines (World Health Organization, 1998).

Phytochemical screening of the extracts

Secondary metabolites such as alkaloid, phenolic, flavonoid, triterpenoids, steroids and saponin in the extracts were identified qualitatively using standard analytical procedures with slight modification. The chemical materials used were Dragendorff and Bouchardat reagents for alkaloids detection; Folin-ciocalteu reagent for phenolics detection, AlCl₃ reagent for flavonoids detection; Liebermann-Burchard reagent for triterpenoids/steroids detection, and gelatine 10% reagent for tannins detection (Hanani, 2015; Ministry of Health Republic of Indonesia, 2008)

Preparation of animals

The experimental design was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia, with ethical approval number: KET-552/UN2.F1/ETIK/PPM.00.02/2019. The design used included Randomized Design. Twenty-five male *Sprague-Dawley* rats aged 2-3 months around 150-250g were obtained from Research Animal Breeder, Bekasi. The animals were divided into four groups, where each group consists of 6 animals.

Table I. Physicochemical properties of beetroot extracts

No.	Parameters	Samples		
		DEBR	EEBR	AF-EEBR
1	Organoleptic			
	a. Form	Thick	Thick	Thick
	b. Odour	Specific	Specific	Specific
	c. Colour	Reddish-brown	Reddish-brown	Reddish-brown
2	Extraction yield (w/w)	1.97%	34.52%	24.55%
3	Total ash (w/w)	2.16%	9.66%	9.75%
4	Loss of drying (w/w)	2.48%	9.89%	10.27%

Note: DEBR = Dichloromethane Extract of Beetroots; EEBR = Ethanolic Extract of Beetroots; AF-EEBR = Alkaloids Free-Ethanolic Extract of Beetroots

Table II. Phytochemical screening of beetroot extracts

No.	Compounds	Samples		
		DEBR	EEBR	AF-EEBR
1	Alkaloids	+	+	-
2	Phenolics	+	+	+
3	Flavonoids	+	+	+
4	Tannins	-	+	+
5	Saponins	-	+	+
6	Steroids	-	-	-
7	Triterpenoids	-	-	-

Note: (+) = detected; (-) = not detected

Before treatment, the animals were acclimatized for seven days. At this stage, the animals received a standard drink and feed *ad libitum*.

Experimental design

Animals were grouped into (1) Normal Control Group: No treatment; (2) Extract Group I: received DEBR; (3) Extract Group II: received EEBR; (4) Extract Group III: received AF-EEBR. Each extract was given at a dose of 200 mg.Kg⁻¹ BW once a day orally for 21 days. On the 22nd day, animals were injected with ketamine intramuscularly at a dose of 40 mg.Kg⁻¹ BW. The blood sample was taken through the eye's orbital sinus and collected in the EDTA tube.

Haematological analysis

Blood tests were carried out at the Center for Primate Animal Studies (Pusat Studi Satwa Primata/PSSP) IPB, Bogor. The examination of erythrocyte, haemoglobin, platelet, leukocyte, MCV, MCH, and MCHC levels in rat blood was carried out using Hematology Analyzer (Nikon Kohden MEK-6450, Tokyo, Japan).

Data analysis

The blood data were analyzed with one-way Analysis of Variance (ANOVA) with a significance level of 95% ($\alpha=0.05$). The analysis continued with the Tukey test.

RESULTS AND DISCUSSION

Extraction yields and extracted compounds

The research of natural ingredients is generally started by extraction procedures. The selection of solvents in the extraction procedure is important to produce target compounds that contribute to pharmacological activities. Ethanolic extract (EEBR) produced the highest percentage of active compounds compared to other extracts (EEBR>AF-EEBR>DEBR) (Table I).

Phytochemical screening results of beetroot extracts can be seen in **Table 2**. Ethanolic solvent has many advantages such as easy to evaporate, low toxicity, and able to extract polyphenol groups better than water. It can extract aglycones such as flavonoids and alkaloids, and also glycosides (Tiwari *et al.*, 2011) Dichloromethane is included in solvents with medium polarity.

Table III. Blood parameters of rats after treated with beetroot extracts

Groups	Blood Parameters						
	Erythrocyte (10 ⁶ /μL)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Leukocyte (10 ³ /μL)	Platelets (10 ³ /μL)
Normal	6.09±0.29 ^a	12.18±0.46 ^a	51.9±0.26 ^a	14.07±0.27 ^a	22.63±0.41 ^a	5.25±0.42 ^a	577.5 ±38.23 ^a
DEBR	7.22±0.22 ^b	12.93±0.48 ^b	54.25±0.36 ^b	15.57±0.53 ^b	25.12±0.47 ^b	8.00±0.36 ^b	641.5 ±38.34 ^a
EEBR	10.41±0.25 ^c	16.23±0.42 ^c	61.00±0.40 ^c	21.17±0.38 ^c	31.08±0.81 ^c	12.93±0.36 ^c	893.5 ±37.42 ^b
AF-EEBR	9.26±0.32 ^d	14.95±0.19 ^d	58.93±0.56 ^d	19.03±0.26 ^d	29.05±0.55 ^d	11.1±0.26 ^d	783.5 ±65.23 ^c
Normal Value*	7.80-9.91	14.50-18.20	48.00-55.60	16.60-23.30	33.40-41.90	3.78-11.75	178.0-940.0

Note: Different letters in the same column indicate differences with significant ($\alpha = 0.05$). * Hematology profile of 10-week-old male Sprague-Dawley rats (Rosidah et al., 2020).

The polarity of these compounds is influenced by the electronegativity of Cl atoms in their structures. These solvents can extract alkaloids, flavonoid aglycones, and volatile compounds (Houghton & Raman, 1998).

Haematopoietic activity

Flavonoid and iron contents in extracts may be related to the haematopoietic activity of the beetroot extracts. According to Hikmawanti *et al.*, (2021), dichloromethane extract, alkaloid-free ethanol extract, and crude ethanol extract contained different amounts of flavonoids and iron (crude ethanol extract > alkaloid-free ethanol extract > dichloromethane extract). This is related to the polarity of the solvent used for the extraction and also the extraction process. The 70% ethanol extract is able to extract flavonoids and iron which is good from beetroot. The weak acidification process of the beetroot dried powder affects the level of flavonoid and iron levels of beetroot in the final extract product, which is thought to have been lost during the alkaloid removal process (Table III).

Red blood cells contain a protein called hemoglobin (Hb), which is expressed in grams per deciliter (g/dl). This protein is responsible for delivering oxygen to the tissues. This protein needs to be maintained at a certain level so that oxygen need by the tissues remains fulfilled. If the Hb is at a low level, it will result in a condition called anemia (Billett, 1990). Flavonoids are polyphenol active compounds that act as antioxidants that can increase erythropoiesis (the formation of erythrocytes) in the bone marrow and have an immunostimulatory effect (Sundaryono, 2011). These antioxidant properties can maintain heme-iron remains in the form of Ferro associated with the production of methaemoglobin. With the presence of flavonoids when ferric-haemoglobin is

formed, it is estimated that they can prevent half of the oxyhaemoglobin molecules from being oxidized to met-Hb. Thus, haemoglobin can still function to bind oxygen because it is still present in the form of oxyhaemoglobin (Gebicka & Banasiak, 2009).

Leukocytes are a heterogenous group of blood cells that play a role in the immune system in fighting infection by pathogenic microorganisms (Blumenreich, 1990). Flavonoids in dates were able to increase immune cells in mice (Cuevas *et al.*, 2013). In the small intestine, there are flavonoid receptor receptors called Toll-Like Receptors (TLRs) receptors located in the small intestinal epithelial cells (Pérez-Cano *et al.*, 2014) Flavonoids in the Ajwa date palm extract are carried to the bone marrow to modulate through the process of haematopoietic stem cell proliferation and differentiation to form each type of leukocytes.

Platelets are an important component in the hemostasis response and are a fragmentation of the cytoplasmic cytoplasm. Flavonoids can work synergistically in increasing platelet counts. Flavonoids have increased platelet activity through the mechanism of stimulation against Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and Interleukin-3 (IL-3). GM-CSF and IL-3 are hormones that function to trigger the formation of megacocyte cells (Prasetyaningsih *et al.*, 2019).

A hematology analyzer tool automatically calculates blood profile values in the form of erythrocyte, leukocyte, hemoglobin, platelets, and is also able to calculate the values of MCV, MCH, and MCHC in one run. MCV is a value (in femtoliters, fl or cubic micron, μm^3) that indicates the size of the red blood cells. MCH is a value (in picograms per cell, pg/cell) that shows the amount of hemoglobin in each red blood cell, while MCHC is a value (expressed as g/dl or percent, %) which shows the relationship between hemoglobin levels and red

blood cell volume. Thus, if the administration of the test material has an effect on the values of hemoglobin, red blood cells, and MCV, then MCH and MCHC will also be affected (Sarma, 1990). In this study, when erythrocytes and hemoglobin increased, there was also a significant increase in MCV, MCH, and MCHC when compared to the normal group of mice.

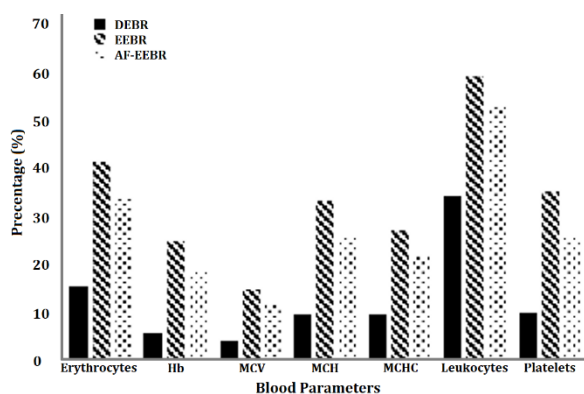


Figure 1. The percentage increase in blood parameters after rats were treated with beetroot extracts

According to (Al-Khazraji, 2018), increasing the dose of beetroot ethanol extract (from 200 to 1600 mg.Kg⁻¹ body weight) was able to affect the hematological profile in Swiss albino mice after treatment for sixteen days. In this study, different rat strains (Sprague-Dawley) were treated using three types of beetroot extract (EEBR, AF-EEBR, and DE) at a dose of 200 mg.Kg⁻¹ body weight. The results showed lower blood profile values due to longer treatment for 21 days. These three types of extract (EEBR>AF-EEBR>DE) are able to improve all blood profiles (except platelets) compared to normal controls (Figure 1.). Previously, it was suspected that tannins and alkaloids in beetroot would cause disturbance to the hematopoietic activity. The weakness of this study is that the total alkaloid and tannin levels were not determined in each extract. However, this research proved that the two compounds (alkaloids and tannins) did not significantly influence the hematopoietic activity of beetroot ethanolic extract.

CONCLUSION

Based on the results of the study, ethanolic extract of beetroot has the potential as a good natural haematopoietic agent.

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REFERENCES

- Al-Khazraji, S. M. (2018). Hemopoietic activity of the beetroot ethanolic extract of beta Vulgaris (Shamandar) in albino rats. *Journal of Global Pharma Technology*, 10(3), 16–20.
- Benouadah, Z., Mahdeb, N., & Bouzidi, A. (2016). Evaluation of acute and sub-acute toxicity of alkaloids from *Datura stramonium* sp. In mice. *International Journal of Pharmacognosy and Phytochemical Research*, 8(11), 1759–1766.
- Billett, H. H. (1990). Hemoglobin and Hematocrit. In H. K. Walker, W. D. Hall, & J. W. Hurst (Eds.), *Clinical Methods: The History, Physical, and Laboratory Examinations* (3rd ed.). Retrieved from <https://www.ncbi.nlm.nih.gov/books/NBK259/>
- Blumenreich, M. S. (1990). The White Blood Cell and Differential Count. In H. K. Walker, W. D. Hall, & J. W. Hurst (Eds.), *Clinical Methods: The History, Physical, and Laboratory Examinations* (3rd ed.). Retrieved from <https://www.ncbi.nlm.nih.gov/books/NBK261/>
- Chung, K.-T., Wong, T.-Y., Wei, C.-I., Huang, Y.-W., & Lin, Y. (1998). Tannins and human health: A review. *Critical Reviews in Food Science and Nutrition*, 38(6), 421–464.
- Cuevas, A., Saavedra, N., Salazar, L. A., & Abdalla, D. S. P. (2013). Modulation of immune function by polyphenols: Possible contribution of epigenetic factors. *Nutrients*, 5(7), 2314–2332.
- Gebicka, L., & Banasiak, E. (2009). Flavonoids as reductants of ferryl hemoglobin. *Acta Biochimica Polonica*, 56(3), 509–513.
- Hanani, E. (2015). *Analisis Fitokimia (In Bahasa)*. Jakarta: Buku Kedokteran EGC, pp: 69, 83, 114-115, 149, 202, 232-233
- Hikmawanti, N. P. E., Dwita, L. P., Wisnunanda, D. W., & Farista, F. (2021). The Effect of Different Extracts of Beetroots as Antioxidant and Anti-Anaemia On Phenylhydrazine-Induced Rats. *European Pharmaceutical Journal*, [in press]. <https://doi.org/10.2478/afpuc-2020-0014>
- Houghton, P. J., & Raman, A. (1998). *Laboratory Handbook for the Fractionation of Natural*

- Extracts. London: Springer Sciences Bussines Media.
- Indhumathi, T., & Kannikaparameswari, K. (2012). Hematopoietic Study of the Methanolic Root Extract of Beta Vulgaris on Albino Rats - an in Vivo Study. *International Journal of Pharma and Bio Sciences*, 3(4), 1005–1015.
- Jaiswal, A., Ganeshpurkar, A., Awasthi, A., Bansal, D., & Dubey, N. (2014). Protective effects of beetroot extract against phenyl hydrazine induced anemia in rats. *Pharmacognosy Journal*, 6(5), 1–4. <https://doi.org/10.5530/pj.2014.5.1>
- Mazhar, M., Faizi, S., Gul, A., Kabir, N., & Simjee, S. U. (2017). Effects of naturally occurring flavonoids on ferroportin expression in the spleen in iron deficiency anemia: In vivo. *RSC Advances*, 7(38), 23238–23245.
- Ministry of Health Republic of Indonesia. (2008). *Farmakope Herbal Indonesia (Indonesian Herb Pharmacopoeia)* (1st Ed.). Jakarta: Ministry of Health Republic of Indonesia, pp: 180-183.
- Neha, P., Jain, S. K., Jain, N. K., Jain, H. K., & Mittal, H. K. (2018). Chemical and functional properties of Beetroot (Beta vulgaris L.) for product development: A review. *International Journal of Chemical Studies*, 6(3), 3190–3194.
- Odoh, U. E., & Okoro, E. C. (2013). Quantitative phytochemical, proximate/nutritive composition analysis of beta vulgaris linnaeus (chenopodiaceae). *International Journal of Current Research*, 5(12), 3723–3728.
- Pérez-Cano, F. J., Massot-Cladera, M., Rodríguez-Lagunas, M. J., & Castell, M. (2014). Flavonoids affect host-microbiota crosstalk through TLR modulation. *Antioxidants*, 3(4), 649–670.
- Prasetyaningsih, Y. ., Sari, N. ., Prasetya, H. R. ., & Wulandari, R. (2019). Potensi Etnomedicine Daun Ubi Jalar Ungu (Ipomoea batatas L. Poir) sebagai Obat Demam Berdarah di Sleman. *Journal of Health*, 6(1), 1–7.
- Pratiwi, R., & Widari, D. (2018). Hubungan Konsumsi Sumber Pangan Enhancer Dan Inhibitor Zat Besi Dengan Kejadian Anemia Pada Ibu Hamil (In Bahasa). *Amerta Nutrition*, 2(3), 283.
- Rieger, M. A. ., & Schroeder, T. (2012). Hematopoiesis. *Cold Spring Harb Perspect Biology*, 4, 1–7.
- Rosidah, I., Ningsih, S., Renggani, T. N., Agustini, K., & Efendi, J. (2020). Profil Hematologi Tikus (Rattus norvegicus) Galur Sparague-Dawley Jantan Umur 7 dan 10 Minggu. *Jurnal Bioteknologi & Biosains Indonesia*, 7(1), 136–145.
- Sarma, P. . (1990). Red Cell indices. In H. K. Walker, W. D. Hall, & J. W. Hurst (Eds.), *Clinical Methods: The History, Physical, and Laboratory Examinations* (3rd ed.). Retrieved from <https://www.ncbi.nlm.nih.gov/books/NBK260/>
- Sundaryono, A. (2011). Uji Aktivitas Senyawa Flavonoid Total Dari Gynura Segetum (Lour) Terhadap Peningkatan Eritrosit Dan Penurunan Leukosit Pada Mencit (Mus Musculus). *Jurnal Exacta*, 9(2), 8–16.
- Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Scientia*, 1(1), 98–106.
- Widiyanti, P., Prajogo, B., & Hikmawanti, N. P. E. (2016). Cytotoxicity of Justicia Gendarussa Burm F. Leaf Extracts on Molt-4 Cell. *Indonesian Journal of Tropical and Infectious Disease*, 6(1), 24–28.
- World Health Organization. (1998). *Quality control methods for medicinal plant materials*. Geneva, Switzerland: World Health Organization.