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SURAT TUGAS

NOMOR: 127 /F.03.01/2021

Pimpinan Fakultas Farmasi dan Sains, Universitas Muhammadiyah Prof. DR. Hamka dengan ini memberi tugas kepada :

Nama	:	Ni Putu Ermi Hikmawanti, M.Farm.
Jabatan	:	Dosen FFS UHAMKA
Alamat	:	Islamic Center Jl. Delima Raya II/ IV, Perumnas Klender – Jakarta Timur
Tugas	:	Sebagai Penulis pada **Indonesian Journal of Pharmacy**: "**Beetroot Extracts as Haematopoietic Agents on Rats**"
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Lain-lain	:	Setelah melaksanakan tugas agar memberikan laporan kepada Dekan atau sama yang memberi tugas.

Demikian surat tugas ini diberikan untuk dilaksanakan dengan sebaik–baiknya sebagai amanah dan ibadah kepada Allah Subhanahu Wata`ala

Jakarta, 05 Maret 2021

Dekan,

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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, $AlCl_3$ 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% (α=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 (α =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 hematopoietic 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 hematopoietic 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 picnograms 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.

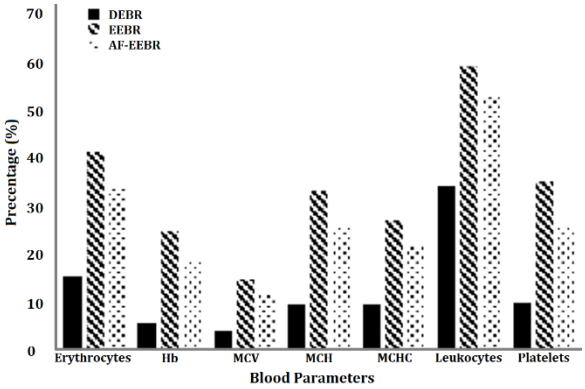

Figure 1. The percentage increase in blood parameters after rats were treated with beetroot extracts

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 Jpurnal 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 Sciencia*, *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.

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Bismillahirrohmanirrohiim,
Yang bertanda tangan di bawah ini

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Jabatan	Dekan
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Memberikan tugas Penelitian dan Publikasi pada **tahun akademik 2021/2022** kepada:

N a m a	**Ni Putu Ermi Hikmawanti, M.Farm.**
NID/NIDN	D.15.1005/ 0309078901
Pangkat /Jabatan Akademik	Penata Muda Tingkat I/ III-B
Jabatan Fungsional	ASISTEN AHLI
Unit Kerja	Fakultas Farmasi dan Sains UHAMKA Jakarta

Untuk Melaksanakan Penelitian dan Publikasi sebagai berikut:

NO	JUDUL PENELITIAN DAN PUBLIKASI
1.	**The Effect of Pre-Extraction Preparation on Antioxidant Compounds of Sauropus androgynus (L.) Merr. Leaves Extracts"**

Demikian surat tugas ini diberikan kepada yang bersangkutan untuk dilaksanakan dengan penuh amanah dan tanggung jawab

Jakarta, 05 September 2021
Dekan,

Dr. apt. Hadi Sunaryo, M.Si.

Tembusan Yth:
1. Rektor UHAMKA Jakarta
2. Wakil Rektor I dan II UHAMKA Jakarta
3. Arsip

Original Article

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The Effect of Pre-Extraction Preparation on Antioxidant Compounds of *Sauropus androgynus* (L.) Merr. Leaves Extracts

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ABSTRACT

Sauropus androgynus (L.) Merr. (Phyllanthaceae) is a green vegetable. It is rich in natural antioxidant compounds such as phenolics and flavonoids. The pre-extraction preparation can affect the results of extracting compounds from natural materials. This study aims to determine the effect of pre-extraction procedures, including sample form (fresh/dried), drying process (oven-drying/indirect sunlight-drying/air-drying), and particle size (ground/powdered) on total phenolic and flavonoid levels of ethanolic extracts of *S. androgynus* leaves and their antioxidant activity against DPPH radicals. *S. androgynus* leaves were extracted using the cold maceration method. The total phenolic and flavonoid contents of the extract were determined by the colorimetric method using a UV-Vis spectrophotometer. Antioxidant activity was measured based on the ability of the extracts to reduce DPPH free radicals. The results showed that the pre-extraction preparation influenced the antioxidant components obtained in *S. androgynus* leaves. According to sample form, fresh leaves contained a higher phenolics and flavonoids (33.66 mg GAE/g and 11.61 mg QE/g, respectively) than dried leaves. Whereas according to particle size, powdered dried leaves have higher phenolics and flavonoids content (45.12 mg GAE/g and 12.54 mg QE/g, respectively) than ground dried leaves. (45.12 mg GAE/g and 12.54 mg QE/g, respectively) than ground dried leaves. The leaves drying method that produced the highest phenolic content was oven-drying (47.08 mg GAE/g), while the one with the highest flavonoid content was air-drying (8.87 mg QE/g). All extracts had IC_{50} values against DPPH radicals around 85.71-93.91 ppm. Pre-extraction preparation in optimum condition makes the extraction process more efficient and effective in obtaining the target compounds with antioxidant activities.

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INTRODUCTION

Substances used in small concentrations to inhibit or reduce oxidation caused by an oxidant are known as antioxidants. One of the mechanisms of action of antioxidants to deactivate radicals is donating hydrogen atoms to free radicals to form a more stable new radicals (Singh & Singh, 2008). Plants are a source of natural ingredients with natural antioxidant potential include phenolic compounds such as phenolic acids (ferulic acid, gallic acid, ellagic acid), flavonoids (quercetin, catechins, hesperidin), tannins, anthocyanins, lignin, carotenoids (beta-carotene, lycopene, lutein), vitamins (vitamins A, C, E), etc. (Moharram & Youssef, 2014; Altemimi et al., 2017).

Green vegetables, such as *Sauropus androgynus* (L.) Merr, are a great source of antioxidants (Andarwulan et al., 2010). This plant, in Indonesia, is known as "katuk". Empirically, *S. androgynus* has been used for antipyretic, the simple evacuation of a retained placenta after childbirth, increasing and accelerating breast milk supply for breastfeeding mother, etc. (Petrus, 2013). The antioxidant capacity of *S. androgynus* leaves is related to its various pharmacological activities has been reported, such as antimicrobial, cytotoxic against cancer cells, anti-inflammatory, and wound healing (Petrus, 2013; Khoo et al., 2015). The pharmacological activity of *S. androgynus* is related to its secondary metabolites. The leaves of *S. androgynus* contain bioactive compounds such as phenolics, tannins, flavonoids, anthocyanins, phytosterols, etc. (Petrus, 2013). Wijono (2004) reported that the phenolic compounds detected in *S. androgynus* leaves were phenolic acid, such as para-hydroxy benzoic acid, ferulic acid, caffeic acid, and vanillic acid. Djamil & Zaidan (2017) reported that the methanol extract of *S. androgynus* leaves contained flavonoids of flavonols and flavones.

The first step in studying the chemical contents of plants is to prepare plant samples for the following extraction process (Azwanida, 2015). Preparation for extraction of phenolic compounds also depends on the sample matrix and the chemical properties of the phenolic compounds (Khoddami et al., 2013). Plant samples can be fresh or dry from the whole plant or parts of plant materials such as leaves, stems, roots, etc. The dried form of the plant sample is preferred because it is more durable

(not easily damaged) and more accessible to be stored than the fresh form. In contrast, the fresh plant material may still contain more chemical compounds than the dried form. Several simple techniques for drying plant material include air-drying, oven-drying, freeze-drying (Khoddami et al., 2013), or indirect sunlight drying. Depending on the part of the plant that has to be dried, air-drying may take 3-7 days or even months. Oven-drying is a faster drying process than the air-drying and indirect-sunlight drying methods (Azwanida, 2015).

Another preparation procedure for extraction is using ground or powdered materials. Ideally, a small and homogenized sample particle size will make the extraction process runs more effectively and efficiently. It is due to the high contact between the sample and the extracting solvent. Resizing of sample particles can be done using a mortar and pestle or an electric blender (Azwanida, 2015).

This study aimed to evaluate the total phenolic and flavonoid contents in the ethanolic extracts of *S. androgynus* leaves. The leaves of *S. androgynus* were prepared under various pre-extraction conditions. In addition, the antioxidant activity of the extracts against DPPH radicals was also determined.

METHODS

Materials

Fresh green leaves of *S. androgynus* were harvested in May 2019 from Unit Konservasi Budidaya Biofarmaka (UKBB), Pusat Studi Biofarmaka Tropika LPPM IPB, Bogor, West Java, Indonesia. Plant determination was performed in the same place with the specimen number BMK0168092016. The specimen was deposited in the Laboratory of Pharmacognosy, Faculty of Pharmacy and Sciences, Universitas Muhammadiyah Prof. DR. HAMKA, Jakarta. Ethanol as solvent extraction in analytical grade was obtained from Merck (Darmstadt, Germany). Gallic acid, quercetin, and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) were obtained from Sigma-Aldrich Co. (St. Louis, USA).

Preparation of Samples

The pre-extraction conditions of *S. androgynus* leaves were classified into three groups. They were given the following information:

1. The first group of samples (G1) were treated differently depending on their form, such as (a) fresh and (b) dried leaves. The fresh leaves were crushed in a mortar (diameter 160 mm). The dried leaves were obtained by drying the leaves using the air-drying method. The dried leaves were then ground using an electric blender.
2. The second group of samples (G2) was treated based on the drying process. The leaves were dried by: (a) oven-drying using a vacuum oven at a maximum temperature of 50 °C for 1-2 days; (b) indirect sunlight by exposing the samples to the sun with a black cloth covered at 32-34 °C for three days; and (c) air-drying indoors with good air circulation at room temperature (25-27 °C) for three days. The method to measure the drying temperature was using the digital room thermometer.
3. The third group of samples (G3) was treated based on particle size. Dried leaves obtained from the drying process using the air-drying method were divided into two groups: (a) ground samples, the sample was chopped in the mortar without sieving; (b) powdered sample, the dried sample was blended and then sieved with a sieve Mesh number 40 so that the particle size is uniform with a size of 425 µm (slightly coarse powder) (Ministry of Health Republic of Indonesia, 2008).

Extraction

Each sample of *S. androgynus* leaves (150.0 g) was extracted by cold maceration using 70% ethanol as the extracting solvent following the procedure in Indonesian Herb Pharmacopoeia. Each extraction process was carried out for 24 hours with stirred occasionally. The filtrate was separated from the residue using Whatman filter paper. The residue was macerated three times. The filtrate from each solvent was collected and evaporated using a vacuum rotary evaporator N-1200 BS series (EYELA, Shanghai, China) at 50 °C and continued using a water bath at 50 °C until a crude extract was obtained (Ministry of Health Republic of Indonesia, 2008).

Characterization of the Extract

The characterization parameters of *S. androgynus* leaves extract were determined, including the organoleptic, percentage of extract yield, and total ash content according to the procedure stated in the Indonesian Herb Pharmacopoeia (Ministry of Health Republic of Indonesia, 2008). The water content was determined using a Moisture Balance Analyzer.

Phytochemical Screening

The compounds of the extracts are identified qualitatively according to Hanani (2015) and Indonesian Herb Pharmacopoeia (Ministry of Health Republic of Indonesia, 2008). The phenolics were identified using 3% $FeCl_3$ reagent, flavonoids using $AlCl_3$ reagent, and tannins using 10% gelatine reagent. The alkaloids were identified using Dragendorff, Mayer, Wagner, and Bouchardat reagents. The steroids/triterpenoids were identified using Liebermann Burchard and Salkowski reagents. Identification of saponins was carried out by frothing testing.

Determination of Total Phenolic Content (TPC)

TPC determination followed a procedure Hikmawanti et al. (2020) with modification using Folin-Ciocalteu reagent (FCR) and gallic acid as standard. Ethanolic extract of *S. androgynus* leaves in ethanol solution was piped as much as 300 µL and then added with 1.5 mL of FCR (1:10) and homogenized. After incubated for 3 min, then 1.2 mL of 7.5% Na_2CO_3 solution was added. Finally, distilled water was added to the mixture to obtain a total volume of 10 mL. The mixture was incubated again for 60 min at room temperature. The absorbance of the solution was measured using a UV-Vis Shimadzu UV-1601 Series (Kyoto, Japan) spectrophotometer with a maximum wavelength of 756.5 nm at room temperature. The maximum wavelength was obtained through optimization using gallic acid (30 µg/mL) in the range of 600-800 nm. The absorbance obtained is plotted into the linear regression equation. TPC is expressed in mg, which is equivalent to gallic acid per g of extract. Each extract was tested for five replication and reported as mean ± SD.

Determination of Total Flavonoid Content (TFC)

The determination of TFC followed the procedure of Chang et al. (2002) with modification. The standard used is quercetin. Ethanolic extract of *S. androgynus* leaves in methanol solution was piped as much as 0.5 mL. Then, 1.5 mL of methanol, 0.1 mL of $AlCl_3$ 10%, and 0.1 mL of 1 M sodium acetate were added. Finally, distilled water was added up to 5 mL. The solution was incubated for 60 min at room temperature. The absorbance of the solution was measured with a UV-Vis Shimadzu UV-1601 Series (Kyoto, Japan) spectrophotometer at a wavelength of 434 nm. The absorbance obtained is plotted into the linear regression equation. TFC is expressed in mg, which is equivalent to quercetin per g of extract. Each extract was tested for five replication and reported as mean ± SD.

Antioxidant Activity Assay

The test for the antioxidant activity of *S. androgynus* leaves extract was carried out using the DPPH method following the procedure Wan et al. (2011) with modification. Quercetin was used as a reference. The extract was dissolved in methanol and diluted into five concentrations (20-100 ppm). Quercetin solution was dissolved in methanol and diluted to 2-10 ppm. Each concentration of the extract or quercetin solution was pipette 1 mL and added 1 mL of 0.5 mM fresh DPPH in methanol. The solution was added with methanol to a total volume of 5 mL. The mixture in a tube covered with aluminium foil paper, incubated for 30 minutes at room temperature in dark conditions. The blank solution was made from 1 mL DPPH mixed with 4 mL methanol. Furthermore, the absorption was measured at a wavelength of 515.5 nm. Each concentration series of each sample was tested triplicate. Results are reported as mean ± SD. The calculation of the percentage of DPPH radical inhibition uses the formula:

$$\text{Inhibition of DPPH (\%)} = \frac{\text{Ab} - \text{As}}{\text{Ab}} \text{ x } 100$$

where Ab is the absorbance of the blank and As is the absorbance of the sample.

RESULTS AND DISCUSSION

The characterization of *S. androgynus* leaves extracts from various pre-extraction conditions can be seen in Table 1. The yield of extract prepared from the dry leaves (35.34%) was higher than the fresh leaves. The yield of extract prepared from the air-drying process (36.26%) showed the highest yield compared to other drying processes (air-drying > oven-drying > indirect sunlight-drying). Extracts produced from powdered (homogenized particle size) leaves had a greater extract yield (36.06%) than those made from ground leaves.

Table 1. The characteristics of *S. androgynus* leaves ethanolic extracts in various pre-extraction conditions.

Pre-extraction conditions	Percentage of extraction yield (%, w/w)	Total ash (%, w/w)	Water content (%, w/w)
Sample Form			
Fresh	10.75 ± 0.60	7.37 ± 0.74	3.81 ± 0.44
Dried	35.34 ± 3.09	6.76 ± 0.79	2.08 ± 0.65
Drying Process			
Oven-drying	31.25 ± 2.41	7.39 ± 1.21	2.55 ± 1.59
Indirect sunlight-drying	25.39 ± 2.21	7.00 ± 1.40	1.75 ± 0.75
Air-drying	36.26 ± 3.38	7.14 ± 1.08	1.97 ± 0.77
Particle Size			
Grinded	35.99 ± 3.41	7.51 ± 1.07	1.88 ± 0.71
Powdered	36.06 ± 0.75	7.94 ± 1.30	1.97 ± 0.56

Note: The test of each extract was carried out five times and reported in mean ± SD.

Table 2. The phytochemical screening of *S. androgynus* leaves ethanolic extracts in various pre-extraction conditions.

Pre-extraction conditions	Compounds of *S. androgynus* Leaves Ethanolic Extracts						
	Alkaloids	Phenolics	Flavonoids	Tannins	Saponins	Steroids	Triterpenoids
Sample form							
Fresh	+	+	+	+	+	+	-
Dried	+	+	+	+	+	+	-
Drying Process							
Oven-drying	+	+	+	+	+	+	-
Indirect sunlight-drying	+	+	+	+	+	+	-
Air-drying	+	+	+	+	+	+	-
Particle Size							
Grinded	+	+	+	+	+	+	-
Powdered	+	+	+	+	+	+	-

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

Based on the results in Table 1, the ash content produced by all extracts was around 6.76-7.94%. Ash content is determined to provide an overview of mineral content derived from plants or from outside of plants (Departemen Kesehatan RI, 2000). The water content of each extract was 1.75-3.81%. The percentage of water content is determined to provide an overview of the maximum limit for water contained in the extract, especially for materials that absorb moisture quickly. The high water content in an extract is a good condition for microbes to grow (World Health Organization, 1998). Determination using a moisture analyzer is an efficient way of measuring with many samples in a short analysis time.

All extracts of *S. androgynus* leaves showed the presence of alkaloid, phenolic, flavonoid, tannin, saponin, and steroid compounds (Table 2). Ethanol is a solvent capable of dissolving polyphenols, tannins, polyacetylene, flavonols, terpenoids, sterols, and alkaloids (Azmir et al., 2013; Pandey & Tripathi, 2014). Simple phenolic compounds, polyphenols, and flavonoids are generally extracted with alcohol (methanol, ethanol, and propanol) solvents, water, acetone, and ethyl acetate (Stalikas, 2007). The amount of phenolic compounds and their derivatives extracted in a solvent depends on the type and chemical properties of the phenolic contained in the extracted plant material (Khoddami et al., 2013). In this study, terpenoids were not detected in all extracts. In contrast, Fikri & Purnama (2020) were reported that the leaves of *S. androgynus* contained terpenoids. In addition, another study by Awaludin et al., (2020) reported that ethanolic extract of *S. androgynus* leaves contained phytol, a terpenoid, which was at a level of 4.03%. Phytochemical screening with a simple method that used in this study has weaknesses. For example, false negative reactions can occur because the presence of compounds identified in the test solution from the extract is too small so it cannot be detected with the reagents used.

The determination of total phenolic and flavonoid contents of *S. androgynus* leaves was carried out by a colorimetric method using a UV-Vis spectrophotometer. Phenolic is the largest compound present in nature, often found in plants. Several ways were used to determine the phenolic content of plant extracts, including Folin-Denis, Folin-Ciocalteu, permanganate titration, colorimetry with iron salts, or UV absorption. However, Folin-Ciocalteu is the most popular method because it is simple and reproducible. This method relies on the transfer of electrons from the alkaline medium of the phenolic compound to the phosphomolybdate/ phosphotungstate to form a blue complex ($PMoW_{11}O_{40})^{4-}$) which is determined using a spectrophotometer at about 760 nm. The total phenolic content was then equated with mg of gallic acid per gram or kilograms or litters of extract (Dai & Mumper, 2010).

Flavonoids are phenolic derivatives with a conjugated aromatic ring system so they can provide absorption in UV rays. As a standard, quercetin is one of the flavonol groups with a hydroxyl group that is substituted on carbon number 5 and 7 in the aromatic ring A and carbon number 3 in C heterocyclic, and a ketone group substituted on carbon number 4 in the heterocyclic C ring. Hydroxyl on carbon number 3 or 5 and ketone at carbon position number 4 is made a complex with $AlCl_3$ will form a bright yellow complex that is stable in visible light (Hanani, 2015).

The concentration range of gallic acid used to determine the standard curve for gallic acid was 18-66 ppm.

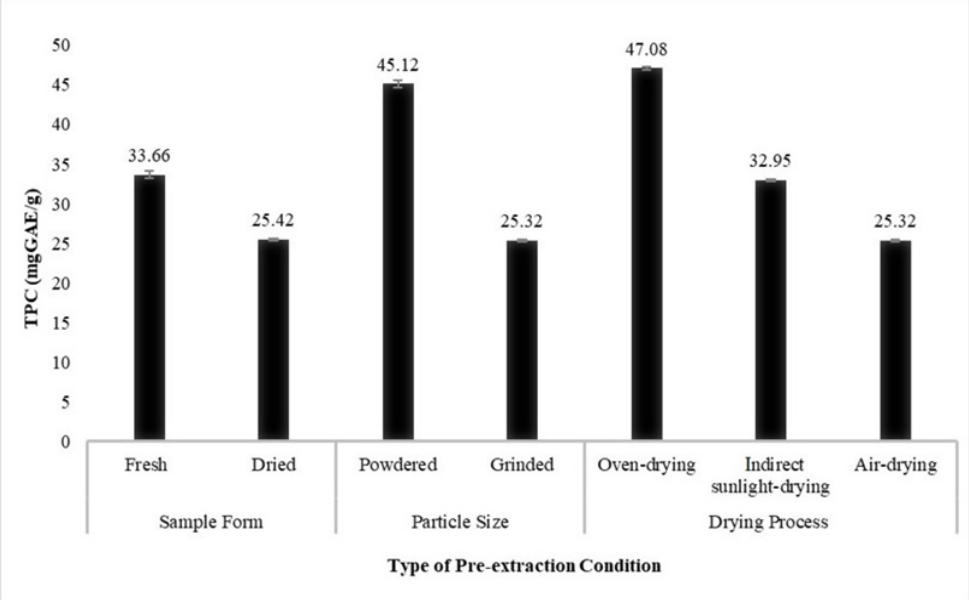

Figure 1. Total phenolic content (TPC) of *S. androgynus* leaves ethanolic extracts in various pre-extraction conditions. The test of each extract was carried out five times and reported as mean ± SD.

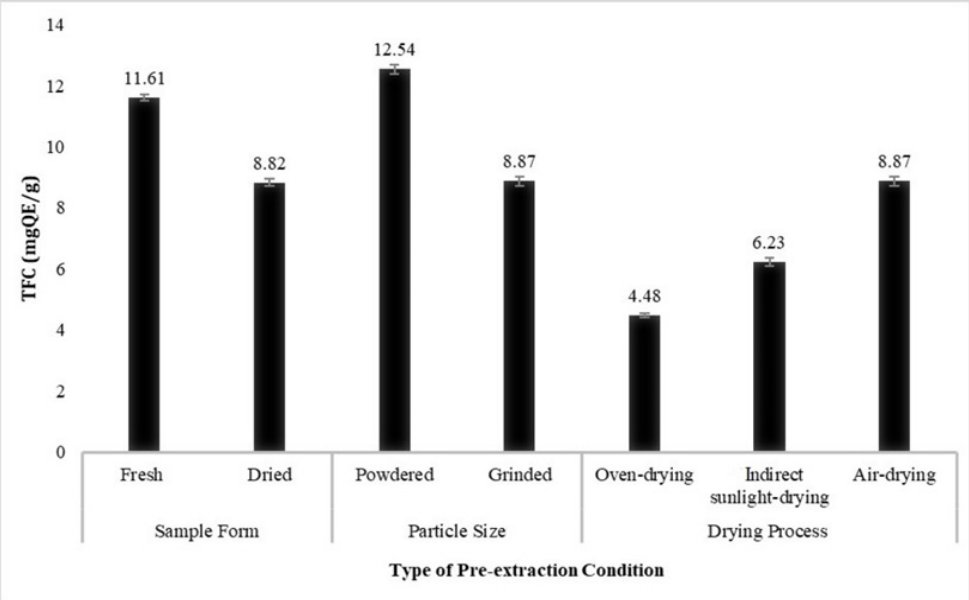

Figure 2. Total flavonoid content (TFC) of *S. androgynus* leaves ethanolic extracts in various pre-extraction conditions. The test of each extract was carried out five times and reported as mean ± SD.

The gallic acid calibration curve produced a linear regression equation y = 0.0093x+0.1824 with r = 0.99. Meanwhile, the concentration range for determining the standard quercetin curve was 33-129 ppm. Quercetin calibration curve produced a linear regression equation y = 0.0042x+0.1897 with a value of r = 0.99. The value of r approaching number 1 indicated the linear line equation. The total phenolic and flavonoid contents of *S. androgynus* leaves extract under various conditions of pre-extraction preparation were presented in Figure 1 and Figure 2. Based on these results, the extract prepared from fresh leaves contained phenolic and flavonoids higher than dry ones. According to Suvarnakuta et al. (2011), the drying process of plant materials can affect the degradation of xanthones (chemical compounds in mangosteen rind) and their antioxidant activity. However, dry plant material has the advantage. It can be stored for a long time before being used in different processes, such as extraction. The drying process can inhibit the degradation of compounds due to enzymatic and microbial growth during storage (Pinela et al., 2012).

Table 3. Antioxidant activities of *S. androgynus* leaves extracts in various pre-extraction conditions.

Pre-extraction Conditions		IC_{50} value of *S. androgynus* Ethanolic Extract (ppm)
Sample Form	Fresh	87.04 ± 0.51
	Dried	90.65 ± 0.41
Drying Process	Oven-drying	85.71 ± 2.06
	Air-drying	90.65 ± 0.41
	Indirect sunlight-drying	93.91 ± 1.57
Particle Size	Grinded	90.65 ± 0.41
	Powdered	89.82 ± 3.24
Quercetin as a reference		8.92 ± 0.72

Note: The test of each extract was carried out five times and reported in mean ± SD.

The extract prepared from the powdered plant materials (homogenized particle size) contains higher phenolic and flavonoid levels than the ground ones. Extract prepared from dried leaves by the oven-drying method contained higher phenolic content than other drying processes (oven-drying > indirect sunlight-drying > air-drying). In contrast, extract prepared from dried leaves by air-drying had the highest levels of flavonoids compared to other drying processes (air-drying > indirect sunlight-drying > oven-drying). This result suggested that the selection of the pre-extraction process affects the levels of antioxidant compounds (phenolic and flavonoids) in the extract. The efficiency of the drying process of plant material is influenced by the temperature used in the drying method. The oven-drying method in this study had a shorter drying time than other methods. This is advantageous because enzymes that degrade polyphenolic compounds, such as polyphenol oxidase, can be deactivated immediately (Rababah et al., 2015). Thus, in this study, the total phenolic content in the extract produced from dried leaves with the oven-drying process had the highest concentration compared to other methods. However, the levels of flavonoids in the extract obtained from dried leaves with the oven-drying process were lower than those with air-drying. The heat used in the oven-drying process can also cause a decrease in the chemical contents (Yuan et al., 2015).

The data in Table 3 showed the antioxidant potential of 70% ethanol extract of *S. androgynus* leaves with variations in pre-extraction conditions. The IC_{50} value of the ethanolic extract of *S. androgynus* leaves was 85.71-93.91 ppm. It means that the ethanolic extract is a potent source of antioxidants (IC_{50} = 50-100 ppm). DPPH method is fast, simple, and relatively more inexpensive, reproducible than other radical scavenging in vitro assays (Alam et al., 2013). Nonetheless, the DPPH radical interacts with other radicals (alkyl) and is sensitive to some Lewis bases. In addition, this method also requires an organic solvent in the test. The presence of oxygen can also react with DPPH directly in conditions without light protection, which causes a decrease in absorption (Singh & Singh, 2008). Evaluation of the antioxidant potential of a sample using this method is based on changes in DPPH free radical absorption after reacting with an antioxidant substrate that can donate a hydrogen atom. This change can be observed from the decrease in the intensity of the dark purple color of the DPPH radical measured using a spectrophotometer at a wavelength in the range 515-517 nm (Alam et al., 2013; Prior et al., 2005).

There are two antioxidant mechanisms of phenolic compounds: (a) the ability of phenol functional groups to donate hydrogen atoms to free radicals (hydrogen-atom transfer, HAT) and (b) single-electron transfer (SET) from phenolic antioxidants (AROH) to free radicals ($R^{\bullet}$ by forming radical cations ($ArOH^{\bullet+}$) (Quideau et al., 2011). Flavonoids have several mechanisms of action as antioxidants, one of them is direct radical reduction. Flavonoids will be oxidized by radicals, resulting in more stable (less reactive) radicals. Otherwise, flavonoids stabilize reactive oxygen species by neutralizing the radicals because of the high reactivity of the hydroxyl groups of flavonoids (Nijveldt et al., 2001).

CONCLUSION

The pre-extraction preparation process, such as sample form, drying process, and particle size, affects the yield of antioxidant compounds, including phenolics and flavonoids in the *S. androgynus* leaves extract. The pre-

extraction procedures need to be prepared in optimum condition to efficiently and effectively extract the target compounds.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, *21*(2), 143–152. https://doi.org/10.1016/j.jsps.2012.05.002

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, *6*(42). https://doi.org/10.3390/plants6040042

Andarwulan, N., Batari, R., Sandrasari, D. A., Bolling, B., & Wijaya, H. (2010). Flavonoid content and antioxidant activity of vegetables from Indonesia. *Food Chemistry*, *121*(4), 1231–1235. https://doi.org/10.1016/j.foodchem.2010.01.033

Awaludin, Kartina, Maulianawati, D., Manalu, W., Andriyanto, Septiana, R., Arfandi, A., & Lalang, Y. (2020). Phytochemical screening and toxicity of ethanol extract of *Sauropus androgynus*. *Biodiversitas*, *21*(7), 2966–2970. https://doi.org/10.13057/biodiv/d210712

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, *117*(4), 426–436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

Azwanida, N. N. (2015). Review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants*, *4*(3), 196. https://doi.org/http://dx.doi.org/10.4172/2167-0412.1000196

Chang, C.-C., Yang, M.-H., Wen, H.-M., & Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of Food and Drug Analysis*, *10*(3), 178–182. https://doi.org/https://doi.org/10.38212/2224-6614.2748

Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*, 7313–7352. https://doi.org/10.3390/molecules15107313

Departemen Kesehatan RI. (2000). *Parameter Standar Umum Ekstrak Tumbuhan*. Departemen Kesehatan RI.

Djamil, R., & Zaidan, S. (2017). Isolasi senyawa flavonoid dari ekstrak metanol daun katuk (*Sauropus androgynus* (L.) Merr), Euphorbiaceae. *Jurnal Ilmu Kefarmasian Indonesia*, *14*(1), 57–61. http://jifi.farmasi.univpancasila.ac.id/index.php/jifi/article/view/50

Fikri, F., & Purnama, M. T. E. (2020). Pharmacology and phytochemistry overview on *Sauropus androgynous*. *Systematic Reviews in Pharmacy*, *11*(6), 124–128. https://doi.org/10.31838/srp.2020.6.20

Hanani, E. (2015). *Analisis fitokimia*. Buku Kedokteran EGC.

Hikmawanti, N. P. E., Hanani, E., Sapitri, Y., & Ningrum, W. (2020). Total phenolic content and antioxidant activity of different extracts of *Cordia sebestena* L. leaves. *Pharmacognosy Journal*, *12*(6), 1311–1316. https://doi.org/10.5530/pj.2020.12.180

Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, *18*(2), 2328–2375. https://doi.org/10.3390/molecules18022328

Khoo, H. E., Azlan, A., & Ismail, A. (2015). *Sauropus androgynus* leaves for health benefits: Hype and the science. *The Natural Products Journal*, *5*, 115–123. https://doi.org/10.2174/2210315505021507021420 28

Ministry of Health Republic of Indonesia. (2008). *Farmakope Herbal Indonesia (Indonesian Herb Pharmacopoeia)* (First Ed.). Ministry of Health Republic of Indonesia.

Moharram, H. A., & Youssef, M. M. (2014). Methods for determining the antioxidant activity: A review. *Alexandria Journal of Food Science and Technology*, *11*(1), 31–42.

Nijveldt, R. J., van Nood, E., van Hoorn, D. E. C., Boelens, P. G., van Norren, K., & van Leeuwen, P. A. M. (2001). Flavonoids: A review of probable mechanisms

of action and potential applications. *The American Journal of Clinical Nutrition*, *74*(4), 418–425. https://doi.org/10.1093/ajcn/74.4.418

Pandey, A., & Tripathi, S. (2014). Concept of standardization, extraction and pre-phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry*, *2*(5), 115–119.

Petrus, A. J. A. (2013). *Sauropus androgynus* (L.) Merrill-a potentially nutritive functional leafy-vegetable. *Asian Journal of Chemistry*, *25*(17), 9425–9433. https://doi.org/10.14233/ajchem.2013.15405

Pinela, J., Barros, L., Dueñas, M., Carvalho, A. M., Santos-Buelga, C., & Ferreira, I. C. F. R. (2012). Antioxidant activity, ascorbic acid, phenolic compounds and sugars of wild and commercial *Tuberaria lignosa* samples: Effects of drying and oral preparation methods. *Food Chemistry*, *135*, 1028–1035. https://doi.org/10.1016/j.foodchem.2012.05.038

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricutural and Food Chemistry*, *53*, 4290–4302. https://doi.org/10.1021/jf0502698

Quideau, S., Deffieux, D., Douat-Casassus, C., & Pouysøgu, L. (2011). Plant polyphenols: Chemical properties, biological activities, and synthesis. *Angewandte Chemie International Edition*, *50*(3), 586–621. https://doi.org/10.1002/anie.201000044

Rababah, T. M., Al-U' Datt, M., Alhamad, M., Al-Mahasneh, M., Ereifej, K., Andrade, J., Altarifi, B., Almajwal, A., & Yang, W. (2015). Effects of drying process on total phenolics, antioxidant activity and flavonoid contents of common Mediterranean herbs. *International Journal of Agricultural and Biological Engineering*, *8*(2), 145–150. https://doi.org/10.3965/j.ijabe.20150802.1496

Singh, S., & Singh, R. P. (2008). *In vitro* methods of assay of antioxidants: An overview. *Food Reviews International*, *24*(4), 392–415. https://doi.org/10.1080/87559120802304269

Stalikas, C. D. (2007). Extraction, separation, and detection methods for phenolic acids and flavonoids. *Journal of Separation Science*, *30*(18), 3268–3295. https://doi.org/10.1002/jssc.200700261

Suvarnakuta, P., Chaweerungrat, C., & Devahastin, S. (2011). Effects of drying methods on assay and antioxidant activity of xanthones in mangosteen rind. *Food Chemistry*, *125*, 240–247. https://doi.org/10.1016/j.foodchem.2010.09.015

Wan, C., Yu, Y., Zhou, S., Liu, W., Tian, S., & Cao, S. (2011). Antioxidant activity and free radical-scavenging capacity of *Gynura divaricata* leaf extracts at different temperatures. *Pharmacognosy Magazine*, *7*(25), 40–45. https://doi.org/10.4103/0973-1296.75900

Wijono, S. H. (2003). Isolasi dan identifikasi asam fenolat pada daun katu (*Sauropus androgynus* (L.) Merr). *Makara, Sains*, *7*(2), 3–9. https://doi.org/10.7554/eLife.23471

World Health Organization. (1998). *Quality control methods for medicinal plant materials World Health Organization Geneva*. World Health Organization. https://apps.who.int/iris/handle/10665/41986

Yuan, J., Hao, L. J., Wu, G., Wang, S., Duan, J. ao, Xie, G. Y., & Qin, M. J. (2015). Effects of drying methods on the phytochemicals contents and antioxidant properties of chrysanthemum flower heads harvested at two developmental stages. *Journal of Functional Foods*, *19*, 786–795. https://doi.org/10.1016/j.jff.2015.10.008