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TOTAL PHENOLIC CONTENT EVALUATION OF TABAR KEDAYAN (*Aristolochia foveolata* Merr) ROOTS EXTRACT

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ABSTRACT

East Kalimantan has a diversity of plants from various Dayak ethnic groups. One of them is the Tabar Kedayan (*Aristolochia foveolata* Merr) plant located in Malinau district. This plant is empirically efficacious as antidiarrhea, antidotum (anti poison). Efficacy of Tabar Kedayan plant has not been scientifically confirmed, this is because there is still little scientific research and information about the content of secondary metabolites and bioactive compounds contained in Tabar Kedayan Root. The presence of secondary metabolite is phenolic which has considerable biological active prospects as antioxidant, antibacterial, antiamuba, anti-inflammatory, antihepatotoxic and antivirus. The aim of this research is to analyze the chemical content and total phenolic content of tabar kedayan root in fractionation with various nonpolar, semipolar and polar solvents. The analysis used in the determination of total phenolic content using visible spectrophotometric method. Data of analysis used standard curve method based on absorbance data and concentration of standard solution. The results of this study obtained the highest total phenolic average on ethyl acetate fraction of 77.74 ±2,633 mg GAE/g then ethanol fraction of water amounted to 38.10 ±0,461 mg GAE/g and the smallest level of n-hexane fraction of 29.36±0,193 mg GAE/g. Conclusions in this study total phenolic content is found in most semipolar fractions.

Keywords: Tabar Kedayan (*Aristolochia foveolata* Merr), total phenolic, spectrophotometry

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INTRODUCTION

East Kalimantan has a very high diversity of plants from various Dayak ethnic groups who have knowledge of medicinal plants from generation to generation to handle the health of the surrounding community. One of the diversity of plants found in East Kalimantan is Tabar Kedayan (Aristolochia), which empirically by ethnic ancestors of Dayak tribes inland in East Kalimantan (in the Malinau area) efficacious as anti-toxins, neutralize the poison of insects, snakes and all kinds of animal bites venomous [1]

Identification of chemical compound of ethanol extract Tabar Kedayan root (*Aristolochia foveolata* Merr) contains alkaloid compounds, tannins and flavonoids [2]. Phenolic compounds have redox properties, which allow them to act asantioxidants [3]. As their free radical scavenging abilityis facilitated by their hydroxyl groups, the total phenolicconcentration could be used as a basis for rapid screening of antioxidant activity [4]

There is still little scientific research and information about the content of secondary metabolites contained in *Aristolochia foveolata* Merr plants that have not been widely publicized, whereas the exploration of the chemical content of plants which are very important is needed to find new therapeutic agents in medicine so that the use of plants for medicinal purposes is not only based on hereditary experience.Separation of compounds from a plant can be done by fractionation. This fractionation process will separate the compounds of a plant according to its polarity level. Research conducted free radical fracture capture activity of ethanol extract of dewandaru leaves (*Eugenia uniflora* L.) along with the determination of total phenol content shows that the more effective a compound in radical catching activity is the greater the content [5]. Given the importance of the function of these compounds, it is necessary to conduct research with the aim of analyzing the total phenolic content which will later be used as an antioxidant agent.

MATERIALS AND METHODS

The materials used are the roots of Tabar Kedayan (*Aristolochia foveolata* Merr), 95% ethanol(onemed), aquadest, gallic acid, sodium carbonate, folin ciocalteu reagent, iron (III) chloride, n-hexane and ethyl acetate.

The equipment used in this research is blender, rotary evaporator, spectrophotometer and glass tools.

1. Processing and making of simplicia

Samples that have been collected and cleaned of impurities, then washed, drained, weighed as wet weight, then dried and weighed as dry weight. The next sample was powdered and sieved with mesh 40.

2. Extraction

The Tabar Kedayan root powder was weighed 200 g and then extracted by maseration by using 95% ethanol solvent 2 liters, extracted until the extract solution was colorless again, then filtered and the solvent was evaporated with a rotary evaporator to obtain a thickened extract such as a paste. The extract is weighed and then calculated of rendement.

3. Fractionation

Extract Tabar Kedayan root of 6 g was dissolved in 95% ethanol by 50 mL and then added n-hexane solvent with volume ratio between n-hexane and ethanol was 2: 1 (v/v) stirred over hotplate and let stand overnight. There are 2 phases of n-hexane and ethanol phase, n-hexane phase is collected. The second fractioned at the ethanol-water phase was added n-hexane of 75 mL stirred over the hotplate for 1 hour, transferred to the separating funnel stays overnight, the n-hexane fraction is dipped. The ethanol-water fraction is sufficient to 30 mL with 95% ethanol and 20 mL water plus 100 mL of ethyl acetate, stirred overnight and fractionated overnight with the addition of 50 ml of ethyl acetate. this fraction will be obtained by two phases namely phase of ethyl acetate and ethanol-water phase which then each concentrated.

4. Identification of Phenolic

Test of Polyphenol Compound was done by each fraction of 10% $FeCl_3$ reagent added 3 drops, blue green color showed polyphenol.

5. Total Phenolic Analysis with UV / Vis Spectrophotometry

a. Determination of maximum λ

300 µL of the 30 µm concentration of the acidic acid solution plus 1.5 mL of Folin-Ciocalteau solution (1:10), were shaken out and allowed to stand for 3 min. To the solution plus 1.2 mL of 7.5% Na_2CO_3 solution, shaken back and let stand for 60 minutes. Measured absorbance of the solution at 600-850 nm of wavelengths.

b. Preparation of standard gallic acid curve with Folin-Ciocalteau reagent

300 µL concentration of 5 µL concentration of the concentration of 5,10,15,20,25 and 30 ppm was added to the test tube, plus 1.5 mL of Folin-Ciocalteau solution (1:10), shaken and let stand for 3 minutes. To the solution plus 1.2 mL of 7.5% Na_2CO_3 solution, shaken back and let stand for 60 minutes. All the solution measured its absorbance at λmak, then made calibration curve relation between the concentrations of gallic acid (ppm) with absorbance.

3). Determination of total phenolic content

A total of 10 mg of each root fraction of the tabar kedayan was dissolved with 95% ethanol to 10 mL volume. the extract solution obtained was piped as much as 300 µL and added 1.5 mL solution of Folin-Ciocalteau (1:10), shaken out and let stand for 3 minutes. To the solution plus 1.2 mL of 7.5% Na_2CO_3 solution, shaken back and let stand for 60 minutes. Absorbance of the extract solution was measured.[6] Repeated 3 times.

DATA ANALYSIS

Data analysis was done by standard curve method, linear regression with equation y = bx + a based on concentration data and absorbance of standard solution.

RESULTS AND DISCUSSION

This study aims to identify and determine the level of total phenolic content from tabar kedayan roots with extraction and fractionation processes. The results of this study found that the crude extract weight was 6 grams with a yield of 3%. Crude extracts are fractionated using n-hexane, ethyl acetate and ethanol-water, aiming to separate the compounds based on their solubility to solvents with different polarity levels. The results are shows in Table 1.

Table 1. Weight and yield of n-hexane, ethyl acetate and ethanol-water fractions

No	Fraction	Weight (g)	Rendement (%)*
1	n-hexane	1.42	23.66
2	Ethyl Acetat	2.84	47.33
3	Ethanol-water	0.75	12.50

* Calculated against crude extract

Table 2. Identification of Phenolic Compounds

Fraction	Result	Description
n-hexane	Yellow-Green	+
Ethyl Acetate	Green-Yellow	+
Ethanol-water	Green	+

In Table 1 above the highest yield of ethyl acetate fraction compared to n-hexane and ethanol-water fractions, it is possible to have ethoxy groups present in ethyl acetate. The presence of ethoxy groups causes ethyl acetate to form hydrogen bonds with the compounds present in the sample [7]. The hydrogen binding formed on ethyl acetate is greater than that of non polar or polar compounds.

Phytochemical tests for polyphenols are characterized by the occurrence of reactions between polyphenolic compounds and ferric chlorides forming complex compounds that are green, purple, blue or black. The results of the preliminary test of phenol compounds based on table 2.

Determination of total phenolic content was performed using the folin-Ciocalteau reagent [8]. The Folin Ciochalteau reagent is used because the phenolic compound forms a blue color complex compound which can be measured at a wavelength (alkali salt) or a hydroxy phenol group reducing the heteropoly acid (fosfomolibdat-phosphotungstat) contained in the folin ciocalteau reagent into a blue molybdenum-tungsten complex. Phenolic compounds only in alkaline atmosphere using Na_2CO_3 7.5% proton dissociation into phenolic ions, the greater the concentration of phenolic compounds the more the phenolic ions that reduce the heteropolyacids into molybdenum-tungsten complex so the blue color becomes thicker [9].

Determination of total phenolic content with standard solution of 5, 10, 15, 20, 25 and 30 ppm concentration of gallic acid obtained in Figure 1.

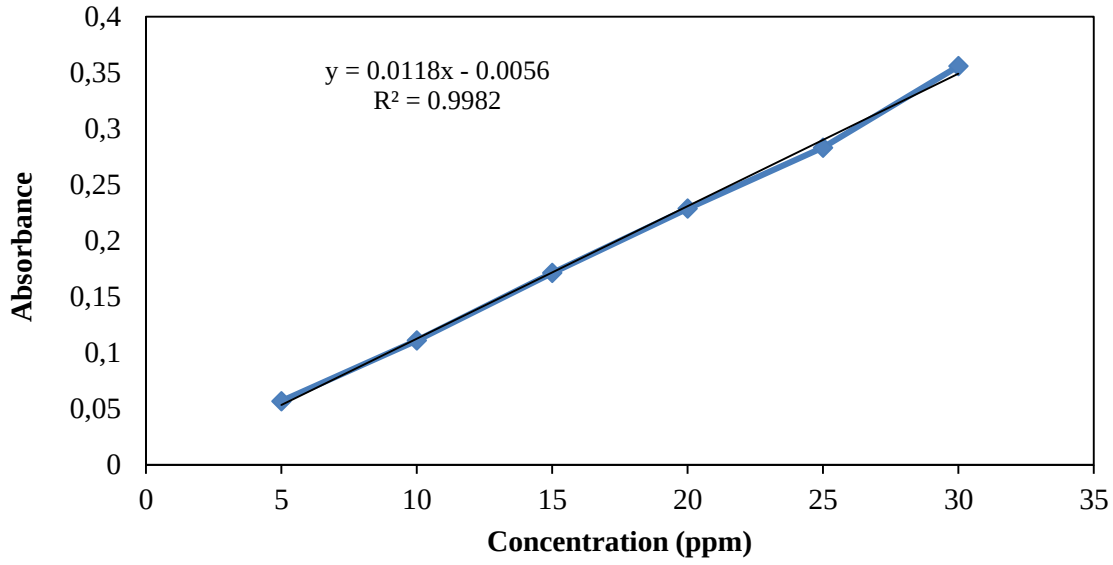

Figure 1. Graph of the relationship between concentration and absorbance of gallic acid solution

Table 3. Result of Determination of Total Phenolic Content of Tabar Kedayan Root

Fraction	Total Phenolic Content (mg GAE/g)	SD
n-hexane	29.36	0.193
Ethyl Acetate	77.74	2.633
Ethanol-Water	38.10	0.461

Determination of total phenolic content was carried out based on the standard gallic acid curve. The use of gallic a.cid as a standard because gallic acid is a derivative of hydrobenzoate which is a simple phenolic acid that is pure and stable [10]. This gallic acid standard curve obtained the correlation coefficient value close to 1 (R2 = 0.998). Data from the measurement of total phenolic content based on the standard curve can be seen in table 3.

The total phenolic content of various fractions at the largest root of beetle is ethyl acetate fraction of 77.74 mg of galic acid equivalent/g, which means in each gram of extract equivalent to 77.74 mg of gallic acid, followed by a water ethanol fraction of 38.10 mgGAE/ g and the smallest is the n-hexane fraction of 29.36 mgGAE/g. The high total phenolic content in the ethyl acetate solvent is presumed to be a class of polyphenols having the same molecular weight as the ethyl acetate solvent such as tannin and flavonol [11].This is consistent reported that the ethyl acetate solvent is particularly suitable for extracting phenolic compounds, so that the ethyl acetate solvent is used to extract the phenolic compounds contained in Morinda citrifolia L [12]. Also reported that the total phenolic content contained in the Indian Plum ethyl acetate extract (*Flacourtia jangomas* L.) was greater than that of methanol and chloroform extracts [13].

In general compounds that have strong antioxidant activity are phenol groups that have hydroxy groups substituted on benzene rings with para positions on the -OH group. These phenolic compounds can counteract free radicals by donating protons so that they can form stable radicals with resonance at aromatic ring which results in the delocalisation of electrons in the free electrons, thus preventing oxidation reactions. It is informed that the ethyl acetate fraction has a large phenolic content and can be used as a source of natural antioxidants.

CONCLUSION

Based on the results of that study it has been done that it can be concluded that the largest total phenolic content of ethyl acetate fraction is 77.74 ± mgGAE/g extract.

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REFERENCES

[1] Liwun, N.M. 2009. Inventarisasi dan Identifikasi Tanaman Obat yang Digunakan Oleh Suku Dayak Lundayeh di Kecamatan Muntarang Kabupaten Malinau Kalimantan Timur. KTI Akademi Farmasi Samarinda. Samarinda.Page 1-2.

[2] Jubaidah, S. Apriliana, A. Wijaya, H. 2015. Uji Bioaktivitas Ekstrak Akar Tabar Kedayan (*Aristolochia foveolata* Merr). Media Sains 2nd edition. Vol 8. Published by Kopertis Wilayah XI Kalimantan. Page 69-75.

[3] M.A. Soobrattee, V.S. Neergheen, A. Luximon-Ramma, O.I.Aruoma, O.T. Bahorun. 2005. Phenolics as potential antioxidant thera-peutic agents: mechanism and actions, Mutat. Res. Fundam. Vol.579. Page 200–213.

[4] S. Geetha, M. Sai-Ram, S.S. Mongia, V. Singh, G. Ilavazha-gan, et al. 2003. Evaluation of antioxidant activity of leaf extract ofsea buckthorn (*Hippophae rhamnoides* L.) on chromium (VI) induced oxidative stress in albino rats, J. Ethnopharmacol. 87 Page 247–251.

[5] Utami, Wahyu., Da'i, Muhammad., dan Negara, D. W. K., 2008. Uji Aktivitas Penangkap Radikal Bebas Fraksi Non Polar Ekstrak Etanol Daun Dewandaru (*Eugenia uniflora* L.) Dengan Metode DPPH (2, 2-diphenyl-1-picrylhidrazyl) Beserta Penetapan Kadar Fenol Dan Flavonoidnya. Jurnal Farmasi Indonesia. 2nd vol.9. Page 71.

[6] Murtijaya, J. Lim Y.Y. 2007. Antioxidant Properties of *Phylanthus amarus* Extracts as Affected by Different Drying Methods, *LWT-Food Sci. Technol*, 40. Page 1664-1669.

[7] Tursiman, Ardini, P. Nofiani, R. 2012. Total Fenol Fraksi Etil Asetat Dari Buah Asam Kandis (*Garcinia dioca* Blume). JKK. 2nd vol.1. Page 45-48.

[8] Kaur, C. Kapoor, C.H. 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. International Journal of Sainces plus Technology. 2nd vol.37. Page 159-161.

[9] Alfian, R. Susanti, H. 2012. Penetapan Kadar Fenolik Total Ekstrak Metanol Kelopak Bunga Rosella Merah (*Hibiscus sabdariffa* Linn) dengan Variasi Tempat Tumbuh Secara Spektrofotometri. Jurnal Ilmiah Kefarmasian. 1nd vol 2. Page 78.

[10] Lee SE, Hwang HJ, Ha JS and Kim JH. 2003. Screening of Medicinal Plant Extracts for Antioxidant Activity. Life Sci.73:167-179.

[11] Nur, A.M., Astawan, M. 2011. Kapasitas Antioksidan Bawang Dayak (*Eleutherine palmifolia*) Dalam Bentuk Segar, Simplisia dan Keripik, Pada Pelarut Nonpolar, Semipolar dan Polar. Skripsi. Bogor: Departemen Ilmu dan Teknologi Pangan Institut Pertanian Bogor.

[12] Rohman, A., Riyanto, S., Utari, D. 2006. Aktivitas Antioksidan, Kandungan Fenolik Total dan Kandungan Flavonoid Total Ekstrak Etil Asetat Buah Mengkudu Serta Fraksi-fraksinya. Jurnal MFI. 3 nd Vol. 17. Page. 136-142.

[13] Rahman, M., Habib, R., Hasan, R., Islam, A.M.T., Khan, I.N. 2012. Comparative Antioxidant Potential Of Different Extracts Of *Flacourtia Jangomas* Lour Fruits. Asian Journal of pharmaceutical and Clinical Research. 5(1):73-75.