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QUANTITATIVE PHYTOCHEMICAL TEST OF METHANOL EXTRACT OF TABAT BARITO LEAVES (*Ficus deltoidea* Jack.)

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ABSTRACT
Background: *Tabat barito is a medicinal plant that has long been used by various ethnic groups in Indonesia, especially* on the islands of Sumatra and Kalimantan. *The people of Kalimantan use tabat barito as a traditional medicine for women who have just given birth,* enhance blood circulation and get energy. *Tabat barito leaves contain alkaloids, flavonoids, steroids, and phenolic compounds. Because of its use only based on the results of* community trials and passed down orally, *scientific research is needed for the chemical content or compounds contained therein. It aims to determine the bioactive profile of plants that play a role in therapy and their use can be accounted for.* ***Purpose:*** *To analyze the quantitative phytochemical test results of the methanol extract of tabat barito leaves (Ficus deltoidea Jack.) 100% concentration.* ***Methods:*** *Non-experimental research with quantitative laboratory examination to determine sample content.* ***Results:*** *The results showed that the total alkaloids content 154.31 mg/ml, flavonoids 62.917 mg/ml, steroids 49.036, and phenolic 99.689 mg/ml.* ***Conclusion:*** *The methanol extract of tabat barito leaves showed the content of alkaloid compounds at 154.31 mg/ml, flavonoids at 62.917 mg/ml, steroids at 49.036 mg/ml, and phenolics at 99.689 mg/ml. The compound with the highest total content was alkaloid at 154.31 mg/ml and the compound with the lowest total content was steroid at 49.036 mg/ml.*

Keywords: *gravimetric, tabat barito leaves, uv-vis spectrophotometry, quantitative phytochemical test*
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INTRODUCTION

Data from the World Health Organization (WHO) shows that approximately 80% of people in developing countries use herbal medicinal as their primary health care.[1] Indonesia is a country that mostly uses traditional medicine because there are 90,000 types of plants with around 9600 of them having medicinal properties and 300 it has been used as an ingredient in traditional medicine.[2]

Because the use of medicinal plants is only based on community trials and passed down orally, scientific research on the substance or chemical compounds contained in it is necessary. It aims to determine the bioactive profile of plants that useful in therapy and their use can be accounted for.[2]

One of the medicinal plants is tabat barito. Tabat barito has long been used by various ethnic groups in Indonesia, especially on the islands of Sumatra and Kalimantan.[3] In Kalimantan, tabat barito is used as a traditional medicine for women who have just given birth, enhance blood circulation and get energy. Tabat barito is known to have various bioactive activitiy that have been tested, such as antimicrobial, anti-diabetic mellitus, anti-cancer, aprosidiac, anti-inflammatory, and antioxidant.[3,4] These various bioactive activities are due to the existence of several secondary metabolites.[5] According to Manurung et al. (2019), methanol extract of tabat barito leaves contains alkaloids, flavonoids, steroids, and phenolic compounds.[6]

Tabat barito leaves will be extracted by the maceration method using methanol as a solvent. Methanol was chosen because it is a universal

solvent used in phytochemical tests and can dissolve both polar and non-polar compounds.[7] Quantitative phytochemical test studies have been carried out on tabat barito leaves from West Java and Malaysia, while none has been conducted from South Kalimantan. Differences in the place of growth and environmental conditions can be affect the content of secondary metabolites.[8]

Based on this, it is necessary to carry out quantitative phytochemical test studies to determine the total content of alkaloids, steroids and phenolic compounds from the methanol extract of tabat barito leave with 100% concentration.

MATERIAL AND METHODS

This research is non-experimental research with quantitative laboratory examination to determine the sample content. This research has received ethical approval from the Health Research Ethics Commission, Faculty of Dentistry, Lambung Mangkurat University with No. 004/KEPKG-FKGULM/EC/II/2021. The research was conducted at the Basic Laboratory Faculty of Mathematics and Natural Sciences, University of Lambung Mangkurat, Banjarbaru for plant determination and the Biochemistry Laboratory, Faculty of Medicine, University of Lambung Mangkurat, Banjarbaru for making extracts and quantitative phytochemical tests. The total content test for contained compounds in the methanol extract of tabat barito leaves will carried out on four compounds, such as alkaloids, flavonoids, steroids, and phenolics. Quantitative phytochemical tests were carried out using gravimetric method for alkaloid compounds and uv-vis spectrophotometric method for flavonoid, steroid, and phenolic compounds. The concentration was measured three times for each compound. Data from the results of quantitative phytochemical tests were analyzed using descriptive statistical methods. Descriptive statistics is a method of data analysis by describing or describing data in tabular form. The obtained results are the average value of the three measurements.

Materials and Tools

The materials for extraction were tabat barito leaves and 98% methanol. Materials for quantitative phytochemical tests consist of acetic acid 10% (E. Merck), aquadest, $NaNO_2$ 5%, $AlCl_3$ 10%, NaOH 4%, sulfuric acid 4N (E. Merck), iron (III) chloride 0.5% (E. Merck), 0.5% potassium hexacyanoferrate (III) solution (E. merck), *Folin Ciocalteau* reagent (E. Merck), 7% Na2CO3, and aquabidestillat.

The tools for extraction consisted of an extractor, oven (Memmert), filter paper (WH40), analytical balance (Precis), Erlenmeyer flask (Iwaki), blender (Philips), rotary evaporator (Heidolph), and water bath (SMIC). Equipment for quantitative phytochemical tests consist of uv-vis spectrophotometry (Shimadzu 1800), glass beaker (Iwaki), volumetric flask (Iwaki), micropipette (Dragon lab), test tube (Iwaki), water bath (SMIC), stir bar, and cuvette.

Extraction of Tabat Barito Leaves

The barito leaves (*Ficus deltoidea* Jack.) was obtained from *UPT BBTPH (Balai Pengembangan Perbenihan Tanaman Pangan dan Hortikultura)* Banjarbaru City. Tabat barito leaves used in this study were selected according to the criteria, which are fresh leaves, green mature leaves, starting from the third leaf from the shoot, no holes, and no pests, and the leaves were taken in the morning before 10:00.

Tabat barito leaves that have been selected according to the criteria are washed with water and then dried using an oven for 2 × 24 hours. The dried leaves are mashed with a blender until simplicia is formed. The simplicia powder then soaked with 98% methanol for 3 × 24 hours, stirring several times during the immersion and the solvent was changed every day. The filtering process is carried out using filter paper. The extract then evaporated using a rotary evaporator with a heating temperature of 40°C, after that a waterbath was used to obtain a viscous 100% concentration of methanol extract of tabat barito leaves without dilution.

Quantitative Phytochemical Test

The total content test of alkaloid compounds was detemined by placing 10 grams of sample into a 250 ml beaker glass, and also adding 200 ml of 10% acetic acid. The beaker glass was closed and kept for 4 hours, then filtered. A quarter of the extract was evaporated with a waterbath and added ammonium hydroxide and then precipitated. The precipitate was washed with dilute ammonium hydroxide and filtered. The residue was evaporated until a constant weight was obtained.

The total flavonoid content was determined by taking samples prepared with a 500 L micropipette and pouring them into a test tube, then adding 2 ml of distilled water. Add 150 L of 5% $NaNO_2$ and kept for 6 minutes, then add 150 L of 10% $AlCl_3$ and kept for 6 minutes. 2 ml of 4% NaOH was added and diluted with distilled water until the volume of the tube reached 15 ml and kept for 5 minutes. The absorbance was measured using uv-vis spectrophotometry at 520 nm.

The test for steroid compounds was carried out using 1 ml of sample extract which was put into a 10 ml volumetric flask then added 2 ml of 4N sulfuric acid, 2 ml of 0.5% iron (III) chloride, and 0.5 ml of potassium hexacyanoferrate (III) solution.

0.5%. Then heated with a waterbath for 30 minutes with occasional stirring. Furthermore, the absorbance measurements were carried out with uv-vis spectrophotometry at 780 nm.

The total phenolic content was determined by adding 1 ml of the extract solution, adding 0.4 ml of *Folin Ciocalteau* reagent, and kept for 4-8 minutes, then adding 4 ml of 7% Na_2CO_3 solution until homogeneous. Then add 10 ml of aquabidestillata and kept for 2 hours at room temperature. Then the absorption measurement was carried out using uv-vis spectrophotometry at 744.8 nm.

RESULT

Table 1. Total Content of Alkaloids, Flavonoids, Steroids and Phenolics Methanol Extract of Tabat Leaves Barito (in mg/ml)

No.	Secondary Metabolite Compounds	Total Content (mg/ml)
1.	Alkaloids	154.31 ± 5,85
2.	Flavonoids	62.917 ± 0,382
3.	Steroids	49.036 ± 0,473
4.	Phenolics	99.689 ± 0,139

Based on table 1, quantitative phytochemical tests carried out on the methanol extract of tabat barito leaves with a concentration of 100% showed the results of the total content of secondary metabolites including alkaloids of 154.31 mg/ml, flavonoids of 62.917 mg/ml, steroids of 49.036 mg/ml, and phenolics of 99.689 mg/ml. From the table, it is known that alkaloids are compounds with the highest levels of 154.31 mg/ml and steroids are compounds with the lowest levels of 49,036 mg/ml.

DISCUSSION

The quantitative phytochemical tests revealed that the total content of alkaloids were 154.31 mg/ml, flavonoids were 62.917 mg/ml, steroids were 49.036 mg/ml, and phenolics were 99.689 mg/ml. This result is different from the result of previous studies such as the research of Siswoyo et al (2016) in West Java. That study showed flavonoid content of 34.5 mg/ml in tabat barito from Gunung Gede Pangrangro National Park and 24.1 mg/ml in tabat barito from Mount Halimun Salak National Park. Different results can also be seen from the research of Mustapha and Harun (2015) in Malaysia, which obtained alkaloid levels of 17.5 mg/ml, flavonoids of 5.8 mg/ml, and phenol of 2.8 mg/ml from tabat barito var. Kunstleri (King) Corner.[9, 10]

The difference in phytochemical content can be influenced by the solvent used for extraction. A compound will dissolve in a solvent that has the same polarity according to the principle of polarization.[11] Research by Siswoyo et al. (2016) used ethyl acetate as a solvent while this study used methanol. Methanol is a universal solvent because it can bind all chemical components in plants, including non-polar, semi-polar, and polar components.[12] Internal factors such as genes and external factors also affect the levels of phytochemical content. External factors are caused by differences in environmental conditions where plants grow which vary from one place to another, for example, such as climate, light, humidity, temperature, pH, rainfall, altitude, soil conditions, nutrient content, and water availability.[13.14]

The difference in total content of tabat barito leaves compounds in South Kalimantan, West Java, and Malaysia is influenced by differences in rainfall in the three regions. Based on data from the *Badan Pusat Statistik*, the rainfall in South Kalimantan is 1917 mm and the rainfall in West Java is 3555.90 mm while the rainfall in Malaysia according to the *Jabatan Meteorologi Malaysia* is 4354.54 mm. The lower rainfall in South Kalimantan causes the levels of compounds contained in tabat barito leaves grown there is higher than the other two places. This is in line with the findings of Ramadhan et al. (2015), who found that rainfall affects the levels of flavonoids in kepel leaves, which tend to decreases as rainfall increases. Differences in compound levels also caused by the temperature difference. According to data from the *Badan Pusat Statistik*, South Kalimantan has an average temperature of 27.2°C while West Java has an average temperature of 26.31°C. The temperature of South Kalimantan which tends to be higher causes the flavonoid content of tabat barito leaves growing in South Kalimantan to be higher than those grown in West Java. This is in line with the research of Utomo et al. (2020) which showed higher levels of flavonoids and phenolics in the kepel plant in areas with higher temperatures.[15,16]

Alkaloids are compounds with the highest levels, the measurement process is carried out by adding 10% acetic acid which aims to extract alkaline alkaloids, and then NH_4OH is added to precipitate them.[17] Tabat barito leaves have the potential to be used as an antibacterial agent due to their high content of alkaloids. This is because alkaloids can interfere peptidoglycan component of bacterial cells, inhibit protein synthesis, and changes genetic balance in the DNA chain. Alkaloids can also act as antifungals by preventing DNA replication so the fungal growth is disrupted.

This compound also acts as an antioxidant by donating H atoms to free radicals.[18,19]

Fenolik menjadi senyawa dengan kadar tertinggi kedua, sebelum diukur dengan spektrofotometri terlebih dahulu ditambahkan reagen *Folin ciocalteu* dan Na_2CO_3. Senyawa fenolat (garam alkali) atau gugus fenolik-hidroksi akan mereduksi asam heteropoli pada reagen *Folin ciocalteau* menjadi suatu kompleks

Phenolic became the compound with the second-highest concentration, before being measured by spectrophotometry, *Folin ciocalteu* and Na_2CO_3 reagent were first added. Phenolic compounds (alkali salts) or phenolic-hydroxy groups will reduce heteropoly acids in *Folin ciocalteau* reagent to a blue molybdenum-tungsten complex which can be measured by spectrophotometry. Because this reaction can only occur in alkaline conditions, Na_2CO_3 is added to cause proton dissociation in phenolic compounds into phenolic ions.[20] Phenolics have antibacterial activity because they can denature cell proteins and cause protein structure to be damaged. Phenolics also act as antifungals by destroying proteins and phospholipids. As antioxidants, phenolics can act as hydrogen donors and reduce reactive oxygen.[21]

The total flavonoids content was determined by adding AlC_{l3}, $NaNO_2$, and NaOH. $AlCl_3$ will react with the keto group at C4 and the OH group at C3 or C5 in flavones or flavonol compounds to form yellow complex compounds. The addition of $NaNO_2$ and NaOH is used to form a complex of NaNO2-$AlCl_3$-NaOH based on the reaction of aluminum ions with flavonoid compounds in an alkaline environment.[22] Flavonoids work as antibacterial agents by inhibiting cell membrane function and nucleic acid synthesis. Flavonoids are also useful as antifungals because they can denature proteins and cause irreversible lysis of cell membranes. As antioxidants, flavonoids can stabilize free radicals by donating hydrogen ions.[23]

Steroids were the compounds with the lowest content among the four compounds tested. Determination of the content is done by adding sulfuric acid so that a reaction occurs that causes the formation of a bluish-green color.[24] Steroids can work as antibacterials because they cause lysosomal leakage, decrease membrane integrity and make cells brittle and lyse. The role of steroids as antifungals is by inhibiting the formation of ergosterol which is a component of the plasma membrane and plays a role in the formation of chitin which is a polysaccharide component of the cell wall.[25]

Based on the research, it can be concluded that the methanol extract of tabat barito leaves showed total content of alkaloid compounds at 154.31 mg/ml, flavonoids at 62.917 mg/ml, steroids at 49.036 mg/ml, and phenolics at 99.689 mg/ml. The compound with the highest total content was alkaloid at 154.31 mg/ml and the compound with the lowest total content was steroid at 49.036 mg/ml.

REFERENCES

1. Ahmad VN, Amin IM. 2017. Anti- Oral Ulcer Activity of Ficus deltoidea Leaves Extract on Animal Model. *Pertanika J. Sci. & Technol*. 25 (S): 41-52.
2. Larassati A, Marmaini, Kartika T. 2019. Inventarisasi Tumbuhan Berkhasiat Obat Di Sekitar Pekarangan Di Kelurahan Sentosa. *Jurnal Indobiosains*. 1 (2): 76-87.
3. Silalahi M. 2019. Pemanfaatan dan Bioaktivitas Tabat Barito (*Ficus deltoidea* Jack). *Jurnal Ilmiah Ilmu Kesehatan: Wawasan Kesehatan*. 6 (1): 29-37.
4. Manurung H, Kustiawan W, Kusuma IW, Marjenah. 2017. Total Flavonoid Content and Antioxidant Activity in Leaves and Stems Extract of Cultivated and Wild Tabat Barito (*Ficus deltoidea* Jack). *The 1st International Conference on Mathematics, Science, and Computer Science* (*ICMSC*). Samarinda. 020007-1 – 020007-6.
5. Agustina A, Zuhud E A.M, Darusman LK. 2015. Karakteristik Habitat Mikro Tabat Barito (*Ficus deltoidea* Jack) Pada Tumbuhan Inangnya (Characteristics of Tabat Barito (*Ficus deltoidea* Jack) Microhabitat on Their Host Plants). *Jurnal Penelitian Hutan dan Konservasi Alam*. 12 (1): 89-104.
6. Manurung H, Kustiawan W, Kusuma IW, Marjenah, Nugroho RA. 2019. Growth, phytochemical profile, and antioxidant activity of cultivated tabat barito (*Ficus deltoidea* Jack) under drought stress. *International Journal of Biosciences*. 14 (1): 366-378.
7. Zhang QW, Lin LG, Ye WC. 2018. Techniques for extraction and isolation of natural products: a comprehensive review. *Chin Med*. 13 (20): 1-26.
8. Tanamal MT, Papilaya PM, Smith A. 2017. Kandungan Senyawa Flavonoid Pada Daun Melinjo (*Gnetum gnemon* L.) Berdasarkan Perbedaan Tempat Tumbuh. *Biopendix*. 3 (2): 142-147.
9. Siswoyo, Batubara I, Aristyanti D. 2016. Tempat Tumbuh dan Kandungan Flavonoid Total Daun Tabat Barito (*Ficus deltoidea* Jack.). *Prosiding Seminar Nasional Tumbuhan Obat Indonesia Ke-50*. Samarinda. 78-86.

10. Mustapha Z, Harun H. 2015. Phytochemical constituents in leaves and callus of *Ficus deltoidea* Jack var. *Kunstleri* (King) Corner. *Walailak Jornal of Science and Technology*. 12 (2): 431-439.
11. Julianti WP, Ikrawan Y, Iwansyah AC. 2019. Pengaruh Jenis Pelarut Terhadap Kandungan Total Fenolik, Aktifitas Antioksidan dan Toksisitas Ekstrak Buah Ciplukan (*Physalis angulata L*). *Jurnal Riset Teknologi Industri*. 13 (1): 70-79.
12. Suryani NC, Permana DGM, Anom Jambe AAGN. 2016. Pengaruh Jenis Pelarut Terhadap Kandungan Total Flavonoid dan Aktivitas Antioksidan Ekstrak Daun Matoa (*Pometia pinnata*). *Jurnal Ilmu dan Teknologi Pangan* (*ITEPA*). 5 (1): 1-10.
13. Katuuk RHH, Wanget SA, Tumewu P. 2019. Pengaruh Perbedaan Ketinggian Tempat Terhadap Kandungan Metabolit Sekunder Pada Gulma Babandotan (*Ageratum conyzoides* L.). *COCOS*. 1 (4): 1-6.
14. Tarakanita DNS, Satriadi T, Jauhari A. 2019. Potensi Keberadaan Fitokimia Kamalaka (*Phyllanthus emblica*) Berdasarkan Perbedaan Ketinggian Tempat Tumbuh. *Jurnal Sylva Scienteae*. 02 (4): 645-654.
15. Ramadhan BC, Aziz SA, Ghulamahdi M. 2015. Potensi Kadar Bioaktif yang Terdapat Pada Daun Kepel (Stelechocarpus burahol). Buletin Penelitian Tanaman Rempah dan Obat. 26 (2): 99-108.
16. Utomo DS, Kristiani EBE, Mahardika A. 2020. Pengaruh Lokasi Tumbuh Terhadap Kadar Flavonoid, Fenolik, Klorofil, Karotenoid Dan Aktivitas Antioksidan Pada Tumbuhan Pecut Kuda (*Stachytarpheta Jamaicensis*). *Bioma : Berkala Ilmiah Biologi*. 22 (2): 143-149.
17. Romansyah E, Dewi ES, Suhairin, Muanah, Ridho R. 2019. Identifikasi Senyawa Kimia Daun Bambu Segar Sebagai Bahan Penetral Limbah Cair. *Jurnal AGROTEK*. 6 (2): 77-81.
18. Azizah AN, Yahya A, Risandiansyah R. 2019. Efek Kombinasi Fraksi Alkaloid Ekstrak *Imperata cylindrica L.* dengan Amoksisilin atau Kloramfenikol Terhadap daya hambat *Staphylococcus aureus*. *Jurnal Kesehatan Islam*. 8 (2): 1-9.
19. Tjandra RF, Fatimawali, Datu OS. 2020. Analisis Senyawa Alkaloid dan Uji Daya Hambat Ekstrak Buah Sirih (*Piper betle* L) Terhadap Bakteri *Staphylococcus epidermidis*. 8 (2): 173-179.
20. Prayoga DGE, Nocianitri KA, Puspawati NN. 2019. Identifikasi Senyawa Fitokimia dan Aktivitas Antioksidan Ekstrak Kasar Daun Pepe (*Gymnema reticulatum* Br.) Pada Berbagai Jenis Pelarut. *Jurnal Ilmu dan Teknologi Pangan*. 8 (2): 111-121.
21. Helilusiatiningsih N. 2020. Aktivitas Antioksidan Ekstrak Metanol dan Air Teh Herbal Pokak (*Solanum torvum*) Terhadap Antibakteri Patogen. *Buana Sains.* 20 (2): 161-170.
22. Fadillah A, Rahmadani A, Rijai L. 2017. Analisis Kadar Total Flavonoid dan Uji Aktivitas Antioksidan Ekstrak Daun Kelubut (*Passiflora foetida* L.). *Proceeding of the 5th Mulawarman Pharmaceuticals Conferences*. Samarinda. 21-28.
23. Waskito H, Apriasari ML, Utami JP. 2020. Antifungal Effect of Mauli Banana Stem Extract, Basil Leaf Extract, and Their Combination On *Candida albicans*. *Dentino* (*Jurnal Kedokteran Gigi*). V (2): 179-184.
24. Sandy FF, Susilawati Y, Ramadhania ZM. 2020. Review: Analisis Kualitatif dan Kuantitatif Kandungan Senyawa Kimia Herba Sasaladaan (*Peperomia pellucid*a (L) H.B.K). *Jurnal Sains dan Kesehatan*. 2 (4): 505-518.
25. Rahayu NT, Nurhasanah AS, Rumidatul A, Fadhila F, Maryana Y. 2020. Pengujian Aktivitas Antibakteri Ekstrak Kulit Ranting Sengon (*Farlcataria moluccana*) Dengan Pelarut Metanol dan N-Heksana. *Jurnal Mitra Kesehatan*. 3 (1); 6-15.