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Research Article

Characteristics Study and Total Flavonoids Quantification of Kareho Leaves (*Callicarpa longifolia* Lam)

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ABSTRACT

The *Callicarpa longifolia* Lam. known as 'kareho' is one of the plants used by several Dayak tribes for traditional medicine such as antiacne, swelling, wounds, diarrhea, diabetes, and lowering cholesterol levels. This study has obtained scientific data on the characteristics of dried leaves and determined the flavonoids levels of methanol extract of *C. longifolia* leaves. Characterization includes both qualitative and quantitative examination on the dried leaves of *C. longifolia* leaves, while the determination of total flavonoid content was determined by the aluminium chloride method and calculated as quercetin equivalent (QE). The results of characteristics observations obtained organoleptic in the form of powder, dark green, bitter taste and have a distinctive smell. Microscopic test of leaves had an actinositic stomata type, ethanol soluble extract content of 17.50 ± 0.10% and water soluble extract content of 16.53 ± 0.37%. Drying loss 8.16 ± 0.25%, total ash content of 5.52 ± 0.06%, and acid insoluble ash content of 0.07 ± 0.01%. The results of phytochemical screening confirmed the presence of saponins, flavonoids, steroids, tannins, glycosides, and phenols. TLC observasions using 254 nm UV light obtained 6 spots and 7 spots appearance on sulphuric acid. It has total flavonoid levels obtained at 7.995 ± 0.050% w/w QE.

Keywords: Kerehau, Sangkareho, Dayak Tribes, Standardization, Specific and Non-Specific Parameters.

I. INTRODUCTION

Kareho (*Callicarpa longifolia* Lam.) is one of the herbs used by the Dayak Bakumpai tribe in Kalimantan Tengah Province; Dayak Tunjung and Dayak Kutai in Kalimantan Timur Province as traditional medicine. Empirically, *C. longifolia* leaves are used as medicine for acne, swelling, external wounds, diarrhea, diabetes, and reduce

cholesterol levels (Kusumawati, E., Apriliana, A., & Khatimah, 2016; Qamariah et al., 2015; Syamsul et al., 2016). Several preclinical tests have also reported that *C. longifolia* leaves have various activities, including antidiabetic by working to increase insulin sensitivity at a dose of 75mg / KgBW (Susilawati et al., 2018), as an analgesic with an effective dose of 150 mg / kgBW which was tested on mice (Syamsul et al., 2016), and its ethanol extract has inhibitory power against the growth of *Escherichia coli* and *Staphylococcus aureus* bacteria (Kusumawati et al., 2016).

The suitability of plant characteristics and the amount of certain compounds contained will affect the activity of *C. longifolia* dry leaves. Flavonoids are active compounds that have an antioxidant, antibacterial, anti-inflammatory and wound healing effect. The research conducted by Semiawan et al. (2015) showed that the methanol extract of *C. longifolia* leaves has anti-inflammatory activity and one of the compounds that provide this activity was flavonoids. Another research also states that flavonoids had activities to accelerate wound healing (Pazry et al., 2017).

Based on the description, it was necessary to determine the dried leaves characteristics of *C. longifolia* leaves and its total flavonoid content so that the feasibility of using *C. longifolia* leaves which is a typical ethnic plant of the Dayak tribe can be explored more deeply. Determination of the characteristics of the dried leaves carried out included organoleptic tests, microscopic tests, ethanol soluble and water-soluble extracts, loss of drying, total ash content, screening for phytochemical compounds, chromatogram analysis from TLC, and total flavonoid determination on methanol extract of kareho leaves.

II. METHOD

A. Sample collection

C. longifolia were taken from natural forests in the Puruk Cahu area, Murung Raya Regency, Kalimantan Tengah Province, Indonesia. All parts of the plant are taken for determination. The plants then identified in the Pharma-cognosy - Phytochemical Laboratory and determinated in the Laboratorium Dasar, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University.

B. *C. longifolia* leaves characterization

Determination of the characteristics of *C. longifolia* dried leaves includes organoleptic tests, microscopy tests on longitudinal and transverse sections, dissolved compounds in certain solvents (ethanol soluble extract and water soluble extract content), loss of drying

determination, total ash content, acid insoluble ash content, screening phytochemical and thin layer chromatographic patterns.

C. *C. longifolia* leaves extraction

The sample was carried out by taking the 3rd to 5th leaf parts from the shoots, and then dried into oven. Five hundred grams of *C. longifolia* dried leaves that had powdered was extracted by maceration method using methanol as a solvent. Extraction was carried out for 3x24 hours with stirring every 6 hours. The filtrate is then collected and then concentrated using a rotary evaporator and evaporated over a waterbath with a temperature of 50 ºC until a thick extract is obtained to a fixed weight.

D. Total flavonoid determination

The standard stock was prepared by weighing 10 mg of quercetin, dissolved with ethanol pro analysis (p.a) in a 10 mL volumetric flask until the limit mark. From the main liquor, 0.4; 0.6; 0.8; 1.0; and 1.2 mL each put into a 10 mL volumetric flask and then add ethanol p.a until the limit mark. Standard solution with a concentration of 40; 60; 80; The 100 and 120 ppm each were taken 0.5 mL and then put into the test tube. The solution then added with 0.1 mL of 10% $AlCl_3$, 0.1 mL of 5% acetic acid and 2.8 mL of distilled water, the solution was measured at its maximum wavelength (416nm) after standing for operating time (28 minutes) at room temperature (Fadlilaturrahmah et al., 2020).

In another hand, methanol extract of *C. longifolia* leaves made a concentration of 1000 ppm. A total of 10 mg of extract was weighed and put into a 10 mL volumetric flask, added with ethanol p.a until the limit mark. A total of 0.5 mL of the solution was taken and then added with 1.5 mL of ethanol p.a then reacted with 0.1 mL of 10% $AlCl_3$, 0.1 mL of 5% acetic acid and 2.8 mL of aquadest. The solution was shaken and allowed to stand for operating time (28 minutes) then done the replication 3 times. The absorbance of the solution was read at the maximum wavelength (416 nm). The blanks used were ethanol p.a which was reacted with 10% $AlCl_3$, 5% acetic acid and aquadest**.**

III. RESULTS AND DISCUSSIONS

A. Determination of *C. longifolia* and sample collection

Determination aims to ensure the identity of the sample species used in accordance with the plant species contained in the literature. Based on certificate number 106b/LB.LABDASAR /V /2019 issued by the Laboratorium Dasar of FMIPA Lambung Mangkurat

University, the results of the determination indicated that the plant used was the *Callicarpa longifolia* Lam species from the Lamiaceae family. Samples were taken by the 3rd to 5th leaf part of the shoot.

The leaves of *C. longifolia* are wet sorted to separate dirt or foreign material. The leaves of *C. longifolia* are then washed under clean running water to remove impurities attached to the material, then carried out to dried using the wind dry method and then continued using an oven with a temperature of 50 ºC, the aim is to reduce moisture content and prevent enzymatic processes.

B. *C. longifolia* leaves characterization

Organoleptic test on *C. longifolia* leaves dried leaves showed the results were in the form of coarse powder, dark green color, distinctive odor and bitter taste. According to Anggraini et al. (2017), the plants of the Lamiaceae family have a distinctive odor from each species. The bitter taste of *C. longifolia* dried leaves is due to the presence of saponin secondary metabolite content (Qamariah et al., 2015), this is evidenced by the results of the phytochemical screening test for the ethanol extract of *C. longifolia* leaves that contain positive saponins. Cross-sectional microscopic examination using an optical microscope with a magnification of 10 times (Figure 1) showed the presence of trichomes, cuticles, epidermis, spongy tissue, xylem and phloem. Trichomes are hairs that grow and come from epidermal cells that function as defense in plants (Dewi et al., 2015).

The longitudinal section with a magnification of 40 times (Figure 2) shows the presence of stomata on the upper surface of the leaf with an actinocytic type, namely stomata with flat neighboring cells surrounding the stomata in a circular arrangement that functions for water evaporation and gas exchange between leaf tissue and the atmosphere. Each stomata pore is surrounded by two special epidermal cells called guard cells which are different from other cells (Handayani et al., 2018).

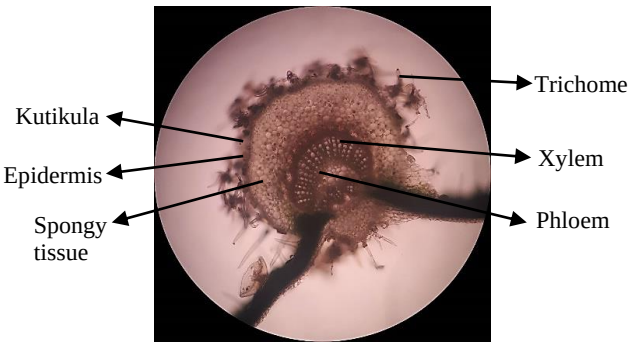

Figure 1. Cross section of *C. longifolia* leaves

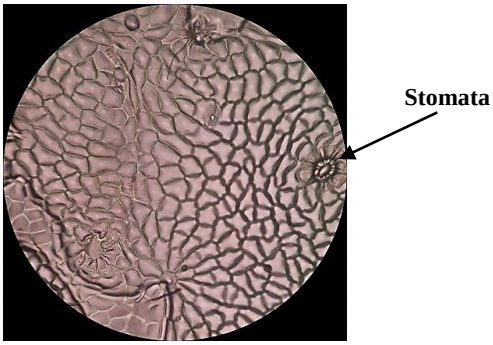

Figure 2. Longitudinal cross section of *C. longifolia* leaves

The test for the ethanol soluble extract and the water-soluble extract content was carried out to provide information regarding the compounds contained in the sample. The results of the determination of the ethanol soluble extract content were 17.50 ± 0.10% and the water soluble extract content was 16.53 ± 0.37%. The value of extract content that dissolves in ethanol is greater than that of extract that dissolves in water, indicating that *C. longifolia* leaves are more soluble in ethanol than in water. The compounds that are thought to be dissolved in ethanol solvent are terpenes, alkaloids, glucosides, lipids (oils) and volatile oils. Compounds that are thought to be dissolved in water solvents are saponins, tannins, quaternary alkaloids, glucosides, amino acids, and several vitamins (Hardiana et al., 2012; Khotimah, 2016; Zainab et al., 2016).

The lost of drying test was carried out by replicating 3 times and the results were 8.16 ± 0.25%, this test aims to obtain information regarding the compounds that are lost and evaporated during drying. The greater the value obtained from the drying loss test, the more compounds will evaporate during the drying process. This is also related to the possibility of fungal and bacterial growth in dried leaves (Rizqa, 2010).

The result of total ash content in *C. longifolia* leaf samples was 5.52 ± 0.06%, this parameter indicates that the powder obtained contains inorganic minerals. While the acid-insoluble ash content shows the amount of impurity or sand that is included, in the test the value is 0.07 ± 0.01%. The presence of acid-insoluble ash content in this test was caused by contamination of impurities such as sand and silicates during the process of taking the leaves into fine powder (Sudarmadji et al., 1989).

Phytochemical screening of *C. longifolia* leaf extract was carried out with the aim of qualitatively determining the content of the secondary metabolite compounds contained in the *C. longifolia* leaf extract (Khoirani, 2013). Information on the content of secondary metabolites in *C. longifolia* leaves can emphasize the compound content in the dried leaves, which is a quality parameter related to pharmacological effects and can be a reference for determining the compound content quantitatively.

Phytochemical screening results of *C. longifolia* leaf samples showed positive saponins, flavonoids, steroids, tannins, glycosides, and phenols. Observation of *C. longifolia* leaf extract on TLC with silica gel GF 254 as stationary phase and n-hexane: ethyl acetate (6: 4) mobile phase showed the separation of compounds after elution. Observations were using 254 nm UV light obtained 6 spots and 7 spots

appearance on sulphuric acid. This shows that the elution process can attract the active ingredient components (Nurani et al., 2015). Compounds that are non-polar will have a low affinity for the stationary phase, so they have a large Rf value, and vice versa, polar compounds have a small Rf value (Sarker & Nahar, 2012).

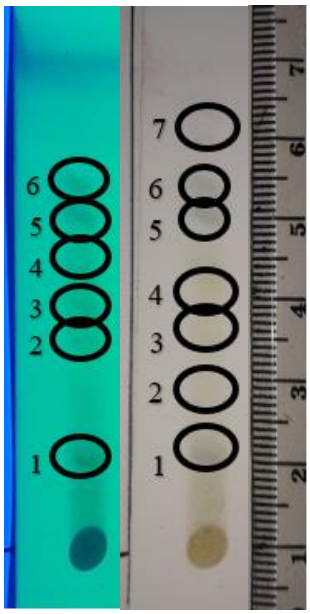

Figure 3. TLC identification of *C. longifolia* leaves extract with n-hexane: ethyl acetate (6: 4) mobile phase in **A**. 254 nm UV light **B**. sulphuric acid

C. Determination of Total Flavonoid Levels

Absorbance values of the standard quercetin series determined respectively. It is resulting the equation y = 0.0033x - 0.0052 with the linearity (r) value obtained by 0.999 (Figure 3). The accuracy obtained 99.9% with an error rate that occurs only 0.1%. Linearity implies a linear relationship between the concentration and the absorbance value of concentration series solution. The closer to 1, the more linear the results obtained. This is in accordance with the literature which states that linearity is good if the correlation coefficient (r) = approaches 1 (Insani et al., 2016) in calculating the total flavonoid content of *C. longifolia* leaf extract, the levels obtained were 7.995 ± 0.050% w/w QE (Quersetin Equivalent), with an RSD value of 0.635 (Table I).

The total flavonoid levels were lower when compared to other Lamiaceae species such as *Ocimum basillicum* L with levels of 48.0 ± 0.73% w/w QE and *Ocimum tenuiflorum* L of 62.1 ± 0.70% w/w QE. The %RSD yield in the methanol extract sample of *C. longifolia* leaves was in accordance with the RSD percent requirements ≤ 4%. This shows that the results have repeatability and those are well accepted so the validity requirements are met (González et al., 2010). Determination of total flavonoid levels has a function to ensure the consistency of the activity of the extract, if the repetition of extracts has consistent levels of total flavonoids, the pharmacological activity of the extract will be consistent (Saifudin, 2014).

Table I. The results of total flavonoid content calculation

Sample Absorbance	Absorb. Average ± SD	Total Flavonoid Content (%w/w QE)	Total Flav. Content Average (%w/w QE) ± SD	RSD (%)
0,236		8,04		
0,235	0,234 ± 0,001	8	7,995 ± 0,050	0,635
0,233		7,94		

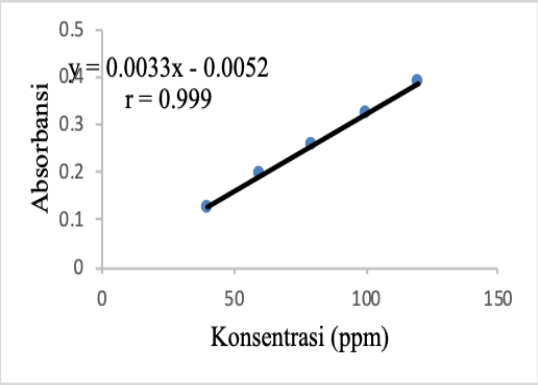

Figure 4. The curves of quercetin standard series with concentration of 40; 60; 80; 100 and 120 ppm

IV. CONCLUSSION

Based on this research, it can be concluded that the results of characterization of kareho leaves from Puruk Cahu, Murung Raya Regency, Kalimantan Tengah have organoleptic and microscopic characteristics, the ethanol soluble extract content was 17.50 ± 0.10% and the water soluble extract content was 16.53 ± 0.37%, the drying loss was 8.16 ± 0.25%, total ash content of 5.52 ± 0.06%, and acid insoluble ash content of 0.07 ± 0.01%. The results of phytochemical screening confirmed the presence of saponins, flavonoids, steroids, tannins, glycosides, and phenols. The results of TLC observation obtained 6 spots at 254 nm UV light and 7 spots appearance on sulphuric acid reagen, determination of the total flavonoid levels was known to be 7.995 ± 0.050% w/w Qurcetin Equivalent.

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