

Spectrophotometric Determination of Total Flavonoid Content in *Biancaea Sappan* (*Caesalpinia sappan* L.) Leaves

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Article info	Abstract
<p>History Submission: 17-06-2020 Review: 06-11-2020 Accepted: 24-02-2021</p> <p>*Email: faradiba.faradiba@umi.ac.id</p> <p>DOI: 10.33096/jffi.v8i3.712</p> <p>Keywords: <i>Caesalpinia sappan</i>; Flavonoid content; Spectrophotometric</p>	<p><i>Biancaea sappan</i> (BS) is traditionally used to treat anticonvulsants, anti-inflammatory, antiproliferative, anticoagulant, antiviral, immunostimulant, antioxidant, and antimicrobial. Flavonoids are found in <i>Secang</i>; flavonoids are secondary metabolites that have antioxidant activity. This study aims to identify the flavonoids using TLC and determination of flavonoids content in BS leaves. Initially, The methanol extract of BS was obtained by maceration with ethanol. The qualitative analysis of flavonoid was using TLC and visualization by sprayed with $AlCl_3$. The determination of total flavonoid content is conducted based on the $AlCl_3$ method with total flavonoids expressed in QE (Quercetin equivalent) at the maximum wavelength of 431 nm. The research results showed that BS leaves contain flavonoids and the total flavonoid content of BS leaf extract is 1.0318 mg QE/g extract.</p>

I. Introduction

Indonesia is one of the mega diversity countries for medicinal plants in the world. Indonesia's tropical forest area has the second-highest biodiversity in the world after Brazil. Of the 40,000 species globally, 30,000 in Indonesia, and 940 have medicinal properties used in traditional medicine from generation to generation by various ethnic groups in Indonesia. The number of medicinal plants covers about 90% of the total number of medicinal plants found in the Asian region (Pertamawati *et al.*, 2017).

Flavonoid compounds are the largest group of phenolic compounds found in nature. These compounds are red, purple, blue and some yellow dyes found in plants (Andersen and Markham, 2005). Several medicinal plants containing flavonoids have antioxidant, antibacterial, antiviral, anti-inflammatory, allergy, and anticancer activities (Miller and Begoña Ruiz-Larrea, 2002). Flavonoid compounds are thought to be very useful in food because, in the form of phenolic compounds, these compounds are potent antioxidants. Many disease conditions are known to be exacerbated by the presence of free radicals such as superoxides and hydroxyl, and flavonoids can virtually eliminate these damaging oxidizing species (Heinrich, M, Bames, J, Gibbons, S, Williamson, E, 2010).

In the previous research, Setiawan had carried out the antioxidant activity test of BS wood extract (Setiawan, Yunita and Kurniawan, 2018). In this test, phytochemical screening was also carried out in which the BS wood was positive for flavonoids. Flavonoid compounds had found in all plants, including seeds, roots, fruits, fruit skins,

leaves, and stems (Harbone, 1987). Based on that theory, the levels of flavonoids contained in BS leaves can be determined. Flavonoids are among the most extensive natural phenolic group antioxidant compounds and are present in all plants to ensure flavonoids in plants.

Quantitative analysis of flavonoids could perform using UV-VIS spectrophotometry. Ultraviolet absorption and visible absorption spectrum are ways to identify flavonoids' structure (Andersen and Markham, 2005) Flavonoids contain a conjugated the aromatic chemical structure can show a strong absorption band in the UV-VIS area (Mukhrani, Nonci and Munawarah, 2015).

Based on the description above, this research was conducted to determine the flavonoid levels of BS leaf extract (*Caesalpinia sappan* L.) to increase scientific data from medicinal plants.

II. Research Method

II.1 Preparation of *Biancaea Sappan* Leaves Powder

Samples of BS (*Caesalpinia sappan* L.) leaves which have been taken, then cleaned by washing with running water, then dried in a drying cabinet with 40-50°C temperature. After that, it is powdered using a blender, and then the sample is ready to be extracted (Rasyid *et al.*, 2017).

II.2 Preparation of Methanol Extract *Biancaea Sappan* Leaves

The fresh leaves were minced into small pieces and macerated with methanol (1:20, w/v) for 72 h at room temperature (28 ± 2 °C) with occasional stirring. The extract was filtered, and the marc was re-macerated with the same solvent until the



extraction was exhausted. The filtrate obtained from the maceration results is combined, then evaporated with a rotary evaporator (Handayani, Kurniawati and Abdul Rasyid, 2020).

II.3 The Qualitative analysis of Flavonoid in Methanol Extract *Biancaea Sappan* Leaves

The qualitative test of flavonoids, thin layer chromatography analysis was performed. BS leaf extract was dissolved with acetone and then spotted on the TLC plate. The plates were inserted in the chamber containing the eluent n-hexane: ethyl acetate (9: 1), then observed under UV254 and UV366 nm. Then sprayed with specific reagents. The reagents often used to identify flavonoids as spray reagents in thin layer chromatography are $AlCl_3$, which give a yellow color (Ahmad, Juwita and Ratulangi, 2015), (R, Dahlia and Ahmad, 2016).

II.4 The Quantitative analysis of Flavonoid in Methanol Extract *Biancaea Sappan* Leaves

II.4.1 Preparation of Standard Solution

10 mg of standard quercetin standard was weighed and dissolved in 10 mL of methanol p.a for 1000 ppm concentration. The stock solution of 1000 ppm quercetin, pipetted 1 mL and dissolved in 10 mL of methanol p.a to obtain 100 ppm, then made several concentrations of 4 ppm, 5, 6 ppm, 7 ppm, and 8 ppm. From each concentration of the quercetin standard solution, 3 mL of methanol, 0.2 mL of 10% $AlCl_3$, 0.2 mL of 1 M potassium acetate were added, and add aquadestilata up to 10 mL, incubated for 30 minutes at room temperature. The absorbance was measured on UV-Vis spectrophotometry with a wavelength of 431 nm. (Aminah, Tomayahu and Abidin, 2017).

II.4.2 Determination of Flavonoid compound of BS Leaf extract

25 mg of the extract was weighed, dissolved in 10 mL ethanol to obtain a concentration of 2500 ppm. 1 mL was pipetted from the solution,

then add 3 mL of methanol, 0.2 mL of 10% $AlCl_3$ and 0.2 mL of 1 M potassium acetate, and 10 mL of aquadestilata. Samples were incubated for 30 minutes at room temperature. The absorbance was measured on UV-Vis spectrophotometry at a wavelength of 431 nm; the samples were made in three replications for each analysis mean absorbance value was obtained. The level of flavonoids can be calculated using the following formula:

$$\text{Total Flavonoid} = \frac{V.\text{Sample} \times \text{Initial Concentration (x)} \times \text{DF}}{\text{Sample weight}} \quad (1)$$

III. Results and Discussion

BS (*Caesalpinia sappan* L.) plant is one of the plants empirically used as a treatment. Based on previous research, BS plants are anticonvulsant, anti-inflammatory, antiproliferative, anticoagulant, antiviral, immunostimulant, antioxidant antimicrobial (Kusmiati, Dameria and Dody Priadi, 2016). Besides, BS is also used to treat diarrhea, dysentery, coughing up blood in tuberculosis, vomiting blood, syphilis, malaria, tetanus, swelling (tumors), and pain due to blood circulation disorders (Dianasari, 2009).

BS leaves have different chemical contents; one of them is flavonoids. Flavonoids are chemical compounds found in almost all plants, namely roots, stems, leaves, fruits, and seeds. Some plants that contain flavonoids have antioxidant, antibacterial, antiviral, anti-inflammatory, antihistamine, and anticancer activity (Ahmad et al., 2015). 100 g of BS (*Caesalpinia sappan* L.) leaf sample extracted by maceration using 2.75 L methanol to obtain 10.063% yield value.

The results of extracting samples of BS leaves (*Caesalpinia sappan* L.) can be seen in the Table 1.

Table 1. Yield value of BS (*Caesalpinia sappan* L.) leaves extract

Sample	Sample weight (g)	Amount of Solvent (L)	Extract result (g)	Yield value
BS Leaves	100	2,75	10,063	10,063

A qualitative test was performed to determine the sample's chemical components using the thin layer chromatography (TLC) method and the result can be seen in the Table 2. The sample extract of BS (*Caesalpinia sappan* L.) leaves dissolved with acetone because acetone is a polar

compound, so it is expected to attract polar flavonoids. Besides, acetone is also volatile so that when it is placed on the TLC plate, the solvent can quickly evaporate (Saputra, 2015).

Table 2. Qualitative test Results of BS leaf (*Caesalpinia sappan* L.) extract flavonoid compound using TLC

Sample	Flavonoid test (AlCl ₃)	Reference (Ahmad, 2015)	Result
BSLeaf	Yellow	Yellow	+

Note: + (contains flavonoid)

Quantitative analysis of total flavonoid compounds using UV-Vis spectrophotometry was carried out to determine the total flavonoid levels contained in BS (*Caesalpinia sappan* L.) leaf extract. Flavonoid analysis was carried out using UV-Vis spectrophotometry because flavonoids have conjugated aromatic systems to show strong absorption bands in the ultraviolet light spectrum and visible light spectrum, because quercetin is a type of flavonoid that is commonly used as a standard in determining levels of flavonoids, quercetin is a class of flavonols that is widespread in plants; besides, flavonols have a keto group found in C-4 and a hydroxyl group on C-3 or C-5 atoms they can form complexes with AlCl₃ (Ipandi, Triyasmono and Prayitno, 2016). In this study, quercetin was

used to determine total flavonoid levels as a standard solution. The result of the quercetin absorbance with concentration 4 ppm, 5 ppm, 6 ppm, 7 ppm and 8 ppm can be seen in the Table 3. Quercetin was used as a standard to establish calibration linear with function, which can be seen in Figure 3.

Table 3. Results of quercetin absorbance measurements at 431 nm

Concentration (ppm)	Absorbance
4	0,398
5	0,505
6	0,593
7	0,709
8	0,829

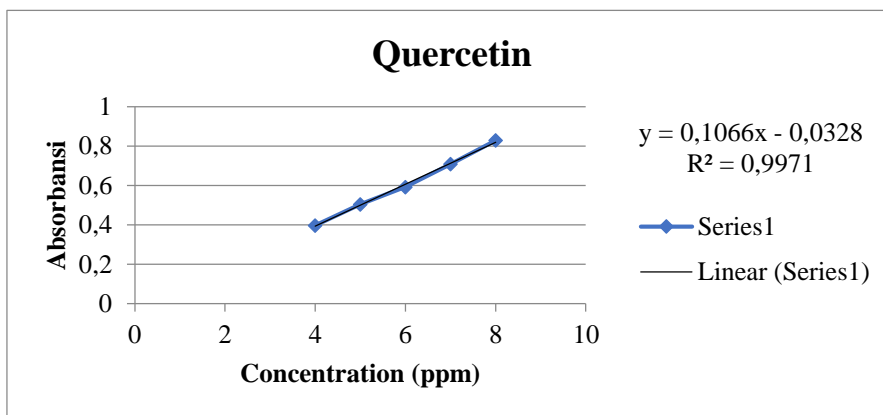


Figure 1. Quercetin calibration curve at a maximum wavelength of 431 nm

In measuring the total flavonoid content of BS (*Caesalpinia sappan* L.) leaf extract, 25 mg of BS leaf extract were weighed then dissolved with 10 ml p.a methanol. Then take 1 mL and then add 3 ml of methanol pa, 0.2 mL of 10% AlCl₃ which can form a complex, resulting in a shift in the wavelength towards the visible (visible) and marked with a solution producing a intensively yellow color, then add 0.2 mL of potassium acetate to maintain the

wavelength in the visible area, then add up to 10 mL of aquadestilata. The mixture is incubated at room temperature for 30 minutes; the mixture is incubated for 30 minutes to make the reaction runs perfectly so that the resulting color intensity is more (Ahmad, Juwita and Ratulangi, 2015). They are then measured at a wavelength of 431 nm. The measurement results can be seen in Table 4.

Table 4. Determination of Total Flavonoid Levels of BS (*Caesalpinia sappan* L.) leaf extract

Sample	Replication	Absorbance	Initial Flavonoid Content (mg/ml)	Total Flavonoid Content (mgQE/g eks)	Total Flavonoid Content average mgQE/g ekss
BSLeaves	1	0,262	0,002773	1,1092	1,0318
	2	0,234	0,002509	1,0016	
	3	0,229	0,002462	0,9848	

The total flavanoid content of BS was calculated from the calibration plot of the linear regression equation (Y = 0.1066 X - 0.0328, R =

0,997) which stated as mg quercetin equivalent (QE)/g of dried plants materials (Chandra *et al.*, 2014). Based on the results, the total flavonoid

content of BS leaf extract (*Caesalpinia sappan* L.) was 1.0318 mg QE/g.

IV. Conclusions

Based on the research results, it can be concluded that the total flavonoid level of BS leaf extract (*Caesalpinia sappan* L.) is 1.0138 mg QE/g.

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