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SPECTROPHOTOMETRIC DETERMINATION OF TOTAL FLAVONOID CONTENT IN BIANCAEA SAPPAN (Caesalpinia sappan L.) LEAVES Nurlinda1, Virsa Handayani1, Faradiba 1* 1Department of Phytochemistry, Faculty of Pharmacy, Universitas Muslim Indonesia, Indonesia *faradiba.faradiba@umi.ac.id Abstract Biancaea sappan (BS) is traditionally used to treat anticonvulsants, anti-inflammatory, antiproliferative, anticoagulant, antiviral, immunostimulant, antioxidant, and antimicrobial treatments. Flavonoids are found in Secang; 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 AlCl3.

The determination of total flavonoid **content is conducted based on** the **AlCl3 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. Keywords: Caesalpinia sappan; Flavonoid content, Spectrophotometric 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, 2014) 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 (Markham, 1988). Several medicinal plants containing flavonoids have antioxidant, antibacterial, antiviral, anti-inflammatory, allergy, and anticancer activities (Miller, 1996). 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, et al. 2010). In the previous research, Setiawan had carried out the antioxidant activity test of BS

wood extract (Setiawan, 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 (Markham, 1988). Flavonoids contain a conjugated the aromatic chemical structure can show a strong absorption band in the UV-VIS area (Mukriani, Nonci & 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 40-50oC temperature. After that, it is powdered using a blender, and then the sample is ready to be extracted (Mukriani et al., 2015) 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. (Martinus, B.V, Verawati, 2015). 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 AlCl3, which give a yellow color. II.4 The Quantitative analysis of Flavonoid in Methanol Extract Biancaea Sappan Leaves 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% AlCl3, 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.

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% AlCl3 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: Total Flavonoid = V.

Sample x Initial Concentration (x) x DF Sample weight III. Result 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, et al., 2014). 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 following table: Table 1. Yield value of

BS (Caesalpinia sappan L.) leaves extract Sample Sample weight (g) BS 100 Leaves Amount of Solvent (L) 2,75 Extract result (g) 10,063 Yield value 10,063 A qualitative test was performed to determine the sample's chemical components using the thin layer chromatography (TLC) method. 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 Reference Result test (AlCl3) (Ahmad 2015)

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. In**

this study, quercetin **was** used **to determine total flavonoid levels**

as a standard solution. 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). 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 Quercetin 0.9 0.8 y = 0.1066x - 0.7 0.0328 Absorbansi 0.6 R^2 = 0.9971 0.5 0.4 Series1 0.3 0.2 Linear 0.1 (Series1) 0 0 5 10 Concentration (ppm) Figure 3. 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 (Azizah and Faramayuda 2014). They **are**

then measured

at a wavelength of 431 nm. The measurement results can be seen **in**

table 4. Table 4. Determination leaf extract of Total Flavonoid Levels of BS(Caesalpinia sappan L.) Sample Replication Absorbance Initial Flavonoid Content (mg/ml) Total Total Flavonoid Flavonoid Content (mgQE/ g eks) Content average mgQE/ g ekss BSLeaves 1 0,262 2 0,234 3 0,229 0,002773 0,002509 0,002462 1,1092 1,0016 0,9848 1,0318 Based on the results, the total flavonoid content of BSleaf extract (Caesalpinia sappan L.) was 1.0318 mg QE /g. IV.Conclusion 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. V. Acknowledgment The authors gratefully thank Pharmacy's faculty, Universitas Muslim Indonesia Makassar, for all the facilities used for this research. References Ahmad, A,R, Juwita, Ratulangi, A, D dan Malik, A 2015, Penetapan Kadar Fenolik dan Flvonoid Total Ekstrak Metanol Buah dan Daun Patikala (Etlingera elatior (Jack) R.M.SM), Pharmaceutical Science and Reseach, Vol. 2 No. 1 Dianasari, N 2009, 'Uji Aktivitas Antibakteri Ekstrak Etanol Kayu BS(Caesalpinia sappan L.) Terhadap Staphylococcus dan Shygella dysentriae serta Bioautografinya', S.Si Skripsi, Universitas Muhammadiyah Surakarta, Direktorat Jendral Pengawasan Obat dan Makanan 2000, 'Parameter Standar Umum Ekstrak Tumbuhan Obat, Departemen Kesehatan Repoblik Indonesia, Jakarta. Dyah N, A, Endang, K, Fahrauk, F 2014, ' Penetapan Kadar Flavonoid Metode AlCl3 pada Ekstrak Metanol Kulit buah Kakao (Theobroma cacao L.)', Fakultas Farmasi Universitas Jendral Achmad Yani, Bandung Gandjar, G. dan Rohman., 2009. Kimia Farmasi Analisis. Pustaka Pelajar : Yogyakarta. Hanani, E 2015, Analisis Fitokimia, EGC, Jakarta, pp. 11 Harbone, J.B 1987, Metode Fitokimia, Penerbit ITB, Bandung Heinrich, M, Bames, J, Gibbons, S, Williamson, E, M 2010, ' 'Farmakognosi dan Fitoterapi', Penerbit Buku Kedokteran, Jakarta Ipandi I, Triyasmono L, Prayitno B 2016, Penentuan Kadar Flavonoid Total dan Aktivitas Antioksidan Ekstrak Etanol Daun Kajajahi (Leucosyke capitellata Wedd.), Jurnal Pharmascience, Vol 3 No. 1, Hal 93-100 Markham, K, R. 1988, 'Cara Mengidentifikasi Flavonoid', Padmawinata K, penerjemah, Terjemahan dari: Techniques of Flavonoid Identification. Martinus, B, V, Verawati 2015, ' Penentuan Kadar Flavonoid Total dan Aktivitas Antioksidan dri ekstrak Daun Bandotan (Ageratium conyzoides L.), Sekolah Tinggi Farmasi Indonesia Perintis Padang, Scientia Vol. 5, No. Miller, A, L 1996, 'Antioxidant Flavonoids, Structure, Function and Clinical Usage,' Alt, Med, Rev 1(2) Mukhriani, Nonci, F, Y, Munawarah, S 2015, ' Analisis Kadar Flavonoid Total Pada Ekstrak Daun Sirsak (Annona muricata L.) dengan Metode Spektrofotometri UV- Vis', JK FIK UINAM Vol. 3, No. 2 Pertamawati, Nuralih, Fahrudin, F 2014, ' Ekstrak BSSebagai Bahan Diuretikum (Pecobaan terhadap Tikus Putih Jantan Galur Spraque Dawley', Jurnal Biologi Vol. 7 No. 2 Saputra,A 2015 'Uji Aktivitas Antiinflamasi Ekstrak Etanol 96% Kulit Batang Kayu Jawa (Lannea coromandelica) Dengan Metode Stabilisasi Membran Sel Darah Merah Secara In Vitro' S.Si Skripsi, Fakultas Kedokteran dan Ilmu Kesehatan, Universitas Islam Negeri, Jakarta.