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Determination of Total Phenol and Flavonoid Contents and Antioxidant Activity from Extract Fraction of Sappan Wood (*Caesalpinia sappan* L.) by Liquid-Liquid Extraction and Vacuum Liquid Chromatography

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This study reported the antioxidant activity of sappan wood (*Caesalpinia sappan* L.) extract and their extract fraction from the liquid-liquid extraction and vacuum liquid chromatography. The study comprises with phytochemical assessments, evaluation of total phenolic content (TPC) and total flavonoid content (TFC). The evaluation of antioxidant activity was carried out by using the DPPH method. The effectiveness of antioxidant activity in sappan wood compared to quercetin. Extract of sappan wood (ESW) yields of 5.40%, extract fraction from liquid-liquid extraction (FE LLE) yields of 52.15% and extract fraction from vacuum liquid chromatography (FE VLC) with silica gel and eluent sequential from *n*-hexane, 5% *n*-hexane/95% ethyl acetate, ethyl acetate and 5% ethyl acetate/45% methanol was produced 60 of fractions extract. The thin-layer chromatography (TLC) was analyzed using silica gel as a stationary phase with the mobile phase of *n*-hexane:ethyl acetate (1:0.55) was obtained 10 of fractions extract in fractions of 6 and 7, which showed the most positive results. The phytochemical test indicated the presence of phenolic and flavonoids compounds. The highest values for TPC was FE LLE (216.67 mg GAE/g), FE VLC 160.83 (mg GAE/g) and ESW (18.33 mg GAE/g), respectively. The highest values for TFC were F VLC (152.48 mg QE/g), FE LLE (66.2 mg QE/g) and ESW (48.4 mg QE/g), respectively. Then, the highest of antioxidants activity were F VLC (5.42 µg/mL), FE LLE (6.41 µg/mL), quercetin (6.52 µg/mL) and ESW (7.82 µg/mL), respectively. The results showed that the highest of TFC was related to the antioxidant activity. However, the highest value of TPC was not showed the highest antioxidant activity. Both ESW, FE LLE and FE VLC with quercetin as control exhibited powerful antioxidants.

Keywords: ***Caesalpinia sappan,*** **Antioxidant, Total phenolic content, Total flavonoid content.**

INTRODUCTION

Oxidative pressure is described as an imbalance of oxygen free radicals or reactive oxygen species (ROS) with the organism's capacity to counteract the oxidation process [1]. ROS is a highly reactive molecule, such as peroxyl, hydroxyl radicals, superoxide anion, hydrogen peroxide and singlet oxygen, which are produced as a byproduct of normal metabolism under aerobic cell condition [2,3]. In general, ROS are produced in medium concentration to maintain normal physiological function in an organism. However, excess production of ROS causes the high concentration, so that it readily reacts with DNA, protein and lipids that cause oxidative damage and cause many chronic diseases [4-6]. Also, oxidative pressure is responsible for decreasing physiological function and food quality, that causes food became toxic, rancid smell and damage crucial biochemical component of food [7].

Antioxidants can prevent chronic disease and protect food ingredients by cleaning free radicals that will inhibit the oxidation reaction process [8,9]. The body naturally obtains antioxidants from food ingredients [10-12], containing vitamins or phytochemicals. Generally, vitamins that function as antioxidants are vitamin A (retinol), C (ascorbic acid) and E (α-tocopherol) [13,14]. Examples of phytochemical compounds known as

antioxidants are phenol or polyphenol and flavonoids [15-17]. There are also synthetic antioxidants which are often used in food additives, namely butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tetrahydroxyquinone (TBHQ). The use of synthetic antioxidants is due to their low price and effectively prevents oxidation reactions in foodstuffs. However, excessive use of synthetic materials will harm health [18].

One of Indonesia's abundant and cheap materials is secang (sappan) wood (*Caesalpinia sappan* L.), which is widely used sappan plants in overcoming diseases [19,20]. Sappan wood consists of flavonoid, brazilin, alkaloid, saponin, tannin, phenyl propane and terpenoid [21]. Traditionally, sappan wood is used as a colouring agent in food [22]. Several studies explained that phenolic compounds, polyphenols and flavonoids, show potential antioxidant activity. Previous research [23] has proven that sappan bark has xanthine oxidase inhibitory activity. A methanol extract of sappan wood has also been shown to deal with the xanthine oxidase enzyme activity [24,25]. However, research on the extract fraction activity, especially as an antioxidant, is still limited. This research was conducted to study the antioxidant activity of sappan wood extract and its extract fraction from the liquid-liquid extraction and vacuum liquid chromatography.

EXPERIMENTAL

The chemicals used in this study *viz. n*-hexane, ethyl acetate, chloroform, acetic anhydride, H_2SO_4, HCl, methanol, ethanol, $FeCl_3$ and Na_2CO_3 (Merck, Germany) were of analytical grade. Silica gel 60 (0.040-0.063 mm, 300-400 mesh), Dragendorf reagent, Mayer reagent, Folin-Ciocalteau reagent, gallic acid, quercetin and DPPH reagent were procured from Sigma-Aldrich, USA.

Sappan wood was obtained from the Kendal area, Central Java and its identification was carried out at the Biology Laboratory, Universitas Negeri Semarang. Sappan wood (*Caesalpinia sappan* L.) was cleaned and dried in the open air. The dry sample was blended and weighed as much as 2 kg.

Extraction of sappan wood: Sappan wood dry powder (2 kg) was extracted by maceration method using *n*-hexane solvent for 5 × 24 h, then filtered. The obtained residue was macerated again with methanol for 7 × 24 h and filtered. The filtrate was concentrated using a rotary evaporator for the extraction process.

Sappan wood fractionation by liquid-liquid extraction (LLE): The liquid-liquid extraction (LLE) was carried out by dissolving methanolic extract with 400 mL of *n*-hexane with constant stirring and then, transferred to a separating funnel containing 400 mL of water. The mixture was then shaken several times and obtained the *n*-hexane fraction. The remaining water fraction obtained was re-partitioned using ethyl acetate to obtain the ethyl acetate fraction. The *n*-hexane and ethyl acetate fractions were then concentrated using a rotary evaporator.

Sappan wood fractionation by vacuum liquid chromatography (VLC): The ethyl acetate extract fraction obtained from LLE was fractionated by vacuum liquid chromatography (VLC). The sample mixed with silica gel 60 (0.040-0.063 mm, 230-400 mesh) until homogenous. Then, put in a vacuum liquid column and compacted. Samples were eluted by *n*-hexane 100%, ethyl acetate (5-95%), ethyl acetate 100% and ethyl acetate: methanol (5:45%). The fraction obtained from separation by VLC was collected in a glass bottle and then concentrated by a rotary evaporator. The fraction obtained was analyzed by the separation pattern and its components using the TLC.

Phytochemical analysis of sappan wood

Alkaloid compound test: The alkaloid test was carried out with Dragendorf reagent. Ethanol (5 mL) was added in the sappan wood extract (1 g) followed by the addition of 2 drops of Dragendorf reagent resulted in the formation of reddish-orange precipitates [26].

Test for steroid and terpenoid: Sappan wood extract (1 g) was added with 5 mL ethanol, 2 mL chloroform and 2 drops of acetic acid anhydride. Then, 2 drops of conc. H_2SO_4 were added through the walls of the test tube. The formation of green or blue colour indicated the presence of steroids. Meanwhile, if a reddish-purple colour is formed, the extract positively contains terpenoids [26].

Flavonoid test: Sappan wood extract (1 g) was added with 5 mL of ethanol. The solution was added with 1 mL of hot methanol and magnesium metal on the spatula's tip and a 0.5 mL of conc. HCl. The formation of a red or orange solution indicated the presence of flavonoid [26].

Phenolic test: Sappan wood extract (1 g) was mixed with 5 mL of ethanol followed by the addition of 1 mL of $FeCl_3$ solution. A purple-blue colour formation confirmed the presence of phenolic compounds [26].

Analysis of extract fractions from vacuum liquid chromatography (VLC) by thin layer chromatography (TLC): The fractions of VLC were analyzed for their components by TLC. Fractions had similar spots or stains and then the TLC analysis was carried out again. The retention factor (R_f) value was calculated.

Determination of total phenolic content (TPC): The maximum wavelength was determined using a UV-vis spectrophotometer using gallic acid as the standard solution. Separately, 0.5 mL of gallic acid and 0.5 mL of sample solution was added 2.5 mL of Folin-Ciocalteau reagent, 30 mL of distilled water and stirred for 5 min. These two solutions were added with 7.5 mL of 7.5% (w/v) Na_2CO_3 solution and distilled water until the volume became 50 mL. The mixture was stirred and incubated for 30 min in the dark at room temperature (28-30 ºC). After 30 min, the sample solution was subjected to a wavelength scan at 600-800 nm and showed the maximum wavelength absorption at 765 nm.

Preparation of standard calibration curves for gallic acid for the determination of TPC: Gallic acid (50 mg) was dissolved in ethanol and made up the final volume of 50 mL. Then gallic acid solution was taken from 0.25 to 5 mL and diluted with distilled water to a final volume of 10 mL so that the concentration of the gallic acid solution was 25-500 µg/mL. Each 0.5 mL of standard gallic acid solution (50-250 µg/mL) was mixed with 2.5 mL of Folin-Ciocalteau reagent in a 50 mL volumetric flask. After mixing for 5 min, 7.5 mL of 7.5%

(w/v) sodium carbonate solution was added and distilled water until the volume was 50 mL. The mixture was stirred and incubated for 30 min in the dark at room temperature (28-30 ºC). The absorbance was measured at a maximum wavelength of 765 nm against the blank solution using a UV-vis spectrophotometer. The calibration curve obtained was expressed in the linear regression equation at concentrations of 25-500 µg/mL. The regression equation is $y = 0.001x + 0.011$; $R^2 = 0.986$. The phenol concentration was expressed in µg/mL on a standard curve. The TPC obtained was expressed as mg GAE (gallic acid equivalent)/g of a sample. All the determinations were made by three times replication.

Determination of total flavonoid content (TFC): Determination of the maximum wavelength was carried out using a UV-vis spectrophotometer using quercetin as standard solution. Quercetin solution (2 mL) and the sample solution (1 mL) were added separately to 3 mL of 0.1 mol/mL solution of $AlCl_3$ solution. Then, the two solutions were mixed and diluted with 70% ethanol solution. After 0.5 h, the sample solution was scanned at 400-500 nm and showed the maximum wavelength absorption at 425 nm [27].

Preparation of quercetin standard calibration curves for the determination of TFC: Quercetin (50 mg) was dissolved in ethanol to a final volume of 50 mL. The quercetin solution was taken 0.25-2 mL and diluted with distilled water to a final volume of 10 mL, hence the concentration of the quercetin solution became 40-180 µg/mL. Each concentration of quercetin solution (2 mL) was added to 2 mL of 2% $AlCl_3$ solution and 2 mL of 120 mM potassium acetate solution. Samples were incubated for 1 h at room temperature (28-30 ºC). The absorbance was measured at a maximum wavelength of 425 nm against the blank solution using a UV-vis spectrophotometer. The calibration curve was expressed in a linear regression equation at a 25-200 µg/mL [27]. The regression equation is $y = 0.005x - 0.025$; $R^2 = 0.992$. Flavonoid concentrations are expressed in µg/mL on a standard curve. The TFC obtained was expressed as mg QE (quercetin equivalent)/g of a sample. All the determinations were made by replication three times.

Antioxidant activity: Determination of antioxidant activity was conducted using free radical scavenging activity based on 1,1-diphenyl-2-picrylhydrazyl ($DPPH^•$) method. DPPH solution (1 mL) was added with 2 mL of methanol and left for 30 min in the dark at room temperature (28-30 ºC) and the absorption was measured with a UV-vis spectrophotometer at a wavelength ranging from 510-520 nm to obtain the maximum wavelength. The absorbance of DPPH was determined before-hand as control at a maximum wavelength of 517 nm.

Each sample (2 mg) of ESW, LLE and VLC and quercetin were weighed and dissolved separately using 10 mL methanol. The solution was mixed a 100 mL volumetric flask and methanol was added to the limit mark so that a solution concentration of 1000 µg/mL was obtained. Antioxidant testing was carried out by taking 3 mL of sample test solution and adding 1 mL of DPPH solution in methanol and diluting it with methanol so that the concentration becomes 10-50 µg/mL with 1 mL of methanol as a blank solution. The mixture was left for 0.5 h in a dark place at room temperature (28-30 ºC). Each antioxidant activity test was carried out three times. The absorbance measurement results have analyzed the percentage of their antioxidant activity using eqn. 1:

$$\text{Inhibition } (\%) = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100 \quad (1)$$

The linear regression line equation obtained was used to evaluate the IC_{50} value.

RESULTS AND DISCUSSION

The extraction process was carried out to take the chemical compounds contained in the sample. The principle of extraction is based on the mass transfer of the dissolved substance components into the solvent. There is a displacement of the interface layer and diffuses into the solvent-extraction of sappan wood (*C. sappan* L.) using the maceration method with *n*-hexane and methanol solvents. The maceration method was chosen since, it is easy and does not use high temperatures, which might damage chemical compounds with antioxidant activity in the sappan wood simplicia. The macerated filtrate was concentrated with a rotary evaporator to obtain 2.1436 g thick yellow *n*-hexane extract with a 0.10718% yield and 108.0855 g thick brown methanol extract with 5.404275% yield.

Fractionation of secang wood extract with liquid-liquid extraction (LLE): The thick extract of sappan wood was then fractionated using the liquid-liquid partition extraction method. The fractionation of the liquid-liquid partition extraction was carried out using *n*-hexane (non-polar), ethyl acetate (semi-polar) and the remaining solvent in water (polar). The purpose of this method is to separate chemical compounds based on their level of polarity. This liquid-liquid extraction process is repeated until clear *n*-hexane and ethyl acetate solvents are obtained, indicating no more sample components dissolved in *n*-hexane ethyl acetate solvents. The results of the extraction of the liquid-liquid partition were then concentrated with a rotary evaporator so that the *n*-hexane fraction was 0.0161 g and the ethyl acetate fraction was 56.37 g with a yield of 52.15% from the initial wood methanol extract.

Fractionation of secang wood extract by vacuum liquid chromatography (VLC): The ethyl acetate fraction was separated by vacuum liquid chromatography (VLC) method. The working principle of VLC is the partition and adsorption of compound components in the extracted sample whose separation is assisted by pressure from a vacuum device. Separation with VLC aims to determine which subfraction has antioxidant activity. The VLC results obtained 60 fractions, which were then analyzed by TLC to see the chromatographic profile.

The fractions that have similar spots are put together and 10 subfractions are obtained. The spot profile on TLC of the 10 subfractions obtained is seen using UV lamps at 366 and 254 mm (Fig. 1). The R_f value for each subfraction is shown in Table-1.

Table-1 shows that the R_f value of the subfraction 1-10 of the VLC yield varies from the sappan wood extract. However, fractions, F6 and F7 have the same R_f values: spot 1 = 0.2, 2 = 0.3, 3 = 0.35, 4 = 0.5 and 5 = 0.8. According to Rusnaeni & Fitria [19], two or more compounds can be considered identical

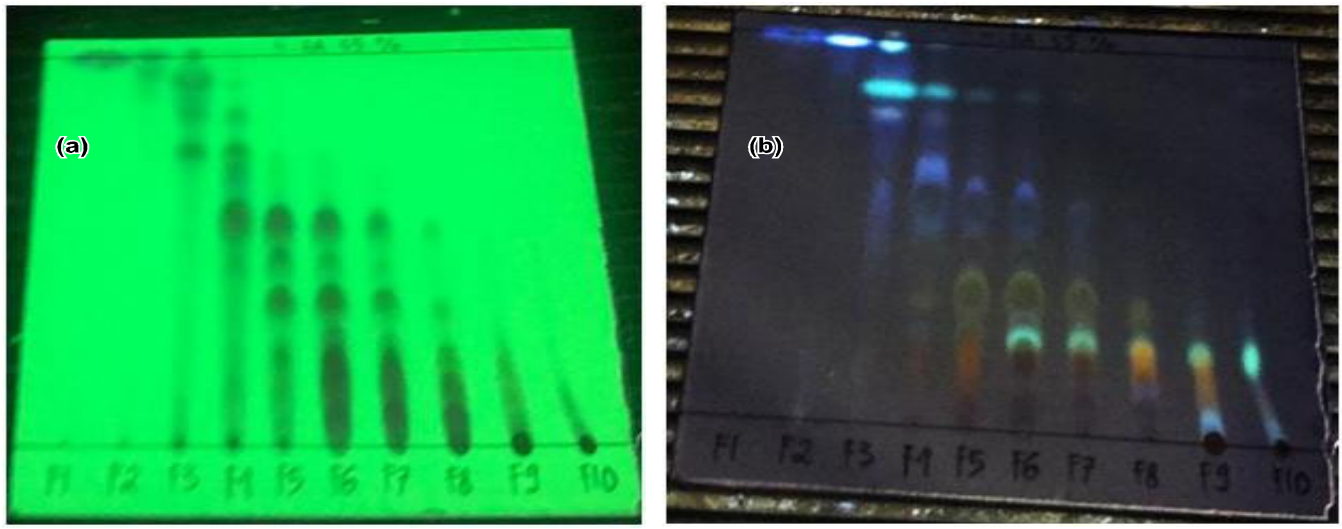

Fig. 1. Thin-layer chromatography (TLC) profile of subfractions 1-10 of sappan wood (*Caesalpinia sappan* L.) extract resulted from vacuum liquid chromatography (VLC) using (a) 366 mm and (b) 254 mm UV lamp

TABLE-1
R_f VALUE, NUMBER OF SPOTS AND SPOT COLOUR IN SUBFRACTIONS 1-10 FROM SAPPAN WOOD (*Caesalpinia sappan* L.) EXTRACT RESULTED FROM VACUUM LIQUID CHROMATOGRAPHY (VLC) USING THE ELUENT RATIO OF *n*-HEXANE AND ETHYL ACETATE (1:0.55)

Subfraction	Number of spots	Retention factor (R_f)	Colour
F1	1	0.9	Purple
F2	1	0.9	Blue
F3	3	0.6	Purple
		0.7	Blue
		0.9	Blue
F4	7	0.2	Yellow
		0.3	Yellow
		0.4	Purple
		0.5	Purple
		0.6	Purple
		0.7	Blue
		0.9	Blue
F5	5	0.2	Orange
		0.3	Yellow
		0.4	Purple
		0.5	Purple
		0.8	Blue
F6	5	0.2	Brown
		0.3	Green
		0.35	Yellow
		0.5	Purple
		0.8	Blue
F7	5	0.2	Brown
		0.3	Green
		0.35	Yellow
		0.5	Purple
		0.8	Blue
F8	3	0.2	Purple
		0.3	Orange
		0.4	Yellow
F9	3	0.2	Blue
		0.3	Orange
		0.4	Green
F10	2	0.2	Blue
		0.4	Green

if they have the same R_f values with the same TLC conditions. Then, F6 and F7 are taken together to test its antioxidant activity because of its similarity to quercetin, which is likely to contain numerous flavonoids.

Phytochemical analysis of sappan wood extract revealed that sappan wood (*C. sappan* L.) can detect secondary metabolites in alkaloid, terpenoid, phenolic and flavonoid compounds. However, it cannot detect steroid compounds. Table-1 presents the results of the phytochemical assay on the detected secondary metabolites inside sappan wood.

The presence of phenolic and flavonoid compounds in sappan wood indicates that it can have antioxidant activities, and can be further analyzed quantitatively for phenolic and flavonoid compounds through the total phenolic and flavonoid contents by using the Folin-Ciocalteu and $AlCl_3$ methods, respectively. Table-2 presents the results of the phytochemical assay of sappan wood.

TABEL-2
PHYTOCHEMICAL ASSAY OF SAPPAN WOOD (*Caesalpinia sappan* L.)

Compound	Phytochemical assay*			Reaction result
	ESW	FE LLE	FE VLC	
Alkaloid	+	+	+	Orange sediment
Steroid	–	–	–	Red
Terpenoid	+	+	+	Red
Phenolic	+	+	+	Blackish blue
Flavonoid	+	+	+	Red

*+: Positive/Detected; –: Negative/Undetected
ESW = Extract of sappan wood; FE LLE = Fraction of sappan wood extract from liquid-liquid extraction; FE VLC = Fraction of sappan wood extract from vacuum liquid chromatography.

For the evaluation of total phenolic content (TPC), phenol compounds were quantitatively analyzed using a UV-visible spectrophotometer to determine the phenol content in the sappan wood extract. The extract fraction from the liquid-liquid partition resulted from vacuum liquid chromatography.

In this study, the Folin-Ciocalteu method was used to determine the TPC of the sample as a standard solution with a concentration series of 50, 100, 150, 200, and 250 µg/mL. The results showed that the maximum wavelength of quercetin is 765 nm. The maximum wavelength used to measure the absorption curve of gallic acid calibration is shown in Fig. 2. These results suggested that the higher the concentration used, the higher the absorbance value obtained. The result of the standard curve of gallic acid obtained is plotted between the level and the absorbance, so that the linear regression equation was obtained ($y = 0.001x + 0.011$) with the R^2 value of 0.986. The gallic acid calibration curve equation can be used as a comparison to determine the the total phenolic compound concentration in the sample extract.

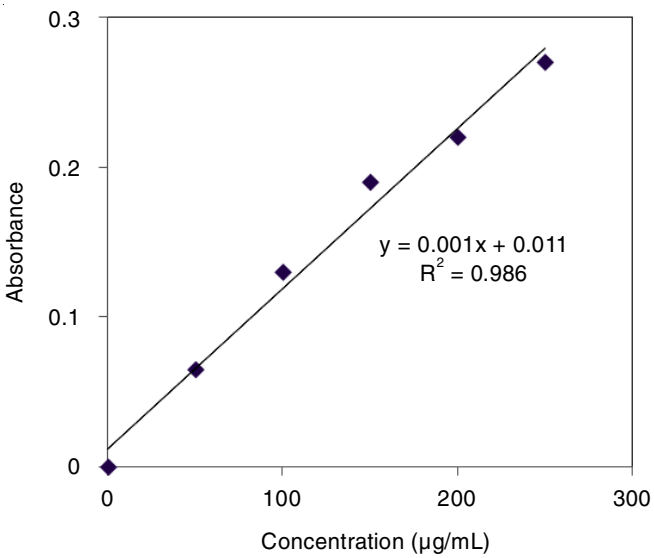

Fig. 2. Calibration curve of gallic acid at a maximum wavelength of 765 nm

Table-3 shows that the highest TPC was found in the extract fraction from the liquid-liquid extraction. The lowest TPC was noted in the extract fraction from VLC. The result indicates that the liquid-liquid extraction process could separate components other than phenolic compounds. Compounds such as polysaccharides from amylose, cellulose, hemicellulose and lignin were not present in the extract fraction from VLC. Then, the TPC in the extract fraction from VLC showed the lowest result, possibly because only certain compounds according to their polarity present in the extract fraction.

TABLE-3
EVALUATION OF TOTAL PHENOLIC CONTENT IN SAPPAN WOOD (*Caesalpinia sappan* L.)

Sample	TPC (mg GAE/g)
ESW	183.33
FE LLE	216.67
FE VL	160.83

ESW = Extract of sappan wood; FE EPC = Fraction of sappan wood extract by liquid-liquid extraction; FE VLC = Fraction of extract sappan wood by vacuum liquid chromatography.

Evaluation of total flavonoid content (TFC): The quantitative analysis of flavonoids was conducted using a UV-visible spectrophotometer using $AlCl_3$ method. This method can be used to determine the flavonoid levels in the sappan wood extract, the extract fraction from the LLE and the extract fraction from the results of VLC. In this study, quercetin was used to determine the TFC in the sample as a standard solution with a concentration series of 40, 60, 70, 80, 100, 120, 140, 160 and 180 µg/mL. The results showed that the maximum wavelength of the standard quercetin is 425 nm. The maximum wavelength used to measure the absorption of the wood extract sample, the extract fraction from the LLE, and the extract fraction from the VLC are shown in Fig. 3, which indicate that the higher the absorbance was obtained at higher concentration. The quercetin calibration curve equation can be used as a comparison to determine the TFC in the sample extract.

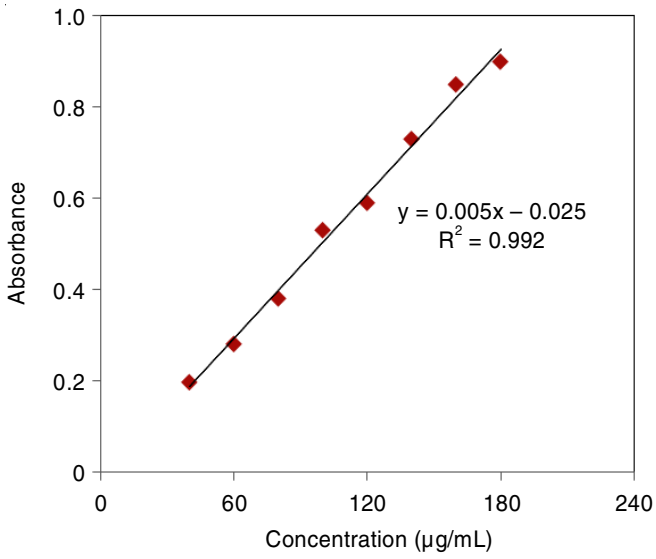

Fig. 3. Quercetin calibration curve at a maximum wavelength of 425 nm

Quantitative analysis with a UV-visible spectrophotometer used a blank solution as control, which functions as a blank to multiply zero compounds that do not need to be analyzed. In the TFC measurement, the sample solution was added to $AlCl_3$, which can form a complex, resulting in a shift in the wavelength towards the visible light, marked with the solution producing a yellow colour. The addition of potassium acetate aims to maintain the wavelength in the visible area. Incubation treatment for 1 h before the measurement was intended to stabilize the resulting colour intensity.

Table-4 shows that the highest yield was found in the extract fraction from the result of VLC. At the same time, the lowest yield was found in the extract. The polarity level of the sample will affect the TFC. These results indicate that the VLC process has most likely succeeded in separating flavonoids based on the polarity degree of other sappan wood components. The TFC results of the extract fraction of the LLE still contained the largest flavonoids other than phenolic compounds.

Antioxidant activity: In this study, the antioxidant activity was performed using the DPPH method. A quantitative antioxidant activity testing was performed for sappan wood extract samples, extract fraction from LLE and extract fraction (F6-F7) from VLC, along with positive quercetin control at a wave-

TABLE-4
EVALUATION OF TOTAL FLAVONOID CONTENT IN SAPPAN WOOD (*Caesalpinia sappan* L.)

Sample	Total flavonoid content (mg QE/g)
ESW	48.40
FE LLE	66.20
FE VL	152.48

ESW = Extract of sappan wood; FE EPC = Fraction of sappan wood extract by liquid-liquid extraction; FE VLC = Fraction of extract sappan wood by vacuum liquid chromatography.

length of 517 nm.The antioxidant activity results of sappan wood extract and extract fraction from LLE and VLC with quercetin compared with TPC and TFC are shown in Table-5.

TABLE-5
COMPARISON OF TOTAL PHENOLIC CONTENT (TPC) AND TOTAL FLAVONOID CONTENT (TFC) WITH THE ANTIOXIDANT ACTIVITY OF EXTRACTS AND EXTRACT FRACTIONS FROM SAPPAN WOOD (*Caesalpinia sappan* L.) WITH QUERCETIN AS A COMPARISON INDICATED BY THE VALUE OF IC_{50} (THE HALF MAXIMAL INHIBITORY CONCENTRATION)

Sample*	TPC** (mg GAE/g)	TFC*** (mg QE/g)	IC_{50} (µg/mL)
ESW	183.33	48.4	7.82
FE LLE	216.67	66.2	6.41
FE VLC	160.83	152.48	5.42
Quercetin	–	–	6.52

*ESW = Ekstract sappan wood; FE LLE = Fraction of sappan wood extract by liquid-liquid extraction; FE VLC = Fraction of sappan wood by liquid vacuum chromatography; **TPC = Total phenolic content which obtained and expressed as mg QE (quercetin equivalent)/g sample; ***TFC = Total flavonoid content which obtained and expressed as mg QE (quercetin equivalent)/g sample.

Table-5 shows that the IC_{50} was the lowest at 5.42 µg/mL concentration of the sappan wood extract from VLC, whereas the lowest was observed at 7.82 µg/mL concentration of the sappan wood extract. A lower IC_{50} value indicated a higher radical scavenging effect. The smaller the IC_{50} value, the higher the antioxidant activity. The sample has powerful, strong, moderate, or weak antioxidants depending on whether its IC_{50} value is < 50, 50-100, 100-150 or 150-200 µg/mL, respectively. The samples with the strongest to the weakest antioxidants are FE VLC (5.42 µg/mL), FE LLE (6.41 µg/mL), quercetin (6.52 µg/mL), and ESW (7.82 µg/mL).

Additionally, present study showed that the higher the TFC, the higher the antioxidant activity (Table-5). The FE LLE sample, which had a TFC of 152.48 mg QE/g, showed the highest antioxidant activity with an IC_{50} value of 5.42 µg/mL. Meanwhile, a high TPC does not necessarily indicate a high antioxidant activity. The FE LLE sample, with a TPC of 216.67 mg GAE/g, had antioxidant activity of 6.41 µg/mL, which was lower than that of FE VLC. In this study, flavonoid compounds in sappan wood are found to be more effective than phenols as antioxidants in inhibiting the free radical activity by up to 50%.

Conclusion

Sappan wood extract fractions of liquid-liquid extraction (LLE) and vacuum liquid chromatography (VLC) were analyzed for antioxidant activity by using the DPPH method. Both FE VLC (5.42 µg/mL) and FE LLE (6.41 µg/mL) fractionates consisted of potent antioxidant activities. The sappan wood extract of TPC was higher than TFC whereas, TFC has a higher antioxidant activity. That means a high TPC count does not necessarily indicate a high antioxidant activity. Moreover, this study showed that sappan wood flavonoid compounds are more effective than phenols as antioxidants in inhibiting free radical activity by 50%.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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