

# Identification Phytochemicals and Antioxidant Activities of Various Fractions of Methanol Extracts from Bark of Kulim Tree (*Scorodocarpus borneensis* Becc.)

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## ABSTRACT

Phytochemicals and antioxidant activity of methanolic extract the Kulim's bark were investigated. The extract of *S. borneensis* barks was partitioned by fractionation of gradient elution, sequentially, i.e., hexane, ethyl acetate, ethanol, methanol, and 70% methanol, then stirred for 24 h. The fractions were evaporated at T 40 °C to gain the concentrated samples. Phytochemical of *Scorodocarpus borneensis* bark fractions were determined qualitatively and quantitatively on the phenolic, flavonoid, and alkaloid compounds. The antioxidant activities of those fractions were investigated by DPPH free radical scavenging activity method. The result showed that all the crude fractions consisted of similar phytochemical substances, except n-hexane fraction, which alkaloid and flavonoid were absent. The fraction of ethyl acetate was identified the most significant flavonoid content and scavenging activity against free radical of DPPH ( $273.13 \pm 2.25$  mg QE/g extract and 57.88 ppm, respectively); while methanol fraction showed the highest total phenolic content and alkaloid content, were about  $2332.64 \pm 59.23$  mg GAE/g and  $19.67 \pm 2.08$  mg BE/g extract, respectively.

**Keywords:** *Scorodocarpus borneensis* Becc., antioxidant activity, phytochemicals

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## INTRODUCTION

Plants have been utilized for food sources and remedies since ancient times. Every part of plants (roots, bark, fruit, and leaf) contains enormous biochemical properties that provide several medicinal effects to the human body. The biochemical properties, such as phenolic compounds are playing a significant role since their antioxidant activity. Each plant possesses different biochemical substances, as well as its medicinal effect. The study about biochemical compounds has been executed extensively due to serious concern to the health issue in modern ages. Various plants, particularly indigenous plants that have not yet discovered its biochemical compounds, could be the alternative, moreover the new source to solve the health issue in the future.

Indonesia is a country with abundant natural resources, especially the diversity of plants. Thus due to the extensive tropical rain forests. The plants in tropical rain forests of the island of Kalimantan/Borneo are believed to have health properties. Kulim tree (*Scorodocarpus borneensis* Becc.) or also known as the Garlic tree, is one of the ancient plants grow in such forest with limited investigation yet. Kulim tree belongs to the Olacaceae family plants, which have phytochemicals consist of flavonoids, tannins, glycoside cyanogenetic, polyacetylene fatty acids, and polysulfide substances (Wiar et al., 2001). The natives use the leaves, barks, and fruits as seasoning and preservation agents since the Kulim tree has a similar polysulfide compound as garlic (Kubota et al., 1994).

According to (Sudrajat et al., 2016), the ethyl acetate extract derived from the bark of the Kulim tree dominated by some compounds, including phenol, fatty acid, fatty alcohol,

phthalate acid, triterpene, and a carboxylic acid. Furthermore, several methanolic extracts of the bark, leaves, and fruit of this tree also performed antibacterial activity toward the colony of *S. aureus* and *E. coli* besides a strong antioxidant activity (Dewi et al., 2020). However, there is no study conducted on various solvent fractionation toward its phytochemical and antioxidant activity. Thus, this study aimed to find out the proper solvent in obtaining the extract of barks of *Scorodocarpus borneensis* possessing the highest phytochemicals and antioxidant activity.

## MATERIALS AND METHODS

### Materials

The main material was the bark of *S. borneensis* were obtained from the forest in Sanggau Regency, Kalimantan Barat, Indonesia ( $0^{\circ}23'16.7''N$  and  $110^{\circ}43'24.8''E$ ). After cleaned, the barks were dried at room temperature. It was then ground finely 80-mesh size and stored at a closed container at room temperature.

### Extraction and Fractionation

The methanolic extract of Kulim bark was prepared by maceration using 70 % methanol following the procedure describe by (Dewi et al., 2020) before submitted to the fractionation stage. Fractionation was executed by gradient elution of 150 ml of n-hexane, ethyl acetate, ethanol, methanol, and 70% methanol, respectively, stirred 24 h then filtered by Whatman No.1 and evaporated at 40 °C by a rotary evaporator. The fractions obtained were stored in the freezer until further analysis (Dewi and Purwayantie, 2019).

### Phytochemical Screening

The crude of each fraction was tested for the presence of alkaloid, flavonoid, phenolic, tannin, and terpenoid, according to (Prabhavathi et al., 2016).

Alkaloid was identified by the following instruction. About 1 ml of each sample added 1 ml of marquis reagent followed by 2 ml of concentrated sulfuric acid, add some drops of 40% formaldehyde, then homogenized. The formation of purple or deep orange color showed the presence of alkaloid components in the sample.

Flavonoid was identified by the following instruction. Each sample taken 2 ml and then put a few drops of 20% sodium hydroxide (w/v), observed the change in yellow color formed. Added a few drops of 37% HCl to remove the yellow color in the mixture. The formation and loss of yellow in the sample showed the presence of a flavonoid component.

Phenolic was identified by the following instruction. Each sample taken 2 ml, added 2 ml of 5% (w/v) aqueous iron (III) chloride. The formation of a blue color indicated the phenol component in the sample.

Tannins was identified by the following instruction. Each sample taken 2 ml, add 2 ml of 10%(w/v) methanolic iron(III) chloride. The formation of black or color indicated the tannin components.

Terpenoids was identified by the following instruction. Each sample taken 1 ml, added 0.5 ml chloroform and continued by drops of concentrated sulfuric acid. The reddish-brown color indicated the terpenoid components in the extract.

### Total phenolic content determination

The determination was performed by the Folin-Ciocalteu method (Farhan et al., 2012) with a minor modification. about 200 µL sample mixed 1 ml folin reagent (1:10 v / v) continued by adding 3 ml sodium carbonate (2% w / v). The solution was homogenized and placed at room temperature for 30 min. The absorbance was determined at 765 nm. The calibration curve was according to gallic acid as a standard solution for a calibration curve. The total phenolic content was presented as a percentage of total gallic acid equivalent per 1 g extract (mg GAE /g).

### Total flavonoid content determination

Total flavonoids were identified by the aluminum chloride method (Chang et al., 2002) with modifications. Briefly,

500 µL sample mixed with 0.1 ml aluminum chloride (10% w/v), 1.5 ml methanol, distilled water 2.8 ml, and 0.1 ml potassium acetate 1 M. The solution was mixed and placed at room temperature for 30 min. The absorbance was measured at λ 415 nm. The calibration curve was following Quercetin as a standard solution. Total flavonoid was presented as a percentage of total quercetin equivalent per 1 g extract (mg QE /g).

### Total alkaloid content determination

Total alkaloid content evaluation was performed following (Rinaldi et al., 2017) with modifications. The sample dissolved in 3 ml phosphate buffer solution of 4.5 pH and subjected to a separatory funnel. The solution then mixed with 3 ml of 0.03% bromocresol green solution. After 30 min, chloroform about 1, 2, 3, and 4 mL was added and stirred for 2 min. The lower layer was separated after 10 minutes, and the extract was collected in a 10 ml volumetric flask and diluted up to the mark with chloroform, the absorbance measured at λ 415 nm. Berberine solution (20 - 140 µg / ml) was prepared with the same procedure to obtain the calibration curve. Total alkaloid content was presented as percentage of total berberine equivalent per 1 g extract (mg BE/g).

### Antioxidant activity identification

Free radical inhibition activity was evaluated by the DPPH free radical scavenging method following (Fadly et al., 2020a) with modification. About 4 ml sample mixed with 2 ml of 0.2 mM DPPH methanolic solution, placed in the dark at room temperature for 30 min. The absorbance was measured at λ 517 nm. The radical inhibition calculated as:

$$\text{Radical inhibition (\%)} = [(A_b - A_e) / A_b] \times 100$$

Note:  $A_b$  = absorbance of the control (blank)

$A_e$  = absorbance of extract.

## RESULT AND DISCUSSION

### Phytochemical screening

The phytochemicals in fraction derived from methanol extract of Kulim bark was shown in Table 1. Following the result, all the *Scorodocarpus borneensis* bark fractions identified the absence of terpenoids. The alkaloid, phenolic, flavonoid, and tannin were found in all fractions, except the n-hexane, which only showed the presence of phenols and tannins.

**Table 1.** Phytochemical of the Fractions from the Bark of *Scorodocarpus borneensis*

Fraction	Alkaloid	Phenolic	Flavonoid	Tanin	Terpenoid
N-hexane	X	√	X	√	X
Ethyl Acetate	√	√	√	√	X
Ethanol	√	√	√	√	X
Methanol	√	√	√	√	X
Methanol 70%	√	√	√	√	X

Note: √ = present, X = absent

Phytochemical extracted from plants were affected by solvent polarity, the ratio of solvent and plant materials, material particle size, extraction method, and temperature (Kannamba et al., 2017; Warsidah et al., 2020). There are several studies reported that solvent polarity affects the phytochemical composition of plant extract (Deepa and Nalini, 2013). Those indicate the phytochemical compounds contributed to the

medicinal effect. For instance, tannins were known and used as an astringent, antidiuretic, antitumor, antiseptic, etc. (Bruyne et al., 1999; Dolara et al., 2005).

### Total phenolic content

Our observation revealed the total phenolic content varied from 183.33 ± 47.74 to 2332.64 ± 59.23 mg GAE/g (Table 2).

The highest total phenolic content was obtained from the methanol fraction, and the lowest was obtained from n-hexane fraction

**Table 2.** Total Phenolic Content of the Fraction from the Bark of *Scorodocarpus borneensis*

Fraction	Total Phenolic (mg GAE/g)	Total Flavonoid (mg QE/g)	Total Alkaloid (mg BE/g)
n-hexane	183.33 ± 47.74	-	13.00 ± 0.49
Ethyl Acetate	478.47 ± 26.21	273.13 ± 2.25	17.17 ± 2.03
Ethanol	1950.69 ± 61.04	79.58 ± 4.16	19.00 ± 2.51
Methanol	2332.64 ± 59.23	86.46 ± 4.07	19.67 ± 2.08
70 % Methanol	2030.56 ± 33.48	85.63 ± 5.34	17.96 ± 1.67

Several studies found similar results in which methanol was the most proper solvent to obtain the phenolic substances from materials (Banerjee and Bonde, 2011; Chigayo et al., 2016). It was also known the phenolic content was affected by the type of the plant or materials, the usage of solvent polarity (Jakopič et al., 2009; Jang et al., 2007), a particular part of the plant, and the physiological age of the plants (Luximon-Ramma et al., 2002). Besides, the polyphenols' solubility depends on the size of the molecule, hydroxyl groups, and hydrocarbon length (Bazzaz et al., 2011). Some previous studies also reveal the benefit of phenolic to health, such as its contribution as free radical scavengers besides its capacity to inhibit the increase of blood glucose, then specifically means for diabetes mellitus patients (Fadly et al., 2020b).

**Total flavonoid content**

According to our observation, the total flavonoid content varied from 79.58 ± 4.16 to 273.13 ± 2.25 mg QE/g extract (Table 2). It was also showed the highest flavonoid content was obtained from ethyl acetate fraction, and the lowest was obtained from ethanol fraction, while flavonoid content in n-hexane fraction undetected. The results correspond to the (Amoussa et al., 2015) study, which has the highest flavonoid content on ethyl acetate extract of *Acacia ataxacantha* bark. Following the study executed by (Miller and Ruiz-Larrea, 2002), the flavonoids mainly exist in external parts of plants and the wood. Flavonoids are known to possess many biochemical impacts, such as antibacterial, antitumor, anti-inflammatory, anticancer, and potent antioxidant activity. Those lead to protect the body against free radicals (Saxena et al., 2013).

**Total alkaloid content**

Total alkaloid content evaluation by acid dye colorimetric method is commonly used, which is simple and no need special equipment. The acid dye reacts with alkaloids that have nitrogen in their structure and forming yellow-colored products (Fazel et al., 2010). The total alkaloid content of Kulim bark fraction is displayed in Table 2. Our investigation showed the total alkaloid varied from 13.00 ± 0.49 to 19.67 ± 2.08 mg BE/g extract, in decreasing order were methanol>ethanol>70%methanol>ethyl acetate>hexane. A similar result has been reported in which the methanol solvent gained the most alkaloid content on *Severinia buxifolia* branches (Truong et al., 2019). Alkaloid is one of the largest groups, which approximately 12.000 natural products(Bribi, 2018). Many of these compounds have pharmacological effects, including analgesic, antimicrobial, and

anticancer(O'Connor, 2010; Salminen et al., 2011) the Previous study showed that alkaloids possessed antioxidant activity (Dalimunthe et al., 2018; Rou et al., 2018).

**Antioxidant activity**

Antioxidant activities measured by DPPH free radical inhibition of each Kulim bark fraction are displayed in Table 3. The observation showed the antioxidant activity (IC<sub>50</sub> value) varied from 57.88 to 1288.19 ppm. The most substantial antioxidant activity was derived from the ethyl acetate fraction, and the lowest was obtained from 70% methanol fraction. Compared to the previous study (Sudrajat et al., 2016) identified the ethyl acetate fraction of *Scorodocarpus borneensis* bark slightly higher than the present study (55.524 ppm). Also, another investigation on the bark of Kulim Tree macerated by 70% methanol showed antioxidant activity in IC<sub>50</sub> about 52.45 ppm (Dewi et al., 2020).

Ethyl acetate fraction of Kulim bark possessed the most substantial flavonoid substances and antioxidant activity. There are several studies that reported a significant correlation between flavonoid and antioxidant activity by DPPH method (Aryal et al., 2019; Yakubu et al., 2014). Among all-natural phenolic compounds, Flavonoids were known to have the most potent radical-scavenging power, even phenolic and alkaloid, also correlated to the antioxidant activity (Masriani et al., 2020; Wojdyło et al., 2007). Many flavonoids have a strong capability in scavenging the free radical since their phenolic hydroxyl groups (Subhasree et al., 2009). The antioxidants compounds work on DPPH free radicals contributed by the ability of antioxidants to donate H<sup>+</sup> (Fadly et al., 2020a). Radical scavenging effectivity of flavonoids determined by double bond at C2-3 conjugated with a carbonyl group at C4 (C circle) hydroxyl group of ortho position in catechol structure (B circle), and the hydroxyl group at C5 (A circle) (Amić et al., 2003; Tapas et al., 2008).

**CONCLUSION**

The study suggests that flavonoids played a substantial role in antioxidant activity. It was revealed that ethyl acetate fraction from *Scorodocarpus borneensis* bark possessed the strongest antioxidant activity due to the highest total flavonoid content. Besides, the methanol fraction of *Scorodocarpus borneensis* bark also showed the most substantial phenolic and alkaloid compounds.

**Table 3.** Antioxidant Activity of the Fractions of the Bark of *Scorodocarpus borneensis*

Fraction	Concentrations (ppm)	Radical Scavenging Activity (%)	IC50
N-hexane	20	2.95 ± 0.08	
	40	5.15 ± 0.13	
	60	6.86 ± 0.16	
	80	7.69 ± 0.25	613.15
	100	8.35 ± 0.09	
	120	10.56 ± 0.29	
Ethyl acetate	140	13.59 ± 0.09	
	20	24.07 ± 7.07	
	40	43.85 ± 2.68	
	60	55.42 ± 1.09	
	80	63.77 ± 1.32	57.88
	100	73.04 ± 0.38	
Ethanol	120	80.64 ± 0.32	
	140	84.53 ± 0.29	
	20	93.58 ± 0.58	
	40	92.84 ± 0.62	
	60	90.98 ± 0.99	
	80	89.69 ± 1.09	569.59
Methanol	100	88.57 ± 1.60	
	120	85.85 ± 1.76	
	140	84.04 ± 2.00	
	20	93.35 ± 0.36	
	40	93.51 ± 0.59	
	60	92.52 ± 0.65	
Methanol 70 %	80	91.41 ± 0.86	821.34
	100	89.83 ± 1.34	
	120	88.72 ± 1.43	
	140	87.13 ± 1.76	
	20	87.98 ± 0.71	
	40	94.73 ± 0.33	
	60	94.02 ± 0.37	
	80	93.47 ± 0.32	1288.19
	100	92.72 ± 0.42	
	120	92.25 ± 0.29	
	140	90.91 ± 0.50	

\*The data are displayed with a mean ± standard deviation of four replications

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