


## PHYTOCHEMICALS, ANTIOXIDANT ACTIVITIES, AND TOXICITY EVALUATION OF SEVERAL FRACTIONS OF *Scorodocarpus borneensis* Becc. LEAVES

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### ABSTRACT

*Scorodocarpus borneensis* Becc is an ancient tree in Borneo island, that produces a unique odor like garlic. Thus, it is utilized to enhance food flavor by the locals. This study investigated phytochemical compounds, antioxidant activity, and toxicity of the *S. borneensis* leaves fractions. The fractions were prepared using several gradient elutions in the sequence: n-hexane, ethyl acetate, ethanol, methanol, and 70% methanol. Phytochemicals were observed qualitatively toward the presence of phenol, alkaloid, tannin, flavonoid, and terpenoid; quantitatively conducted toward phenolic, flavonoid, and alkaloid contents. DPPH free radical scavenging activity method was conducted to identify the antioxidant activity. The BSLT Test determined the toxicity. The result revealed that all the leaves' fractions showed the absence of terpenoids. The rest of the phytochemicals screened positively in all fractions. The ethyl acetate fraction owned the most prominent phenolic, alkaloid, and flavonoid content ( $614.86 \pm 35.82$  mg GAE/g,  $462.25 \pm 5.12$  mg BE/g  $271.67 \pm 1.30$  mg QE/g of extracts, respectively). The antioxidant activity ( $IC_{50}$  value) varied from  $100.24 \pm 19.69$  to  $237.89 \pm 2.66$   $\mu$ g/mL. All fractions obtained were classified as non-toxic, except methanol fraction showed low toxicity against nauplii of *Artemia salina* L.

**Keywords:** *Scorodocarpus borneensis* Becc., Phytochemicals, Total Phenolic Contents, Antioxidant Activity, Toxicity

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### INTRODUCTION

Every part of the plant, including root, bark, fruit, seed, flower, and leaf, is already exploited as medicinal substances decades ago. It is known that secondary metabolites produced from plants provide a therapeutic effect on the human body. Secondary metabolites are diverse chemical groups, including alkaloids, steroids, glycosides, amines, flavonoids, and some correlated composites, which have been utilized widely in the medicinal industries.<sup>1</sup> Secondary metabolites have various biological effects such as an antibiotic, antifungal, and antiviral to protect the plants from pathogens. Those have been used as a scientific base for medicinal plants for ancient civilizations.<sup>2</sup> The study about secondary metabolite (referred to as phytochemical compound) of medicinal plants has been rapidly increased due to the serious concern about health in this industrial age.

Meanwhile, mostly indigenous plants yet unknown its phytochemical compounds, possibly used as a new source of medicines. These substances could be derived from plant materials through the solvent extraction mechanism. The appropriate solvent and extraction method selection is key to herbal medicine efficacy.<sup>3-5</sup> One country well-known for its abundance of natural resources, particularly flora diversity, is Indonesia. This country is located in an equatorial area with a tropical climate with extensive tropical rain forests. There is a rarely unknown plant that has been used by natives and believed might be used as a source of medicinal plants in the tropical rainforest. One such plant is *Scorodocarpus borneensis*, which is called a Kulim tree. It is a tall tree plant with a pungent smell like garlic called "Garlic tree" by the natives. From leaves, roots, and barks, every part of this plant has a garlic odor. Kulim plant is classified in the Olacaceae family, which has phytochemical compounds, including tannins, flavonoids, polyacetylene fatty acids, glycoside cyanogenetic, and polysulfide compounds.<sup>6</sup> Its leaves and bark have been used by the native as a

seasoning agent. A report showed that infusion of kulim bark on palm oil could increase the value of the sensory aroma as well as block peroxides formation.<sup>7</sup> Besides, a previous investigation was focused on its bark revealed a significant phytochemical content, including alkaloid, phenols, and flavonoid, which contributes to its potent antioxidant activity.<sup>8</sup>

Investigation on leaves of *S. borneensis* had been conducted before but still limited. Kulim leaves have several phytochemical compounds like flavonoids and methylthiomethyl. Methylthiomethyl sulfide compound has similar to *allium* species, which has anti-cancer properties.<sup>9,10</sup> Also, the leaves extract can inhibit the growth of *Candida albicans* and *Salmonella thypii* in tilapia filets.<sup>11</sup> However, scientific evidence is still more needed. Exploring Kulim leaves phytochemical compounds through solvent extraction is essential to understand its potential as medicinal plants. This study exhibits the proper solvent to obtain the fraction from the leaves, which possess substantial phytochemical and antioxidant activity while determining its toxicity level.

## EXPERIMENTAL

### Materials

A primary material was the leaves of *Scorodocarpus borneensis* gathered from a tropical woodland in Sanggau District, West Kalimantan, Indonesia (0°23'16.7"N and 110°43'24.8"E). Before being subjected to the chemical analysis, the materials were dried by placing them at room temperature. The dried materials were cut and ground using a blender to get a fine powder (80-mesh).

### Extraction

Fifty-gram powdered samples were produced through the extraction process by several gradient elutions in the sequence with 150 ml of n-hexane, ethyl acetate, ethanol, methanol, and 70% methanol. The filtration was carried out by applying Whatman No.1 paper, then concentrated at T 40 °C by a rotary evaporator. All fractions are frozen until further analysis.

### Phytochemical Screening and Quantification

The crude extracts each fraction obtained was analyzed to identify alkaloid, flavonoid, phenolic, tannin, and terpenoid based on the color produced following the chemical reaction against a particular reagent.<sup>12</sup> Simultaneously, the quantifications were conducted on a total of phenolic, flavonoid, and alkaloid contents. The evaluation of total phenols was performed through the Folin-Ciocalteu reagent.<sup>13,14</sup> In brief, 200 µL of the fractions added with 1 ml folin-ciocalteu reagent (1:10 v/v) and 3 ml of Na<sub>2</sub>CO<sub>3</sub> (2% w/v), and homogenized. Then it was kept for 30 min at room temperature. The absorbance read at λ 765 nm. The standard was gallic acid (20-140 µg/ml).

The quantification of total flavonoids was performed by the aluminum chloride method.<sup>15</sup> In brief, 500 µL of the fractions obtained were added with 1.5 ml methanol, 0.1 ml of AlCl<sub>3</sub> (10% w/v), 0.1 ml CH<sub>3</sub>COOK 1 M, and 2.8 ml of aquades. Then, this solution was placed at room temperature for about 30 min. After, the absorbance read at wavelength 415 nm. This study used quercetin solution (20-140 µg/ml) as standard. Total alkaloid content evaluation was based on Li *et al.* 2015 with some modifications. A part of the residue (extracts) was mixed into a 3 ml phosphate buffer solution of 4.5 pH and subjected to a separatory funnel.<sup>16</sup> The solution was added with 3 ml of bromocresol green solution 0.03%. After 30 min, about 1, 2, 3, and 4 ml of chloroform mixed in, then shaken for 2 min. Then, the base layer was distinct after 10 min. The extract was put into a 10 ml volumetric flask and diluted up to the mark with chloroform. The extract's absorbances read at 415 nm. Berberine solution (20 - 140 µg/ml) was the standard.

### Determination of Antioxidant Activity

Radical scavenge capability was evaluated through the DPPH method following Dewi *et al.* with modification.<sup>11</sup> About 4 ml fraction obtained mixed with 2 ml of 0.2 mM DPPH methanolic solution and placed in the dark for about 30 min. The mixture absorbance read at 517 nm wavelength. The determination of IC<sub>50</sub> was using linear equation calculation derived from the free radical inhibitory percentage curve.<sup>17</sup>

### Determination of Toxicity

A bioassay observed the toxicity with Brine Shrimps Lethality Test (BSLT) following Fadly *et al.*, with modification.<sup>18</sup>

The preparation was initially by hatching *Artemia salina* L. eggs. Next, ten nauplii were placed into each vial containing the samples, and seawater was added up to 5 mL. Then, it mixed a bit of dry yeast suspension (3 mg in 5 ml seawater) as food for the nauplii. Survivors were counted after 24 hours. The LC<sub>50</sub> was then determined using antilogarithms linear equation calculation derived from the sample's curve and percent mortality of nauplii.

### Data Analysis

The antioxidant activity and toxicity were analyzed using SPSS for windows through One Way ANOVA with DMRT.

## RESULTS AND DISCUSSION

### Phytochemicals

The essential information of plants regarding the chemical constituents is generally obtained through phytochemical identification of the extracts. The existence of phytochemicals, i.e., alkaloids, phenols, flavonoids, tannins, and terpenoids in these Garlic tree leaves, is presented in Table 1. All the leaves fractions showed no terpenoids available. Chemical structure and dielectric constant belonging to the solvent used in extraction, properties of phytochemical, and particular parts of the plant may affect the phytochemical obtained.<sup>19,20</sup> Some investigation proved that solvent polarity affects plant extract's phytochemicals.<sup>21-23</sup>

Table-1: Phytochemical Screening of *Scorodocarpus borneensis* Becc. Leaves Fraction

Fraction	Phenolic	Alkaloid	Tanin	Flavonoid	Terpenoid
n-hexane	1	1	1	1	0
Ethyl Acetate	1	1	1	1	0
Ethanol	1	1	1	1	0
Methanol	1	1	1	1	0
70% Methanol	1	1	1	1	0

Note: (1) Present; (0) Absent

The total phenolic contents of the Kulim leaves fraction can be seen in Fig.-1. Those were varied from  $245.07 \pm 22.25$  to  $614.86 \pm 35.82$  mg GAE/g, in decreasing order from ethyl acetate > ethanol > methanol 70% > methanol > n-hexane. Previous studies also identified similar results; the most significant and powerful solvent to draw phenols from plants.<sup>24-26</sup>

The total flavonoid contents of the Kulim leaves fraction are displayed in Fig.-1. The total flavonoid content varied from  $61.67 \pm 2.37$  to  $271.67 \pm 1.30$  mg QE/g extract; also, ethyl acetate > ethanol > methanol > n-hexane > methanol 70%. A study on the kulim bark fraction also revealed that ethyl acetate solvent extracted the highest flavonoid content.<sup>11</sup>

The total alkaloid contents of the Kulim leaves fraction can be seen in Fig.-1. Those were valued from  $15.75 \pm 1.95$  to  $462.25 \pm 5.12$  mg BE/g extract; also, ethyl acetate > ethanol > hexane > methanol > methanol 70%. Alkaloids are chemical compounds with basic nitrogen atoms included with neutral and even weakly acidic properties.<sup>27</sup> Several studies showed that alkaloids possess antioxidant activity.<sup>28,29</sup>

### Antioxidant Activity

Our observation revealed the antioxidant activity (IC<sub>50</sub> value) varied from  $100.24 \pm 19.69$  to  $237.89 \pm 2.66$  µg/mL (Fig.-2). The most powerful antioxidant activity was obtained from methanol 70%, and it was significantly different among other fractions at  $\alpha=0.05$ ; methanol 70% > ethanol > methanol > n-hexane > ethyl acetate. The oxidation inhibitory capability of plant extract may be correlated to the phenolic substances.<sup>30</sup> Phenolic compounds have hydroxyl groups, ideal structure chemistry to scavenge the free radical by electron or hydrogen atom donation to free radical or unpaired electrons.<sup>31</sup> Even though methanol 70% was not the best solvent to extract phenolic compounds; however, it showed the most potent antioxidant activity. This result corresponds to Bhebe *et al.*, who found that most phenolic content does not possess the highest antioxidant activity.<sup>32</sup> Antioxidant activity is not contributed only to the concentration but also to the phenolic compounds.<sup>31</sup>

According to the Molyneux classification, the antioxidant activity of *S. borneensis* classified as medium to very weak antioxidant activity.<sup>33</sup> The 70% methanol fraction was the highest among those leaves fractions

and classified as a potent antioxidant. The ethanol and methanol fractions were classified as a medium antioxidant, and the n-hexane fraction was classified as a weak antioxidant. The lowest antioxidant was owned by ethyl acetate fractions and considered a very weak antioxidant. While other investigations on a fraction of the kulim bark found that the fraction of ethyl acetate possesses the highest antioxidant capacity (vigorous antioxidant activity) followed by ethanol, n-hexane, methanol, and 70% methanol fractions (very weak antioxidant activity).<sup>8</sup>

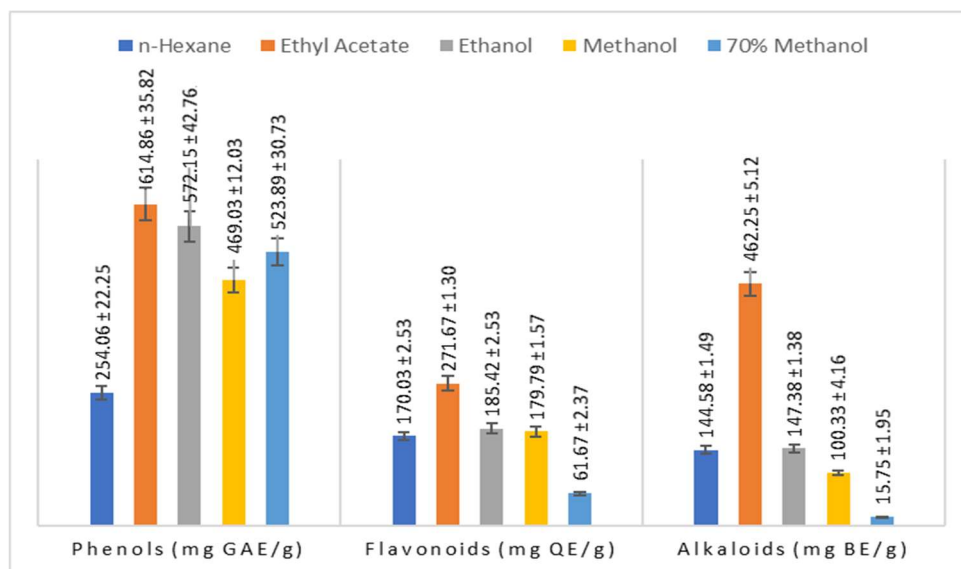


Fig.-1: Total Phenolic, Flavonoid, and Alkaloid Contents of the Leaves Fractions of *Scorodocarpus borneensis* Becc.

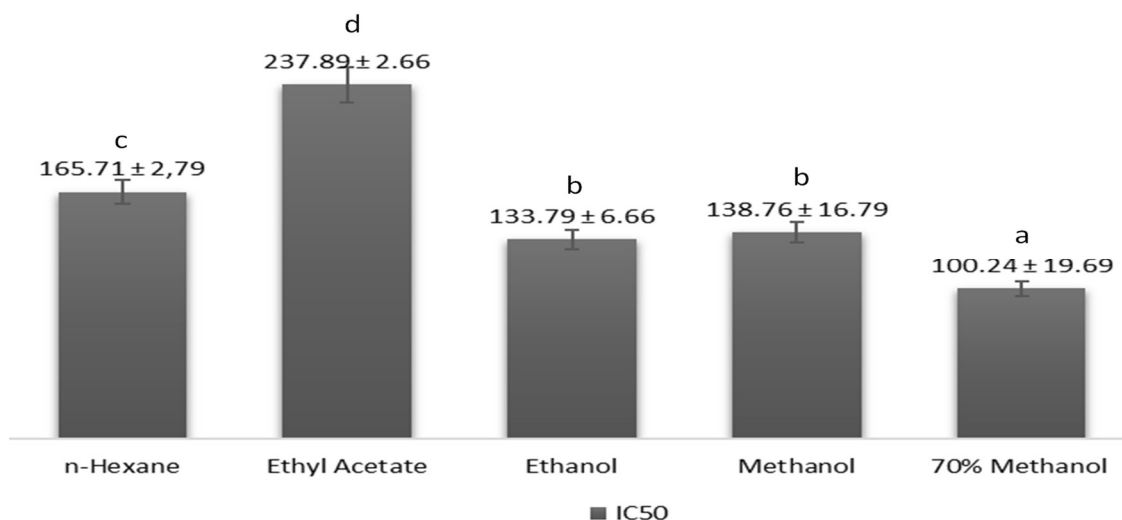


Fig.-2: IC<sub>50</sub> of *Scorodocarpus borneensis* Becc. Leaves Fractions against DPPH Free Radical (µg/mL)

Note: Different letters shows a significant difference at  $\alpha = 0.05$ .

### Toxicity

The BSLT, as a bioassay, is a preliminary method to predict the cytotoxic level.<sup>34</sup> The toxicity observation was tested at several concentrations, which the result presented in Table 2. According to Clarkson et al. 2004, the methanol fractions were low toxic, while the rest were considered non-toxic.<sup>35</sup> The results also show the significant differences in toxicity level at  $\alpha = 0.05$ ; the gradient elution in the sequence may contribute to the different toxicity levels.

Table-2: LC<sub>50</sub> of *Scorodocarpus borneensis* Becc. Leaves Fractions

Extract	Percent of Lethality in 24 hours (%) (means $\pm$ stdev)						LC <sub>50</sub> ( $\mu$ g/mL)
	25 $\mu$ g/mL	50 $\mu$ g/mL	100 $\mu$ g/mL	200 $\mu$ g/mL	500 $\mu$ g/mL	1000 $\mu$ g/mL	
n-Hexane	20 $\pm$ 1.22	27 $\pm$ 2.35	37 $\pm$ 0.71	43 $\pm$ 1.41	63 $\pm$ 0.71	100 $\pm$ 0.00	32 645 <sup>d</sup>
Ethyl acetate	30 $\pm$ 2.92	43 $\pm$ 1.22	53 $\pm$ 2.92	67 $\pm$ 2.35	83 $\pm$ 0.71	100 $\pm$ 0.00	4 237 <sup>c</sup>
Ethanol	20 $\pm$ 0.71	43 $\pm$ 1.41	73 $\pm$ 3.16	93 $\pm$ 2.12	100 $\pm$ 0.00	100 $\pm$ 0.00	1 778 <sup>b</sup>
Methanol	33 $\pm$ 1.87	43 $\pm$ 1.87	90 $\pm$ 0.71	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	9 84 <sup>a</sup>
70% Methanol	13 $\pm$ 0.58	23 $\pm$ 0.58	60 $\pm$ 1.00	100 $\pm$ 0.00	100 $\pm$ 0.00	100 $\pm$ 0.00	3 957 <sup>c</sup>

Note: Different superscript in the same column shows a significant difference at  $\alpha = 0.05$ .

The compounds' good biological activity in the four fractions of the Kulim leaves fractions. Those including n-hexane, ethyl acetate, ethanol, and 70% methanol illustrate an excellent bioactive potential antioxidant activity while considered non-toxic.<sup>35</sup> These findings serve as a basic description for developing in-depth studies on bioactive compounds' potential and biochemical advantages from Kulim leaves, an endemic plant in West Kalimantan, Indonesia.

### CONCLUSION

Solvent polarity plays a significant part in the phytochemical content that affects the extract's oxidation inhibitory capability. Following this study, methanol 70% fraction possesses the highest antioxidant activity. In contrast, the fraction of ethyl acetate contains the most substantial phenolic, flavonoid, and alkaloid content. The most distinguished antioxidant activity was obtained from methanol 70%. This study found the fractions of n-hexane, ethyl acetate, ethanol, and 70% methanol of *Scorodocarpus borneensis* Becc. Leaves were classified as non-toxic, but methanol fraction was considered low toxic.

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