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Phytocemical Screening and Toxicity Test of Ethanolic Extracts *Phanera semibifida* (Roxb.) Benth. Stem and Leaves from West Sumatra

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Abstract

Indonesian people use plants as medicine because they are considered to have fewer side effects. *Phanera semibifida* (Roxb.) is a climbing plant that has been used by the community to make therapeutic drugs. Phanera semibifida, especially species in western Sumatra, lacks information on phytochemical composition and toxicity capabilities. So the purpose of this study was to determine the phytochemical content, toxicity (BSLT), and biological activity of *Phanera semibifida* growing in western Sumatra. The BSLT approach was used in this study to conduct phytochemical screening and toxicity studies on leaves and stems. Phyto chemical results were negative for alkaloids and steroids but positive for flavonoids, tannins, saponins, and terpenoids. Based on the BSLT experiment, it can be concluded that both ethanol extracts are highly toxic and show anticancer properties

Keyword: BSLT; *Phanera semibifida*; phytochemical; toxicity

INTRODUCTION

Indonesia is a country with high natural wealth, this causes the closeness of people's lives to nature. Nature is utilized well for the needs of Indonesian people's lives as clothing, food, and shelter. In addition, nature, especially plants, are utilized by the community as medicinal plants (Masriana *et al.*, 2023; Safitri *et al.*, 2023; Nisa *et al.*, 2024). Plants have secondary chemical compounds that can benefit human life, particularly in terms of health. Using medicanal plants for treatment is the utilization of biodiversity that exists around us, Indonesian people have long used medicinal plants to treat various conditions because they have the potential for pharmacological actions such as antineoplastic, antibacterial, antioxidant, anti-inflammatory, analgesic, antidiabetic, antyhypertensive, antidiarrheal, and other functions (Dewantari *et al.*, 2018; Makalalag *et al.*, 2019; Shaikh & Patil, 2020). Therapy through natural ingredients is considered more beneficial and has fewer or even no side effect when compared to chemical drug, therefore the approach and search for herbal medicines in the treatment of diases continues (Yuruk *et al.*, 2016; Kumontoy *et al.*, 2023; Parinding *et al.*, 2024).

Traditional herbal medicine is often used by various cultures, including the Malay ethnic community. *Phanera semibifida* is a forest plant with liana-like leaves that are split like butterflies. This tropical plant grows along open forest areas and forest edges, this plant is a raw material used in the process of making medicines (Mackinder & Clark, 2014; Roza *et al.*, 2019; Fitmawati *et al.*, 2022). Several *Phanera* species have been extensively studied to contain antioxidants and immunomodulators (Zakaria *et al.*, 2016; Shahana *et al.*, 2017; Fitmawati *et al.*, 2022). Previous research by Hazimi *et al.* (2018) found flavonoids, terpenoids, saponins, and tannins in the stems of *Phanera semibifida*, which

are used as raw materials for making herbal medicines. Because of its benefits, this plant is thought to have secondary metabolite compounds.

Secondary metabolite compounds are bioactive compounds that can be used in medicine. Phytochemical screening can reveal the secondary metabolic capabilities of a plant. This method is simple, fast, and highly selective, and can be used to identify groups of compounds and active substances in plants (Makalalag *et al.*, 2019; Sekhon-loodu & Rupasinghe, 2019; Brahmana *et al.*, 2022). Toxicity tests are also needed to assess the safety of *Phanera* plants that will be used as medicinal plants. In addition, biological activity tests of plants are also needed. This biological activity can be used as a reference in the standardization of traditional medicines based on natural ingredients so that standardized herbal medicines can be produced (Setiasih *et al.*, 2016; Cahyani & Mita, 2018; Farhany *et al.*, 2024). The purpose of this study was to determine the phytochemical content, toxicity (BSLT), and biological activity of *Phanera semibifida* growing in western Sumatra.

METHOD

The object used in this study was taken from West Sumatra, with a primary focus on the vine *Phanera semibifida*. Fresh *Phanera semibifida* stems and leaves were collected from Lima Puluh Koto district. Plant morphological description data were collected and documented in the field, whereas experimental data in the form of phytochemical content from *Phanera semibifida* were obtained through laboratory testing in Botany Laboratory of the Faculty of Mathematics and Natural Sciences, Riau University. The sampling area can be seen in Figure 1.

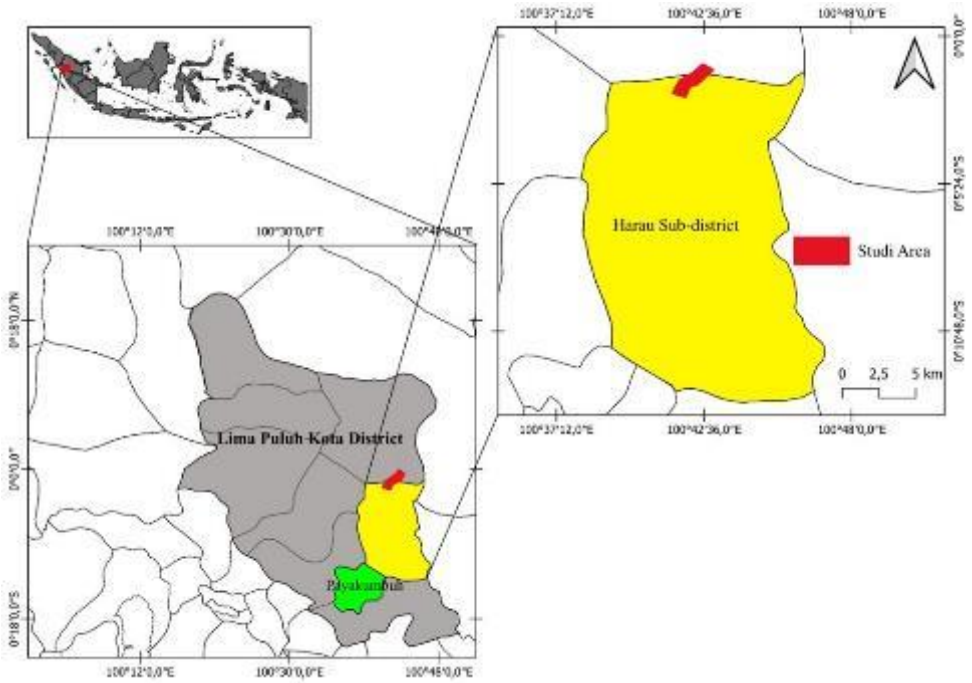

Figure 1. *Phanera semibifida* sampling area

Several basic qualitative examinations were conducted to analyze and screen the presence of various secondary metabolites and phytochemical content of dry *Phanera semibifida*'s stems and leaves, including the examination of alkaloids, flavanoids, saponins, tannins, steroids, and terpenoids following Sangi *et al.* (2008) method. To determine the quality of the stem and leaf powders, a moisture content test is also conducted. Both stem and leaf powders were extracted by maceration method using ethanol, and their toxicity values were evaluated by the BSLT (Brine Shrimp Lethality Test) method,

which included larval preparation, cytotoxic testing, and LC_{50} calculation. Test for alkaloids, a total of 0.5 grams of stems and leaves powder is mixed with 2 mL of chloroform, 6 mL of ammonia, and 10 drops of H_2SO_4. The mixture is beaten and allowed to form two layers. A total of 2.5 mL was formed in two layers; the top layer of H_2SO_4 was transferred and tested using the Dragendroff reagent. The formation of a red or orange color indicates the presence of alkaloids. Test for flavonoids, a total of 0.5 grams of stems and leaves powder is used. 5 mL of 70% ethanol is heated for 5 minutes before adding 10 drops of concentrated HCl and 0.2 grams of magnesium powder. The presence of flavonoids is indicated by the transition from brownish-red to black, yellow, or dark orange.

Test for saponins, a total of 0.5 grams of stems and leaves powder is added to 10 mL of aquades, vigorously beaten for 1 minute, and allowed to stand for a duration of ten minutes. Bubbles or foam are then observed. The presence of saponins in the sample is indicated by the appearance of stable foam. Test for tannins, a total of 0.5 grams of stems and leaves powder is mixed with 10 ml of hot water and dripped with 15 drops of 1% $FeCl_3$. Tannins are indicated by the appearance of a blackish-green color. Test for steroids and terpenoids, a total of 0.5 grams of stems and leaves powder were dissolved in chloroform, followed by 0.5 mL of glacial acetic acid and 2 mL concentrated sulfuric acid. Terpenoids are identified by the formation of orange to dark red solutions. Steroids, on the other hand, produce green to blue solutions. Determination of moisture content 2 grams of stem and leaf powder is heated in the oven at 100°C for 2 hours, after which the weight is calculated and this step is repeated three times to obtain a constant weight of stem and leaf powder. The percentage of moisture content is calculated using the following formula:

$$\%\ Moisture\ Content = \frac{W0\ (g) - W1(g)}{W1\ (g)}\ X\ 100\%$$

where, W0 indicates the weight in grams of the stem and leaf powder prior to moisture content measurement, while W1 indicates the weight in grams of the stem and leaf powder after the oven.

Extraction, each stem and leaf powder weighing up to 100 grams is soaked in a 70% technical ethanol solvent, with a powder : solvent ratio of 1:10. The extraction process takes 2 x 24 hour periods. The first 24 hours of soaking are filtered in a glass container. The filtered pulp is then re-soaked for 24 hours in 70% technical ethanol at a 1:10 ratio, followed by re-filtration. The filtered solution from the first and second 24 hours is combined and evaporated on a rotary evaporator at a maximum temperature of 40 °C. Larval preparation, fish salt is dissolved in 2 L of aqueduct to produce a solution with a salinity of 30 ppm and a pH of 8-9. The egg hatchery aquarium contains 1 L of seawater and is divided into two parts: open and closed. *Artemia salina* eggs weighing up to 1 gram are added to the closed part, while the open area is illuminated with a lamp or light to attract the larvae that hatch successfully. This process takes 48 hours. Test for cytotoxic, a standard solution with a concentration of 10,000 ppm for each sample was prepared by dissolving 40 mg of stem and leaf extracts of *P. semibifida* in 4 mL of aquades. The cascade concentration series (1000 ppm, 100 ppm, and 10 ppm) is diluted from the parent solution by adding 0.5 mL of concentration solution and 5 mL of aquades. Each series of solution and aqueous solution (0 ppm) is mixed with 5 mL of seawater and 50 µL of DMSO. A total of 10 *A. salina* larvae were placed in a test tube containing a series of solutions for each of the stem and leaf extracts, with three replications. The number of dead *A. salina* larvae at each concentration was counted after 24 hours. The percentage of *A. salina* larval mortality is then calculated using the following formula:

$$\%\ Larval\ Death = \frac{Total\ amount\ of\ dead\ larvae}{Total\ amount\ of\ larva\ count}\ X\ 100\%$$

LC_{50} calculation, the LC_{50} value is calculated using probit analysis in Microsoft Excel. The scatter chart is generated by entering the concentration log on the x-axis and the probit of larval mortality percentage on the y-axis. Linear trendline options, equation display, and R-squared value are

added to the chart. The number 5 (the probability of 50% *A. salina* larval mortality) is substituted as y in the equation y = ax + b that appears on the graph. The value of log x, or the LC_{50} value of stem and leaf extracts against *A. salina* larvae, was sought. Determination of toxicity levels (LC50) and their categories can be seen in table 1.

Table 1. Categories of toxicity based on LC_{50} value

Clarkson (2004)		**Mc Laughlin (1991)**	
LC_{50} (ppm)	**Categories**	**LC_{50} (ppm)**	**Categories**
LC_{50} > 1000	Non-toxic	LC_{50} >1000	Non-toxic
LC_{50} 500 – 1000	Low toxicity	LC_{50} < 1000	Toxic
LC_{50} 100 – 500	Moderately toxic	LC_{50} 30 – 200	Antimicrobe potential
LC_{50} < 100	Strongly toxic	LC_{50} < 30	Anticancer potential

RESULT AND DISCUSSION

Phanera semibifida phytochemical content screening was carried out using the method of Sangi *et al.* (2008). The stem extract was dark brown while the leaf extract was green with a herbal-like aroma. The stem yield was obtained at 10.03% based on dry weight with an air correction factor of 5.39%. Meanwhile, the leaf yield was obtained at 8.92% based on dry weight with an air correction factor of 45%. The results of the phytochemical test can be seen in table 2.

Table 2. Phytochemical of steam and leaves ethanolic extract of *Phanera semibifida*

Phytochemical Test	**Stem**	**Leaves**	**Description**
Alkaloid	-	-	Orange precipitation
Flavonoid	+	+	Yellow flourescence
Tanin	++	+	Formation of a precipitation
Saponin	+	++	Permanent foam
Steroid	-	-	Greeny ring
Terpenoid	++	+	Brown ring

Note: Sign + there are indication of bioactive compounds and the sign - there are no indication of bioactive compounds

In the flavonoid test, it showed positive results with a dark red color change, flavonoids can act as antioxidants, antivirals, antimicrobials, and antidiabetics (Fadhli *et al.*, 2019; Saputri & Pertiwi, 2021; Praparatana *et al.*, 2022). The tannin test showed a blue color change and the presence of sediment, this shows that the plant has an antioxidant effect. The saponin test showed the formation of foam, The content of saponin compounds has the potential to be an anticancer agent that works by inducing cell cycle arrest and cell apoptosis. Moreover, the terpenoid test showed the formation of a brown ring, this shows that the plant has analgesic, anti-inflammatory, and antitumor effects. The alkaloid and steroid tests produced negative results because there was no adhesion or color change when the reaction was added (Tambunan *et al.*, 2016; Khasanah *et al.*, 2020; Aryantini, 2021). Toxicity tests of ethanolic steams and leaf extracts of *Phanera semibifida* conducted to determine the level of toxicity of the extracts against larvae shrimp *A. salina*. The test results showed that the steams and leaf extract of *Phanera semibifida* has different effect based on concentration levels. Impact on mortality and larval toxicity of this case is shown in table 3.

Table 3. LC_{50} value of both leaves and stems ethanolic extract of *Phanera semibifida*

Parts of Plant	Concentration (ppm)	Mortality ± SD (%)	LC_{50} (ppm)	Description
Leaves	1000	33.3 ± 1.87	0.119	Strongly toxic, anticancer potential
	100	30.0 ± 2.22		
	10	43.3 ± 2.20		
	0	0.0 ± 2.27		
Stems	1000	36.7 ± 2.27	0.541	Strongly toxic, anticancer potential
	100	33.3 ± 2.17		
	10	46.7 ±2.65		
	0	0.0 ± 2.27		

Leaf extract has an LC_{50} value of 0.119, whereas stem extract has an LC_{50} value of 0.541. The lower the LC50 value, the greater the toxicity effect, and vice versa. Extracts with LC_{50} values < 1000 g/mL are considered strongly toxicity and have potential as anticancer. Advanced tests on its anti-cancer capabilities using in vivo or in vitro methods targeting specific types of cancer can be conducted to provide more anticancer potential data for this plant. The demise mechanism of *A. salina* larvae is closely related to the activity of high antioxidant compounds found in *Phenera semibifida* leaves and stems, including, alkaloid, flavonoids, terpenoid, saponins, and tannins. These antioxidant compounds are antifeedant, which means they can inhibit the nutrition of *A. salina* larvae. The compound, particularly flavonoids, has stomatch poisoning properties that can reduce the activity of digestive enzymes and food absorption, causing *A. salina* larvae to die from starvation. Flavonoids are polyphenol compounds that have a mechanism as an anticancer through the activation of the apoptosis pathway of cancer cells, inhibiting tumor/cancer proliferation, reducing tumor resistance to chemotherapy agents and can inhibit DNA topoisomerase I/II activity. Based on the benefits of this compound, Flavonoids are promising to be used as anticancer agents (Khasanah *et al.*, 2020; Mawardi *et al.*, 2021; Razoki *et al.*, 2023). Terpenoids, flavonoids, saponins, and tannins are among the compounds in *Phanera semibifida* responsible for this phenomenon.

CONCLUSION

Based on the results of the study using phytochemical tests on *Phanera semibifida* plants, there are 4 groups of compounds identified in the stems and leaves of *Phanera semibifida*, namely flavonoids, tannins, saponins and terpenoids. Toxicity tests using the BSLT method obtained a higher LC50 value for leaf extract than stem extract and are highly toxic. This study is useful for the community because it can provide more knowledge about the content of the *Phanera semibifida* medicinal plant. In addition, this study can be used as initial data for researchers to see the anticancer potential of the *Phanera semifibida* plant. Therefore, this data can be useful for the national agenda in discovering the potential of natural ingredients as medicines.

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