TOXICITY TEST USING THE BRINE SHRIMP LETHALITY TEST (BSLT) METHOD ON EXTRACTS OF STEM BARK, STEM WOOD, AND LEAVES ON BAYUR (*Pterospermum diversifolium* B. Rob.)

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Abstract: Ethnobotanically, the leaves of *P. diversifolium* are used as a medicine for itching and the root bark is used as fish poison. The traditional use of natural materials should be followed by scientific studies. This study aims to determine the toxicity of the stem bark, stem wood, and leaf tissue extracts of *P. diversifolium* using the Brine Shrimp Lethality Test (BSLT) method. Extraction using maceration method with ethanol solvent for 3 x 24 hours. The macerate was filtered and the extract obtained was evaporated until a crude extract was obtained. The three ethanol extracts obtained were tested for toxicity and obtained LC_{50} values of stem bark = 4,753 ppm, stem wood = 97,723 ppm, and leaves = 27,797 ppm. All extracts were declared non-toxic because the LC_{50} value was more than 1000 ppm.

Keywords: *P. diversifolium;* toxicity; bayur

Abstrak: Secara etnobotani, daun *P. diversifolium* digunakan sebagai obat gatal serta kulit akarnya sebagai racun ikan. Penggunaan bahan alam secara tradisional seharusnya diikuti kajian ilmiah. Penelitian ini bertujuan untuk mengetahui toksisitas pada ekstrak jaringan kulit batang, kayu batang, dan daun *P.diversifolium* dengan metode *Brine Shrimp Lethality Test* (BSLT). Ekstraksi menggunakan metode maserasi dengan pelarut etanol selama 3 x 24 jam. Maserat disaring dan ekstrak yang peroleh dievaporasi sampai didapatkan ekstrak kental. Ketiga ekstrak etanol yang diperoleh diuji toksisitasnya dan diperoleh nilai LC_{50} kulit batang = 4.753 ppm, kayu batang = 97.723 ppm, dan daun = 27.797 ppm. Semua ekstrak dinyatakan tidak toksik karena nilai LC_{50} lebih dari 1.000 ppm.

Kata kunci: *P. diversifolium*; toksisitas; bayur

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Introduction

Indonesia's tropical forests are one of the forests that have the widest level of biodiversity in the world and are second only to Brazil. Around 250,000 species of higher plants in the world, and an estimated 30,000 - 40,000 species of flowering plants (Ministry of Environment and Forestry Republic of Indonesia, 2018). Tropical plants can engineer a variety of chemical compounds that have a variety of interesting bioactivities. Each plant species is a very special producer of secondary metabolites so biodiversity is a source of molecular diversity with their respective properties and benefits. This ability is caused by a self-defense mechanism, considering that these plants live under hard climatic conditions and disturbances from herbivores, insects, and pests (Whitehill et al., 2023).

Tropical plants are widely used in traditional medicine. Several studies have reported that different extracts from traditional medicinal plants have been tested and shown to be effective against microorganisms so plants become one of the basic ingredients of modern medicine (Altamish et al., 2022). Plants have been used for the treatment of diseases around the world before the advent of modern clinical medicine. Natural phytochemicals are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful new drugs. As much as 50% of modern medicine is derived from natural products and therefore these natural products play an important role in drug development in the pharmaceutical industry. The use of plants as a source of medicine has been inherited and is an important component of the healthcare system (Whitehill et al., 2023).

One of the tropical plant genera of the Malvaceae family is *Pterospermum*. This plant lives widely in the tropics and sub-tropics with a topology of tall trees. Since ancient times this group of plants has been widely used as building materials, pulp raw materials, and some are used as traditional medicine. *P. diversifolium* leaves are used as an itching medicine and the root bark is used as fish poison (Ogata, 1995). The leaves and bark are rich in tannins, used in traditional medicine for example as a poultice against itching, to treat wounds, and drunk to treat dysentery. *P. diversifolium* leaves are also given to livestock that suffer from gastric disorders (Kamble et al., 2010).

Literature studies of the bioactivity of leaf and stem bark extracts from *P. diversifolium* have been reported to have antibacterial activity against several pathogenic microorganisms, namely *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginous, Shigella flexneri and Staphylococcus epidermidis* (Madhuri et al., 2011; Hidayathulla et al., 2011). However, the literature on toxicity to *P. diversifolium* plants is still minimal. Thus, this study aimed to determine the toxicity of the bark, stem, and leaf tissue extracts of *P. diversifolium* using the Brime Shrimp Lethality Test (BSLT) method.

Research Methods

Tools and Materials

Materials used in this study plant *P. diversifolium*, ethanol solvent, thin layer chromatography plate, filter paper, *Artemia salina* leach, and seawater. The tools used are a set of distillation equipment, grinding machine, maceration equipment, evaporator, aerator, Buchner funnel, TLC chamber, capillary tube, analytical balance, test tube, micropipette, thermometer, fluorescent lamp, UV lamp.

Sample Preparation

P. diversifolium as the sample was obtained in Topoyo Village, Topoyo District, Central Mamuju Regency, West Sulawesi Province. The sample was identified at the Herbarium Bogoriense, Center for Biological Research, LIPI Indonesia. The bark tissue, stem wood, and leaves were dried by aerating at room temperature, and not exposed to direct sunlight for 6 days. The dried sample was shaved and then re-dried in the same way for 3 days and then ground with a grinding machine to a fineness level of 80 mesh.

Production of *P. diversifolium* Extract

A total of 2 kg of samples (bark tissue, stem wood, and leaves) were extracted by immersion technique at room temperature (maceration) using 10 L ethanol solvent for 3 x 24 hours. The extraction was stopped when no more stains were visible under the UV lamp on the results of the thin layer chromatography (TLC) test. Thin layer chromatography (TLC) analysis was performed with a Silica gel-coated plate of Merck Kiesegel 60 F254 0.25 mm. The obtained maserate was filtered and the filtrate was concentrated with a rotary evaporator until a total concentrated extract was obtained which was then tested for toxicity using the Brine Shrimp Lethality Test (BSLT) method.

Toxicity Testing

Artemia salina shrimp larvae were hatched in 500 mL seawater containers equipped with lights and aerators. The container used is divided into two sides, namely the dark part and the light part with a partition that has been given a hole and under constant aeration for 48 hours. After hatching, active *nauplii* free from eggshells were collected and used for testing. Each test tube contained sample extract with concentrations of 10, 100, and 1000 ppm (Potu, 2021; Rafiqah, 2019; Winahyu, 2024) and ethanol as a controlled solvent. Next, 2 mL of seawater and 10 *A. salina* were added, then added with seawater until the volume reached 5 mL. The conditions were maintained at room temperature for 24 hours. Seawater was used as a negative control. The LC_{50} value was calculated using a curve method based on probit analysis (Dali et al, 2022).

$$\%\ \text{larval mortality} = \frac{[\text{Number of deaths larvae} - \text{Number of control deaths}]}{[\text{Number of test larvae}]} \times 100\% \quad \ldots\ldots\ldots\ldots\ (1)$$

Analysis of the percentage of mortality (death) in *A. salina*, a graph of the relationship between the log of methanol extract concentration and the probit value was obtained. The graph produces a linear regression equation

$$Y=bX+a \quad \text{.....(2)}$$

Where: X = log concentration, and
Y = Probit number
a = Intersep/constant
b = regression coefficient
In the linear regression equation, the Y value (Pecoraro, 2021)

Results and Discussions

Toxicity testing is the first step in the safety parameters of a drug before it becomes a product used in humans (Jabbar et al., 2019). Scientifically, new plants require standardization, biological activity, and toxicity of each plant material. In addition, experimental toxicity assessment has been used as a standard safety study with efficacy trials (Pitakpawasutthi et al., 2021). One method is the Brine Shrimp Lethality Test (BSLT). This method can identify the toxicity of natural materials (Saragih et al., 2020). The BSLT method was carried out by calculating mortality by extracts or isolates of *Artemia salina* Leach shrimp larvae for 24 hours. The results obtained by the value of LC_{50} (Lethal Concentration) is the number of doses that can cause shrimp death.

The Brine Shrimp Lethality Test (BSLT) method was chosen because it does not take long, is easy, cheap, accurate, and requires a small sample (Mastura et al., 2022). The test animal used was *Artemia salina* because it has a response to chemical compounds similar to mammals, such as DNA-dependent RNA polymerase and this organism has a Na^+ and K^+ dependent ATPase transport system (Riaz et al., 2018). The hatching process of *A. salina* requires an aerator as a source of oxygen and is carried out at room temperature. Hatching takes 24-48 hours (AlEnazi, 2018).

The test sample used in this study was the ethanol extract of the bark, ethanol is a polar solvent and is a versatile solvent and is very good for use as a preliminary extraction. Ethanol toxicity occurs as a result of consuming large amounts of ethanol, usually in the form of alcohol. It affects many organ systems in both the acute and chronic phases (Anthony J. L., 2020). stem and leaf tissue of *P. diversifolium* with concentrations of 10, 100, and 1000 ppm, respectively. This concentration was chosen because it follows the statement that if the LC_{50} of the tested extract is less than 1000 mg/L then it is considered toxic (Oluwaniyi and Obi, 2018). The result obtained from the difference between concentration 1000 and concentration 100 is only 0.5% so we determine the range (Nahrum, 2020). The references we use are: Meyer et al. (1982) stated that the determination of bioactive

potential was carried out by comparing the LC_{50} of each extract with the provisions of McLaughin (1991) said to be toxic when the LC_{50} value 1000. From the results of the research, the BSLT test on ethanol extract obtained an LC_{50} value of 1.788 µg / ml with larvae mortality at a concentration of 1000 ppm = 95% and probit value = 6.64; % of larvae mortality at a concentration of 100 ppm = 85% and probit value = 6.04; and % of larvae mortality at a concentration of 10 ppm = 5% and probit value = 3.36. This shows that LC_{50} values have potential as an antitumor for the class of compounds contained, namely flavonoids, steroids, alkaloids, and terpenoids. Conclusion Ethanol extract of red algae Euch.

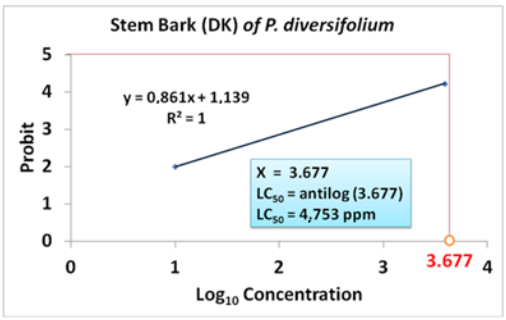
The results of the toxicity test of the ethanol extract of the stem bark, stem wood, and leaves of *P. diversifolium* against shrimp (*Artemia salina* Leach) larvae can be seen in Table 1.

Table 1. Observation data on mortality of shrimp larvae (*A. salina* Leach) on Ethanol extract of stem bark (DK), stem wood (DB), and leaves (DD) of *P. Diversifolium*

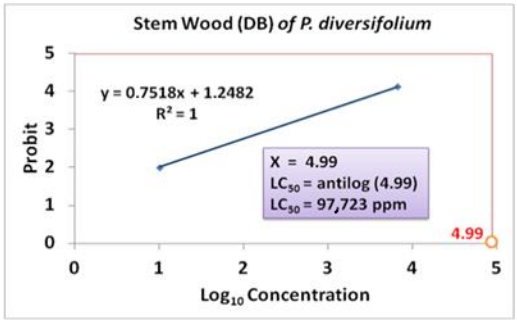
Sample	Conc. (C) (ppm)	Log_{10} C	Number of Live Larvae	Number of Dead Larvae				Mortality (%) (48 hours)	Corrected Mortality (%)	Probit	LC_{50} (ppm)
				1	2	3	Tot				
		1	30	1	2	1	4	13.33	-	-	
Control		2	30	0	2	1	3	10.00	-	-	-
		3	30	9	10	10	29	96.67	-	-	
	10	**1**	30	1	2	3	6	20.00	8	**3.59**	
DK	100	**2**	30	6	3	0	9	30.00	22	**4.23**	**4,753**
	1000	3	30	10	10	10	30	100	100	-	
	10	**1**	30	2	2	3	7	23.33	12	**3.82**	
DB	100	**2**	30	4	3	1	8	26.67	19	**4.12**	**97,723**
	1000	3	30	10	10	10	30	100	100	-	
	10	**1**	30	1	2	2	5	16.67	4	**3.25**	
DD	100	**2**	30	1	4	2	7	23.33	15	**3.96**	**27,797**
	1000	3	30	10	10	10	30	100	100	-	

Table 1 was used the negative control, the purpose of the control that the solvent used as a diluent did not affect the antibacterial test results of the extract to be tested and that used as a comparison for each concentration (Mubarak, 2016) and (Daud, N.S, 2023)

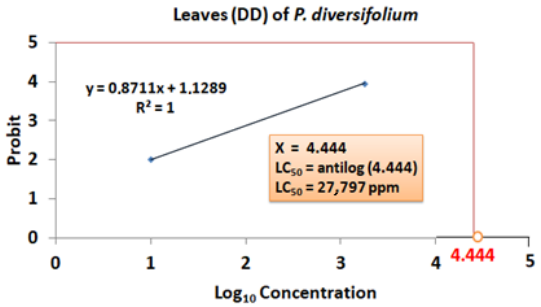
The results of the ethanol extract of the bark, stem wood, and leaves of *P. diversifolium* showed that the higher the concentration of the extract, the higher the percentage of mortality of *A. salina* leach. The percentage of mortality values that obtained in Table 1 and analyzed using linear regression equation (Figure 1) to obtain the LC_{50} value. LC_{50} is the concentration at which an extract can cause the death of 50% of test animals (Rasyid and Angraeni, 2020).



(a)



(b)



(c)

Figure 1. (a) Graph of the relationship between probit and the log concentration of the ethanol extract of the. *P. diversifolium* stem bark; (b) Graph of the relationship between probit and the log concentration of the ethanol extract of the *P. diversifolium* stem wood; and (c) Graph of the relationship between probit and the log concentration of the ethanol extract of the *P. diversifolium* leaves.

In Figure 1. (a) the linear regression equation y = 0.861x + 1.139 is obtained. The y parameter represents the probit number and the x parameter represents the log concentration of the *P. diversifolium* stem bark ethanol extract. The y value of 5 is entered in the linear equation y = 0.861x + 1.139, the x value is 3.677. The LC_{50} value is the antilog of the x value, which is 4,753 ppm. Figure 1. (b) obtained a linear regression equation y = 0.7518x + 1.2482. The y parameter represents the probit number and the x parameter represents the log concentration of the *P. diversifolium* stem ethanol extract. The y value of 5 is entered in the linear equation y = 0.7518x + 1.2482 and the x value is 4.99. The LC_{50} value is the antilog of the x value, which is 97,723 ppm. Figure 1. (c) obtained a linear regression equation y = 0.8711x + 1.1289. The y parameter represents the probit number and the x parameter represents the log concentration of the *P. diversifolium* leaves ethanol extract. The y value of 5 is entered in the linear equation y = 0.8711x + 1.1289 and the x value is 4.444. The LC_{50} value is the antilog of the x value, which is 27,797 ppm.

The calculation of the LC_{50} value obtained in this study can be said that all ethanol extracts of *P. diversifolium* showed insignificant toxicity to shrimp larvae

with an average value of LC_{50} > 1000 ppm (24 hours). Based on the LC_{50} value, the toxicity category of the extract was divided into three groups, very toxic (LC_{50} value < 30 ppm), toxic (LC_{50} value 30–1000 ppm), and non-toxic (LC_{50} value > 1000 ppm) (Dali et al., 2022). That was due to the presence of phenolic content of stems which is much higher than stem wood and leaves on bayur (Hidayat, 2017) and (Mani, 2020). On the other hand, the antibacterial activity of leaves and stem bark extracts of *P. divesifolium* against several pathogenic microorganisms has been widely reported (Hidayathulla, et al., 2011). Although the ethanolic extract of *P. diversifolium* showed insignificant toxicity to shrimp larvae, this study can be a source of information for other researchers to conduct further research. Even though the same genus (Marzuki; 2019) shows the results of research on the toxic power of ethanol extract *P. celebicum* with LC_{50} value = 15.15 µg/ml (strong).

Conclusion

The LC_{50} values of the ethanol extract of the stem bark, stem wood, and leaves of *P. diversifolium* were 4,753 ppm; 97,723 ppm; and 27,797 ppm, respectively. It can be concluded that *P. diversifolium* extract has insignificant toxicity to shrimp larvae (> 1000 ppm). The types of secondary metabolite compounds that have been isolated from Bayur **(*Pterospermum diversifolium* B. Rob.)** include phenols, terpenoids, alkaloids, and steroids. (Andila, 2021). The side effects caused by traditional medicine using plants are almost non-existent. This is very different from chemical drugs which, when used in the long term, will have negative effects.

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