ABSTRACT

The Pagoda (*Clerodendrum paniculatum* L.) is a medicinal plant that is spread widely in the Asian region. The Pagoda is known to have the activity as antioxidant and anti-inflammatory activity, and have other activities that are believed to be potential medicinal plants. To determine the strength of potential activities possessed by pagoda plants needs to be tested using the Brine Shrimp Lethality Test (BSLT) method. Potential activities test was carried out on ethanol, ethyl acetate and n-hexane extracts from pagoda leaves by observing the ability from each extract to kill 50% of the tested sample (LC$_{50}$). LC$_{50}$ values obtained from ethanol, ethyl acetate and n-hexane extracts were 43.501, 29.182 and 41.919 ppm. Based on the results shown from each extract, pagoda leaves have a very strong activity potential based on the LC$_{50}$ value <100 ppm of each extract.

Keywords: Brine Shrimp Lethality Test, *Clerodendrum paniculatum* L., Potential Bioactivities.

INTRODUCTION

The pagoda plant (*Clerodendrum paniculatum* L.) is a medicinal plant that is often used in the Asian region for traditional medicine. This plant is one of the most frequently encountered species of Clerodendrum because it is usually grown for ornamental purposes in home gardens and is also used as traditional medicine. Pagoda plant is known as ethnomedicinal because it is used as medicine to treat ailments such as wounds, typhus, snake bites, jaundice, dizziness, malaria, anemia and hemorrhoids. This plant also has biological activities such as antimicrobial, anti-inflammatory, antioxidant, anthelmintic, anti-diabetic, anti-cholesterol, insecticide and anti-aging. In line with the increasing use of medicinal plants as alternative treatments, experimental examinations, namely the toxicity test of these plants, need to be carried out to ensure safety and assess the potential effectiveness of these natural sources. The early stage of the method commonly used to measure the safety and potential activity is by using Brine Shrimp Lethality Test (BSLT) on *Artemia salina* larvae. The test method using brine shrimp (*Artemia salina*) as the research object is considered a simple bioassay for natural product research, particularly in assessing the potential toxicity and bioactivity of natural products. The procedure determines LC$_{50}$ values in ppm of active compounds and extracts in the brine medium. Activities of active compounds commonly known based on the toxicity to shrimp larvae. The potential activity of the ethanol extract of the pagoda flower was carried out by testing its toxicity against *Artemia salina* by using the BSLT method and the results obtained were LC$_{50}$ value = 49.415 ppm. It is known that the anti-inflammatory activity of pagoda leaves is better than flowers. Another research showed that the antioxidant activity of the leaves is better than flowers. Based on the better activity of pagoda leaves, it is needed to determine the potential activities of pagoda leaves by using the BSLT method. In this study, the potential activities or toxicity tests of the pagoda leaves extracted using three organic
solvents were ethanol, ethyl acetate and n-hexane respectively. This study aimed to compare the potential activities of pagoda leaves based on the type of extract in organic solvents.

EXPERIMENTAL

Materials and Instruments
The materials used in this study were pagoda leaves obtained from Deli Serdang district, North Sumatra, Indonesia and have been determined by the Research Center for Biology, Indonesian Institute of Science. The solvents used were ethanol 96%, ethyl acetate and n-hexane. Other materials were dimethyl sulfoxide (DMSO), distilled water and iodine-free salt.

The instruments used were drying cabinet, blender, analytical scales, pH-meter, glassware, rotary evaporator, aerator and magnifying glasses.

General Procedure
Pagoda leaves were dried using a drying cabinet for two days and being ground by the blender. The obtained powder was divided into three parts each was extracted using ethanol 96%, ethyl acetate and n-hexane solvents. The extraction process used was maceration method by soaking for three days while stirring occasionally. Each liquid was separated from its pulp, then the liquid was concentrated using a rotary evaporator to separate the solvent from the dissolved extract. The extracts obtained were diluted in 20, 40, 80 and 160 ppm with DMSO (2%) into water solvent to be tested further against *Artemia salina*.

Tested animals used were *Artemia salina* shrimp larvae. Shrimp eggs were first hatched in an aerator-filled container containing artificial seawater (iodine-free salt and water) with a pH of 8-9. pH value of the seawater is a very important factor for hatching the Artemia eggs and the most optimal pH range is 8.0 ± 0.5. A total of 1 gram of *Artemia salina* egg was put in a container for 48 hours. After 24 hours, the eggs hatched into larvae and move towards a bright space. The 24-hours-old larvae were transferred into other plastic containers until they were 48-hours-old and ready to be used as a tested animal. The best *Artemia salina* used in BSLT is 48-hours-old larvae, because if more than 48 hours, the death of larvae was not due to the toxicity from extracts, but because of lacking food supply.

Detection Method
About ten 48-hour-old shrimp larvae were put into each glass container (test tube) that contained test sample. Test samples were ethanol, ethyl acetate and n-hexane extracts of pagoda leaves with concentrations of 20, 40, 80 and 160 ppm respectively. A mixture of 2% DMSO was used as a negative control. The total of dead larvae was observed after 24 hours. Lethality concentration (LC$_{50}$) was analyzed by using the Probit analysis method based on the amount of dead larvae from various extracts concentration.

RESULTS AND DISCUSSION

The mortality percentage of shrimp larvae can be seen in Figure 1 and LC$_{50}$ values can be seen in Table-1. Based on the graph, the extract concentration and the number of dead *Artemia salina* larvae were directly proportional. This condition showed in all ethanol, ethyl acetate, or n-hexane extracts. There are no dead larvae shown in the negative control, which indicated that dead larvae were caused by the activities of the active substances in the extracts.

The number of death or mortality percentage of shrimp larvae was analyzed using the Probit method to obtain LC$_{50}$. The values obtained were used to describe the ability in causing the death of 50% of the total population of shrimp larvae.

The LC$_{50}$ value of ethyl acetate extract was the lowest compared to the other two extracts. It concludes that the toxicity of ethyl acetate extract is more toxic than ethanol and n-hexane extracts. The more toxic a compound, the higher its activity and the more potential it can be used as medicine. The ethanol, ethyl acetate and n-hexane extracts of the pagoda leaves, as well as the ethanol extract of the pagoda flowers that have been studied previously, are in the high toxic category. Based on the Meyer toxicity index, extracts with an LC$_{50}$ <1000 ppm were considered toxic and an LC$_{50}$ > 1000 ppm were considered non-toxic. The toxicity index based on the Clarkson criteria of plant extracts is classified in the following order: extracts with LC$_{50}$ above 1000 ppm are non-toxic, LC$_{50}$ of 500-1000 ppm is low toxic,
extracts with LC50 of 100-500 ppm are medium toxic, while extracts with LC50 of 0-100 ppm are highly toxic.\textsuperscript{22-24}

![Fig.-1: Mortality Percentage of *Artemia salina* Larvae.](image)

| No  | Sample                | LC50 (ppm) |
|-----|-----------------------|------------|
| 1.  | Ethanol Extract       | 43.501     |
| 2.  | Ethyl Acetate Extract | 29.182     |
| 3.  | n-Hexane Extract      | 41.919     |

Toxicity to shrimp larvae is often associated with the activity of a bioactive as an antibacterial and cytotoxic activity. That potential activity should be investigated further as a new anticancer agent.\textsuperscript{23–27} The brine shrimp mortality bioassay is a simple method that has been used extensively in the primary screening of crude extracts. This method is used to evaluate the toxicity value of the bioactive tested, and the toxicity value is closely related to the bioactivity potential.\textsuperscript{23,25-27}

**CONCLUSION**

Ethanol, ethyl acetate and n-hexane extracts from pagoda leaves have high potential bioactivities assessed from LC50 values of the three extracts in the highly toxic category (0-100 ppm).

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