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Acute Toxicity Assay from Seeds and Flesh of Tarap Fruit *(Artocarpus odoratissimus Blanco)* Ethanolic Extract against *Daphnia magna* Larvae

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Abstract

Tarap (Artocarpus odoratissimus Blanco) is one of the plants in the tropics that are consumed by dayak tribe in East Kalimantan. Toxicity tests on seeds and bark have been done but there is no data regarding the acute toxicity of Artocarpus odoratissimus Blanco seeds and flesh of fruit causing the need for acute toxicity tests. This Research to know the acute toxic effects of tarap (Artocarpus odoratissimus Blanco) seed and flesh extracts on larvae of Daphnia magna. Tarap seeds and flesh (Artocarpus odoratissimus Blanco) was taken from dayak market in Samarinda, is East Kalimantan, Indonesia. The seeds and flesh of the tarap fruit are extracted by maceration with ethanol solvent. An acute toxicity test was performed by exposing Dapnia magna larvae aged ≤ 24 hours with a solution of the experimental group and the control group for 48 hours. Toxicity test results are expressed in percentage of immobilization of larvae of Daphnia magna calculated by probit test to obtain EC50 (Half maximal effective concentration) values. Extracts are toxic if the EC50 value > 1000ppm. EC50 Ethanol extract of tarap seeds obtained values (3922,301 ± 324,590) for EC50 24h and (2964,498 ± 412,498) for EC50 48h. The EC50 value of ethanol extract from flesh of tarap fruit is (12224.514 ± 2186. 899) EC_{50} 24h and (6165,235 ± 1940,006) EC_{50} 48h.Ethanol extract of tarap fruit and flesh is non toxic to larvae of Daphnia magna.

Keywords: *Artocarpus odoratissimus* Blanco, acute toxocity, *Daphnia magna*

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■ Introduction

Indonesia is a tropical country that has megabiodiversity. Indonesia is recorded to have 30,000-40,000 species of flora (15.5% of the total flora in the world) and because of its unique geology, Indonesia has a high endemicity of flora

and fauna. Kalimantan Island has 9,956 species of flora and has 40% endemicity [1].

Tarap (*Artocarpus odoratissimus* Blanco) is one of the plants that grow in tropical regions such as Kalimantan. Tarap (*Artocarpus odoratissimus* Blanco) is a plant of the family moraceae which has 60 genera and 1400 species. Genus Artocarpus is a genus that is frequently used or exploited [2]. The community usually processes the juices and seeds of the young tarap as vegetables and when they are ripe, The flesh of fruit is consumed directly and the seeds can also be eaten after boiling or baking [3].

There are several studies that have been done on the content of the plant parts from tarap. Research on the safety of the compound in parts of the plant have also been done that tarap safety testing of leaf flavonoid compounds tarap conducted by [4] and safety tests conducted on tarap bark methanol extract by [5]. Both studies were carried out on Artemia salina larvae by the Brine Shrimp Lethality Test (BSLT) method and showed that tarap leaves flavonoid compounds and methanol bark of tarap bark were toxic against Brine shrimp.

Research conducted by [6] suggest that tarap extract has the potential to be developed as a medicinal plant because of the content of phenolic and flavonoid compounds contained in the seeds and flesh of tarap fruit which has pharmacological properties which include antibacterial, anti-inflammatory, and antioxidant properties. Tarap seeds and meat are most often consumed by the community directly or after being processed but until now there has been no research conducted on the safety of tarap fruit and flesh. This study aims to determine the acute toxicity of tarap (*Artocarpus odoratissimus* Blanco) seeds and flesh extracts against Daphnia magna larvae.

■ Experimental

The research design used was this study is the Posttest Only Control Group Design. The subjects of the test animals in this study were Daphnia magna larvae which were bred in the Pharmacology Laboratory of the Faculty of Medicine. The larvae used were larvae that were jam 24 hours old and showed active movements before the experiment with a total of 540 larvae of Daphnia magna used.

Each study used 6 concentrations of ethanol extract of seeds, fruit flesh and control and replication carried out three times. Larvae are taken randomly assuming each larvae found in the population are homogeneous. The independent variable of this study was the concentration of ethanol extract of seeds and flesh of tarap fruit (*Artocarpus odoratissimus* Blanco) and the dependent variable was the number of immobilized larvae of *Daphnia magna*.

Acute toxicity test for Daphnia magna larvae is an acute toxicity test using the acute immobilization test method by observing the immobilizing effect of Daphnia magna larvae after being exposed to the extract for 48 hours.

The ethanol extract seeds and flesh tarap tarap obtained after extraction by maceration method using ethanol and made in 6 concentrations are concentrations of 10 ppm, 50 ppm, 100 ppm, 500 ppm, 1000 ppm and 2000 ppm. The value of 48h EC50 is the concentration of tarap seed extract and tarap fruit meat extract causing immobilization of $\geq$ 50% of Daphnia magna larvae after 48 hours of exposure. Daphnia immobilization is Daphnia which does not show active movement within 15 seconds after a mild stirring.

Data on the number of immobilized larvae were compared with extract concentrations, then analyzed using a probit test to obtain EC50 values of extracts of tarap seeds and flesh of fruit (Artocarpus Odoratissimus Blanco).

■ Results and Discussion

The extraction of tarap seeds and flesh of fruit that has been carried out by maceration method and using ethanol solvent is obtained as a result of concentrated seed extracts and fruit flesh as much as 4.67 g and 140.11 g and the yield value as in Table 1.

Table 1. The yield of ethanol extract of seeds and flesh of tarap fruit.

	Simplicia weight (g)	Weight of Concentrated extract (g)	Yield (%)
Tarap seeds	299	4,67	1,56
Flesh of tarap fruit	284	140,11	49,33

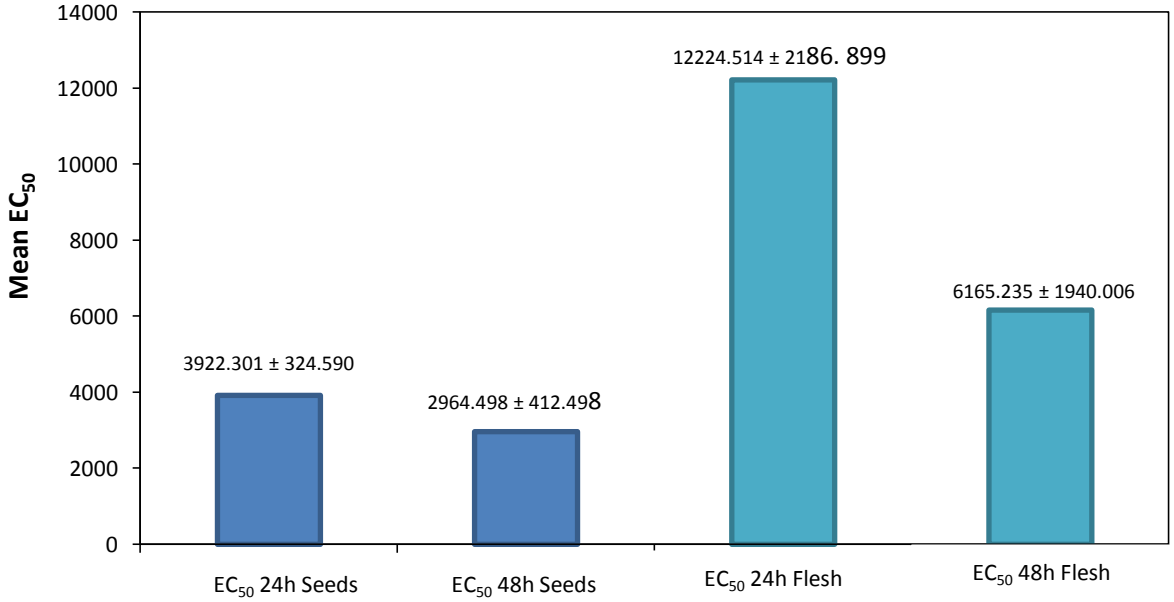

Figure 1. Mean and Standard Error EC 50 seeds and tarap flesh

Figure 1. The mean and standard error values of EC50 seeds and flesh of tarap fruit showed that the second 24 hour EC50 value of the extract was higher than the EC50 value at 48 hours. The results of the calculation of the mean value of EC50 in tarap seed extract at 24 hours were 3922,301 ppm ± 324,590 and 2964,498 ppm ± 412,498 at 48 hours, while in the extract of tarap flesh of fruit results obtained mean EC50 at 12224,514 ppm ± 2186. 899 and 48 hours amounted to 6165,235 ppm ± 1940,006.

These results indicate that ethanol extract of tarap seeds at a concentration of 2964,498 ppm ± 412,498 and ethanol extract of tarap flesh of fruit at a concentration of 6165,235 ppm ± 1940,006 at 48 hours exposure could cause 50% immobilization of Daphnia magna larvae population. a compound is declared to have the potential for acute toxicity if it has a 48h EC50 value of less than 1000 ppm [7].

Research on the seeds and flesh of the fruit is different from the study of other parts of tarap tree leaves and bark are conducted by [4,5]. Brine Shrimp Lethality Test (BSLT) test results showed that the leaves and bark of tarap are toxic with LC50 values 110.51 ppm for leaves and 462.38 ppm for bark. This difference can occur because the parts of the plant are used in different studies, which in this study researchers used the seeds and pulp are also frequently used by the community than the leaves and bark. Classes of compounds contained in seeds and fruit flesh are phenolic, flavonoid, keratenoid and anthocyanin groups [6,8]. The leaves are known to have steroids, phenolics and flavonoids [4], while the bark contains a class of alkaloid, phenolic, flavonoid, tannin and saponin compounds [9]. Differences in the content of secondary metabolites are suspected to cause differences in toxicity test results on tarap tree parts.

EC50 values obtained in this study cannot be used as a basis for proving the safety of an ingredient or preparation in humans, because the

toxicity test for Daphnia magna is an initial screening of a test material [10,11]. To determine the acute toxicity of a plant or material used as food or medicinal material in humans, it is necessary to carry out further toxicity tests in accordance with Indonesian Drug and Food Control Agency recommendations.

Conclusion

The ethanol extract of the seeds and flesh of tarap fruit (Artocarpus odoratissimus Blanco) has no toxic potential to the larvae of Daphnia magna.

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