



COMPARISON OF ACUTE TOXICITY TEST OF CARDAMOM EXTRACT COMBINATION (*Amomum compactum* Soland. Ex Maton) AND CURCUMA (*Curcuma xanthorrhiza* Roxb) WITH A COMBINATION OF CARDAMOM EXTRACT (*Amomum compactum* Soland. Ex. Maton) AND GALANGAL (*Alpinia galanga* L) AGAINST *Artemia salina* Leach BY BRINE SHRIMP LETHALITY TEST (BSLT) METHOD

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Abstract

*Cardamom, ginger, and galangal are one of the plants that are often used as herbal medicine. Similar studies that have been carried out previously only conducted single extract tests and have not combined extracts, and compared the results of using two combinations of extracts. However, because there is a possibility of interactions between compounds in a single extract that can increase their toxic effects, it is necessary to conduct acute toxicity testing of the combination aimed at determining the potential toxicity. This study aims to determine the comparison of the potential acute toxicity of cardamom and curcuma extract combinations with cardamom and galangal extract combinations seen from LC₅₀ values using the Brine Shrimp Lethality Test (BSLT) method. The research conducted was an experimental study, extracted by maceration method and using BSLT method for toxicity testing. In this study using 5 variations in concentration, but the concentration of extracts used in the combination of extracts is different, namely the combination of cardamom and curcuma extracts using variations in concentrations of 12.5ppm, 25 ppm, 50 ppm, 100 ppm, while the combination of cardamom and galangal extracts uses variations in concentrations of 500 ppm, 250 ppm, 100 ppm, 50 ppm, 10 ppm. The LC₅₀ value was obtained from the number of larvae that died after 24 hours of treatment with probit analysis. The results showed that the combination of cardamom extract and curcuma had a highly toxic level against *Artemia salina* Leach larvae with an LC₅₀ value of 12.98 ppm, and a combination of cardamom extract (*Amomum compactum* Soland. ex Maton) and galangal (*Alpinia galanga* L) had an LC₅₀ of 18.08 ppm. The results showed that both combinations are highly toxic due to LC₅₀ ≤ 30 ppm values.*

Keywords : cardamomum, curcuma, galangal, BSLT method



Background

Traditional medicine research in Indonesia includes research on traditional medicines in single or in combination form. Based on preclinical data of cardamom, ginger, and galangal plants, the use of a combination can be developed as an anticancer agent. Brine *Shrimp Lethality Test* (BSLT) is an initial study and is included in the acute toxicity test group to determine the potential of a plant as an anticancer ingredient. This BSLT method was chosen because it is easy to work, fast, quite accurate, cheap and uses a small amount of test material. This method has been shown to have a correlation with anticancer activity and has confidence levels of up to 95% (Meyer *et al.*, 1982).

The BSLT method uses *Artemia salina* Leach larvae as test samples. These larvae are thought to represent zoological organisms for in vivo mortality testing. The test results showed a positive correlation of the toxicity properties of the test compound to proliferation barriers. The BSLT test was carried out by observing deaths that occurred after giving extracts to *A. salina* shrimp larvae for 1x24 hours. The results obtained are then calculated as *Lethal Concentration* (LC₅₀) values, namely the concentration of extracts that can cause 50% death of *A. salina* larvae. Active compounds will produce high mortality (Chusniasih *et al.*, 2020).

Cardamom (*Amomum compactum* Soland. ex Maton) contains active compounds, namely *flavonoids*, *phenolics*, *saponins*, *alkaloids*, *terpenoids*, and negative *steroids* (Muna *et al.*, 2019). In previous studies, the toxicity activity of cardamom extract obtained LC₅₀ of 25.52 ppm (Muna *et al.*, 2019). The LC₅₀ value indicates that cardamom extract has a very toxic toxicity activity, because according to Mayer., *et al* (1982) the LC₅₀ value below 30 ppm is included in the very toxic category. Curcuma (*Curcuma xanthorrhiza* Roxb) contains xanthorrhizol which has anticancer activity on the skin by inhibiting the expression of *ornithine decarboxylase*, *cyclooxygenase-2* and *inducible nitric oxide synthase* through *mitogen-activated protein kinases* and / or *nuclear factor KB*. Xanthorrhizol can also inhibit liver cancer through *induces apoptosis via the up-regulation of bax* and *p* in *HeLa cells* (Udin, 2013).

Another plant that has toxicity activity is galangal (*Alpinia galanga* L). The results of phytochemical screening of Galangal extract show the presence of *alkaloid* compounds, *flavonoids*, *saponins*, and *tannins* (Khusnul, 2017). Galangal has toxicity activity with an LC₅₀ value of 62.4979 ppm (Jayanti *et al.*, 2019). The results of phytochemical screening of Galangal extract show the presence of *alkaloid* compounds, *flavonoids*, *saponins*, and *tannins* (Khusnul, 2017). The potential acute toxicity of each extract is well known, but because there is a possibility of interactions between compounds in a single extract that can increase their toxic effects, it is necessary to conduct acute toxicity testing from a combination of the two in order to determine the potential toxicity.

Method

Toxicity test treatment was carried out 3 times on each sample extract. *Artemia salina* Leach is then inserted 10 tails into a test tube that has contained each extract concentration. Negative controls are created in the same way except for the addition of extracts. Negative control was carried out to see if the death response of the test animals really came from the sample and was not caused by the solvent used.

Result and Discussion

Table 1. Toxicity test results of cardamom and curcuma extract combination using Brine Shrimp Lehality method

Waktu (Jam)	Konsentrasi (ppm)					
	0	10	50	100	250	500
0	0	0	0	0	0	0
1	0	0	0	0	1	4

2	0	0	0	3	3	2
3	0	0	0	1	4	3
4	0	0	3	1	2	0
5	0	0	1	0	0	5
6	0	0	1	1	4	1
7	0	0	0	1	5	4
8	0	1	0	0	1	6
9	0	0	0	3	5	2
10	0	1	3	0	1	0
11	0	0	3	0	2	3
12	0	0	0	1	1	-
13	0	2	0	0	1	-
14	0	0	1	2	-	-
15	0	0	0	0	-	-
16	0	0	0	0	-	-
17	0	0	0	0	-	-
18	0	0	0	0	-	-
19	0	3	3	4	-	-
20	0	0	0	1	-	-
21	0	2	2	2	-	-
22	0	0	1	4	-	-
23	0	3	3	2	-	-
24	0	0	0	2	-	-
Total kematian	0	12	21	28	30	30
% Kematian larva	0%	40 %	70%	93,3%	100%	100%

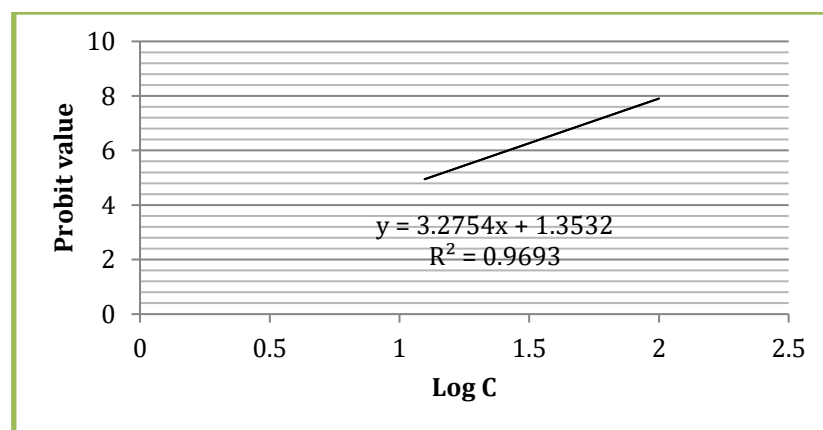


Figure 1. Probit Value Graph of Cardamom and Curcuma Extract Combination

Based on the results of research that has been presented in Table 1, it shows that the highest percentage of larval mortality is found at a concentration of 100 ppm and the lowest at a concentration of 12.5 ppm. The percentage of larval mortality is also aligned with an increase in the concentration of the extract. In the table it can also be seen that in the negative control that was not given a combination of cardamom extract and curcuma no dead larvae were obtained, and the increase in the percentage of deaths based on the use of concentration was very significant. So that the death of *Artemia salina* Leach larvae is purely due to the administration of extracts.

Table 1 also shows that over time the more larvae die. In previous studies, cardamom toxicity tests at concentrations of 100 and 50 ppm had a mortality percent of 76.7% and 60% and curcuma toxicity tests reported by prasetyo at concentrations of 100 and 50 ppm had a mortality percent of

100% and 83%. This shows that the combination of cardamom extract and curcuma has more toxic properties than single extract preparations. An extract is declared toxic if LC₅₀ is below 1000 ppm and is declared highly toxic if LC₅₀ is below 30 ppm and above 1000 ppm is declared non-toxic (Meyer *et al.*, 1982). The statement shows that the combination of cardamom extract and curcuma is very toxic because it has an LC₅₀ value below 30 ppm, which is 12.98 ppm.

Table 2. Toxicity Test Results Of Cardamom And Galangal Extract Combination Using Brine Shrimp Lethality Test method

Waktu (Jam)	Konsentrasi (ppm)				
	0	12,5	25	50	100
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	0	0	5
4	0	0	0	2	2
5	0	0	0	0	0
6	0	2	3	4	7
8	0	2	6	4	8
10	0	0	0	3	6
12	0	3	2	6	2
16	0	6	6	7	-
20	0	0	0	0	-
24	0	4	6	2	-
Jumlah Kematian	0	17	23	28	30
% Kematian	0	56,66%	76,66%	96,66%	100%

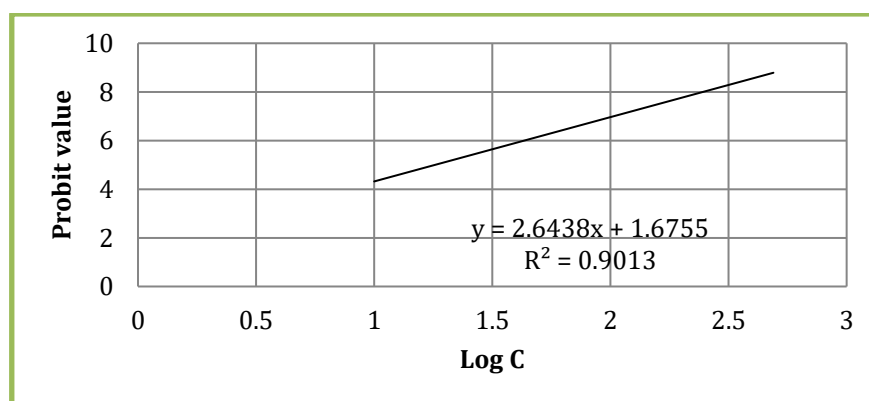


Figure 2. Probit Value Graph of Cardamom and Galangal Extract Combination

Table 2 shows the highest larval mortality at concentrations of 500 ppm as well as 250 ppm, and the lowest at 10 ppm. In addition, there was an increase in the mortality of *Artemia salina* Leach larvae in line with an increase in the concentration of a combination of Cardamom (*Amomum compactum* Soland. Ex. Maton) and Galangal (*Alpinia galanga* L.). In the negative control, no dead larvae were obtained, so the death of larvae was purely due to the extract given not due to the influence of sea water. The standard deviation for each death is also still within the normal limit of less than 2.

In probit analysis, an LC₅₀ value of 18.08 ppm was obtained which showed that the combination of galangal and cardamom extracts was very toxic to shrimp larvae because the LC₅₀ value was ≤ 30 ppm. Acute toxicity tests of cardamom and galangal extracts in previous studies

conducted by Muna *et al.*, (2019) and Jayanti *et al.*, (2019) obtained LC_{50} values of 25.51 ppm and 62.4979 ppm, respectively. The results of the LC_{50} of the two studies were smaller than the results of this study, meaning that the combination of cardamom and galangal extracts was more toxic than each extract. Because according to Meyer *et al.*, (1982) the smaller the LC_{50} value, the more toxic the compound, and a compound that has antitumor and anticancer activity correlates with the high toxic content in the compound (L, Vivi *et al.*, 2006).

Conclusions

The combination of cardamom and curcuma extract with galangal cardamom extract has highly toxic properties with LC_{50} values of 12.98 ppm, and 18.08 ppm, respectively. The greater the concentration of the combined extract of cardamom extract and curcuma, the greater the death of larvae.

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