BIOACTIVITY *Brine Shrimp Lethality Test* EXTRACT AND FRACTINATION FROM *KALANCHOE PINNATA* LAM PERS

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Abstract

Bioactivity test of ***Kalanchoe pinnata Lam. Pers.*** *has been done using Brine Shrimp Lethality Test method. Toxicity preliminary test of ethanol and methanol extracts of K. pinnata showed that ethanol extract (LC50 176,20 µg/mL) more toxic than methanol extract (481.98 µg/mL). K.pinnata leaves extracted with 70% ethanol and further fractionated with n-hexane, ethyl acetate, n-butanol and water. Toxicity test results showed that ethyl acetate and n-butanol fractions of K. pinnata leaves have activity against Artemia salina Leach. larvae with LC50 values 53.09 µg/mL and 183.65 µg/mL, respectively.*

Key word : *Kalanchoe pinnata Lam. Pers, Brine Shrimp Lethality Test. Artemia salina Leach. Toxicity.*

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INTRODUCTION

Biological activity test (toxicity test) of *Kalanchoe pinnata* (Lamarck) Persoon (*Bryophyllum pinnatum*) leaves extracts has been done, however, has not been found in the literature examined the potential of anti-cancer activity indicated by LC_{50} values of toxicity of *K. pinnata* plant. *K. pinnata* is an annual that grows medicinal plants (perennial) and is widely used as anti-inflammatory, antileishmania (Muzitano, M.F, *et.al*, 2006), antiulcer (Pal, S.*et.al* 1991)), antibacterial (Akinpelu, D.A. *et al*.,2000), a natural insecticidal (Supratman, U, *et al*.,2000), hepatoprotective (Yadav.*et al*.,2003), antihistamine (Cruz, E.A.*et al*.,2008), and antitumor (Supratman, U, *et al* .,2001). Secondary metabolites compounds contained in this plant are primarily group of bufadienolida, terpenoids and flavonoids (Muzitano, M.F, *et.al* .,2006).Such as bersaldegenin-1,3,5-orthoacetate,bufadienilide-bryophylin B, bryophollone, bryophollenone, bryophynol, 2(9-decenyl) phenanthrene and 2-(undecenyl)-phenanthrene (Yadav, et al., 2003).

Bioactive compounds in high doses are (almost) always toxic. Thus, in vitro killing power of the compounds against the animal organism can be used to screening plant extracts which has bioactivity, and to monitor bioactive fraction during the fractionation and purification. One of organism suitable for toxicity animal testing is a brine shrimp (crayfish) (B.N. Meyer, et.al.,1982). To determine toxicity and antioxidant activity of leaf extract K. pinnata, we used BSLT (Brine Shrimp Lethality Test) method.

The measurement results indicate the level of toxicity and potential antioxidant activity of the samples in general. In the second method, *brine shrimp* serves as animal tester to determine toxicity levels of the extracts, that calculated based on the number of dead shrimp larvae correlated with the extract concentration used (Juniarti, *et.al*.,2009).

MATERIALS AND METHODS

Materials : 932 gram of sample used in this study is cocor bebek leaves (*K. pinnata* Lam. Pres) obtained from Serpong area.

Methods: ***Toxicity Test Method BSLT*** (*Meyer's method*) (Artanti, N *et.al* 2003) used to study the general toxicity of samples using shrimp eggs (*Artemia salina Leach*). Two vessels prepared for shrimp hatcheries and for larvae. In one vessel in the vessel was placed lamp to warm temperatures in the hatchery, while in the other vessel next to the sea water was not given. Into the sea water entered ±50-100 mg shrimp eggs for hatching, incubated for 48 hours. Extracts to be tested are made in a concentration of 10, 100.500 and 1000 ppm in sea water. If the sample does not dissolve, add 10 mL of DMSO. Procedure: About 100 mL of sea water containing 10-11 larvae of the shrimp, inserted into the test container. Added sample solution to be tested 100 mL each, with concentrations of 10, 100, 500 and 1000 ppm, respectively. Each concentration was carried out three repetitions (triplicate). Solution was stirred until homogeneous. To control is done by adding 10 mL of DMSO. Solution was left for 24 hours, counted the dead and live number of larvae in every hole. Amount of died larvae number summing the dead larvae that died in each concentration (3 holes), the same way to count the live larvae number based on the life larvae in each concentrations. Accumulated dead number calculation was carried out in the following way: the accumulation of dead to 10 µg/mL = number dead at these concentrations, the accumulation of dead to 100 µg/mL concentration = number of dead at 10 µg/mL + number of dead at 100 µg/mL concentration, accumulation of dead to 500 µg/mL concentration = number of dead at 10 µg/mL + number dead at concentrations of 100 µg/mL + number dead at a concentration of 500 µg/mL. Accumulated number of dead counted up to 1000 µg/mL. Calculation of accumulated life of each concentration was carried out in the following manner: accumulation of life to 1000 µg/mL = number living at 1000 µg/mL, the accumulation of life for the concentration of 500

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µg/mL = number living at 1000 µg/mL + number of living at a concentration of 500 µg/mL, the accumulation of life for the concentration of 100 µg/mL = number living at 1000 µg/mL + figures life at a concentration of 500 µg/mL + number of living at a concentration of 100 µg/mL. Accumulated number of life calculated to 10 µg/mL. Furthermore, mortality is calculated by: the accumulation of dead divided by the accumulation of life and death (total) multiplied by 100%. Graphics made with the log concentration as the x-axis on mortality as the y-axis. LC_{50} value is the concentration of a substance which causes the death of 50% obtained by using linear regression equation y = a + bx. An active or toxic substance to say when the value of LC_{50} <1000 µg/mL to extract and <30 µg/mL for a compound.

RESULTS AND DISCUSSION

Fractionation of ethanol extract of *K. pinnata* leaves with *n*-hexane, ethyl acetate, *n*-butanol and water, obtained various yield of each fractions. The yield of fraction were 40.13%; 0.20%; 0.84% dan 2.11%, respectively.

Table I and figure 1 showed that ethanol extract has LC_{50} value higher than methanol extract with LC_{50} 176.19 µg/ml and 481.98 µg/mL, respectively. Means that extraction using ethanol will obtain extract with a toxicity activity value higher than using methanol.

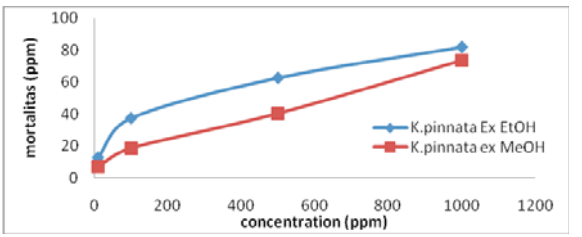

Figure 1. Brine Shrimp Lethality Test Result (Ethanol Extract vs Methanol Extract of *K. Pinnata*)

A substance has activity or toxicity figures if they have LC_{50} value < 1000 µg/mL for an extract and < 30 µg/mL for a pure compound.

Toxicity test of each fractions obtained from ethanol extract fractionated with different solvent (Table 2 and Figure 2.) showed that only ethyl acetate (LC_{50} 53.09 µg/mL) and *n*-butanol (LC_{50} 183.65 µg/mL) fractions were active with LC_{50} values lower than 1.000 µg/mL, compared with *n*-hexane (LC_{50} 7144.96 µg/mL) and water fractions (LC_{50} 4285.49 µg/mL) with LC_{50} values higher than 1000 µg/mL (LC_{50} > 1.000 µg/mL). It's means that in ethyl acetate and *n*-butanol fraction contained toxicity active chemical compounds.

Table I. Brine Shrimp Lethality Test Result (Ethanol Extract vs Methanol Extract of *K. Pinnata*)

K. pinnata	concentration ppm	Life value	Dead Value	Live accumulation	Dead accumulation	Dead accumulation/total	Mortalitas	LC_{-50}
Methanol Extract	10	24	6	81	6	6/87	6.897	481.98 Active
	100	23	7	57	13	13/70	18.571	
	500	20	10	34	23	23/57	40.351	
	1000	14	16	14	39	39/53	73.585	
Ethanol Extract	10	20	10	64	10	10/74	13.51	176.20 Active
	100	20	10	44	20	20/64	31.25	
	500	15	15	24	35	35/59	59.32	
	1000	9	21	9	56	56/65	86.15	

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Table II. Brine Shrimp Lethality Test Result (Fractions of *K.pinnata* Ethanol Extract)

K. pinnata	concentration ppm	Life value	Dead Value	Live accumulation	Dead accumulation	Dead accumulation/total	Mortalitas	LC_{-50}
n-hexane Extract	10	28	2	101	2	2/103	1.942	7144.96
	100	23	7	73	9	9/82	10.976	
	500	26	4	50	13	13/63	20.635	Non active
	1000	24	6	24	19	19/43	44.186	
Ethyl acetate Extract	10	17	13	39	13	13/52	25.000	
	100	9	21	22	34	34/56	60.714	53.09
	500	8	22	13	56	56/69	81.159	Active
	1000	5	25	5	81	81/86	94.186	
BuOH Extract	10	26	4	64	4	4/68	5.882	
	100	15	15	38	19	19/57	33.333	183.65
	500	15	15	23	34	34/57	59.649	Active
	1000	8	22	8	56	56/64	87.500	
Water Extract	10	25	5	98	5	5/104	4.854	4285.49
	100	25	5	73	10	10/83	12.048	
	500	24	6	48	16	16/64	25.000	Non active
	1000	24	6	24	22	22/46	47.826	

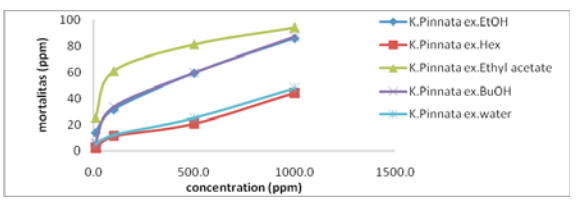

Figure 2. Brine Shrimp Lethality Test Result

Figure 2. Brine Shrimp Lethality Test Result (Fractions of *K.pinnata* Ethanol Extract) Further investigation are needed to isolation one or more active compounds from ethyl acetate and *n*-butanol fractions, suitable chromatographic and spectroscopic methods compilation will lead to the chemical structure elucidation. Chemical active compounds isolated from each fraction could be developed and used as drug precursor (lead compound) or drug it's self.

CONCLUSION

Ethanol extract of *K. pinnata* has toxicity activity higher than methanol extract. Different fractions obtained from *K. pinnata* ethanol extract fractionation showed different level of toxicity activity. Ethyl acetate fraction with LC_{50} 53.09 µg/mL is the highest toxicity activity fraction compared with the other fraction (*n*-butanol LC_{50} 183.65 µg/mL; *n*-hexane LC_{50} 7144.96 µg/mL; and water fractions LC_{50} 4285.49 µg/mL).

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