Fractionation Ethyl Acetate Extract of Stem Bark Soursop(*a. Muricata*. Linn) Potential Anticancer

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Abstract.. Influence the toxicity of the ethyl acetate extract of the bark of the soursop (Annona muricata. Linn) at Brine Shrimp Lethality Test Test (BLST) against Artemia salina. The test is to observe the lethal concentration of one of the active fraction. Fractionation and characterization is done by thin layer chromatography, liquid chromatography column vacuum (KKV) and column chromatography press (of the summit). The ethyl acetate extract on one of the main fraction showed activity with LC_{50} value is 16.688 ppm.

Keywords: soursop, *A. muricata* Linn., Toxicity, *Artemia salina*

INTRODUCTION

One family of plants are used as medicinal plants in various countries including Indonesia are family Annonaceae. Annonaceae is a large plant consisting of 126 genera and 2500 species (Simbala, 2009).

One species of medicinal plants of the family Annonaceae are often used as traditional medicine by the public is Annona muricata L. or better known as soursop. Along with the development of technology, content and properties of plants soursop began to unfold. Various studies show that the soursop plant contains many properties as medicines. Soursop plant parts, ranging from leaves, flowers, fruits, seeds, root and bark can be used as a drug. In general, the parts of the soursop plant widely used to treat diseases such as hypertension, diabetes, cough, fever, ulcers, and other diseases (Mardiana, 2012).

Research on this plant have been done, including the discovery of alkaloids in the leaves and seeds of soursop (Idrus, 2012), the content of the antioxidant and anti-inflammatory in the ethanol extract of leaves of the soursop (Suharyadi, 2013). Flavanoid compounds are also found in the chloroform extract of soursop fruit flesh and the n-butanol extract of leaves of the soursop is an active compound that can lower uric acid levels (Artini, 2011); of the ethanol extract was found leucoanthocyanins nature toxicity to larvae Aedesaegypti to be useful as a medicine for dengue fever (Torres, 2014); of the methanol extract compounds found kaempferol potential as anti-cancer (Silmi, 2014). Therefore he other class of compounds such as flavonoids, steroids and alkaloids can also be found on the bark. Theoretically chemical compounds that are bioactive in higher plants can be found in all parts of the plant (Suhando, 2013). Soursop plants are plants with a variety of health benefits. This plant can be used as a medicine to cure various diseases, ranging from mild illness such as itching of the skin to severe diseases such as tumors and cancer. In addition to cure cancer, soursop fruit also acts as an antibacterial, antifungal effective against various types of parasites / worms. Soursop leaves contain active ingredients, such as saponins, flavonoids, and tannins. As drugs made from plant material, it will be more safe if consumed. (Ardraviz, 2012). Based on the above formulated question of whether the fraction of the ethyl acetate extract of the bark of the soursop can potentially anticancer.

RESEARCH METHODS

Equipment and Materials

The tools used in this research are: a set of distillation equipment, Buchner funnels, TLC chamber, a capillary tube, a tool for fractionation include vacuum column chromatography (KKV) and press column chromatography (of the summit). Then some equipment such as: analytical balance, evaporator and means of determining the melting point ("melting point Krüss.") And FT-IR

For solvent extraction and chromatography used p.a and technical quality distilled beforehand, namely: n-hexane, chloroform, ethyl acetate, acetone and methanol. Column chromatography vacuum (KKV) was performed using Si gel Merck 60 7730, column chromatography press (of the summit) with silica gel Merck 60 7734 (0063-0200 mm), silica gel Merck 60 7733 (0.2 - 0.5 mm) to inpregnasi, and analysis thin layer chromatography (TLC) is performed by Si gel-coated plates Merck Kieselgel 60 F254 (0.2 - 0.5 mm). Cerium sulfate solution 1.5% in 2 N sulfuric acid is used to penampak stains. To test BLST used dimethyl sulfoxide (DMSO), and fry shrimp Artemia salina

Sample Preparation

Plant material used is the bark of the soursop (A. muricata Linn) obtained from Pinrang South Sulawesi. Bark of A. muricata Linn. cleaned and dried with aerated at room temperature in open air to dry. The bark is dried cut into small pieces and then smoothed using a smoothing machine and weighed.

Test Brine Shrimp

One mg of sample in Eppendorf tubes made with as much as 100 mL of DMSO and then diluted with akuabides. The dilution was taken to 200 mL diluted with 600 mL concentration akuabides that the concentration of the sample into 1000 mL / mL.

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Further dilution is done in mikroplate with varying concentrations and sample volume of 100 mL each hole in triplo. Shrimp seed was 48 hours pipette as much as 100 mL by the number of 7-15 tail fries, included in mikroplate containing samples were then incubated for 24 hours. To control the same treatment without the use of samples. Furthermore, shrimp counted the dead and the living, and determined LC_{50}. Stating toxicity LC_{50} value of extracts and pure compounds each less than 500 ug / mL and 200 mg / mL. The toxicity values are divided into two categories, namely toxicity (high toxic) to LC_{50} <100 pg / mL and low toxicity (low toxic) to LC_{50}> 100 pg / mL (Anderson, 1990).

RESULTS AND DISCUSSION

Extraction

A total of 2.5 kg of fine powder of bark A. muricata Linn. macerated with ethyl acetate as many as 10 L. Maceration technical performed for 3 times 24 h with shaking / stirring. Maserat ethyl acetate obtained is then filtered with a Buchner funnel lined Whatman filter paper No. 41. The extract obtained as much as 3 L concentrated using an evaporator at a low temperature (40 ℃) to obtain a thick extract of 15.48 grams.

Fractionation

Ethyl acetate fraction further fractionated by column chromatography initial vacuum (KKV) with eluent n-hexane, EtOAc: n-hexane, EtOAc, acetone, and methanol with enhanced polarity sequence. Fractions obtained from fractionation stages identified by TLC, fractions that have the same chromatogram are combined and produces eight major fraction (A - H).

Test Brine Shrimp

The toxicity test on shrimp larvae ethyl acetate extract fraction D fractionation result, the bark of the soursop can be seen in Table 1. The results graph the relationship between probit versus log concentration of the ethyl acetate extract fraction D fractionation results bark of the soursop can be seen in Figure 1.

From the images obtained linear regression equation is Y = -0.765x + 4:52, in order to obtain the LC_{50} value is calculated based on the formula, then:

Y = -2.41x + 7.96
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$$\frac{y - 7.96}{-2.41} = x$$

$$\frac{5 - 7.96}{-2.1} = 1.222$$

So, log x = 1.222
X= antilog 1.222
= 16.688 ppm

So LC_{50} for the ethyl acetate extract fraction D fractionation results bark sirsak to larva *Artemia salina* is = 16 688 mg / mL.

Table 1. Test Results shrimp (*Artemia salina* Leach) of the Ethyl Acetate Extract fractionation results fraction D Leather Trunk Soursop *(A. muricata* Linn)

Concentration (ppm)	Log concentration	Number of dead	Number of living	percent Response	Probit
10	1	30	0	43	4.82
100	2	30	0	33	4.56
1000	3	29	1	-3	0

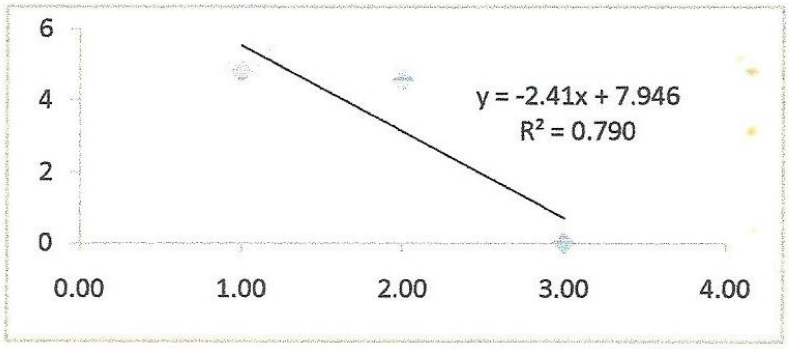

Figure 1. Graph The relationship between the log Probit Concentration Ethyl Acetate Extract fractionation results fraction D Leather Trunk Soursop (*A. muricata* Linn)

CONCLUSION

Based on the results of toxicity tests to fry shrimp *Artemia salina* Leach obtained LC_{50} for the ethyl acetate extract fraction D fractionation results bark of the soursop is 16 688 mg / mL.

ACKNOWLEDGEMENTS

We thank you To DIPA Ditlitabmas of Higher Education on the cost of research given in Fundamentals Grant Program 2016.

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