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*“Conserving Sumatran Wildlife Heritage
for Sustainable Livelihood”*



**Institute for Research and Community Service
University of Lampung**

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“Conserving Sumatran Wildlife Heritage for Sustainable Livelihood”

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THE COMPARISON OF TOXICITY PURIFIED ISOLATE OF WATER AND METHANOL EXTRACTS OF PAWDER LEAF *GLIRICIDIA MACULATA* ON MORTALITY SOURSOP MEALYBUG *PSEUDOCOCCUS CRYPTUS*

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ABSTRACT

Soursop production in Indonesia continues to decline from year to year. One cause is the mealybug pest (*Pseudococcus cryptus*). The white lice suck the young fruits soursop to dry and stunted. To control the pest, using botanical insecticides more safety than synthetic insecticides. One of the plants that can be used is *Gliricidia maculata*. The leaves of *G. maculata* contain plenty of an active compound flavonoid. The purpose of the study to compare the toxicity of the purified isolates of water and methanol extracts of *G. maculata* leaves on mortality soursop mealybugs (*P. cryptus*). Extraction was done by maceration series using various organic solvents (n-hexane, dichloromethane, methanol and water). Fractionation and purification of flavonoids from polar extracts were done by Chromatography Coloum. A set of laboratory experiment was conducted by using block design. Water and Methanol extracts (WE and ME) with 5 levels concentration i.e. 0%, 0.02%, 0.04%, 0.06% and 0.08%, and 3 replications. ANOVA was conducted to obtain the means and standarrd deviations of the experimental study, and Tukey's test at $\alpha = 5\%$ was peformed in order to obtain the different among the expelimental groups. Analisis Probit were used for compare the efectivities the extracts. The result indicated the was toxic to mealybug pest (*P. cryptus*) with LC50 72 hours water extract 0,061% and metanol extract 0,096%. Therefore water extract more toxic than metanol extract.

Keywords : extract water , methanol , powder leaves (*G.maculata*) , soursop mealybug (*P.cryptus*)

1. INTRODUCTION

Soursop plant is one of the agricultural commodities. Soursop production in Indonesia has declined to 15% from the previous year (Agricultural Statistics 2009). One reason is that pests and diseases in plants soursop fruit. One of the pests that cause a decline in the production of soursop is mealybug (*Pseudococcus cryptus*). The existence of mealybugs can reduce the production of soursop fruit up to 58% (Ivkdalam, 2010).

To overcome the mealybug pest attacks on crops soursop typically used synthetic insecticides. The use of synthetic insecticides are not appropriate and for a long time will bring bad effects. That requires insecticide safer and environmentally friendly, such as using vegetable insecticides derived from plants (Priyono, 2005; Siswanto and Karmawati, 2012). One of the plants that can be used as an insecticide plant are the leaves of *Gliricidia* (*Gliricidia maculata* Hbr.). *Gliricidia* leaves has coumarin active ingredients that are insecticides, rodenticides and bactericidal (Manglayang Farm, 2006).

The purpose of this study was to determine the comparative toxicity of water extract and isolate pure methanol extract of leaves of *Gliricidia* against mealybugs on soursop.

2. RESEARCH METHODS

Tools and Materials

The tools used machetes and treasure, Disk Mill machine, scales, jars, gauze pads. Materials used in this research that plant leaves Gamal and mealybugs (*P. cryptus*). The tools used are glass jars, filter Bunchner, Freezedrayer, TLC (Thin Layer Chromatography), electric heating, capillary pipette, digital cameras stationery. N-hexane, dichloromethane (DCM), and methanol with a brand J.T Beker, distilled water. AmberliteXAD KK-4, Plat TLC (thin layer chromatography),. TLC Reagent SbCl₃

Section, AlCl₃, and CeSo₄, aluminum foil, Erlenmeyer flask, test tubes, spatulas, analytical balance, oven, beaker, beakers, pipettes, funnels, filter paper, and hot plate.

The course of study

Materials prepared for bioassay ie 1x pistil soursop fruit as much as 30 pieces measuring 2-3 cm and are free of pests. Preparation for the bioassay ie plastic cups and containers to soak test medium. Gauze to cover plastic cups. Brush and a pin to move and put the test insects in the test medium. Extracts used to bioassay that extracts polar (water and methanol) Gliricidia leaf powder, sucking insects soursop fruit (*P. cryptus*) adult stage females who are already acclimatized for 1 day prior to treatment, soursop fruit as a test medium.

Gliricidia leaves that have been dried for milled using Disk Mill machine to a powder and wrapped in plastic, solvents Hexana, DCM, methanol and water.

Gliricidia leaf powder extract water maceration results that show the deposition of amorphous form is filtered with filters Buncher, in order to separate the sediment (EA) and the filtrate (FA) it. Furthermore EA drayer freeze dried. Pasta obtained do bioassay against mealybug pest. The filtrate (FA) was purified by fractionation by column chromatography method using KK-AmberliteXAD-4 and isolated slope (gradient elution). Fractions were collected based on volume. Each fraction was tested content of flavonoids by TLC method and positive fractions containing flavonoids will do bioassay against mealybug pest.

The methanol extract of dried Gliricidia leaves contain lots of chlorophyll, chlorophyll with a method to separate column chromatography (CC) using silica and isocratic. The filtrate methanol been cleared of chlorophyll (FM) direfraksikan using the method of using the column AmberliteXAD KK-4. Fractions were collected based on the volume and content of each fraction was analyzed using the method of flavonoids thin layer chromatography (TLC). The fraction of the isolated flavonoid-rich test keserangga tested pest mealybugs (*P. cryptus*) in plants soursop. The selected active fraction is the fraction rich in flavonoids with a low amount of matrix and provide a high activity against test insects.

Each compound found on the fractionation stages of bioassays performed against pest infestation white female adult stage and the test medium used was pistil soursop. This is done to screen for compounds active insecticide. Bioassays were carried out is the test of mortality with residual effect (residual effect).

Residue testing conducted by soaking soursop with 5 degree of concentration that is (0%, 0.05%, 0.010%, 0.015% and 0.020%) for 10 minutes, 10 heads mealybugs (*P. cryptus*) female adults who had been acclimatized for 1 days before treatment is placed on the pistil of soursop fruit that has been soaked with gliricidia leaf extract and maintained in test containers. Observations of insect mortality trial conducted at 12, 24, 48 and 72 hours after treatment. These trials were conducted each with 3 replications.

3. RESULTS AND DISCUSSION

A. Extraction Methanol Extracts Water And Leaf Extract Gamal

Results maceration storey of 500 grams of powdered leaves of Gliricidia, obtained methanol extract as much as 6 liters. The evaporated 6 liters of extract obtained 80 grams of crude extract. Results frezdrayer 80 grams of crude extract, obtained 23 grams of extract in the form of pasta. Results frezdrayer 500 ml of water extract obtained 6 grams of water extract, the rest of which is not in frezdrayer stored in the refrigerator.

Test the water extract of crude extract and methanol extract of leaves of Gliricidia LC₅₀ value obtained for 0.034% water extracts and methanol crude extract of 0.025%. The LC₅₀ value is used as the determination of the concentration on testing water and methanol extract of leaves of Gliricidia against mealybug pest death.

a. Purification of the crude extract water (hydrolysis)

Hydrolysis of 2.5 grams of water extract obtained precipitate (EA) in crystalline form as much as 1 gram and filtrate (FA) in the form of the aqueous phase as much as 35 ml of yellowish and phase as much as 15 ml of ethyl acetate brownish (Figure 1).



Figure 1. Results of hydrolysis of water extract: a. aerobic phase. ethyl acetate phase, c. crystal water phase

Hydrolysis extract water to the water phase test TLC, the TLC plate looks are still many spots on the chromatogram indicates there are still many other compounds. Crystals to the water phase TLC test was done and the result is only one spot on the chromatogram and obvious, it is expected that the compound is already used as a pure and bioassays on mealybugs (*P.cryptus*) on soursop plant.

b. Purification of the crude extract methanol (fractionation)

Fractionation results obtained as much as 1 gram of methanol extract using KK Amberlite XAD-4 obtained 29 fractions. The same visual merged into one in order to obtain 6 fractions. The test results obtained TLC 6 fraction 1 fraction (19) is thought to be a pure compound content of flavonoids (Figure 2).



fractions 19

The test results purity done by monitoring by TLC with eluent same that DCM: methanol (4: 1), the plate TLC seen the content of flavonoid compounds in extracts of pure methanol and pure extract of leaf water gamal shown their patches of yellow and brown on TLC plate (chromatograms) after being sprayed visualization $CeSO_4$, $AlCl_3$, $NaOH$ and H_3BO_3 (Figure 3).

The test results show only a fraction of the 19 flavonoids continued to test the purity by TLC, the results of purity testing that has been done is obtained in the form of chromatogram shows a single spot. These results indicate that the isolate is relatively pure in TLC (Suteja et al, 2016).

From the testing that has been done can be seen that the methanol extract and water *Gliricidia* leaves can be used as a vegetable insecticides to control pests on crops soursop mealybugs because they contain secondary metabolic compounds. Seen from RF Value (Retention Factor) of methanol extract and water extract of leaves of *Gliricidia* (Table 1).

Rf value of flavonoid compounds in the extract water is higher than the value of the methanol extract of leaves of *Gliricidia* RF (Table 1). This shows the polar extract more water from the methanol extract is seen that the yellow spots on the methanol extract thicker than the water extract caused during the process of hydrolysis do not break the glucoside flavonoid glycoside compounds. Water

extracts and methanol extract showed almost identical Rf values in any comparison with the 4 visualization reagent, it indicates that the compound is a compound identified. According to Khopkar, (1990), the value of Rf indicates the identification of a substance that is sought.

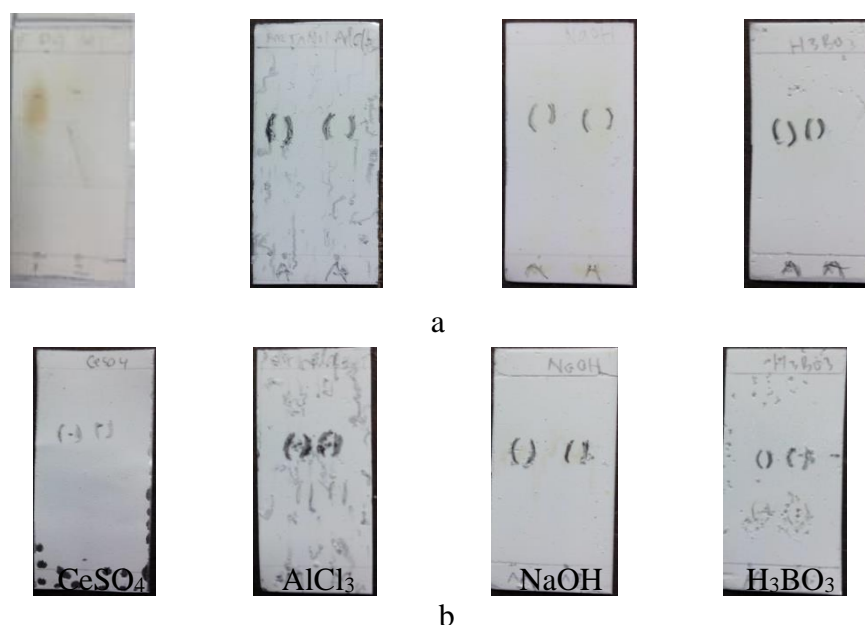


Figure 3. TLC chromatogram analysis results of water extracts (a) and the methanol extract (b) *Gliricidia* leaves with visualization CeSO_4 solvent, AlCl_3 , NaOH , H_3BO_3 .

Table 1. Values of Rf on the analysis of secondary metabolites (flavonoids) with TLC methods methanol extract and water extract of leaves of *Gliricidia* with a developer solution DCM: methanol (4: 1)

visualization reagent	RF Extract Pure water	RF Pure Methanol
CeSO_4	0,87	0,82
AlCl_3	0,80	0,80
NaOH	0,80	0,75
H_3BO_3	0,75	0,70

c. Mortality Hama White Lice treated with water and methanol Leaf Extract Gamal

The percentage of deaths mealybug pests on crops treated with methanol extract of soursop leaves of *Gliricidia* can be seen in Figure 4.

In Figure 4 and 5 seen from the death of mealybugs (*P. cryptus*) on soursop plant extract with water occurs at 12 hours of treatment reaches 6.66%, while the methanol extract of 3.33% at a concentration of 0.02%. At the methanol extract at a concentration of 0.04% at 12 hours and 24 hours have not experienced death, so also the water extract at a concentration of 0.06% and 0.08% at 12 hours. This is presumably because at 12 hours and 24 hours has not happened death in white lice because the insecticide has not been taken into the body mealybug is very little that has not been damage organs mealybugs, but in the next hours as at 24 hours, 48 hours, and 72 hour at each concentration there have been many deaths (Figure 4 and 5).

Death of test insects is increasing along with the increase of time and concentration of observation, the higher the concentration of extract used, the percentage of deaths caused also higher due to the longer time and high concentrations into the body test insects it will cause a lot of damage to the body mealybug.

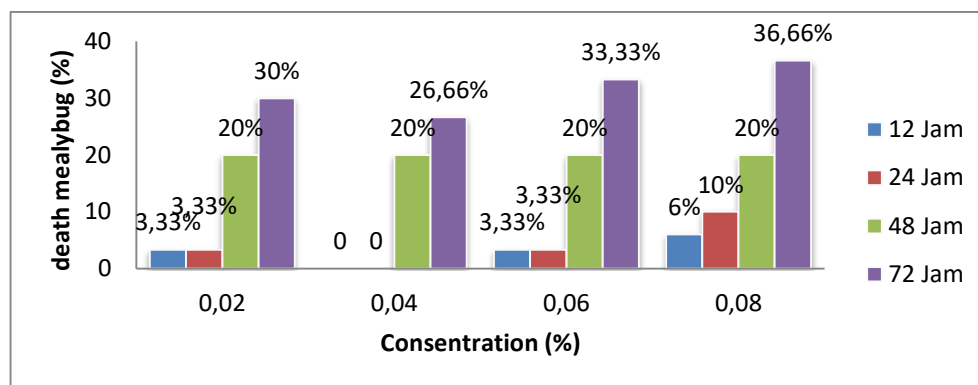


Figure 4. Percentage of deaths pest mealybugs (*P.cryptus*) on soursop plant (*A.muricata*) treatment of the methanol extract of leaves of *Gliricidia* at concentrations and at different times.

The percentage of deaths mealybug pest with *Gliricidia* leaves water extract treatment can be seen in Figure 5.

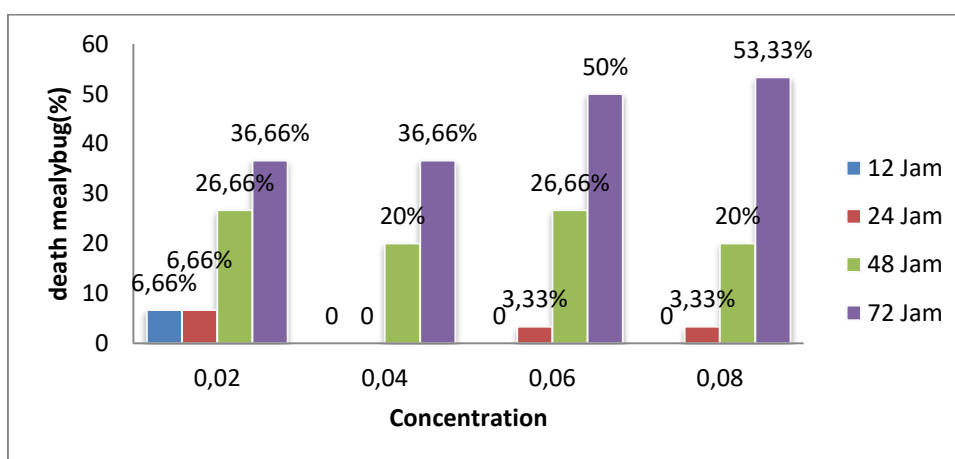


Figure 5. Percentage of deaths pest mealybugs (*P.cryptus*) on soursop plant (*A.muricata*) *Gliricidia* leaves water extract treatment at different concentrations and time.

The methanol extract and water extract of leaves of *Gliricidia* began to kill insects after 12 hours of treatment and at each treatment results of different test insect mortality. Water extract more lethal test insects between 10% - 20% compared with the methanol extract of leaves of *Gliricidia* (Figure 4 and 5).

Death mealybug treated water extract 16.67% higher than the methanol extract at 72 hours (Figure 15 and 16), it is suspected the existence of the resistance mechanism in plants soursop mealybug mealybugs due to be taken from a field while testing is done on a laboratory scale, Insect resistance to insecticides can be defined as the development of the ability of strains of insects to tolerate doses of poison that can kill most of the individuals in the normal population in the same species.

Results of analysis of variance showed a significant difference between treatments. Concentration of observation time, and the interaction of concentration and time, showed a significant difference ($P < 0.001$). While the average mortality mealybug when seen from a comparison between the extract, the extract concentration and, between the concentration, time and the extract was not significantly different ($P = .0,030 - 0,753$).

The results of Tukey's test at α level of 5% of the average death mealybug pests are treated extracts of methanol and water extract of leaves of *Gliricidia* at 72 hours after treatment can be seen in Table 3.

Table 3. Average mortality mealybug (*P.cryptus*) on soursop plant (*A.muricata*) (tail \pm SD) after treatment with the methanol extract and water extract of leaves of *Gliricidia*.

Concentration (%)	The average mortality mealybug (tail) \pm SD%	
	Extract metanol	Extract water
0,00	0,00 \pm 0,000 a	0,00 \pm 0,000 a
0,02	3,00 \pm 1,000 b	3,66 \pm 0,577 b
0,04	2,66 \pm 0,557 b	4,66 \pm 1,115 b
0,06	3,66 \pm 2,082 b	5,00 \pm 1,732 b
0,08	3,33 \pm 0,577 b	5,33 \pm 1,528 b

Description: The average value followed by the same letter are not significantly different line on the level of $\alpha = 5\%$ by HSD test

Table 4. Average mortality mealybug (*P.cryptus*) on soursop plant (*A.muricata*) (tail \pm SD) after treatment with the methanol extract and water extract of leaves of *Gliricidia* at a concentration of 0.08%.

Hours (time)	The average mortality mealybug (tail) \pm SD%	
	Extract metanol	Extract water
12	0,66 \pm 0,577 a	0,00 \pm 0,000 a
24	1,00 \pm 1,000 a	0,33 \pm 0,577 a
48	2,00 \pm 1,000 b	2,00 \pm 0,000 b
72	3,33 \pm 0,577 c	5,33 \pm 1,527 c

Description: The average value followed by the same small letter in the column are significantly different at the level of $\alpha = 5\%$ by HSD test

Water extracts and methanol extract at a concentration of 0.02%, 0.04%, 0.06% and 0.08% showed no significantly different results against the average death mealybugs but significantly different from the concentration of 0.00% (Table 3). Tukey's test results showed that the methanol extract and water extract not significantly different in the deadly mealybug. When viewed from different concentrations and time after treatment at the level of $\alpha = 5\%$ was significantly different, it is suspected that a very small time difference. While both extracts significantly different when seen from the time difference. This is presumably the higher the concentration, the more toxins that enter the body that damage mealybug.

Death mealybugs were treated with an extract of water and methanol at 12 hours after treatment was not significantly different at 24 hours after treatment but significantly different with 48 and 72 hours after treatment. It shows that the effect on the time of death mealybug, the longer observation time after the treatment the higher the death rate mealybug.

Comparison of the average mortality mealybug between water extracts and methanol extract at all concentrations and at different observation showed no significant differences. This is presumably because the water extracts and methanol extract were treated against mealybugs have the same concentration between the extract.

Tukey's test results showed that the methanol extract and extract water in the deadly mealybug significantly different at the level of $\alpha = 5\%$ at a concentration of 0.08%. It can be seen the number of deaths on mealybugs on plants soursop, in other words, a pure extract water more effectively kill pests mealybug than pure methanol extract (Table 4).

LC50 and LT50 Values probit analysis result of pure methanol extract and pure extract water can be seen in Table 5 and Table 6.

Based on the LC50 value, the average death mealybug pest with menggunakan extracts more water than the methanol extract of leaves of *Gliricidia*. Seen from the water extract LC50 lower than methanol extract of leaves of *Gliricidia*. LC50 value of the pure extract water is lower than 0.035% pure methanol extract. whereas LC50 at 12,24 and 48 hours have not detected due to the death of white pest infestation on plants soursop has not reached 50% (Table 5).

Table 5. The value LC50 probit analysis result of pure methanol extract and pure extract water *Gliricidia* leaves 12-72 hours after treatment.

Hours after treatment	Value LC ₅₀ (%)		
	Extract metanol	Extract water	Difference (%)
12 hours	**	**	**
24 hours	**	**	**
48 hours	**	**	**
72 hours	0,096	0,061	0,035

** Note: You can not be detected due to the death of mealybug is less than 50%

LC50 values below 5% concentration showed that the extract pure water more effectively kill pests on plants mealybugs soursop compared to pure methanol extract. If the vegetable insecticides kill insects by organic solvents with concentrations <5%, it is said to be effective (Priyono, 2005).

Table 6. Value LT50 probit analysis result of pure methanol extract and pure extract of leaves of *Gliricidia* water at different concentrations.

Concentration (%)	ValueLT ₅₀ (hours)		
	Extract Metanol	Extract Water	Difference (%)
0,00	**	**	**
0,02	91,288	84,912	6,376
0,04	86,406	71,887	14,519
0,06	83,210	69,411	13,799
0,08	95,876	69,296	26,58

Extract the water faster kill mealybug pests compared to the methanol extract of leaves of *Gliricidia*, as seen in the LT50 values at various concentrations (Table 6).

Based on the value of water extract lower LT50 26.58 hours of pure methanol extract, meaning that the power to kill time faster than the water extract of the methanol extract (Table 6). Based on the LC50 and LT50 values results of tests performed, extract pure water and pure methanol extract can be used as an insecticide can kill pests of vegetable and mealybugs on plants soursop. LC50 and LT50 values (Table 6 and Table 7) water extract is more effective than the methanol extract of leaves of *Gliricidia*.

4. CONCLUSION

Gliricidia leaves extract water more effectively kill pests on plants mealybugs soursop than methanol extract of leaves Gamal. The higher the concentration and the longer the time the more the higher the death mealybug.

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