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THETOXICITY OF PURIFIED ISOLATE OF POLAR EXTRACT POWDER LEAFS *GLIRICIDIA MACULATA* HBR. TO CACAO MEALYBUG (*PLANOCOCCUS MINOR* MASKELL)

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ABSTRACT

One contributing factor in decreasing productivity of cocoa in the last years is due to pest attack. Cacao mealybug (*P. minor*) attack the young cacao fruits, by sucking them until dry and die. Therefore it should be controlled. Alternatives pest control of the insecticide has been widely searched. *G. maculata* leaves consist of rich flavonoid that potencial as botanical insecticide . In oder to get the purified isolate of polar extract pouder leaf *G. Maculata* that named (PIGR), and test its toxicity to cacao mealybug (*P. minor*), the powder leaf of *G. maculata* were extracted by using various organic solvents (n-hexane, dichloromethane, methanol and water). A set of laboratory experiment was conducted to test the toxicity by bioassay, and to know the type and structure of PIGR by spectroscopic analysis. Five different concentrations (0 % , 0,015 % , 0,030 % , 0,045 % and 0,060 %) of PIGR with each of 3 replications were tested to cacao mealybug mortality. Mortality observed at 12 , 24 , 48 and 72 hours after treatment. Probit analysis was conducted to obtain LC_{50} . The result indicated the PIGR was toxic to cacao mealybug (*P. minor*) with LC_{50} 72 hours metanol extract 0,054% and water extract 0,047%. Therefore water extract more toxic than metanol extract. The toxic compound of methanol extract and water extract *G. maculata* is flavon with the structural frame is 2 - phenyl - 1,4 - benzopiron .

Keywords : polar extract , powder leaf, cacao maelybug, flavon.

1. INTRODUCTION

Cocoa (*Theobroma cacao* L) is one of the important tree crops in Indonesia, which has many beneficial value. Production of cocoa in Indonesia is often decreased. The cause of the decline in the production of cocoa beans is due to pest attack mealybugs (Wijaya, 2007). Mealybug (*Planococcus minor* Maskell) was sucking immature fruit causing dry and die (Sumarno, 2015). The use of synthetic insecticides are not right will bring bad effects, more harm than benefit resulting. Leaves gamal (*Gliricidia maculata* Hbr.) Has the active ingredient coumarin which are insecticides, rodenticides and bactericide that can be used as environmentally friendly insecticide plant (Kementerian Pertanian Ditjen Peternakan dan Keswan, 2009). Gliricidia leaves contain toxins that dikumerol main compound, a compound capable of binding vitamin K to clot blood. Dikumerol represents the conversion of coumarin due to bacterial activity when it ferments. Coumarin is suspected flavonoid compounds can irritate the skin and can inhibit the transport of amino acids leucine (Nukmal et al, 2010)

The results of the study Nukmal et al., (2009 and 2010) also proved that extracts polar (water and ethanol) Gliricidia leaves can cause 100% mortality in the pest imago boils dadap (*Quadrastichus erythrinae*) after 72 hours of treatment at the laboratory scale.

From the results of studies have been done to prove that the *G. maculata* leaf powder extract has potential as botanical insecticide. Purposes this study to know the toxicity, the type and structure of the gliricidia (*G. maculata*) pure isolates powder leaf are effective in control (*P. minor*) to the cocoa (*T. cacao*).

2. RESEARCH METHODS

A. Tools and Materials

The tools used this research are Machete, sacks, grinding machines, scales, glass jars, filter paper, Rotary evaporation, separating funnel, Freezedrayer, UV light, aluminum foil, erlenmeyer flask, test tubes, spatulas, analytical balance, oven, beaker, beakers, pipettes, funnel, hot plate, electric heating, capillary pipette. The tools used to prepare the test insects is a plastic knife, jars, gauze pads, rubber bands, brushes and pins.

The materials are Gliricidia leaves, solvent n-hexane, dichloromethane (DCM), methanol, distilled, KK Amberlite XAD-4, Plate TLC cellulose, H_2SO_4 as an ingredient identifier solution, and HCl is used to adjust the pH at the time of fractionation. $CeSO_4$ visualization solvent, $AlCl_3$, H_3BO_3 and NaOH. HCl, NaCl and ethyl acetate to extract water hydrolysis, H_2SO_4 as an ingredient in a solution of identifiers when TLC. Cocoa mealybugs (*P. minor*), cocoa fruits.

B. Isolation and Purification of Flavonoid Compounds Group Methanol extracts

500 gr of Gliricidia powder leafes macerated using a solvent Hexana 1.500 ml, DCM solvent 1000 ml, methanol 1.200 ml and 1.200 ml water. Total filtrate of methanol was evaporated and then recrystallization method using freezedryer for 72 hours. Furthermore, to the purification of the methanol extract conducted by fractionation using column chromatography (CC) Amberlite XAD-4. Amberlite XAD-4 5 gr was added to column chromatography. Fractions that have been obtained are evaporated and TLC analyzed to obtain the active fraction rich in flavonoids.

Water filtrate was concentrated Gliricidia leaf powder by recrystallization method using freezedryer for 72 hours. After that extract water hydrolysed. The results of hydrolysis showed two-phase extraction, the water phase and ethyl acetate phase. Furthermore, the results of hydrolysis was monitored by TLC.

Bioassays were carried out is the test of mortality with residual effect (residual effect) Bioassay made by soaking the test medium with 5 level of concentration levels (0%, 0.015%, 0.030%, 0.045% and 0.060%) for 10 minutes, 10 test insects tail (*P. minor*) females who already acclimatized for 1 day before the treatment is placed on the test medium. Observations of insect mortality trial conducted at 12, 24, 48 and 72 hours after treatment. The percentage of deaths for each extract will be analyzed with probit analysis EXE program ,test ANOVA and continued with Tukey's test was used to determine an effective solution as botanical insecticide to determine the relationship with the concentration of insect death. Test solution is said to be effective if the solution is giving the LC50 value ≤ 5% (Prijono, 2005). These trials were conducted each with 3 replications. The data were then analyzed using probit analysis to determine LC_{50} values. Determination Pure Active Compounds Structure using spectroscopic methods.

3. RESULTS AND DISCUSSION

A. Isolation and Purification of Flavonoid Compounds

The resulting methanol extract as much as 53 gr. The resulting crude extract freezedryer process is as much as 20 grams in the form of pasta that is solid green.
Results fractionation methanol crude extract obtained 44 fractions. Of the 44 factions only 4 fractions which showed spots on the chromatogram as an indication of flavonoid compound that is the fraction 1, fraction 2, fraction 26 and fraction 27. Fraction 1 and 2 has a Rf value of 0,700. Fraction 26 and 27 have a value of Rf 0,875. Rf equal value can be said that the compounds identified as having the same or similar characteristics (Khopkar, 1990).

TLC analysis results after the evaporated fraction flavonoid addressing their spots on the chromatogram that the fraction 1, fraction 2, fraction 26 and fraction 27 with a value of Rf 0,725

whereas other fractions did not indicate the presence of flavonoid compounds. The same RF value is the active fraction methanol and allegedly have the same classes of compounds.

Extract the water produced as many as 10 grams of crude extract water in the form of a brown paste. Pure water extract obtained by hydrolysis with the aim to break bonds glucosides in the extract.Hydrolysis form crystalline precipitate 2 grams, water phase of 20 ml and ethyl acetate phase as much as 15 ml. Based on the analysis chromatogram TLC Rf value of the water phase amounted to 0,625 while on crystal or precipitation does not show stains on the TLC plate as an indication of flavonoid compounds therefore used during the bioassay is to extract the water phase.

B. Mortality *Planococcus minor* Maskell

Table 1. The average percentage mealybug pest mortality (*Planococcus minor* Maskell) treatment with the methanol extract and water extract leafs *G. maculata* with different concentration and time observation.

Time observation after treatment (hours)	Concentration (%)	The death of insects (%)	
		Methanol Extract	Water Extract
12	0	0,00	0,00
	0,015	3,33	0,00
	0,030	0,00	3,33
	0,045	3,33	3,33
	0,060	0,00	0,00
24	0	0,00	0,00
	0,015	3,33	3,33
	0,030	3,33	10,00
	0,045	6,67	10,00
	0,060	6,67	10,00
48	0	0,00	0,00
	0,015	13,33	13,33
	0,030	23,33	20,00
	0,045	13,33	26,67
	0,060	23,33	26,67
72	0	0,00	0,00
	0,015	33,33	30,00
	0,030	33,33	40,00
	0,045	36,67	46,67
	0,060	53,33	56,67

At 12 hours after treatment methanol extract and water extract can kill insects already test (*P. minor)* as much as 3.33%. But death occurs on lower concentration than the high concentration. This could be due to several factors. According Raini (2007) there are several factors that influence the toxicity of a compound of one of them is endurance test animals. Possibilities that occur in test animals (*P. minor*) were treated extract higher concentrations have more endurance stronger. The higher the concentration of the extract is treated persentation higher death rate insects. When compared with the methanol extract, extract the water was shut off more test insects between 3,34% - 13, 34%. This tends to occur due to the influence of several factors among which the dose of the extract, endurance test animals, and exposure times. Low dose will give the effect of compound toxicity is low. While high doses at the time of initial exposure will force the body to continuously defend themselves from substances that are toxic, but exposure times would make these toxic substances accumulate in the body resulting in chronic poisoning and death (Raini, 2007).

Results of analysis of variance showed a significant difference among the treatments. The average mortality mealybug by treatment concentration and time showed a significantly difference. While the

average mortality mealybug if seen from a comparison between extracts, concentration and extract, and extract the time, the concentration, time, and the extract did not show significant differences.

The results Tukey's at the level α 5% average mortality pest infestation white treatment of methanol extract and water extract at 72 hours after treatment showed that the methanol extract and extract water with a concentration of 0% was significantly different from the concentration of 0,015%, 0,030%, 0,045 and 0,060%. 0,015% concentration was significantly different from the concentration of 0,060%, but not significantly different from the concentration of 0,030% and 0,045%. The concentration of 0,030% and 0,045% is not significantly different from the concentration of 0,060%. The difference is not noticeable due to the difference in mortality rates between white lice are very small concentrations. There are real differences between the control and treatment groups showed that the concentration significantly affect mortality mealybug.

The results Tukey's at the level α 5% of the average number of deaths pest mealybugs were treated extracts of methanol and water extract of leaves of *G. maculata* at a concentration of 0,060%, ie death mealybugs were treated with methanol extract and extract water at 12 hours after treatment was not significantly different with 24 hours after treatment but significantly different with 48 and 72 hours after treatment. This suggests that the effect on the time of death mealybug pests. The longer the period of observation after treatment also increase the mortality rate of mealybugs.

Statistically methanol extract and water extract did not show significant differences but when seen from the effectiveness of the extract showed significant differences. It can be seen Table 2 and Table 3.

Table 2. Value LC_{50} results probit analysis of methanol extract and water at 12-72 hours after treatment.

Time (Hours)	LC_{50} value (%)		Difference (%)
	Methanol Extract	Water Extract	
12	-	-	-
24	-	-	-
48	0,104	0,082	0,022
72	0,054	0,047	0,007

LC_{50} value of the water extract of between 0,007 to 0,022 lower than the methanol extract of the same treatment period. This indicates that the water extract is more effective than the methanol extract to kill 50% of test animals.

Table 3. Value LT_{50} probit analysis results of methanol and water extracts at different concentrations

Concentration (%)	**LT_{50} value (hours)**		Difference (hours)
	Methanol Extract	Water Extract	
0,015	89,567	87,777	1,790
0,030	81,707	82,399	0,692
0,045	87,676	73,535	14,141
0,060	68,785	66,248	2,537

LT50 values were also lower water extract between 0,692- 14,41 hours than methanol extract. This indicates that the water extract is more effective than the methanol extract with the same concentration takes longer than methanol extracts even at concentrations of 0,045% difference very long time is 14,141 hours, it indicates the water extract is more effective than the methanol extract.

The ability of the power to kill the methanol extract and water extract of leaves of *G.maculata* caused their compounds which are toxic secondary metabolites. One of them is of secondary metabolites flavonoid. The content of flavonoid compounds in leaf extracts gliricidia seen from the TLC analysis using several solvents visualization (Table 4).

Table 4. Rf value on the analysis of secondary metabolites (flavonoids) methanol extract and water extract of leafs *G. maculata* with TLC methods using several solvents and solvent developers visualization DCM and methanol (4: 1)

Extract	Solvent Visualization				Average value of Rf
	$CeSO_4$	$AlCl_3$	NaOH	H_3BO_3	
Methanol Extract	0,727	0,750	0,675	0,850	0,750
Water Extract	0,625	0,828	0,750	0,625	0,707

Rf Value flavonoid compounds using some solvent visualization shows varying values, the value of Rf methanol extract using solvents visualization $CeSO_4$ and H_3BO_3 higher than the value of the Rf in the water extract but the value of Rf methanol extract using solvents visualization $AlCl_3$ and NaOH lower than the value of Rf on the extract water. This shows that the solvent visualization has the ability to identify different as the principle of KLT according Soebagio (2002) in which the adsorption is absorption at the surface, while the partition is the spread, or the ability of a substance or substances present in the solution to separate and move upwards depending on plate TLC and the solvent used. From the results of the average value of Rf obtained by the use of various solvents visualization of the average value of Rf extract water smaller than the average value of Rf in methanol extract. According to Yazid (2005) the higher the Rf value obtained, the lower the level of the polarity of a substance that, because the concept of the higher polarity of a substance, then a stationary phase which is polar compounds will bind to each other and form a very strong bond so that the distance spot or stains on the TLC plate gets smaller and the lower Rf value. Thus the degree of polarity of the water extract is higher than the methanol extract.

C. Types and Chemical Structure of Extract Leafs *G.maculata*

Based on the results of spectroscopic analysis of the methanol extract of leaves of *G. maculata* has absorbance value of 0,585 -0,849. The presence of flavonoid compounds can be seen from the maximum wavelength of between 230,00 295,00 nm- nm (Neldawati et al, 2013). The methanol extracts were analyzed to give two maximum absorption is from 310,00 to 350,00 nm and 250,00 to 280,00 nm (Figure 1).

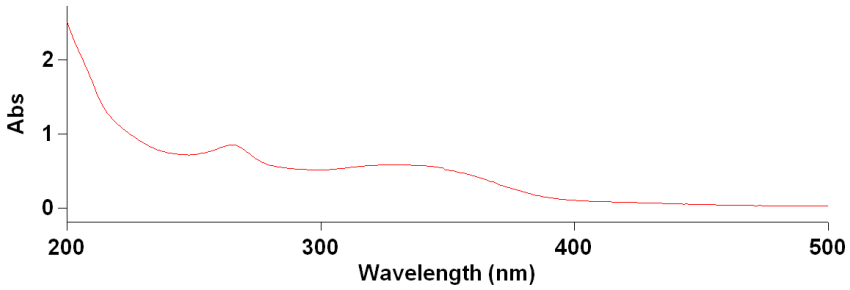

Figure 1. The spectrum of the methanol extract powder leafs *G. maculata*

The results of spectroscopic analysis of aqueous extracts were analyzed giving the maximum absorption is 250,00-280,00nm with the absorbance value of 0,736 (Figure 2).

According Neldawati et al, (2013) from 310,00 to 350,00 nm wavelength and 250,00-280,00nm included into the class of flavonoids types of flavonoids. Thus both polar leaf powder extract containing flavonoids gliricidia kind flavones. Demand Tapas et al, (2008) the characteristics of the flavonoids is almost the same as flavonoid compounds that have the basic structural framework C6-C3-C6 consisting of two C6 aromatic rings (A and B) and heterocyclic ring (C) (Figure 3).

Flavones are flavonoid class consists of the structural frame 2-phenyl-1,4-benzopiron. In chemistry, a phenyl or phenyl ring are one functional group in a chemical formula. In this group, six carbon atoms arranged in a cyclic ring structure. This ring is very stable, and is part of a group of aromatic compounds. The phenyl ring is hydrophobic (water repellent) and aromatic hydrocarbons. These

groups can be found in many organic compounds. This ring is estimated to be derived from benzene, (C_6H_6). Phenyl bergugus simplest compound is phenol, C_6H_5OH. While benzopiren is an organic compound with the formula $C_{20}H_{12}$ polycyclic aromatic hydrocarbons .Benzopiren itself is a chemical that is toxic (Marais, et al., 2006; Kumar and Pandey, 2013).

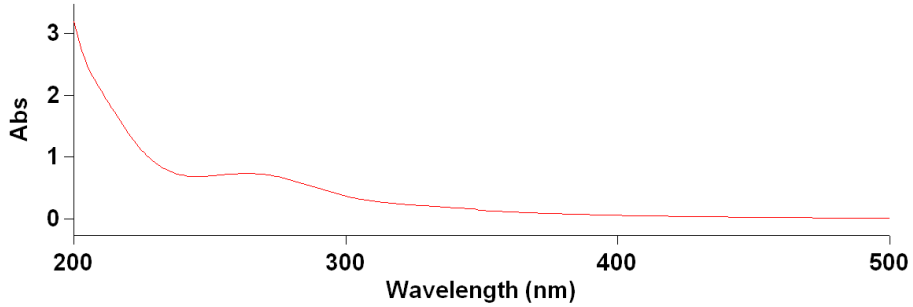

Figure 2. The spectrum of water extract leafs *G. maculata*

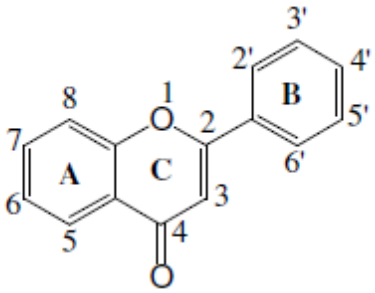

Figure 3. The structure of the flavonoids
(Source: Tapas et al, 2008)

4. CONCLUSION

Based on the research results obtained the following conclusions:

1. Methanol extract and water extract of powder leafs Gamal (*Gliricidia maculata* Hbr.) Has the power insecticides against mealybugs (*Planococcus minor* Maskell) on the cocoa plant (*Theobroma cacao* L).
2. Methanol extract the and water extract of powder leafs gamal of containing flavonoids, with type flavone that the structural frame 2-phenyl-1,4-benzopiron.

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