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Antifeedant test extracts of Hutun seeds against caterpillar pests *Plutella xylostella* on Sawi Plant

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Abstract— Application of the bioactive extract of the hutun seed (*Barringtonia asiatica* L.kurz) as an antifeedant in the *Plutella xylostella* Lcaterpillar pest on sawi plants (*Brassica juncea* L.) was carried out. This study aims to determine the antifeedant activity of the hutun seed extract in controlling the caterpillar sawi *Plutella xylostella*. The method used in this research is a completely randomized design (CRD) method with 4 treatments and 3 repetitions. The concentrations used are 50 ppm, 100 ppm, 500 ppm and 1000 ppm as well as positive control and negative control. The results of the study were tested with one-way ANOVA and continued with the Least Significant Difference test (LSD). Results of the study show that there are differences antifeedant activity caterpillar pests *Plutella xylostella* on sawi plants that significant at different levels of concentration. The study was conducted in several stages starting from the stage of extraction of the hutun seed, phytochemical test, preparation of caterpillar test, antifeedant activity testing. As the treatment is the level of methanol concentration of 50 ppm, 100 ppm, 500 ppm and 1000 ppm. The parameter observed was the percentage of Feeding Reduction (FR). The test results showed antifeedant activity against caterpillars of *Plutella xylostella* supreme contained at a concentration of 500 ppm and 1000 ppm because it can hinder eating caterpillars test amounted to 27.80 % and 40.93 %.

Keywords— *Barringtonia asiatica* seed, antifeedant, *Plutella xylostella*, sawi.

I. INTRODUCTION

An important problem often faced by farmers or agricultural and agribusiness practitioners in cultivating crops, both food crops, plantations and horticulture is pest attacks. Pest attack is a limiting and even determinant factor in efforts to cultivate plants. Pest attacks occur from the beginning are still in the nursery or nursery until harvest time even in storage even pests are inevitable so that these pests can reduce crop production both in quantity and quality, not infrequently even pests of agricultural crops can thwart the harvest resulting in large losses (Rumape, 2013). Until now, the most common control used by cabbage / sawi greens (*Brassicaceae*) vegetable farmers is spraying synthetic insecticides with high application frequency. Cabbage farmers usually control pests that attack cabbage plants by spraying synthetic insecticides on average more than 10 times in one growing season (Rauf *et al.*, 2005). Besides the lack of interest of farmers to use non-chemical control methods, the selection of control methods is carried out because synthetic insecticides are considered more effective and efficient in their use.

Plants have been widely known to produce various types of secondary metabolites such as flavonoids, terpenoids, alkaloids, saponins and others that can act as an attractant, repellent and as an antifeedant that is useful as a means of self-defense (Bernays and Chaman 1994; Prijono, 2008) which can harm the organisms that attack the plant. This shows that plant secondary metabolites have the potential to be used as plant protection agents. Plants that have been isolated by researchers containing active compounds of vegetable insecticide are soursop seeds (*Annona muricata*) with $LC_{50} = 117.27$ ppm (Komansilan *et al.* 2012), and tubal roots (*Barringtonia asiatica* Kurz) with Lethal Concentration $LC_{50} = 44,75$ ppm (Komansilan *et al.* 2017). Starting from the problem of using synthetic insecticides and increasing awareness of environmental sustainability, a safer control method is now being sought, one of which is the use of plants as botanical insecticides. The use of botanical insecticides has several advantages, including being easily biodegradable in the environment and relatively safe against parasitoids (Dono & Prijono, 1998; Schmutterer, 1997). One of the plants that has the potential

as a plant-based insecticide is *Barringtonia asiatica* L. Kurz.

Several types of plants *Meliaceae* and *Annonaceae* have been known to have insecticidal activity. Lately there have been many reported types of plants from other families whose insecticide activity has only been limited reported. Syahputra *et al.*, (2010) reported that several species of plant species from *Clusiaceae*, *Lecythidaceae* and *Sapindaceae* were active against insects. Information on the insecticide activity of plant preparations from the three families is less than the information on the activity of plant preparations *Meliaceae* or *Annonaceae*.

From *Barringtonia asiatica* L. Kurz seed extract, the active ingredients formulas are *liquid* (L) and *Wetable Powder* (WP). Both of these formulas can be used as components in a vegetable insecticide formulation. Vegetable insecticides need to be made in the form of formulas to facilitate storage, transportation and application in the field (Kardinan, 2005). In the formulation of *B. asiatica* seed extract, it is necessary to know the resistance of the active ingredients contained in the formula of *B. asiatica* seed extract, especially against abiotic factors such as rainfall and exposure time in the field. Both of these abiotic factors affect the degradation of active compounds of pesticides (Moniharapon, 2001; Syahputra, 2005), thus potentially reducing their effectiveness. Information about this resistance is needed to determine the interval of application of these pesticides and compare the effectiveness between formulas and with other insecticides such as microbial-based biological insecticides or synthetic insecticides. Therefore, it is necessary to conduct research on the residual activity of *B. asiatica* seed extract formulations as they have been applied to sawi plant pests.

II. RESEARCH METHODS

A. Research Location and Time

This research was conducted at the Laboratory of Integrated Sciences, Laboratory of Chemistry and Physics Laboratory, Faculty of Science, Manado State University. The study was conducted from May to September 2019, starting from the sampling phase, phytochemical screening extraction and testing of antifeedant activity.

B. Materials and tools

The material used is hutun seeds (*Barringtonia asiatica* L. kurz) taken from the coast of the bay of Manado, Malalayang city , North Sulawesi Province. The materials

used are 70% ethanol and 95% for maceration of hutun seed sampling, technical methanol, acetic acid, sulfuric acid, chloroform, 5% FeCl₃ % solution, Dragendorf reagent, Meyer reagent, tissue, cotton, whattman filter paper no. 42, aluminum foil, plastic samples, sawi leaves, and caterpillars *Plutella xylostella*. The tools used are analytical scales, petri dishes, vial tubes, Erlenmeyers, goblets, measuring cups, volumetric pipettes, fillers, test tubes and tube racks, drop pipettes, 50 mL and 100 mL measuring flasks and *rotary vacuum evaporators (Heidolph-Laborota 4000/4001 efficient)*.

C. Experiment Design and Data Analysis

This study uses a Completely Randomized Design (CRD) as a treatment that is the concentration level of methanol solvent 50 ppm, 100 ppm, 500 ppm and 1000 ppm as well as positive control / negative 0 ppm. Each treatment was repeated 3 times. The parameters observed were the percentage of *Feeding Reduction* (FR) or % antifeedant and phytochemical screening / screening tests for ethanol extracts of hutun seeds (*Barringtonia asiatica* L. kurz). The data obtained were analyzed using one-way analysis of variance (ANOVA). If the treatment has a significant effect on the inhibition of eating *Plutella xylostella* caterpillars on sawi plants (*Brassicajuncea*), then further testing of LSD or LSD at 5% significance level.

D. Research procedure

Hutun Seed Extraction (*Barringtonia asiatica* L. kurz)

Forest seed samples were obtained from Malalayang Dua beach coasters, Malalayang District, Manado City of North Sulawesi. The extract material used in this study was the seeds of the hutun plants which grew along the coast of Malalayang Dua. Making the hutun seed extract is done by weighing 1400 grams of the hutun seeds that have been dried at room temperature, then immersed in ethanol (maceration) for 1 x 24 hours in the maserator. Maceration is done several times until it is extracted. The solution was the result of extraction then filtered using Whatman 42 filter paper.

The filtrate obtained was then put into a *vaccum evaporator* at 40°C until the ethanol solvent evaporated to obtain a thick ethanol extract. Further extraction results obtained were weighed using analytical scales. To make the test solution, a dilution was carried out using technical methanol solvent which was re-distilled. The concentration of the hutun seed extract used in this study was 50; 100; 500 and 1000 ppm. While the control (0 ppm). Each treatment was repeated three times.

E. Preparation of Test Larvae / Caterpillars

Plutella xylostella caterpillar obtained from sawi plantations in the village of Rurukan, East Tomohon District, Tomohon City, North Sulawesi. *Plutella xylostella* caterpillars are maintained and propagated in the Integrated Science Laboratory, Faculty of Mathematics and Natural Sciences, Manado State University, in a wooden cage with screen walls where the *Plutella xylostella* caterpillar is placed in a plastic container placed in a cage. *Plutella xylostella* caterpillars are fed pesticide-free sawi leaves during maintenance. Sand mixed soil is used as a medium for the *Plutella xylostella* caterpillars when they turn into pupae. On the top of the culture box a cotton swab is hung which is tied to a rope and has been dipped in a 1 mL honey mixture with 10 mL water. Honey solution serves as a food source for *Plutella xylostella* imago. The pupae then hatch into moths that will reproduce and lay their eggs on sawi plants. The eggs will hatch into larvae instar I to instar III. Furthermore, third instar larvae will be used in *antifeedant* activity testing.

F. Antifeedant Activity Testing

The test was carried out using the leaf disc method according to (Atta *et al.* 2001). On sterile petri dishes are placed wet filter paper / tissue and gauze and the filter paper is coated with transparent plastic that has been perforated. Leaf discs are made with a circle the size of a petri dish on sawi leaves that have not been given synthetic pesticides. Leaf discs to be made are the same in size, shape and thickness. Leaf discs were dipped in each extract sample and compared with positive control. The study was conducted with three repetitions. Leaf discs are dipped / applied for 5 minutes then aired for 5 minutes. After aerating, the leaf disc will be weighed and put into a prepared petri dish.

Plutella xylostella caterpillars were added as much as 1 caterpillar in each petri dish, petri dishes containing leaf discs and test caterpillars would be observed to avoid the caterpillar avoidance response to leaf discs that had been given each extract concentration. Observations are made after 24 hours. Antifeedant activity testing is done by looking at the nature of the *Feeding Reduction* of the sample. The parameter to be observed is the weight of the remaining leaves that are not eaten by the larvae or *Feeding Reduction* (FR). Leaf discs were then weighed, to find out the weight of sawi leaf discs eaten by *Plutella xylostella* caterpillars, the percentage of *Feeding Reduction* (% FR) was used. The percentage value of *Feeding Reduction* is measured by the formula (Atta *et al.*, 2001):

$$\% FR = \left\{ 1 - \frac{\text{Weight of the treated leaves that are eaten}}{\text{Weight of control leaves eaten}} \right\} \times 100\%$$

G. Phytochemical Screening

Phytochemical Test Work Procedures (Ayoola, *et al.*, 2008 & Farnsworth, 1966)

A certain amount of viscous extract was carried out by phytochemical tests which aimed to determine the class of compounds contained in the seeds of the forest (*Barringtonia asiatica L. kurz*). Phytochemical tests were carried out on the group of Alkaloids, Flavonoids, Phenols, Saponins, Triterpenoids, Steroids, Terpenoids, and Tannins.

a. Alkaloid Test

One gram of ammonia extract was added to 10% and then extracted with chloroform and added 1 N hydrochloric acid. The extraction results will be divided into two layers. The upper layer (acid layer) is divided into two tubes. In one tube Meyer reagent was added, while in the other tube Dragendorff reagent was added. Yellow indicates a positive alkaloid.

b. Flavonoid Test

Two methods are used to test Flavonoids.

1. Dilute ammonia (5 mL) is added to the aqueous filtrate portion of the extract. Then concentrated sulfuric acid (1 mL) is added. A missing yellow indicates flavonoids.
2. A portion of the extract is heated with 10 mL ethyl acetate which has been evaporated for 3 minutes. The mixture is then filtered and 4 mL of the filtrate is shaken with the addition of 1 mL of aqueous ammonia solution, the formation of a yellow color indicates the presence of flavonoids.

c. Phenol Test

To one gram of extract was added 1% iron (III) chloride. Green / red / purple / blue / black colors indicate positive phenols.

d. Saponin Test

One gram of extract is added to water then boil in a water bath for 5 minutes, after which it is shaken vigorously. Saponin is positive if foam forms stable for ± 30 minutes.

e. Triterpenoid and Steroid Test

Anhydrous acetic acid was added to the extract until it was submerged; leave for ± 15 minutes. After that, add 1 drop of concentrated sulfuric acid. Green / blue deposits indicate steroids, while red / orange deposits indicate triterpenoids.

f. Terpenoid Test

A number of extracts were added with 2 mL chloroform. Then carefully added concentrated H₂SO₄ (3 mL) to form a layer. The formation of a brownish red color indicates terpenoids.

III. RESULTS AND DISCUSSION

Insects will face two things to start eating activities, first there are stimuli to initiate feeding activities (feedingstimulants) in plants that provide input signals for the introduction of food types and maintain eating activities. The second is detecting the presence of foreign compounds (foreigncompounds) which are as a food inhibitor so that it can shorten the activity of eating or stop eating altogether. Based on the results of interviews with farmers spraying the area of sawi plants where pest control of *Plutella xylostella* caterpillars still relies on the use of chemical pesticides. Spraying interval with chemical pesticides is carried out for

3-4 days, while the recommended use of pesticides is ideally once a month. This results in faster selection of insect resistant to insecticides. The use of botanical insecticides was also not carried out because given the vast land area making it less practical to apply. According to *Dono et. al.*1988, insect resistance to synthetic insecticides can be broken using botanical insecticides, due to the different mechanism of action of the two insecticides. In addition, one of the advantages of botanical insecticides is that it is difficult to cause an immune (resistant) reaction on the target pest so it is safe for the balance of the ecosystem. Based on the results of research the influence of ethanol extracts of hutun seeds (*Barringtonia asiatica L. kurz*) produces data that is the activity of eating / food inhibitors (*FeedingReduction*). The results of eating obstacles *Plutella xylostella* caterpillars can be seen in Table1.

Table 1. Effect of hutun (*Barringtonia asiatica L. kurz*) seed extracts on decreasing the feeding activity of *Plutella xylostella* caterpillars on sawi plants.

Treatment	Repeat	Area of leaves eaten (gr) within 24 hours	Percentage of Food Obstacles (%)	Average
P1 (50 ppm)	1	2.55	1	9.33
	2	2.13	18	
	3	2.35	9	
P2 (100 ppm)	1	1.92	25.3	25.56
	2	1.91	25.7	
	3	1.91	25.7	
P3 (500 ppm)	1	1.89	26.5	27.8
	2	1.87	27.3	
	3	1.81	29.6	
P4 (1000 ppm)	1	1.76	32	40.93
	2	1.41	45.2	
	3	1.40	45.6	
control (0 ppm)	1	2.57	0	0

Based on the table above, data on eating activity can be seen from the percentage of food resistance at a concentration of 50 ppm for 1,2,3 replications of 1 %, 18 % and 9 %, while for a 100-ppm concentration of 25.3 %, 25.7 % and 2 5.7 %. At concentrations of 500 ppm and 1000 ppm the percentage of food barriers increased by 26.5 %, 27.3 % and 29.6 %, while for concentrations of 1000 ppm by 32 %, 45.2 % and 45.6 %. The higher the value of eating barriers means a decrease in eating activity of *Plutella*

xylostella caterpillars on sawi plants. The average percentage of food resistance (*FeedingReduction*, %) results in the following diagram:

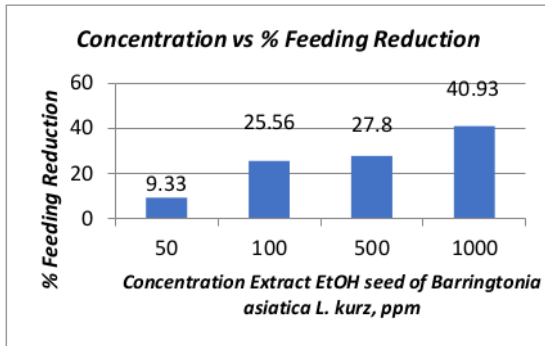


Fig.1: Diagram of the average decrease in feeding activity (% Feeding Reduction) of *Plutella xylostella* caterpillars on sawi plants.

Based on the test results of hutun seed ethanol extract (*Barringtonia asiatica* L.Kurz)effect with three repetitions provide resistance values at different from each

concentration. The higher the concentration, the higher the percentage of eating obstacles will be and this means a decrease in eating activity. The highest value of eating resistance is at a concentration of 500 ppm and 1000 ppm by 27.8% and 40.93%. The results of this study were lower than the antifeedant activity of tuba root extract at concentrations of 500 ppm and 1000 ppm which were able to inhibit feeding power by 30.16% and 44.00%.(Komansilan *et al.* 2019)

Normality test is a test used to determine the distribution of data obtained is normal or not. The normality test is a prerequisite for the one-way ANOVA test. If the number of samples > 50 used is Kolmogorov-Smirnov, whereas if the number of samples < 50 then what is used is Shapiro-Wilk. The results in table 2 are then tested for normality as follows:

Table 2. Test the normality of eating activity of *Plutella xylostella* caterpillars on sawi plants.

Tests of Normality

Treatment	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistics	df	Sig.	Statistics	df	Sig.
Antifeedant 1.00	.182	3	.	.999	3	.935
2.00	.385	3	.	.750	3	.000
3.00	.289	3	.	.928	3	.480
4.00	.376	3	.	.772	3	.499

a. Lilliefors Significance Correction

If in the Shapiro-Wilk column the Sig value > 0.05 then the treatment data are normally distributed, whereas if the Sig value < 0.05 then the treatment data are not normally

distributed. The conclusion of the normality test on the above eating activity data meets the normal requirements because the Sig. > 0.05 for treatment.

Table 3. Homogeneity test of feeding activity of *Plutella xylostella* caterpillars on sawi plants.

Test of Homogeneity of Variances

Antifeedant

Levene Statistics	df1	df2	Sig.
3,723	3	8	.061

Data for each treatment is said to be homogeneous if the Sig value > 0.05 and vice versa the treatment data is said to be homogeneous if the Sig value < 0.05. Based on the homogeneity test table above the activity data of each treatment was declared homogeneous because the Sig value > 0.05 so that the ANOVA test could be performed. ANOVA test table can be seen in Table 4.

After ANOVA (variance) test was carried out at a 5% confidence level, the results showed that the treatment had a significant influence on the antifeedant of *Plutella xylostella* caterpillar on sawi plants (*Brassica juncea L.*). can be seen in the Table below:

Table 4. ANOVA test of eating activity of *Plutella xylostella* caterpillar on sawi plants

ANOVA

Antifeedant

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1512,529	3	504,176	14,953	.001
Within Groups	269,740	8	33,718		
Total	1782,269	11			

If the Sig. Value < 0.05, the treatment was stated to have a significant effect. Based on the ANOVA test table above shows that there is a significant effect of ethanol extract of hutun seeds (*Barringtonia asiatica L. kurz*) on the eating activity of *Plutella xylostella* caterpillar on sawi plants.

Based on the ANOVA test results above, the treatment data can be further tested to find out more specific effects. Further tests used were those with the smallest significant difference (LSD) or LSD (Least Significant Different) to show differences between each treatment individual.

Table 5. Average% of leaves eaten and decreasing the feeding activity of hutun seeds on *Plutella xylostella* caterpillars on sawiplants.

Concentration of hutun seed ethanol extract.ppm	Average food resistance (%), Feeding Reduction (%)	Average food resistance at 24 hours after application (ppm) ± SD	Average food resistance at 24 hours after application (ppm) ± SEM
P1 (50)	9.33	9.33 ± 8.50	9.33 ± 4.91 ^a
P2 (100)	25.56	25.56 ± 0.23	25.56 ± 0.13 ^b
P3 (500)	27.80	27.80 ± 1.60	27.80 ± 0.92 ^b
P4 (1000)	40.93	40.93 ± 7.73	40.93 ± 4.46 ^c

Note: the treatment followed by the same letter shows no significant difference and the treatment followed by different letters shows significantly different.

Values are expressed as Mean ± SD and Mean ± SEM from the triplicate determination.

Prijono (2008) explains how antifeedants work in insects can work in two ways, namely 1) influencing the behavior of insects such as: inhibiting feeding activity, interfering with host discovery, inhibiting spawning activities, and 2) influencing insect physiology, such as: influencing egg development to failure become pre-adult insects (larvae or

nymphs and adults / imago), inhibit the formation of chitin, interfere with reproduction. Judging from the entry of secondary metabolites (antifeedant) into the body of an insect, Rompas (2010) said that, the modus operandi of these chemicals in the body and poisoning organisms, some attack the brain (neurotoxicity), blood (hematoxicity), liver (hepatotoxicity), skin (dermatotoxicity), eyes (ophthalmotoxicity), kidney (nephrotoxicity) and lungs

(pneumotoxicity), with different ways of working depending on the type of compound.

Mitcell and Sufcliff, (1984), explained that insects have receptors including chemoreceptors on antennas, mouth parts, tarsus, and palpuses that can distinguish various chemical compounds from alkaloids, terpenoids which work to inhibit the response of sugars to the galeal sensilla in Coleoptera. According to Dadang (1999) *R. communis* gives a high rejection effect of spawning and feeding activities on beetles, especially Coleoptera: Bruchidae.

Furthermore, the workings of secondary metabolite compounds (antifeedant) in the nervous system, according

to Prijono (2008) allegedly take place through the following series: 1) the interaction of insecticides with certain macromolecules in the nervous system, 2) causing interference with the functioning of the nervous system, 3) causing paralysis muscular system and behavioral abnormalities, 4) there will be a failure in the respiratory system (air exchange), 5) resulting in an imbalance in the substance content in body fluids, 6) cell poisoning will occur, and 6) finally the insect comes to death.

Phytochemical Test Results

Table 6. Phytochemical screening test results of ethanol extract (EtOH) from hutun seed plants (*Barringtonia asiatica L. kurz*)

No.	Group	Observation result	
1	Alkaloids	Hager (-)	Meyer (+)
2	Flavonoids	(+)	
3	Phenol	(++)	
4	Saponin	(+++)	
5	Steroids / triterpenoids	(+))	
6	Terpenoids	(+))	

Note: +++ = Compounds that are contained a lot

++ = Medium contained compounds

+ = The compound contained is small

- = The compound contained does not exist

Table 7. Phytochemical Testing of Ethanol Extract (EtOH) of hutun seeds (*Barringtonia asiatica L. kurz*)

Phytochemical Test	Results (color)	Standard Result (color)
Saponin	Shaped foam (+++)	Formed foam ± 15 minutes stable
Phenol	Purple color (++)	Bluish purple
Steroids / triterpenoids	Green or blue (++)	Brown sediment
Terpenoids	Chocolate (+)	Reddish brown deposits
Flavonoids	Yellow (-)	Chocolate
Alkaloids	Light Brown (+)	Brown sediment

Based on the results of phytochemical tests, ethanol extracts of hutun seeds (*Barringtonia asiatica L. kurz*) are included in the saponin, steroid, phenol, and alkaloid classes. Saponins are generally bitter and also toxic to some cold-blooded animals such as fish and amphibians. The use of saponins as an antidote to predator attacks, the media to fight over the scope, and help the process of reproduction (Liang & Guo, 2013). The bitter taste issued by saponins is thought to inhibit the feeding activity of test larvae.

The content of triterpenoid compounds in ethanol extract was characterized by the formation of reddish-brown deposits in the extracts tested. The terpenoid compounds

contained in the tuba roots function as fish poisons to fight predators that threaten their survival (Handayani *et al.*, 1997). Alkaloids can inhibit the response of cyanogenic glycoside sugars, which are sugars formed from bonds between sugar and toxic compounds stored in plants so that the toxic compounds are lost in toxicity.

IV. CONCLUSIONS

Based on the results of BNT further tests at 5% significance level showed significantly different from the administration of ethanol extract of hutun seeds to the eating activity of *Plutella xylostella* caterpillar on mustard plants. In

treatment P1 was significantly different from treatment P2, and also significantly different from treatment P3 and P4. In treatment P2 was not significantly different from P3 but significantly different from P1 and P4. In treatment P3 was not significantly different from P2, but significantly different from P1 and P4. Whereas the treatment of P4 was significantly different from P1 and P2 also significantly different from P3. Giving ethanol extract from hutun seeds to the eating activity of *Plutella xylostella* caterpillar on mustard plants, the highest value of eating inhibition was at concentrations of 500 ppm and 1000 ppm by 27.8% and 40.93%.

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