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Isolate Bioactivity of Castor Leaf *(Ricinus communis* L) as Antifeedant on Beetle *Epilachna varivestis* Mulsant.

Opir Rumape

Department of Chemistry, Faculty of Mathematics and Science
State University of Gorontalo, Indonesia

Abstract: Castor leaves *(Ricinus communis* L) of 2013 grams that are taken from residential areas in the Huangobotu Village, West City District, Gorontalo City, are mashed in a blender, and macerated for 3 x 24 hours using 3 L of methanol and it obtains crude extracts of 164, 67 grams.
The results of the leaf crude extract as much as 35 gr are partitioned with ethyl acetate and n-hexane to obtain 6.67 g of ethyl acetate fraction, n-hexane fraction of 7.37 grams and 9 grams of methanol extract. Three fractions that represent the level of polarity (i.e., non-polar fraction n-hexane, ethyl acetate fraction of semi-polar and polar fraction of methanol extract), are tested the bioassay on the larvae of *Epilachna varivestis Antifeedant.* Antifeedant activity test results obtains the methanol extract that shows the highest Antifeedant activity (67%) followed by n-hexane fraction (66%), and ethyl acetate fraction (62%).
The isolation process of methanol extract is done and then purified by column chromatography and thin layer chromatography. The column process is performed up to two times. The first column obtains three fractions, i.e. leaf fraction 1 (the result of fraction D1 = 7.15 grams), leaf fraction 2 (the result fraction D2 = 1.07 g) and the fraction of leaf 3 (the result of fraction D3 = 1.4 grams). From the test results of Antifeedant activity of the obtained three fractions, the fraction D1 shows the highest inhibitory activity (fraction D1 = 68%, fraction D2 = 51%, and fraction D3 = 52%); these three fractions are further tested by TLC and fractions D1 shows two spots, so it must be in column again. The second columning uses n-hexane eluent: ethyl acetate obtained two fractions: D1.1 (4.05 g) and D1.2 (2.16 g). D1.1 fraction is tested with TLC and shows a single stain. Then to ensure the result, the two-dimension TLC test is done again and it shows the single stain also.
At the antifeedant testing activity (inhibition of eating), the fraction D1.1 gives higher Antifeedant (D1.1 = 71%) while the Fractions D1.2 only gives antifeedant (D1.2 = 53%).
The results of the analysis and identification of the IR is known that the castor leaf contains compound which has a functional group O-H, C-H, C=O, C=C, C-O cyclic and =C-H. The [1] RMI ^{1}H dan RMI ^{13}C test on leaf isolates find the compounds of triterpenoids group.
Keywords: Activities, compounds, Antifeedant, *Ricinus communis, Epilachna varivestis.*

I. Introduction

Indonesia is agriculture country where most people are farmers. The issue often faced by farmers and agricultural practitioners when cultivating various crops is how to cope with pests [1]. Various strategies are done starting using pest-resistant seeds, using natural enemies and using synthetic pesticides, but the farmers prefer to use synthetic pesticides on the grounds the result is faster and more practical. Indonesia as a country that is rich in plants that have vegetable insecticidal properties, the postscript is safer to use the user's health, for beneficial insects, and the environment. Through this study the author wants to use the plant such as the castor plant which, according to some literatures, is a good seed, as well as the leaves have insecticidal properties.

One of the Coleoptera family from Coccinellidae Order including insects that dominates life in different ecosystems is generally predators, only a few species are herbivores including *E. varivestis* that is very damaging crops, especially legumes such as soybeans that are hosts [2].

Various strategies of pest control are performed, e.g. the use of pest-resistant seeds, the use of natural enemies and the use of synthetic insecticides, but the use of synthetic pesticides is still the main choice of farmers for practical reasons and fast result [3]; but does not consider the effects of synthetic chemicals on health and the environment because it is difficult to synthetic insecticides degraded in nature, so the need to find an alternative that is safe for the environment [4].

Indonesian state has various plants that produce active compounds as insecticidal, repellent and AntifeedanT that are readily biodegradable and leaves no residue [5,6]. Castor *(R. communis)* is one of the plants that are often used as crop protection other than the destruction of reality is not damaged by insects and other animal species, indicating that these plants contain secondary metabolites which have bioactivity that can be as Antifeedant [7].

The purpose of this study is to find Antifeedant compounds of castor leaves *(Ricinus communis)* on insect *E. varivestis.*

II. Material and Methods

A. Sample Preparation

The castor leaves cut into pieces with a knife of approximately 0.5 cm and then smoothed by using a blender. A total of 2 kg of powdered leaves is macerated 4 x 24 hours with 3.0 L of methanol, each 1 x 24 hours is filtered and the residue is macerated again with a new methanol. Methanol filtrate is evaporated at a temperature of 30-40°C. The leaf extracts (crude extract) result as much as 35 grams is partitioned with n-hexane (3 x 120 mL) and ethyl acetate (3 x 120 mL), and methanol extract thus it obtains fraction of n-hexane, acetate ethyl fraction and methanol extracts that are tested the antifeedant activity on *varivestis Epilachna* larvae.

B. Antifeedant Activity Test of Factions

Ethyl acetate fraction, the fraction of n-hexane and methanol extracts are made into solution with various concentration of 0.01%, 1%, 2.5%, 5% and 10% and are tested on larvae *of E. varivestis* who have fasted for 6-8 hours. Fraction which showed the highest antifeedant activity is isolated and purified.

C. Isolation of Antifeedant Active Compounds

The isolation process of active compounds from the extraction and fractionation is performed using column chromatography and thin layer chromatography technique using n-hexane eluent: ethyl acetate. Isolation is done at several times of columning and tested by thin-layer chromatography. If it showed a stain pattern, then it is tested again with a two-dimensional thin-layer chromatography and when it provides a single stain patterns at different eluent, it can be named pure compounds and can be analyzed and its structure can be determined.

D. Application of Pure Isolate at Larvae *E. varivestis*

Pure active isolates obtained from the isolation and purification (D1.1 leaf extract = 4,05gr) are made into solution with five variations of the concentration of 0.01%, 1%, 2.5%, 5% and 10% and applied to larvae *E. varivestis.* This is done to see which one gives the concentration of the highest antifeedant activity.

E. Analysis and Identification of Structures

Pure compounds D1.1 = 4,05 gr of isolation and purification results are analyzed and identified by IR and NMR- ^{1}H and NMR-^{13}C tests.

III. Results And Discussion

A. Fractionation Results of Castor Leaves.

Crude extract of the leaves as much as 35 grams is dissolved in 30 mL of methanol + 60 mL of water. This extract is partitioned with ethyl acetate and n-hexane to obtain fractions based on the polarity level. This is done to make it easier at the separation and purification. These fractions are n-hexane fractions which are the

fraction of non-polar, semi-polar ethyl acetate fraction and polar fraction methanol. The results of partitioning and fractionation are shown in Table 1 below:

Table 1. Results of seed fractionation *(R. communis)*

No	Fraction	Weight (grams)
1	*n*- Hexane	7.32
2	Ethyl-Acetate	6.66
3	Methanol-wather	9.01

Based on Table 1 above, the fractions of leaf tissue *of R. communis* gives similar volume. It is suspected that the active compounds contained in the leaves are semi-polar or polar.

B. The Test Results of Antifeedant Activity

Three fractions, i.e. fraction of ethyl acetate, n-hexane fraction and the methanol extract of the leaves *R. communis* that are applied to larvae *E. varivestis* who had fasted for 6-8 hours give results that methanol extracts gave inhibition value of 67%, while the fraction of 66% ethyl acetate and n-hexane fraction of only 62%.

The histogram of the castor leaves fraction test results can be seen in Figure 1.

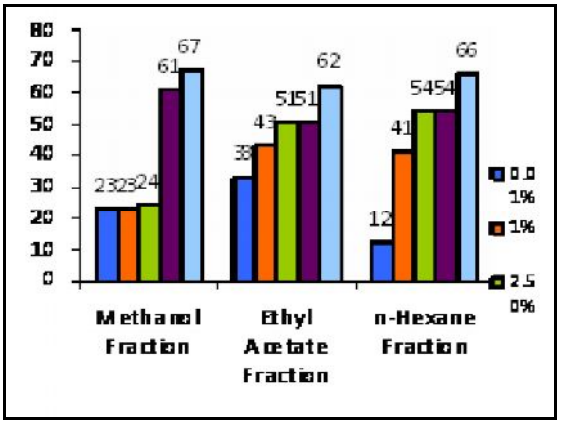

Figure 1. Methanol, Ethyl Acetate and n-hexane Fractionate Test of castor leaves *(RCommunis)*

The results of the tests conducted in this study indicate that the compounds which have antifeedant bioactivity on insects *E. Varivestis* on castor leaves *(R. communis)* is a secondary metabolite of a group of triterpenes which showed eating inhibition on *Epilachna varivestis*.

C. Isolation and Purification

The results of column chromatography and thin layer chromatography of leaf active fractions obtain pure compound that are indicated by two-dimensional TLC final test with a single stain patterns and bioassay of pure isolates on *E. varivestis,* and indicate the value of the inhibition of eating (FR) by 71%. The process of isolation and bioassay on larvae *Epilachna varivestis* is shown in Figure 2 and Figure 3.

The separation and purification of 9 grams of leaf extract are carried out by gravity column chromatography with 3 cm diameter column using silica gel of 300-400 mesh with the solvent n-hexane-ethyl acetate is gradiently obtained in 3 fractions. The bioassay are conducted in these three fractions (Fr.D1 = 68%, = 51% and Fr.D2 Fr.D3 = 52%) which shows fraction Fr.D1 in the crystal form. In this crystal (Fr.D1), TLC test is conducted by the chloroform : methanol (9:1) developer (eluent). The results show there are two stains. Then the second columning is conducted using a stationary phase of silica gel of 70-230 mesh and mobile phase of chloroform-methanol obtains two fractions (Fr. D1.1) and (D1.2 Fr.) which showed the crystal is fraction D1.1, then the TLC with eluent chloroform: methanol (9.5: 0.5) is conducted, indicating the white needle crystals. To confirm the result, the two-dimensional TLC is performed and the results show one stain. This means it can be said to be pure. Then the bioassay is conducted, the Fr D1.1 shows 71% inhibition value.

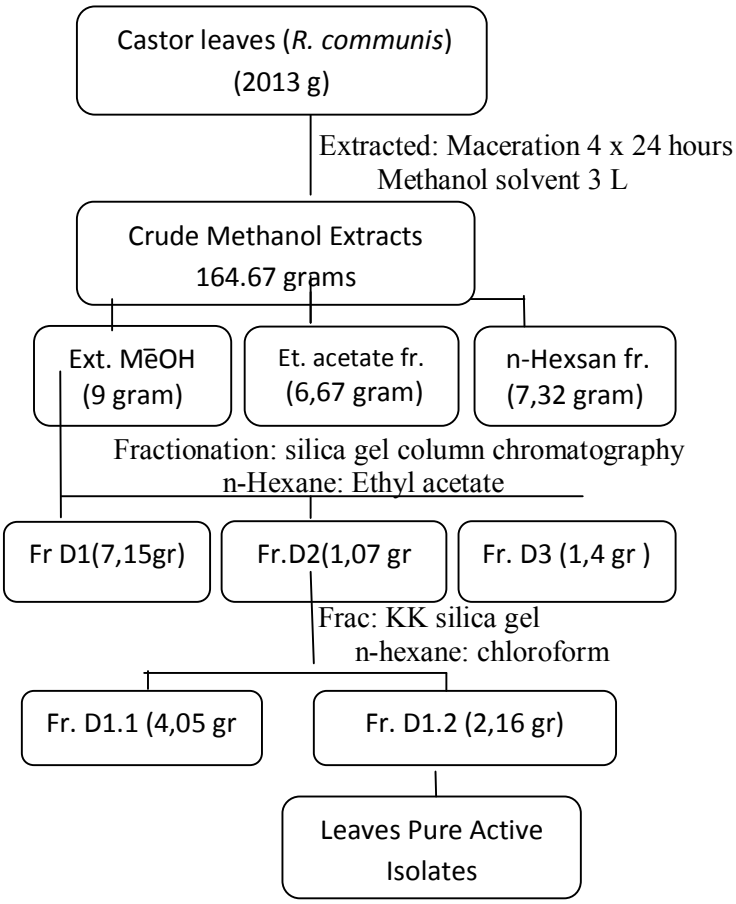

Figure 2. Separation and Purification Scheme of Active Fraction of Castor Leaf Extract

D. Application of Leaf Pure Isolate

The results of column chromatography and thin layer chromatography of leaf active fractions obtains pure compound leaves (4.05 g) as indicated by two-dimensional TLC final test with a single stain patterns and the bioassay test of pure isolates on *E. varivestis,* shows eating inhibition values of 71%. The bioassay test results of isolation pure compounds are shown in Figure 3.

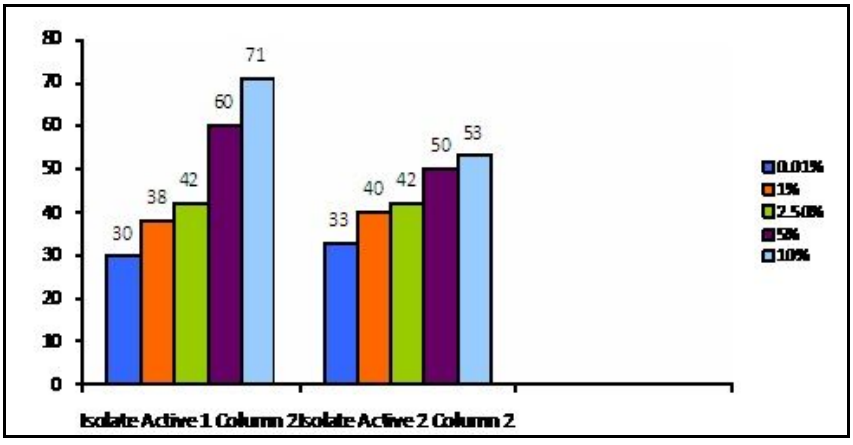

Figure 3. Biological Test Results of Pure Isolate of castor leaves *(R. communis)*

Functional groups -OH, C-H, C = C, C = O, C-OH cyclic and =C-H

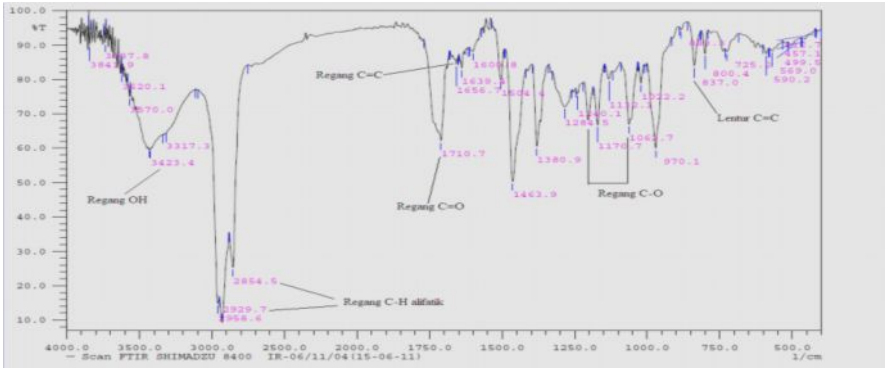

Figure 4. Infrared Spectrum of Castor Leaves Isolate

F. Structure Determination of castor leaves Isolates With NMR Spectroscopy

The structure determination of castor leaf isolates is conducted using nuclear magnetic resonance spectrum analysis of proton and carbon-13 (RMI- ^{1}H and ^{13}C RMI-, Jeol 500 and 100 MHz). RMI-^{1}H and RMI-^{13}C isolates spectrum of castor leaves are presented in Table 2 and Table 3 (RMI-^{1}H spectrum at Figure 4 and the spectrum of RMI-^{13}C at Figure 5).

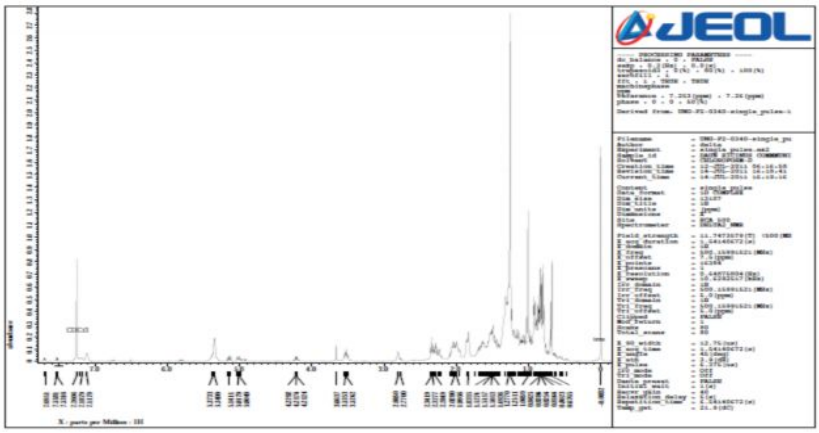

Figure 5. RMI^{1}H spectrum of castor leaf isolates.

The spectrum analysis data as shown in Table 2 and Table 3 show that the leaf isolates have groups of protons and aromatic carbon and aliphatic, polycyclic, as well as the ester carbonyl group. Table 3 shows the ethylenic proton signal at the chemical shift (δ) of 5.13 ppm and is supported also by the presence of the ethylenic carbon (δ) of 121.9. The presence of seven metal angular signals in Table 3 indicate that the isolate compounds from the castor leaves contain a range of aliphatic polycyclic core, in this case, is the triterpene core.

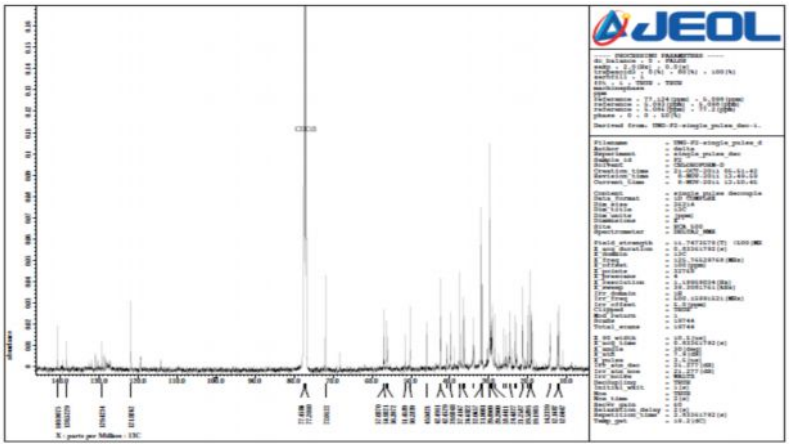

Figure 6. Isolates RMI- ^{13}C Spectrum of Castor Leaf.

Table 2. Isolate RMI-^{1}H Spectrum Tabulation of Castor Leaf.

(δ)(ppm)	Multiplicity *J* Hz	estimated
7,70	Dd	H (aromatic)
7,52	Dd	H (aromatic)
7,11	s (width)	H (N-methyl)
5,13	M	H (etilenic)
4,21	M	H (C-oxygenated)
3,53	M	H (C-oxygenated)
0,67-2,80	M	H (alicyclic)

Table 3: Isolate RMI-^{13}C Spectrum Tabulation of Castor Leaf.

(δ)(ppm)	DEPT	Estimated
185,8	C	C (carbonyl)
140,9	C	C (aromatic
139,4	CH	C (aromatic)
138,5	CH	C (aromatic)
129,4	CH	C (aromatik)
121,9	CH	C (aromatik)
71,9	CH	C (oxygenated)
57,0	CH	C (alicyclic)
56,9	CH_2	C (alicyclic)
56,1	CH_2	C (alicyclic)
51,4	CH_2	C (alicyclic)
50,3	CH_2	C (alicyclic)
45,9	CH_2	C (alicyclic)
42,5	CH_2	C (alicyclic)
42,4	CH_2	C (alicyclic)
40,7	CH	C (alicyclic)
39,9	CH_2	C (alicyclic)
39,8	CH_2	C (alicyclic)
37,4	CH_2	C (alicyclic)
36,7	CH_2	C (alicyclic)
29,9	CH_3	C (alicyclic)
29,3	CH_3	C (alicyclic)
24,5	CH_3	C (alicyclic)
21,3	CH_3	C (alicyclic)
19,5	CH_3	C (alicyclic)
12,2	CH_3	C (alicyclic)
12,0	CH_3	C (alicyclic)

The presence of the ester carbonyl group is indicated by the carbonyl carbon signal at the chemical shift of δ 185.8 ppm (Table 3). Based on the discussion above, it may be proposed that the compound structure on the isolate of the castor leaves is shown in Figure 8. Based on the Table 2 and Table 3, it can be estimated that the isolate compounds of the castor leaf have aromatic core and triterpenoids core which have an ester carbonyl group as shown in Figure 7.

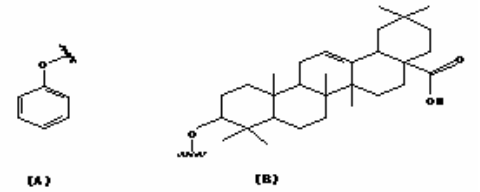

Figure 7. Aromatic (A) core and the triterpene (B) core in isolates from castor leaves *(R. communis)*

Tables 2 and 3 also show the proton and carbon aliphatic groups so that both compound structure in Figure 7 above have aliphatic substituent. Based on the discussion above, it may be proposed to isolate compound from the castor leaves that is shown in Figure 8.

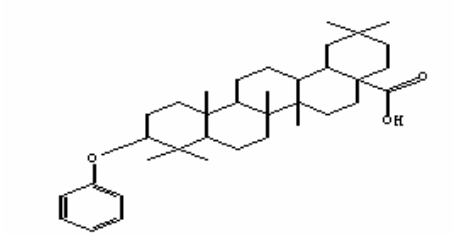

Figure 8. The compound structure of isolated from the castor leaves, 2,2,6a, 6b, 9,9,12a- heptametil-10-phenoxy - 1,2,3,4,4a, 5,6,6a, 6b, 7 , 8,8a, 9,10,11,12,12a, 12b, 13,14b- ikosahidropisen-4a-carboxylic acid.

Identification by IR spectrum of *Ricinus communis* leaf isolates, find that aromatic triterpenoid compounds have characteristics of functional groups O-H, C-H, C=O, C=C, C-O cyclic and =C-H that are supported by UV-Vis test with absorption at a wavelength of 214.5 nm which is a band I thought to be an $n \rightarrow \pi^*$ transition by a chromophore C = O. This conjecture is supported by the presence of peaks that appear with the intensity of the wave number of 11710.7 cm-1 in the IR spectra.

Based on the research and identification, it finds the active secondary metabolites compound as antifeedant on insect *E. varivestis* which is environmentally friendly. Presumably it is advised that this Antifeedant can be utilized as control agents of plant eater pests and it is also recommended for further research to test mass spectroscopy and two-dimensional NMR for ensuring the isolate structure of these results.

Conclusion

Extraction and maceration process that are conducted in 2013 grams of castor *(R. communis)* leaf powder using methanol obtains crude extract of 164.67 grams. Crude extract of the castor leaves is partitioned with n-hexane and ethyl acetate and obtain ethyl acetate fraction, the n-hexane fraction and methanol extracts and these fractions are tested with bioassay test on the larvae *E. varivestis*. The results show that these three fractions gave inhibition values that are not much different which is the highest methanol extract (67%), n-hexane fraction of 66% and ethyl acetate fraction of 62%.

The methanol extract fractionation results are isolated and purified using column chromatography techniques and thin layer chromatography. It obtains 4.05 grams of pure isolates by two-dimensional thin-layer chromatography test and gives a single stain and the Antifeedant activity test shows an increase, reaching (71%). The results of the analysis of the IR spectrum and NMR- ^{1}H and ^{13}C NMR- showed that the active compounds are a class Antifidan isolated triterpenoids.

References

1. Prijono, D.D. 2008. Bio Insecticides. Principles, Utilization and Development. Department of Plant Protection. Faculty of Agriculture, IPB. Bogor
2. Borror D. J Ch., A. Triplehornand, N. F. Johson., 1992. Introduction to Insects Lesson sixth edition. Translated by Soetiyono Partosoedjono. Faculty of Animal Medicine, IPB.
3. Hassan S. A. 1998 Defining the problem: Introduction. In: Haskell, PT, McEwen P, editors. Ecotoxicology: Pesticides and Beneficial Organisms. Dordrecht: Kluwer Academic Publishers. P 55-68
4. Robinson T. 1991, *The Organic constituents of higher plant.* Department of Biochemistry University of Massachusetts. Translated by Kosasisih Padmawinata, High Plant Organic Content. Sixth edition. ITB Publisher. Bandung.
5. Anurag Sharma and Rakesh Gupta, 2009 Biological activity of plant extracts against Prieris Some brassicas (Linn). *Journal of biopesticides, 2 (1): 26-31.*
6. Sinaga, E. 2009. *Ricinus communis Linn* E. *2009.* Pdf Research Report. Available on: December 8th, 2010. www.bpi.da.gov.ph/Publications/mp/pdf/t/tangan-tangan.pdf
7. Bernays E. A. and R. F. Chapman. 1994. Host Plant Selection by Phytophagous Insects. New York: Chapman & Hall.

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