

## Isolating Antifidan Compounds of Kepyar Castor seeds (*Ricinus communis* L) to the Beetle *Epilachna varivestis* Mulsant, (Coleoptera: Coccinelidae)

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**Abstract:** As much as 2 kg kepyar castor beans (*Ricinus communis* L) that has been granulated, then it was macerated for 3 x 24 hours using 3.0 L of methanol solvent got crude extract obtained 387.2 grams. Methanol crude extract was partitioned and fractionated with ethyl acetate and n-hexane to obtain ethyl acetate fraction, the fraction of n-hexane and methanol extracts. Three fractions were tested antifidan activity against larvae *Epilachna varivestis*. Antifidan test results showed that the methanol extract gave the highest antifidan activity (65%) followed etyl acetate fraction (59%) and n-hexane fraction (58%). Methanol extracts were isolated and purified by chromatography column techniques and thin layer chromatography. The first column got three fractions as Fraction B1 = 1.23 grams, as Fraction B2 = 12.34 grams and a fraction B3as much = 1.42 grams). The results of antifidan activity test of three fractions obtained B2 fraction showed the highest inhibitory activity (Fr. B2 = 70%), (Fr. B1 = 53%) and Fr. B3 = 52.72. The three fractions was TLC tested and B2 fractions showed crystals with two stains, so it must be columned again. In the second column used eluen n-hexane: ethyl acetate fraction obtained two B2.1 (1.31 grams) and Fraction B2.2 (6.019 grams). Fraction B2.2 was tested by TLC showed a single stain then tested the two-dimensional TLC showed a single stain. Fraction B2.2 antifidan activity assays provide meals inhibition (antifidan) higher (B2.2 79%) whereas only give a fraction B2.1 inhibition of eating (B2.1 = 58%). Analysis and identification of the IR and NMR note that there are two compounds of the seed that have the same core but different in substituen and compound classes of alkaloids and terpenoids indicated.

**Keywords:** Isolation, compound, antifidan, *Ricinus communis*, *Epilachna varivestis*.

### I INTRODUCTION

The important problem often faced by the farmers in cultivating crops is pests attack <sup>[1]</sup>. Pest attack occurred early in the nursery until the time of harvest even in the storage we cannot avoid it. Coccinellidae (Ordo Coleoptera) Family including insects dominates life in a variety of ecosystems as a predator <sup>[2]</sup> only a few species are herbivores such as *E. Varivestis* is very damaging crops, especially legumes such as soybeans are a host <sup>[2]</sup>.

Various control strategies do but the use of synthetic pesticides is still the primary choice of the farmers for practical reasons, and fast results <sup>[3]</sup>, 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 a safe alternative the environment <sup>[4]</sup>.

Indonesia is rich with a variety of plants that produce active compounds as insecticidal, repellent and antifidan that are easily biodegradable and leaves no residue <sup>[4], [5], [7]</sup>.

*Jatropha kepyar* (*R. communis*) is one of the plants that are often used as protective of other plants, and the reality is not damaged by insects and other animal species indicate that these plants contain secondary metabolites with bioactivity can be as antifidan <sup>[6]</sup>.

The research was conducted with the aim to discover compounds against insects antifidan *E. varivestis* from castor beans kepyar (*Ricinus communis*).

## II. MATERIALS AND METHODS

### A. Sample Preparation

Castor seeds that have been removed from their shells and have been smoothed by 2 kg 3 x 24 hour macerated with 3.0 L of methanol, each 1 x 24 hours filtered and the residue was macerated again with a new methanol. Methanol filtrate evaporated at a temperature of 30-40°C. Results seed extract (crude extract) of 20 grams partitioned and fractionated with n-hexane (3 x 120 mL and ethyl acetate (3 x 120 mL), and methanol extracts were made to obtain the fraction of n-hexane, ethyl acetate fraction and methanol extracts antifidannya activity was tested on larvae *Epilachna varivestis*.

### B. Test Activities Antifidan Factions

Ethyl acetate fraction, the fraction of n-hexane and methanol extracts made solutions with various concentration of 0.01%, 1%, 2.5%, 5% and 10% and was tested on larvae of *E. varivestis* who have fasted for 6-8 hours. Fraction showed highest activity antifidan isolation and purification.

### C. Isolation of Active Compounds Antifidan

Isolation of active compounds extracted and fractionated done with column chromatography techniques and thin layer chromatography using n-hexane eluent: ethyl acetate. Insulation made and tested several column times by LCT when showing a single stain, tested again with two-dimensional TLC when it provides a single stain on different eluents, it can be said of pure compounds and can be analyzed and determined its structure.

### D. Applications Isolate Pure At Larva

Active isolates obtained from the isolation and purification (B2.2 seed extract = 6.019 g) is a solution with a concentration variation of 0.01%, 1%, 2.5%, 5% and 10% and applied to the larvae of *E. varivestis*. To see which one gives the concentration of the highest inhibitory activity of eating (antifidan).

### E. Spectrum Analysis and Structure Determination

The results of the isolation and purification B2.2 analysis and identification via test IR and NMR-<sup>1</sup>H and NMR-<sup>13</sup>C.

## III. RESULTS AND DISCUSSION

### A. Fractionation results Castor Seeds Kepyar.

Seed crude extract as much as 20 grams dissolved in 30 mL of methanol + 60 mL of water. The extract was partitioned with ethyl acetate and n-hexane to obtain fractions based on the level of polarity, this is done to make it easier at the time of separation and purification. Fractions are fractions of n-hexane is a non polar fraction, ethyl acetate fraction of semi-polar and polar fraction is the fraction of methanol. The results of partitioning and fractionation as shown in Table 1 below:

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

No	Fraction	Weight(grams)
1	<i>n</i> - Hexane	2,82
2	Ethyl Acetate	5,15
3	Methanol-water	3,09

Based on Table 1 above seed tissue fractions *R. communis* ethyl acetate fraction showed the results provide more volume. It is suspected that the semi-polar solvent and a polar fraction more dissolved compounds contained by seed kepyar. It can be said that the active compounds present in these fractions have a polar nature.

## B. Activity Assay results Antifidan Factions

Three factions that ethyl acetate fraction, the fraction of n-hexane and methanol extracts of seeds *R. communis* larvae were applied to *E. varivestis* who had fasted for 6-8 hours gave results that methanol extracts gave inhibition values of 65%, whereas the ethyl acetate fraction 59 and n-hexane fraction of only 58%.

The results of biological testing are presented in Figure 1. These results suggest that the fraction of methanol dissolves more compounds that inhibit feeding activity *E. varivestis* compared to two other factions fraction n-hexane and ethyl acetate fraction.

These results indicate that the compounds contained by antifidan kepyar castor seeds is polar. When you consider the results of this test that at concentrations of 10% and 5% for each of the fractions that showed significant inhibition of eating, whereas lower concentrations of 2.5%, 1% and 0.01% showed very low inhibition.

Histogram test results factions kepyar castor seeds can be seen in Figure 1.

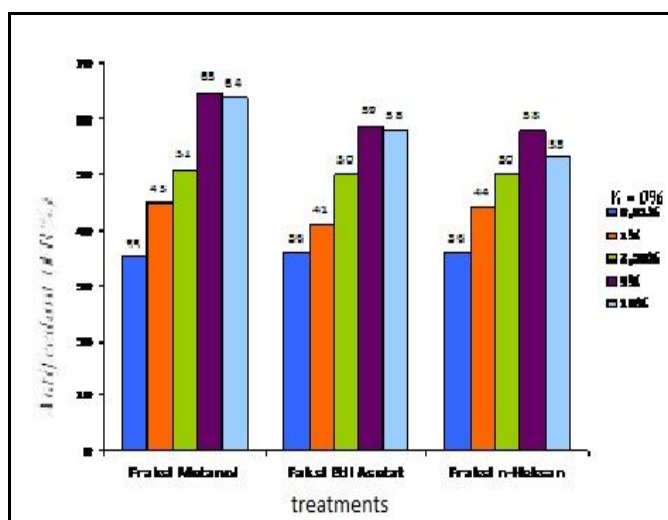


Figure 1. Test Fraksinat Methanol, Ethyl Acetate and n-hexane Castor Seeds Kepyar (*R. communis*)

Test results Factions kepyar seed (*R. communis*) in Figure 1 above shows that the fraction of methanol gave the highest value of inhibition of eating, followed by ethyl acetate fraction, and lowest fraction of n-hexane. Methanol fraction will continue to the isolation process and purification to obtain pure isolates kepyar seed.

The results of the tests conducted in this study showed that the compounds have bioactivity against insects antifidan *E. Varivestis* on kepyar seed (*R. communis*) are secondary metabolites of classes and class terpenoid alkaloid that showed inhibition against *Epilachna varivestis* eat.

## C. Isolation and Purification

The results of column chromatography and thin layer chromatography active fraction obtained seeds pure compound shown by the final test of a two - dimensional TLC with a single stain patterns and biological testing of pure isolates of the *E. varivestis*, demonstrates the value of inhibition of eating (FR) by 79%.

Isolation and purification of seed extract, a total of 14.137 g separated by gravity column chromatography with a diameter of 3 cm column with silica gel 300-400 mesh size with the solvent n-hexane-ethyl acetate in gradient fractions obtained 3. Three fractions was tested antifidan (Fr.B1 = 53%, = 70% and Fr.B2 Fr.B3 = 52.72%) fraction Fr.B2 showing crystal form. In these crystals (Fr.B2) KLT test conducted by the developer (eluent) chloroform: methanol (9:1). The test results showed apparently still has two stains. Then do the second column, using a stationary phase silica gel 70-230 mesh and mobile phase chloroform-methanol

obtained two fractions (Fr. B2.1) and (B2.2 Fr.) showing crystal. Fr. B2.2, in doing TLC with eluent chloroform: methanol (9.5: 0.5), showing the form of white needle crystals. Then Fr B2.2 biological test performed showed 79% inhibition value. To check performed two-dimensional TLC and the results showed a stain spots. This means it can be said to be pure.

#### D. Applications Isolate Pure Seed

The results of column chromatography and thin layer chromatography active fraction obtained seeds pure compound (6.019 g) as indicated by the final test of a two-dimensional TLC with a single stain patterns and biological testing of pure isolates of the *E. varivestis*, shows the value by 79% inhibition of eating. The test results of biological isolation of pure compounds is shown in Figure 3.

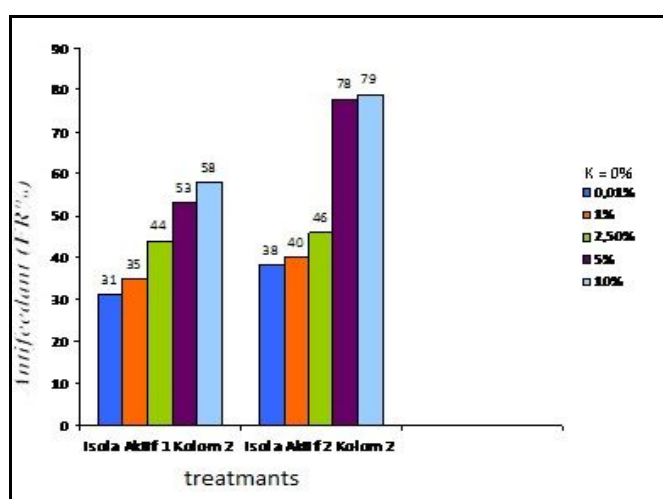


Figure 3. Biological Assay results Isolate Pure Castor Seeds Kepyar (*Ricinus communis* L)

Figure 3 shows that the active isolates B2.2 provide inhibitory eat the insects *E. varivestis* by 79%. The test results on the thin layer chromatography of this fraction showed a single stain. Having tested again through a two-dimensional thin-layer chromatography showed single spots with different eluents.

#### E. Analysis Result and Identification Spectrum IR

The functional groups –OH, C –H, C = C, C –OH cyclic and = C –H

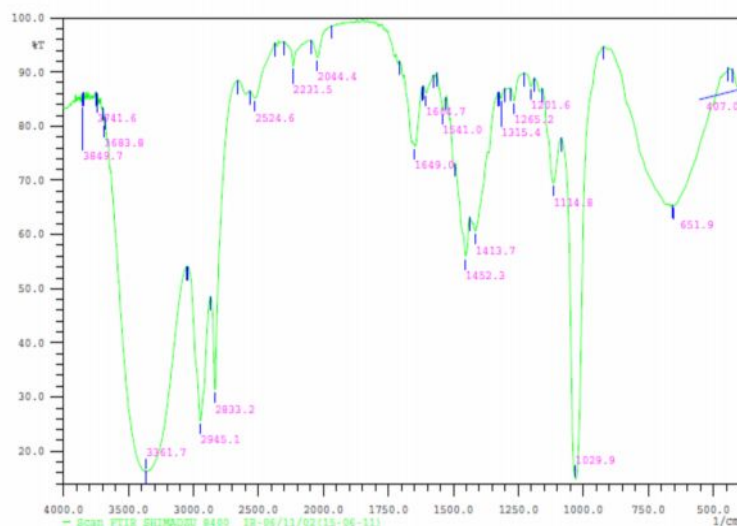


Figure 4. Infrared Spectrum of Isolates Kepyar Castor Seeds (*R. communis*)

## F. Structure Determination of Castor Seeds Isolates Kepyar With NMR

Spectroscopy Determining the structure of isolates from castor beans kepyar performed with analysis of proton nuclear magnetic resonance spectra and carbon-13 (NMR- $^1\text{H}$  and NMR- $^{13}\text{C}$ , JEOL 500 and 100 MHz). NMR- $^1\text{H}$  spectrum and NMR- $^{13}\text{C}$  isolates from castor beans each presented in Table 8 and Table 9 (NMR- $^1\text{H}$  spectrum of Figure 4 and NMR- $^{13}\text{C}$  spectrum in Figure 5.).

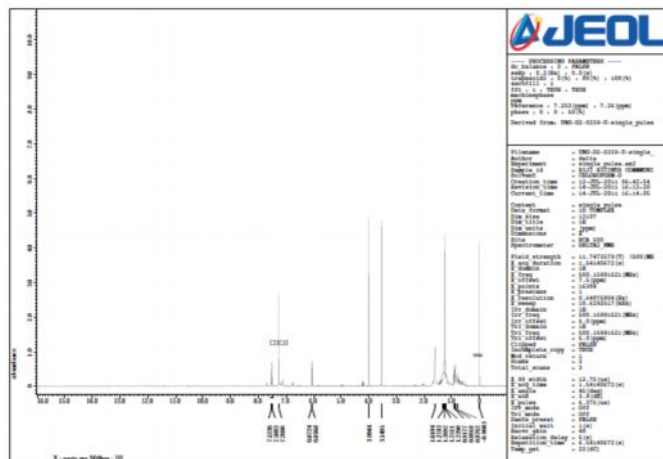


Figure 5. Spectrum NMR $^1\text{H}$  isolat from Castor Seeds

Data analysis of the spectrum as shown in Table 8 and Table 9 show that isolates groups of protons and aromatic and aliphatic carbon, as well as the N-methyl group and methoxy group. Especially for the data in Table 9 gives a hint of the carbonyl group. Based on data from Table 9 also can be concluded that the isolates from castor beans contain two compounds with adjacent carbon chemical shift. Both of these compounds can be expected to have the same core structure and differ in substitue-substituens.

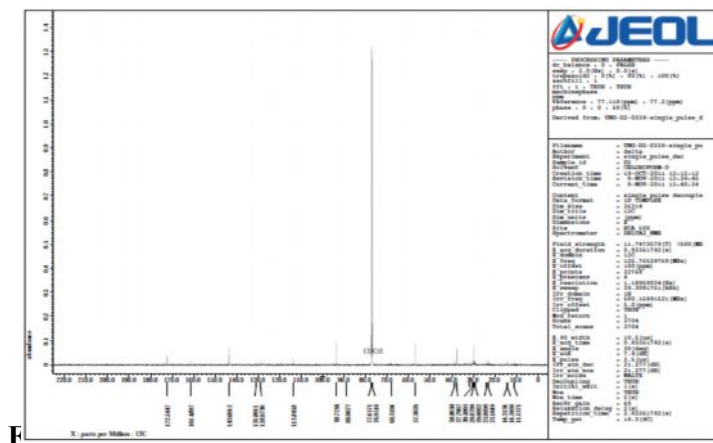


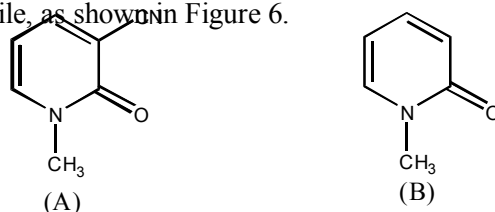
Table 8 . Spectrum tabulation NMR- $^1\text{H}$  isolat from Castor seed.

( $\delta$ )(ppm)	Multiplisitas $J$ Hz	Estimate
7,51	d	H (aromatik)
6,06	d	H (aromatik)
3,99	S	H (N-metil)
3,55	S	H (metoksi)
2,27	T	H ( alifatik)
2,11	d(1.3 Hz)	H (alifatik)
0,87-1,61	M	H (alifatik)

**Table 9. Spectrum tabulation NMR-<sup>13</sup> C isolat from castor seed**

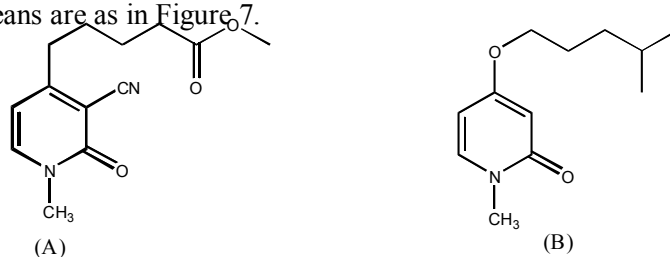
( $\delta$ )(ppm)	DEPT	Estimate
172,5	C	C (karbonil)
161,4	C	C (karbonil)
143,6	CH	C (aromatik)
131,1	CH	C (aromatik)
128,9	CH	C (aromatik)
119,4	CH	C (aromatik)
113,8	C	CN
93,7	CH	C (aromatik)
88,8	C	C (aromatik)
68,3	CH <sub>2</sub>	C (alifatik)
57,3	CH <sub>3</sub>	O-CH <sub>3</sub> (ester)
38,8	CH <sub>3</sub>	N-CH <sub>3</sub>
37,7	CH <sub>3</sub>	N-CH <sub>3</sub>
32,1	CH <sub>2</sub>	C (alifatik)
30,5	CH <sub>2</sub>	C (alifatik)
29,8	CH <sub>2</sub>	C (alifatik)
29,5	CH <sub>2</sub>	C (alifatik)
29,1	CH <sub>2</sub>	C (alifatik)
23,8	CH <sub>2</sub>	C (alifatik)
23,1	CH <sub>2</sub>	C (alifatik)
22,8	CH <sub>2</sub>	C (alifatik)
14,3	CH <sub>3</sub>	C (alifatik)
14,2	CH <sub>3</sub>	C (alifatik)
11,1	CH	C (alifatik)

Based tabulated in Table 8 and Table 9 can be estimated that the compound of isolates castor beans are two compounds, each of which has a nitrogen-containing aromatic core and each core is substituted with a carbonyl group, methyl and nitrile, as shown in Figure 6.



**Figure 6. Aromatic core to isolate compounds from castor beans, (A) core substituted carbonyl, methyl, and nitrile, (B) core with carbonyl and methyl substitution.**

Tables 8 and 9 also indicate the proton and carbon aliphatic compounds so that the second structure in Figure 6 above has Substituents aliphatic. Based on the above two structures can be proposed to isolate compounds from castor beans are as in Figure 7.



**Figure 7. The structure of the two compounds on isolates from castor beans, (A) methyl 5 - (3- cyano-1-methyl-2-oxo-1 ,2-dihydropyridine-4-il) pentanoat; (B) 1-methyl-4-(4- metilpentiloksi) pyridine-2 (1H)-on.**

The results of the identification of the infrared spectrum is known that isolates classes of seed are secondary metabolites of aromatic terpenoid alkaloid class that has the characteristics of functional groups-OH,

C-H, C = C, C-OH and = CH supported cyclic test results with the UV-Vis absorption at long 253.5 nm wave of the transition  $\pi \rightarrow \pi^*$  indicates a double bond in the benzene system. Test NMR<sup>1</sup>H and NMR<sup>13</sup>C to isolate compounds from seeds, there are two classes of alkaloids and terpenoids. Based on the research and identification of secondary metabolites found two compounds are active against insects antifidan *E. varivestis* environmentally friendly. Suggested presumably antifidan compounds can be used as a pest control agent herbivores and presumably also recommended further research to test mass spectroscopy and two-dimensional NMR structure of the isolates to ensure these results.

#### IV. CONCLUSION

Maceration extraction conducted at 2 kg powder kepyar seed (*R. communis*) with methanol crude extract obtained 387.2 grams. Do partitioning with n-hexane and ethyl acetate to obtain ethyl acetate fraction, the fraction of n-hexane and methanol extracts were tested against larvae of *E. varivestis* results showed that the methanol extract gave the highest value of inhibition of eating (65%), 59% ethyl acetate fraction and n-hexane fraction of 58%.

Methanol extracts were isolated and purified by using column chromatography and thin layer chromatography 6.019 gram of pure isolates obtained by two-dimensional TLC assay provides a single stain and antifidan activity assay showed an increase (79%) of the value of antifidan still in fractions (65%).

Analysis of IR spectra and NMR-<sup>1</sup>H and NMR-<sup>13</sup>C showed that the isolated compound is active antifidan group alkaloids and terpenoids.

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