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The bioactive alkaloid from *Derris elliptica* (Roxb.) as biopesticide agents of *Scotinophara coartata* E on rice crops

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ABSTRACT

Discovering effective compounds to control insects is not the only focus of the development of plant pesticides. Recently, the trend has been shifted to addressing one or more particular types of phytophagous insects. This study aims to conduct a lab-scale biological study on the function of the root of *Derris elliptica* (Roxb.) as a pesticide to *Scotinophara coartata E1* from attacking rice crops. This study has been carried out by isolating *Derris elliptica* (Roxb) guided by a biological test. The result of the active extract is alkaloid with concentration 0.1% which can act as biopesticide agents of the *Scotinophara coartata* E.

Key words *: Isolation, Characterization, Alkaloid, Derris elliptica, Scotinophara coartata E*

Introduction

Plants contain chemical compounds with bioactive ability to prevent bugs and diseases from its environment. In addition, specific kinds of the plant can be used to replace the synthetic pesticide in protecting plantation as well.

There are a number of plants known to exhibit secondary metabolic compounds in Indonesia, particularly Gorontalo. One of these examples is *Derris elliptica* (Roxb (Benth)) root or known as Tubile. Widely used as a fish poison, this plant is also effective as biopesticide at farms by mixing the plant with liquid fertiliser. Farmers use this pesticide to fight insects and pests.

A number of studies have been carried out to reveal the secret within *Derris elliptica* (Roxb (Benth)) plant. In a study by Dardenne and Marlier (1975), 2.5-dihydroxymethil-3.4-dihydroxymethil had been isolated successfully. Furthermore, Wu *et al.* (2012) was able to isolate nine flavonoid compounds from the Derris plants. Lu *et al.* (2008) succeeded isolating two 2 new retinoid compounds from the same plant with the results 4′, 5′-dihydroxy-6a, 12a-dehydrodeguelin (1), 11, 4′, 5′-trihydroxy-6a, 12a-dehydrodeguelin (2).

Derris plant contains *rotenone* for its main active compounds. The other active ingredients are *deguelin*, *elliptone*, and *toxicarol*. According to Adharini Gus (2008), both rotenone and deguelin can serve as larvacida. In addition to the four compounds, Lu *et al.* (2008) discovered other compounds, such as: (2*S*,3*S*,4*R*,8*E*)-2*N*-[(2′*R*,3′)-2′,3′-dihydroxyhexanoyl]-1,3,4-trihydroxy-8-octadecane, 1), (2*S*,3*S*,4*R*,8*E*)-2*N*-[(2′*R*,3′)-2′,3′-dihydroxyhexanoyl (2*S*,3*S*,4*R*,8*E*)-2*N*-[(2′*R*,)2′,-dihydroxyhexanoyl]-1,3,4-trihydroxy-8-octadecane (2), 1-′-D-(2*S*,3*S*,4*R*,8*E*)-2*N*-[(2′*R*,3′)-2′,3′-dihydroxyhexanoylglulocopyranosyl- 3,4-dihydoxy-8-octadecane,(3). Wu (2012) were able to isolate 12

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compounds, such as: 6,4'-dihydroxy-7,5-dimethoxy-cumaronchromone (1), 7,4'-dihydroxy-,5'-methoxy-cumaronchromone (2), 7-hydroxy-4'-methylenedioxy-petrokarpan (3), 3-hidroxy-8,9-methylenedioxypetrokarpan-6a-ene (4), flemikapparin-B (5), gene stein (6), prune tin (7), formononetin (8), apegeni (9), lutiolin (10), apegenin 7-0-˜-D glucoside (11) and 5'*R*-6a,12a-dehidrorotenone (12). The compounds 2, 4, 7, 8, and 9 harness toxic characteristic for the agent *Spodoptera Litura* (SL) and *Trichoplusia* Ni BTI-Tn-5B1-4 (Hi-5) have rotenone as its controller.

The previous studies above reported that the active compound, which serves as the pesticide, is from flavonoid group. Consequently, these findings provide rationales for this study to know the effectiveness of the active compound in Derris plant. This compound is used to fight the *Scotinophara coartata E* from attacking rice crops.

Methods

Test Plant and Test Insect Pest

The test plant and test insect pest were from two different sites in Gorontalo. This study uses the root part of the Tubile plant (*Derris elliptica* (Roxb)). The test plant is taken from the district of Biluhu, Gorontalo regency, Gorontalo province. The test insect, *Scotinophara coartata E*), was obtained from Desa Permata, Gorontalo regency, Gorontalo province.

Extraction and Isolation

Dried Derris root is inserted into a container jar, and it is further macerated by methanol (2 x 24 hours). The methanol extract is evaporated by using a rotary evaporator with the temperature at 45 °C. This process will produce a concentrated extract of methanol of 68 gramme. Furthermore, the extract is treated through columnar chromatography by using 30 g of silica gel at height 20 cm. In the next step, this extract is eluted with ethyl acetate : methanol gradually (9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9) until the methanol is at 100%. The products of this process are three fractions of isolate, such as A1, A2, dan A3.

Based on the results of thin-layer chromatography (TLC), to find pure isolate, a further separation process is carried out on fraction A2. This is because the outcome from the fraction is easier to separate compared to other fractions. In the second columnar chromatography, the silica gel is the stationary phase and the ethyl acetate: methanol with ratio 8:2 is the mobile phase. Consequently, two fractions are produced from the process, namely fraction A2.1 and fraction A2.2.

The result of TLC on fraction A2.2 reveals only one stain yet there are some on the baseline; the obtained isolate is not the pure one. This indicates that another columnar chromatography is necessary to be carried out. In the third columnar chromatography, the silica gel is the stationary phase while the chloroform: methanol with ratio 8:2 is the mobile phase to obtain 25 fractions. These obtained fractions are further analysed using the TLC with the eluant chloroform: methanol with ratio 8:2.

Three fractions, namely A2.2.1, A2.2.2, dan A2.2.3, are obtained from the third chromatography. Due to exhibiting the character of the pure isolate, the characterization process is carried out on the fraction A2.2.1. This is based on the outcome of the purity test. The spectrometer UV-Vis and IR are used throughout the characterization.

Pure Isolate Phytochemical Test

In order to recognise the new compound within the isolate, the obtained pure isolate is further examined through a phytochemical test. The test result yields positive result if the white sedimentation was detected. Indicates that the isolate contains alkaloid.

Biological Activity Test

The *Scotinophara coartata E* are collected for a lab-scale biological activity test. This is because farmers are still struggling to prevent the insect pest that attacks rice crops. Table 1 provides information on the outcome of the 24-hours biological test ranging from the complete columnar results of fractions to the isolate.

Results and Discussion

The isolate from the purifying process was in the form of a crystal. The phytochemical test revealed that the pure isolate contains alkaloid. The sediment formation on the dragendorff reagent resonates the finding. On the other hand, both the steroid and terpenoid in the flavonoid test show a negative result; there are no colour changes in each reagent.

The data of spectrum UV-Vis give maximum absorption on the 208.10 nm-wavelength. The continu-

ous electronic transition from n-π* and n-σ*, which indicates the presence of C-N group, might be the trigger of the absorption. Compounds with the transition n-π* absorb the light at the range of wavelength 200-400 nm, while compounds with the transition n-σ*, due to the unconjugated chromophore, absorb the light with 200 nm wavelength (Creswell, *et al.*, 2005). Due to the visibility on the transition n-σ* of spectrum of aromatic compounds contained the transition n-π*, the transition n-π* shifts towards a higher wavelength with lower absorbance.

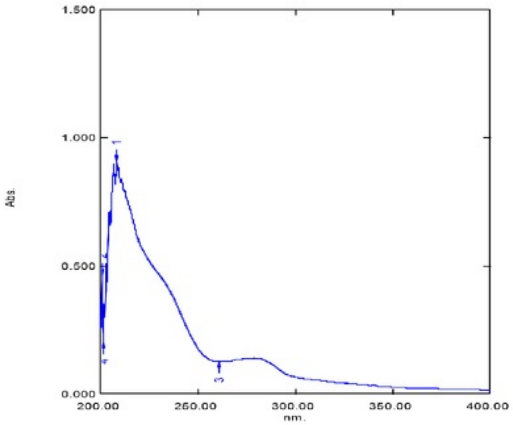

Fig. 1. Spectrum UV-Vis for the Isolate of Derris plant

The IR spectrum of isolate displays that the obtained compound showed wavenumber 3426,5 cm-1 stretched strong absorbance possibly due to bound N-H functional group. Wavenumber 2981,0 cm-1 showed a sharp and vigorous intensity absorbance, possibly due to the stretch absorbance of C-H groups seen at 2850-2950 cm^{-1} (Silverstone *et al.*, 1984). While the low rate of stretch absorbance at wavenumber 1640,6 cm^{-1} possibly due to C-H functional groups by the existence of tertiary amine C-N functional groups on 1300-1476 cm^{-1} area (Creswell *et al.*, 2005). Moreover, it is supported by aromatic tertiary amine functional group at 1300-1475 cm^{-1} (Creswell *et al.*, 2005) and sharp absorbance with

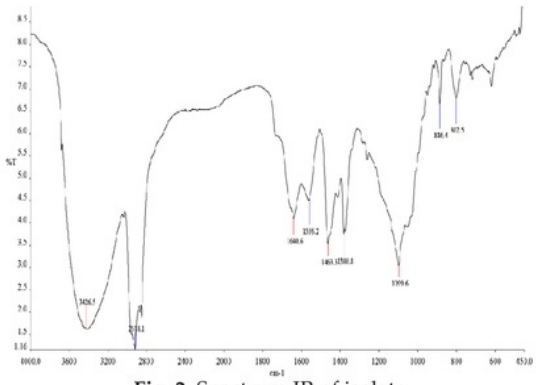

Fig. 2. Spectrum IR of isolate

Table 1. The Result of 24 hours-Observation of Biological Test on Fractions from Columnar Fraction and Isolate of Tubile Plant from the *Scotinophara coartata E*

Fractions and Isolate	Concentration (%)	Insect pest conditions	Leaf conditions	
1.	AT1	0.1%	Dead	Withered & turned yellow
		0.05%	Alive	Withered
		0.01%	Alive	Withered
2.	AT2	01%	Alive	Withered
		0.05%	Alive	Withered
		0.01%	Alive	Withered
3.	AT3	0.1%	Dead	Withered
		0.05%	Alive	Withered
		0.01%	Alive	Withered
4.	AT4	0.1%	Alive	Withered
		0.05%	Alive	Withered
		0.01%	Alive	Withered
5.	Controlled (Leaf + Me OH)		Alive	Withered
6.	Controlled (Leaf)		Alive	Withered
7.	Controlled (No Leaf)		Dead	Withered

Description:
AT1 : Isolate of Derris elliptica (Roxb.)
AT2 : Combined column fraction 1
AT3 : Combined column fraction 2
AT4 : Combined column fraction 3

moderate intensity at wavenumber 1009,6 cm^{1}. This condition is possibly caused by the existence of a C-N bent functional group which showed an existence of aliphatic tertiary seen at 1020-1250 cm^{-1} area (Silverstein *et al.*, 1984). The previous elucidation is presented in following Table 4.7.

The results of the biological test showed that the pure isolate with concentration at 0.1% provides a significant protease inhibitor and the percentage of mortality at 100% compared to the concentration 0.05% and 0.01%. The results were drawn after 24 hours of observation.

Conclusion

The most effective pesticide formula is the methanol extracted from Derris elliptica (Roxb.) with concentration at 0.01%. The phytochemical test produced from this methanol extract is alkaloids. This is supported by the IR spectrum with N-H, C-H, and C-N functional group. The UV-Vis test on the 208.10 nm wavelength reveals the continuous electronic transition from n-σ* and n-π*, with isolate contains C-N and C=O functional group.

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