



**STUDY OF BIOPESTISIDE POTENTIALS OF SECONDARY METABOLITE FROM  
*Toona Sinensis* (Meliaceae) AGAINST *Bactrocera dorsalis***

GEBI DWIYANTI<sup>1</sup>, HAYAT SOLIHIN<sup>1</sup>, ANNA PERMANASARI<sup>1</sup>, ZACKIYAH<sup>1</sup>, SUHARA<sup>2</sup>,  
SITI AISYAH<sup>1\*</sup>

<sup>1</sup>)Departement of Chemistry Education, Faculty of Mathematics and Natural Science Education,  
Indonesia University of Education

<sup>2</sup>) Departement of Biology Education, Faculty of Mathematics and Natural Science Education,  
Indonesia University of Education, Jalan Setiabudi 229 Bandung 40154 Indonesia

Corresponding author, tel. / fax: 62-22-2000579, email: sitiaisyah@upi.edu

**ABSTRACT**

Five secondary metabolite compounds were isolated from steam bark of *Toona sinensis* (Meliaceae). Base on data of functional group test, and spectroscopic UV and IR analyses, all of them show the characteristic of the terpene groups. Some of the isolated compound were examined for their pesticide activities and the result show that some of compounds posses mortality effect against *Bactrocera dorsalis*.

**Keywords** : pectiside, *Toona sinensis*, Meliceae, *Bactocera dorsalis*

**INTRODUCTION**

Indonesia is well-known as agraris coutry as the most of the people live as farmer for their living. To improve the productivity of farming, they use pesticide either natural and synthetic. But recent advance research indicates that synthetic pestiside causes new problems to environment and surrounding living things. Consequently, the use of synthetic pesticide begin to be substituted by natural pesticide. Some of natural pesticide traditionally utilized by the farmers are nimba seed (*Azadirachta indica*, Meliceae) and suren plant (*Toona*, Meliceae). In some Indonesian regions, stems, stem barks, leaves, flowers and the seeds of suren plant are used as biopesticide. Chemically, the use of traditional substances as mentioned above are related with secondary metabolite contents inside.

The literature examination about phytochemistry of *Toona* determines that this kind of genus is enriched by non polar compound from nortetraterpenoid and also phenolic from kunion and flavanoid group. The advance examination indicates that the compound of nortetraterpenoid has *antifeedant* activity (Chowdhury, R., et al., 2003; Chowdhury, R., 2004; Govindachari, T. R., et al. 2000). The researches of pesticide activity of *Toona* plants are mostly conducted toward nortetraterpenoid. Whilst, the research on phenolic compound are largely committed to figure out the activity of antioxidant and sitotoxic against human throath cancer (Chia, Yi-Chen., et al. 2005; Whang, W. K., et al., 2005).

In this article, we will report the research result concerning the biopesticide potential of secondary metabolite compound successfully isolated from stem barks of *Toona sinensis* against *Bactocera dorsalis*.

## MATERIALS AND METHODS

### General experimental procedure

UV and IR spectra were measured with Cary Varian 100 Conc. and Perkin Elmer Spectrum One FT-IR spectrophotometer. Vacuum liquid chromatography was carried out using Merck Si-gel 60 GF254, flash chromatography with Merck Si gel 60 (60-70 mesh), and TLC analysis on precoated Si gel plates (Merck Si gel Kieselgel 60 F254 0,25 mm).

### Plant Material

Steam bark of *Toona sinensis* (suren) was collected from the tree that growing in Lembang, Jawa Barat. The plant was determinated at herbarium on Biology Departement Institute Technology Bandung.

### Extraction and Isolation

The Steam bark of *Toona sinensis* (suren) was first air dried, then cuted to small pieces and crushed to fine powder. Powder of steam bark (5 Kg) was soaked in methanol (3 x 9L). After evaporation of the solvent under reduced pressure, methanol crude extract of steam bark (98 g) was sequentially partitioned in n-hexane and CHCl<sub>3</sub> and EtOAc. Each extract was fractionated by vacuum liquid and flash chromatography techniques and guided by assay using the *Bactocera dorsalis* to monitor mortality effect (pesticide effect). The crude extract (methanol extract), n-hexane, CHCl<sub>3</sub>, and EtOAc fractions were measured of pesticide effect and showed that the

pesticide mainly contained in the EtOAc. Even though further investigation was focused on this fractions, the others fraction are still investigated. The EtOAc fraction (13,7 g) was fractionated using vacuum liquid chromatography on Silica gel 60 GF254 eluted by n-hexane-EtOAc to give six fractions (A-F). All of fractions were measured of pesticide effect, and showed that the mainly pesticide contained in fraction A Further fractionation of fraction A (150 mg) using flash chromatography on silica gel 60 (60-70 mesh) led to isolation of compound **1** (7 mg). Using the same methods, from fractions B led to isolation of compound **2** (16,5 mg). Investigation of n-hexane fraction led to isolation of compound **3** (2 mg) and **4** (30 mg). Beside investigation on CHCl<sub>3</sub> fraction led to isolation of compound **5** (13 mg).

**1** was obtained as a yellow amorphous solid; UV (MeOH)  $\lambda$ maks: 213, 253.3, and 283 nm; IR(KBr)  $\nu$ maks(cm-1): 3349.2, 2854.5, 2927.7, 1708.8, 1461.9

**2** was obtained as a yellow amorphous solid; UV (MeOH)  $\lambda$ maks: 205 nm; UV (MeOH+NaOH)  $\lambda$ maks: 284 nm; IR(KBr)  $\nu$ maks(cm-1): 3402.2, 2854.5, 2923.9, 1627.8,

**3** was obtained as white amorphous solid; UV (MeOH)  $\lambda$ maks: 202 nm; UV (MeOH+NaOH)  $\lambda$ maks: 202 nm; IR(KBr)  $\nu$ maks(cm-1): 3298.0, 2923.9, 2854.5, 1720.4, 1639.4, 1454.2, 1230.5.

**4** was obtained as a brown oil; UV (MeOH)  $\lambda$ maks: 201, 0 nm; UV (MeOH+NaOH)  $\lambda$ maks: 201,0 nm; IR(KBr)  $\nu$ maks(cm-1): 3440.8, 3417.6, 2927.7, 2869.9, 1712.7, 1670.2, 1461.9, 1377.1, 1245.9, 1056.9.

**5** was obtained as a brown oil; UV (MeOH)  $\lambda$ maks: 201,0 nm; UV (MeOH+NaOH)  $\lambda$ maks: 201,0 nm; IR(KBr)  $\nu$ maks(cm-1): 3417.6, 2927.7, 2854.5, 1728.1, 1461.9, 1276.8.

### **Pesticide in vivo assays**

The “lalat buah” (*Bactrocera dorsalis*), at imago stage (four days after pupas), was used as insect to determine pesticide effect of fraction.

### **DISCUSSION**

This research is sequel done by biopesticide group to investigate potency of *Toona sinensis* against insect. Formely research has focused on potency of *Toona sinensis* against *Spodoptera litura*. Latter we focused on potency of *Toona sinensis* against *Bactrocera dorsalis* which is more harmful than *Spodoptera litura*. We focused on EtOAc showing the highest pesticide activities compared to n-hexane and CHCl<sub>3</sub> fraction.

Table 1. Pesticide assay of fraction of *Toona sinensis*

Fraction	The number of death of <i>Bactrocera dorsalis</i> at hour									
	0	1	6	12	18	24	30	36	42	48
MeOH	-	-	-	1	1	2	5	7	9	10
N-Hexane	-	-	-	-	4	4	5	9	10	10
CHCl <sub>3</sub>	-	-	-	1	1	3	4	4	4	5
EtOAc	-	1	3	4	5	6	9	10	11	12

Guided by pesticide assay, we isolated two compounds from EtOAc active fraction. Compound **1** was obtained as a yellow amorphous solid. Group functional identification with Lieberman-Burchad test exhibited that **1** is terpene group, supported by UV and IR data. The UV spectrum showed maximum absorption at 213, 253.3, and 283 nm. While the IR spectrum indicated the presence of hydroxyl ( $3349.2\text{ cm}^{-1}$ ), stretching C-H ( $2854.5$ , and  $2927.7\text{ cm}^{-1}$ ), and  $\alpha,\beta$ -saturated carbonyl ( $1708.8\text{ cm}^{-1}$ ). Based on data analysis of functional group tests and UV and IR spectrum, it is concluded that **1** is terpene compound with hydroksil and  $\alpha,\beta$ -saturated carbonyl, as same as limonoid skeleton usually founded at *Toona* genus. However, the absence of an IR absorption band at ca.  $1780\text{ cm}^{-1}$  indicated that the five-membered 4,7-lactone did not exist in **1**.

Compound **2** was obtained as a yellow amorphous solid. Group functional identification with Lieberman-Burchad test exhibited that **1** is terpene group, supported by UV and IR data. The UV spectrum showed maximum absorption at 205 nm. While the IR spectrum indicated the presence of hydroxyl ( $3402.2\text{ cm}^{-1}$ ), stretching C-H ( $2854.5$  and  $2923.9\text{ cm}^{-1}$ ), and C=C ( $1627.8\text{ cm}^{-1}$ ). Based on data analysis of functional group tests and UV and IR spectrum, it is concluded that **1** is terpene compound with hydroksil and alkene groups. However, the absence of an IR absorption band at ca.  $1708\text{ cm}^{-1}$  indicated that the  $\alpha,\beta$ -saturated carbonyl did not exist **2**. In the same way, based on data analysis of functional group tests and UV and IR spectrum, it is concluded that **3**, **4**, and **5** are included terpene compounds as same as **1**.

In the research, it is conducted the pesticide assay to determine the biopesticide potential of secondary metabolite compound that is successfully isolated from EtOAc active fraction against *Bactrocera dorsalis* at imago stage. Compounds that is tested are **1** and **2** using 0,5%

(concentration) and metanol as eluent. Base on the analysis of biopesticide analysis test, it is acquired that the death percentage of each compounds are as follow:

Table 2. Pesticide assay of **1** and **2**

Compound	The number of death of <i>Bactrocera dorsalis</i> at hour										the death percentage (%)
	0	1	6	12	18	24	30	36	42	48	
<b>1</b>	-	-	-	-	-	1	1	1	1	2	20
<b>2</b>	-	-	-	-	-	-	-	1	1	1	10
<b>control</b>	-	-	-	-	-	-	-	-	-	-	0

Based on the death percentage, it can be concluded that both of compounds caused mortality of *Bactrocera dorsalis* compared to control. Instead of that, from the corrected death percentage data, it also can be figured out that the activity of both compounds are less than EtOAc fraction, and the activity of **1**, terpene had  $\alpha,\beta$ -saturated carbonyl group, is more active than **2**.

#### CONCLUSION

Guided by pesticide assay, we isolated, from steam bark of *Toona sinensis*, two compounds from EtOAc active fraction, two compounds from  $\text{CHCl}_3$  fraction, and one compound from n-hexane fraction. Base on the data of functional groups test, and spectroscopic UV and IR analyses, all of compounds showed the characteristic of the terpene groups. Two isolated compounds caused mortality of *Bactrocera dorsalis* compared to control.

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