

**INSECTICIDAL ACTIVITY OF EXTRACTS OF *AGLAIA* SPP. (MELIACEAE)
AGAINST THE CABBAGE CLUSTER CATERPILLAR,
CROCIDOLOMIA BINOTALIS (LEPIDOPTERA: PYRALIDAE)**

**AKTIVITAS INSEKTISIDA EKSTRAK *AGLAIA* SPP. (MELIACEAE)
TERHADAP ULAT KROP KUBIS, *CROCIDOLOMIA BINOTALIS*
(LEPIDOPTERA: PYRALIDAE)**

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INTISARI

Aktivitas insektisida ekstrak sebelas spesies *Aglaia* (Meliaceae) dan rokaglamida (senyawa aktif dari *A. odorata*) diuji di laboratorium terhadap ulat krop kubis, *Crocidolomia binotalis*. Perlakuan melalui makanan selama 48 jam terhadap larva instar-2 *C. binotalis* dengan ekstrak etanol ranting *A. odorata* (pacar cina, culan) pada konsentrasi 0,5% mengakibatkan kematian larva sebesar 98,7%; ekstrak daun dan ranting *A. elaeagnoidea* masing-masing mengakibatkan kematian sebesar 17,3% dan 6,7%; ekstrak ranting *A. argentea*, *A. formosana*, dan *A. latifolia* masing-masing hanya mengakibatkan kematian sebesar 1,3%; sedangkan ekstrak 6 spesies *Aglaia* lainnya tidak aktif (kematian 0%). Kajian lebih lanjut dengan *A. odorata* menunjukkan bahwa ranting memberikan ekstrak yang paling aktif dibandingkan bagian lainnya (daun, bunga, dan akar), dan pengeringan bahan tumbuhan selama 2 minggu pada suhu kamar menurunkan aktivitas ekstrak yang diperoleh secara nyata. Ekstrak yang aktif juga memperlambat perkembangan larva yang bertahan hidup. LC_{50} fraksi etil asetat *A. odorata* dan senyawa aktif utamanya, rokaglamida, terhadap larva *C. binotalis* masing-masing 310,2 dan 31,4 ppm. Keaktifan senyawa aktif ini sekitar 8,7 kali lebih rendah dibandingkan azadirakhtin (LC_{50} 3,6 ppm).

Kata kunci: *Aglaia*, *Crocidolomia binotalis*, insektisida botani

ABSTRACT

Insecticidal potential of eleven species of *Aglaia* (Meliaceae) was evaluated in the laboratory against the cabbage cluster caterpillar, *Crocidolomia binotalis*. The feeding treatment of second-instar larvae *C. binotalis* for 48 hours with ethanol twig extract of *A. odorata* at 0.5% caused 98.7% larval mortality; leaf and twig extracts of *A. elaeagnoidea* caused 17.3% and 6.7% mortality, respectively; twig extracts of *A. argentea*, *A. formosana*, and *A. latifolia* caused only 1.3% mortality each; whereas extracts of the other six *Aglaia* species were inactive (0% mortality). Further tests with *A. odorata* showed that twigs gave

the most active extract compared to other plant parts (leaves, flowers, and roots), and air-drying of plant materials for 2 weeks markedly decreased the activity of the derived extracts. The active extracts also delayed the development of surviving larvae in similar degree to the level of their lethal effect. LC_{50} of ethyl acetate fraction of *A. odorata* twig extract and its main active compound, rocaglamide, against *C. binotalis* larvae were 310.2 and 31.4 ppm, respectively. This active compound was about 8.7 times less potent than azadirachtin (LC_{50} 3.6 ppm).

Key words: *Aglaia*, botanical insecticides, *Crocidolomia binotalis*

INTRODUCTION

Synthetic insecticides until now still play an important role in modern crop production system. Past experiences, however, taught us that the sole reliance on synthetic chemicals in controlling crop pests could cause undesirable side effects such as pest resistance and resurgence, annihilation of pest natural enemies, insecticide residues in food, and general environmental contamination (Meltcalf, 1986).

In attempts to alleviate the problems associated with the use of synthetic insecticides, in the past three decades there has been a resuscitated interest in the search for natural insect control agents from plants. Among potential sources of botanical insecticides that have been intensively studied in the last ten years are plants in the genus *Aglaia* - family Meliaceae (Janprasert *et al.*, 1993; Satasook *et al.*, 1994; Ewete *et al.*, 1996; Nugroho *et al.*, 1997a, 1997b; Nugroho *et al.*, 1999; Prijono *et al.*, 2000), important components of tropical rainforest in the Indo-Malesian region (Pannell, 1992).

Since the first report on insecticidal activity of a benzofuran compound rocaglamide from *Aglaia odorata* in 1993 (Janprasert *et al.*, 1993), the knowledge on *Aglaia* as promising sources of botanical insecticides has been rapidly expanding. It is now known that rocaglamide derivatives represent the primary insecticidal compounds in most *Aglaia* species that have been studied (Nugroho & Proksch, 1999b). To date, more than 40 insecticidal

rocaglamide derivatives have been isolated from some species of *Aglaia*, including *A. argentea*, *A. duperreana*, *A. elliptica*, *A. forbesii*, *A. harmsiana*, and *A. odorata* (Dumontet *et al.*, 1996; Nugroho & Proksch, 1999b). Some of the compounds, notably rocaglamide and didesmethylrocaglamide, exhibited insecticidal activity comparable to azadirachtin (Ewete *et al.*, 1996; Nugroho *et al.*, 1997a), a potent botanical insecticide from the widely-known neem tree, *Azadirachta indica* (Schmutterer, 1995).

There are about 70 species of *Aglaia* in Indonesia (Pannell, 1992), but only about one-third of them have been evaluated for their insecticidal property. Among the species that have been studied, some species such as *A. elliptica*, *A. harmsiana*, *A. odorata*, and *A. odoratissima* have been identified as potential sources of botanical insecticides (Nugroho *et al.*, 1997a, 1999; Prijono, 1998; Prijono *et al.*, 2000). Of those four potential species, *A. odorata* is commonly cultivated in Indonesia as an ornamental plant and its flowers are commonly used for scenting tea (Ba *et al.*, 1995). Given the number of *Aglaia* species that have not been studied, opportunity is still wide open to find further new sources of botanical insecticides among the species of *Aglaia* in Indonesia.

This study was conducted to evaluate: (1) the insecticidal activity of ethanol extracts of eleven species of *Aglaia* against *C. binotalis* larvae; (2) the insecticidal activity of rocaglamide, the main active substance from *A. odorata*; and (3) the insecticidal activity of extracts of various parts (twigs,

leaves, flowers, and roots) of *A. odorata* in an attempt to identify the most appropriate part that can be used in mass-production of insecticidal materials.

MATERIALS AND METHODS

Test insect. Second-instar larvae *Crocidolomia binotalis* were used in all bioassays. The larvae were obtained from a laboratory *C. binotalis* colony maintained at the Laboratory of Insect Physiology and Toxicology (LIPT), Bogor Agricultural University (BAU). The insect colony has been reared in the laboratory since September 1992 under ambient conditions (25–31.5 °C, 65–85% RH, and ca. 12 L:12 D regime). The larvae were fed pesticide-free broccoli leaves and the adults were fed 10% honey solution in cotton swab as described by Basana & Priyono (1994).

Experiment 1. Screening of insecticidal activity of Aglaia extracts:

- a. **Plant materials.** Leaves, twigs and/or stem barks of eleven species of *Aglaia* were collected from Bogor (including Bogor Botanical Garden), Sukabumi and Jasinga (West Java) in 1998. Plant materials from outside Bogor Botanical Garden were identified by a botanist at the National Herbarium in Bogor.
- b. **Extraction.** Plant materials were cut into small pieces and then extracted with four changes of ethanol in a soxhlet extractor (60–70°C). After extraction was completed, the solvent in the extract was evaporated in a rotary evaporator (rotavapor) at 50°C under reduced pressure. The extract obtained was kept in refrigerator ($\leq 4^{\circ}\text{C}$) until used in the bioassay.
- c. **Bioassay.** Crude ethanol extracts of eleven species of *Aglaia* were tested against second-instar larvae *C. binotalis* at a concentration of 0.5% (w/v) using

leaf-residual method. A particular extract was dissolved in a mixture of acetone and methanol (3:1), then an emulsifier Triton X-100 was added, and the mixture was diluted in water to the desired concentration. The concentrations of acetone, methanol and the emulsifier in the final dilution were 1.5%, 0.5% and 0.25%, respectively. Water containing the solvents and emulsifier at the same concentrations as in the test preparations served as control solution. Portions of broccoli leaves (ca. 5 cm \times 5 cm each) were dipped one by one in particular extract preparations to the complete wetness and then air-dried. Treated and control leaves were placed separately in glass petri dishes (9 cm in diameter) lined with absorbent paper, then 15 second-instar larvae of *C. binotalis* were introduced into each dish. Each extract treatment and control was replicated five times. The larvae were allowed to feed on treated or control leaves for 48 hours, then were provided untreated leaves and maintained until they reached the fourth-instar stage. The number of dead or molting larvae was recorded daily from the second to fourth instar. Developmental time of the surviving larvae from the second to fourth instar was also recorded.

Experiment 2. Further tests with the active extract:

- a. **Extraction.** *Aglaia odorata* twig extract was revealed as active in Experiment 1. Ground twigs of *A. odorata* were extracted with methanol by repeated mixing and filtration, then the solvent was evaporated. The methanol extract obtained was partitioned between ethyl acetate and water. The ethyl acetate phase was collected, then the solvent was evaporated to leave an ethyl acetate fraction of *A. odorata*.

b. Isolation of rocaglamide. For the isolation of rocaglamide, the main insecticidal compound in *A. odorata* (Nugroho *et al.*, 1999), the ground leaves and twigs were extracted with methanol by repeated mixing and filtration, then the solvent was evaporated. The methanol extract obtained was partitioned between n-hexane and aqueous methanol 95%, then the methanol fraction was partitioned between ethyl acetate and water. Rocaglamide was isolated from the ethyl acetate fraction by chromatographic methods as described by Nugroho *et al.* (1997a). The compound was identified by comparing its retention time and absorption spectrum with those of the authentic standard as recorded with a high performance liquid chromatography (HPLC) equipped with a photodiode array detector.

c. Bioassay. Ethyl acetate fraction of *A. odorata* and rocaglamide were tested at seven concentration levels to bracket ranges of concentrations that were expected to give 0–100% larval mortality as determined in preliminary tests. Azadirachtin (Roth, Germany) was included in this test as a positive control.

Ethyl acetate fraction of *A. odorata* was dissolved in a mixture of acetone-methanol (3:1) to the desired concentrations. Rocaglamide and azadirachtin were dissolved in acetone. Test material solution of a particular concentration was applied uniformly on both sides of broccoli leaf disks (3 cm in diameter) using a microsyringe at a rate of 25 μ l/side. Control leaf disks were treated with solvent only. After the solvent evaporated, two treated or control leaf disks were placed in a glass petri dish (9 cm in diameter) lined with towel paper, then 15 second-instar

larvae were introduced into each dish. After 24 hours, treated or control leaf disks were added as necessary, and after additional 24 hours, leftover leaf disks were removed and replaced with untreated leaves. Each treatment was replicated 7 times. Larval mortality was recorded daily until the surviving larvae reached the fourth-instar stage, and the data were analyzed by the probit method (Finney, 1971).

Experiment 3. Insecticidal activity of various parts of *A. odorata*:

a. Extraction. Fresh and air-dried parts (twigs, leaves, flowers, and roots) of *A. odorata* were ground and extracted with methanol by repeated mixing and filtration as above, then the solvent was evaporated. The methanol extract obtained was partitioned between ethyl acetate and water to obtain ethyl acetate fraction.

b. Bioassay. Each extract was dissolved in a mixture of methanol and acetone (3:1) and tested at concentrations of 0.05% and 0.25% by leaf residual method as above. Each treatment was replicated five times with 15 second-instar larvae *C. binotalis* per treatment. The number of dead or molting larvae was recorded daily until the larvae reached the fourth instar. Larval mortality in each treatment was corrected with control mortality using Abbott's formula (Abbott, 1925).

RESULTS

Screening of insecticidal activity of *Aglaia* extracts. The results of initial screening showed that 0.5% ethanol leaf extracts of all *Aglaia* species tested, except *A. elaeagnoidea*, were inactive against *C. binotalis* larvae. *A. elaeagnoidea* leaf extract at 0.5% caused only 17.3% mortality in *C. binotalis* larvae (Table 1).

Initial screening with ethanol twig and stem bark extracts revealed that *A. odorata* twig extract at 0.5% was active against *C. binotalis* larvae with mortality of 98% (Table 2). Extracts of the other test species at 0.5% were only weakly active or inactive.

In addition to lethal effect, the active extracts also delayed the development of *C.*

binotalis larvae. For example, the treatment with leaf extract of *A. elaeagnoidea* and twig extract of *A. odorata* at 0.5% prolonged developmental time of *C. binotalis* from the second to fourth instar by 3 and 3.6 days, respectively, as compared to controls (Table 1 and 2).

Table 1. Insecticidal activity of ethanol leaf extracts of *Aglaia* spp. (0.5%) against *C. binotalis* larvae

Extract	Larval mortality (%) ^a	Developmental time \pm SD (days) (n) ^b
<i>A. elaeagnoidea</i>	17.3	6.4 \pm 0.8 (62)
<i>A. eusideroxylon</i>	0	3.4 \pm 0.5 (74)
<i>A. oxypetala</i>	0	3.9 \pm 0.4 (74)
<i>A. formosana</i>	0	4.0 \pm 0.2 (75)
<i>A. latifolia</i>	0	3.4 \pm 0.5 (75)
<i>A. glabrata</i>	0	3.4 \pm 0.5 (74)
<i>A. argentea</i>	0	3.4 \pm 0.5 (75)
<i>A. ganggo</i>	0	3.5 \pm 0.5 (76)
<i>A. odorata</i>	0	3.9 \pm 0.2 (74)
Control	0	3.4 \pm 0.5 (74)

Note: ^a Mortality from second to fourth instar; average of five replications with 14–16 larvae per replication.

^b Development from second to fourth instar; n = number of survivors to fourth instar.

Table 2. Insecticidal activity of ethanol twig and stem bark extracts of *Aglaia* spp. (0.5%) against *C. binotalis* larvae^a

Extract	Larval mortality (%)	Developmental time \pm SD (days) (n)
Twig		
<i>A. elaeagnoidea</i>	6.7	4.0 \pm 0.5 (70)
<i>A. eusideroxylon</i>	0	3.4 \pm 0.5 (75)
<i>A. oxypetala</i>	0	3.5 \pm 0.5 (75)
<i>A. formosana</i>	1.3	4.0 \pm 0.2 (74)
<i>A. latifolia</i>	1.3	3.4 \pm 0.5 (74)
<i>A. glabrata</i>	0	3.4 \pm 0.5 (75)
<i>A. argentea</i>	1.3	3.4 \pm 0.5 (74)
<i>A. ganggo</i>	0	3.3 \pm 0.5 (75)
<i>A. odorata</i>	98.7	7.0 (1)
<i>A. grandis</i>	0	3.5 \pm 0.5 (75)
<i>A. tomentosa</i>	0	3.5 \pm 0.5 (75)
Control	0	3.4 \pm 0.5 (74)
Stem bark		
<i>A. argentea</i>	0	3.9 \pm 0.3 (74)
<i>A. ganggo</i>	0	3.7 \pm 0.4 (77)
Control	0	3.4 \pm 0.5 (74)

Note: ^a Mortality from second to fourth instar; average of five replications with 14–16 larvae per replication.

Insecticidal activity of *A. odorata* extract and its main active component. LC₅₀ of ethyl acetate fraction of *A. odorata* twig extract was 310.2 ppm (Table 3). This plant species has been intensively studied during the past decade and rocaglamide has been identified as the primary insecticidal principle in this species (Nugroho & Proksch, 1999a). Nugroho *et al.* (1997a) reported that rocaglamide had comparable activity to azadirachtin against *Spodoptera littoralis*. In this study, however, this compound was about 8.7 times less potent than azadirachtin against *C. binotalis* (Table 3). The target site for rocaglamide in *C. binotalis* is probably less sensitive than that in *S. littoralis*. The exact mode of action of this compound, however, is yet to be studied.

Comparative insecticidal activity of various parts of *A. odorata*. For all plant parts extracted, except roots, fresh materials gave higher extract yield and more active extracts compared to air-dried materials (Table 4). Extracts of both fresh and air-dried roots at 0.05% and 0.25% were inactive against *C. binotalis* larvae. The order of activity of extracts of fresh parts of *A. odorata*, in decreasing order of

toxicity against *C. binotalis* larvae, was as follows: twig > young leaf > old leaf > flower > root, and the order for the air-dried parts was as follows: twig > flower > old leaf ≥ young leaf > root (Table 4). The test extracts also caused a delay in the development of *C. binotalis* larvae from the second to fourth instar. The extent of delay was proportionately related to the degree of lethal effect of the extracts.

Air-drying of plant materials could markedly reduce the yield and activity of the derived extracts. Such deleterious effect can be clearly seen in extract of young leaves, in which drying reduced extract yield from over 11% to about 4.4% and decreased lethal effect of the extract from 100% to only about 17% (Table 4).

It can be suggested from the above data that fresh twigs and young leaves may serve as good sources of materials for mass production of insecticidal ingredients from *A. odorata*. If this species is planted on a large scale, harvest of young leaves along with some distal parts of twigs can be started from a certain block of plantation and subsequent harvests can be rotated among different blocks.

Table 3. Toxicity of *A. odorata* extract and rocaglamide to *C. binotalis* larvae

Test material ^a	b ± SE ^b	LC ₅₀ (ppm) ^c (95% CI)	LC ₉₅ (ppm) ^c (95% CI)
<i>A. odorata</i>			
EtOAc fraction	2.89 ± 0.41	310.2 (232.9–375.9)	1150 (838–2160)
Rocaglamide	3.86 ± 0.49	31.4 (25.5–37.2)	83.6 (65.0–131.1)
Azadirachtin	4.02 ± 0.68	3.6 (2.7–4.6)	9.2 (6.5–19.7)

Note: ^a Azadirachtin as a positive control.

^b b: slope of probit regression; SE: standard error.

^c Against mortality from second to fourth instar; CI: confidence interval.

Table 4. Insecticidal activity of extracts of various parts of *A. odorata* against *C. binotalis* larvae

Extract	Yield of extract (%) ^a	Test concentration (%)	Larval mortality (%) ^b	Developmental time \pm SD (days) (n)
Fresh materials				
Old leaves	8.05	0.25%	46.7	6.2 \pm 0.8 (32) ^c
		0.05%	1.7	3.9 \pm 0.7 (59) ^c
Young leaves	11.20	0.25%	100.0	-
		0.05%	48.3	6.1 \pm 0.5 (31) ^d
Twigs	4.46	0.25%	100.0	-
		0.05%	100.0	-
Flowers	8.68	0.25%	40.0	6.4 \pm 0.9 (36) ^c
		0.05%	0	4.1 \pm 0.6 (60) ^c
Roots	1.51	0.25%	0	4.1 \pm 0.3 (60) ^f
		0.05%	0	4.0 \pm 0.1 (60) ^f
Air-dried materials				
Old leaves	5.38	0.25%	23.3	4.6 \pm 0.7 (46) ^c
		0.05%	0	4.2 \pm 0.4 (60) ^c
Young leaves	4.43	0.25%	17.3	6.2 \pm 0.7 (48) ^e
		0.05%	0	5.2 \pm 1.0 (60) ^e
Twigs	1.44	0.25%	98.3	9.0 (1) ^e
		0.05%	46.7	6.2 \pm 0.9 (32) ^e
Flowers	3.27	0.25%	30.0	5.8 \pm 0.8 (42) ^c
		0.05%	1.7	4.9 \pm 0.4 (59) ^c
Roots	3.87	0.25%	0	4.8 \pm 0.4 (60) ^f
		0.05%	0	4.0 \pm 0.1 (60) ^f

Note: ^a On a dry-weight basis.

^b Mortality from second to fourth instar; average of four replications with 14–16 larvae per replication.

Developmental time of control larvae: ^c 3.2 \pm 0.4 (60); ^d 3.0 \pm 0 (60); ^e 4.1 \pm 0.2 (60); ^f 4.0 \pm 0.3 (60).

DISCUSSION

This study shows high variation in the insecticidal activity of ethanol extracts of eleven species of *Aglaia*. The high activity of *A. odorata* twig extract is consistent with other reports (Janprasert *et al.*, 1993; Nugroho *et al.*, 1999; Nugroho & Proksh, 1999a).

More than 40 insecticidal benzofuran compounds, including rocaglamide, have been isolated from various parts of *Aglaia* (Janprasert *et al.* 1993; Ishibashi *et al.* 1993; Nugroho *et al.* 1997a, 1997b, 1999; Nugroho & Proksch, 1999b). The content of insecticidal rocaglamide derivatives is typical of the genus *Aglaia*. Test larvae poisoned by rocaglamide did not show any

sign of molting interference nor neurotoxicity. Instead, the poisoned larvae showed loss of mobility and eventually they died. The precise biochemical lesion of this compound, however, has not been known.

Fifteen insecticidal rocaglamide derivatives have been isolated from various parts of *A. odorata* including leaves (8 compounds), twigs (7 compounds), stem barks (9 compounds), and flowers (6 compounds) (Janprasert *et al.*, 1993; Ishibashi *et al.*, 1993; Güssregen *et al.*, 1997; Nugroho *et al.*, 1999). They reported that some compounds could be isolated from different parts of the plant but other compounds were present only in certain parts. For example, Nugroho *et al.* (1999)

reported that rocaglamide could be isolated from the stem barks but was absent in the twigs, flowers, and leaves of *A. odorata* from the same source. Previously, Janprasert *et al.* (1993) reported the isolation of rocaglamide from the twigs and Ishibashi *et al.* (1993) did so from the leaves. Twigs are continuous with stem barks, and therefore, it is plausible that twigs also contain rocaglamide as do stem barks.

There are wide variations in the level of insecticidal activity among different rocaglamide derivatives depending on the type of functional groups present in their structure. For example, the substitution with an acetyl moiety at position that is normally occupied by a hydroxyl group in rocaglamide structure could decrease the toxicity of the derivatives up to ten times, whereas the substitution of methyl with hydrogen or amine with methyl ester group at the amide position did not markedly affect the activity of the derivatives (Nugroho & Proksch, 1999a). Thus, varied composition of active compounds in different plant parts could explain the different insecticidal activity of extracts of various parts of *A. odorata* as reported in this study.

In conclusion, insecticidal activity of *Aglaia* spp. varies widely among species, and *A. odorata* stood out as the most active species among the eleven species studied. In mass-production of insecticidal materials from *A. odorata*, fresh twigs and young leaves-compared to other plant parts-serve as good sources of raw materials.

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