Antifeedant properties of fractionation *Lantana camara* leaf extract on cabbage caterpillars (*Crocidolomia pavonana* fabricius) larvae

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**Abstract.** *Lantana camara* is known as a noxious weed but it has the potential for pest control. Currently, the insect pest of cabbage (*Crocidolomia pavonana*) larvae reported has been resistant to certain synthetic insecticides. The fractionation *L. camara* leaf extract has been extensively studied to find out the phytochemical active constituents and bioactivity on *C. pavonana* larvae. The antifeedant active fraction and the minimum effective concentration were investigated. The fractionation using polar (ethanol), semipolar (ethyl acetate), and non-polar (*n*-hexane) solvents were tested on 4th instar of *C. pavonana* larvae. Experimentally choice and no-choice antifeedant tested was carried out with 7 concentration levels: 0 ppm (control) and 500-5000 ppm (treatments) exposed 24 hours with 4 replications. The parameters were mean leaf areas consumed and analyzed using Mann-Whitney U non-parametric. The results were showed that ethyl acetate fraction was an active antifeedant fraction on the 3rd instar larvae of *C. pavonana*, the minimum effective concentration was 1000 ppm in both of antifeedant test, and the ethyl acetate fraction at 1000-5000 ppm was considered in good deterrents category. The phytochemical constituents of ethyl acetate fraction including alkaloids, saponins, and steroids that potentially as antifeedant against the 4th instar larvae of *C. pavonana*.

**Keywords:** Antifeedant, Ethyl acetate fraction, *L. camara*, *C. pavonana*

1. **Introduction**

Insecticidal compounds are needed to control insect pests, so their existence is very important in agriculture [1]. The adverse impacts of synthetic insecticides on the environment have stimulated researchers to investigate natural sources from plant metabolites that prospect as new natural insecticides are being safe for humans and natural enemies, specific to target insect, less persistence
and low resistance, that’s more environmentally friendly [2]. One of the promising natural products from *Lantana camara* (Verbenaceae), which is a noxious weed, and so far has been studied in the pharmaceutical field, namely for the antibacterial, antifungal, and anti-inflammatory [3]. Its uses as traditional medicine are related to the content of its active ingredients, which makes *L. camara* is interesting to investigate including for their insecticidal activity. The screening of phytochemical constituent of methanolic plant extracts of *Lantana camara* showed the presence of alkaloids, triterpenoids, saponins, steroids, flavonoids, tannins, glycosides, phenolic compounds, and carbohydrates [4]. Leaf extracts and essential oils of *Lantana camara* reported having a variety of biological activities [5], i.e. as growth and developmental inhibitor on *Dysdercus koenigii* (Heteroptera) [5], as repellent, moderate toxic and antifeedant properties on *Reticulitermes flavipes* (Kollar) [6], as toxicant and antifeedant properties on *S. litura* [7-8] and as antifeedant on larvae of *Plutela xylostella* [9].

The advantages of antifeedant compounds are specific in feeding deterrent bioactivity and indirectly killing insect pest, this systemic mechanism helps prevent resistance [2]. Thus the study is focused on antifeedant properties of *L. camara* as an environmentally friendly biopesticide. Meanwhile, *C. pavonana* has reported through a number of investigations have been resistant to several synthetic insecticides, including Profonefos [10]. Thus, the study for alternative environmentally friendly biopesticides is an important thing to investigate.

A number of studies show that crude extract has stronger bioactivity compared to pure active compounds [11]. This is due to the interaction between active compounds that produce a synergistic effect [12]. The synergistic properties of active compounds can be traced through screening large groups of active compounds based on the character of the polarity of the compounds (polar, semipolar, and non-polar fractions) to get active fractions. The *n*-hexane leaf fraction of *L. Camara* by in-vitro test on *S. litura* showed the antifeedant activity [7]. The ethyl acetate leaf fraction of *L. camara* is known to have the best antifeedant activity on *P. xylostella* larvae [8]. Through a preliminary test showed that crude extract (in ethanolic solvent) of *L. camara* leaves had attractant activity on larvae of *S. litura* and had antifeedant properties on larvae of *C. pavonia* (with the category of medium antifeedant activity) [13]. Referring to the result of the preliminary test, further studies were carried out to find the active fraction of *L. camara* which has antifeedant synergistic properties against cabbage pests, *C. pavonana* larvae. The results of this study are expected to the finding of an antifeedant active fraction of *L. camara* that is prospective in controlling *C. pavonana* larvae.

2. Materials and methods

2.1. The insect preparation

The bioassay test using the 4th instar larvae of *C. pavonana* that was collected from the Vegetable and Crops Research Institute (BALITSA) of Indonesia. The *C. pavonana* larvae were kept in an instrument of rearing cabinet 63.5 × 64 × 186.2 cm³ in size and with the systematic controlled external factors (Temperature 25°C; Humidity at 70%; Photoperiodism 12 night-light: 12 day-light), fresh cabbage leaves used for larvae feed and 10% honey in aquadest for imago feed [13, 14].

2.2. The preparation of *L. camara* leaf extraction followed by fractionation

The extraction was carried out by maceration simplicia of *L. camara*’s leaves in 95% ethanolic solvent for 72 hours. The crude ethanolic extract was obtained through evaporation by a vacuum rotary evaporator (*Buchi Rotavapor R-300*) 40°C 100 atm. Crude extract partitioned with *n*-hexane (nonpolar solvent), the results are *n*-hexane filtrate and ethanolic-aquadest fractions, then the filtrate evaporated into *n*-hexane fractionation. Ethanolic-aquadest partitioned with ethyl acetate (semi-polar solvent), each filtrate was evaporated and obtained ethyl acetate and ethanolic fractions (figure 1).
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Figure 1. The flow chart of extraction followed by fractionation of L. camara leaf extract.

2.3. The phytochemical analysis test of fractionations L. camara leaf extract
Phytochemical screening analysis was carried out in the Analytical Test Laboratory of Research Center Padjadjaran University, consists of alkaloids identification test uses reagent Dragendorff’s reagents and Mayer’s reagent, color identification test to detect flavonoids or tannins using specific reagents, foaming identification test for detect saponins content and Lieberman-Burchard’s analysis for steroids and triterpenoids identifications [13].

2.4. The choice and no-choice antifeedant test
The antifeedant bioassay test was carried out with the choice and no-choice antifeedant method to determine the parameters of the mean leaf area of larvae consumed [13, 15]. The 4th instar larva of C. pavonana that have been fasted (3 hours) was exposed on leaf disc by choosing leaves that contain extract (treatment) or without extract (control) for the choice antifeedant test. The 4th instar larva of C. pavonana in the no-choice test was exposed to the separate treatment and control leaves [13]. The concentration of each fractionation (n-hexane, ethyl acetate & ethanolic fractionations) that tested were: 0 ppm (as a control), 500 ppm, 1000 ppm, 2000 ppm, 4000 ppm, 5000 ppm, (5 replications for each fractionation).

2.5. The analysis of data
The parameters observed in the study were the area of leaf consumed by the 4th instar larva of C. pavonana in choice and no-choice antifeedant test. The area leaf consumed was captured from the photograph of leaves before and after consumed by the larvae and analyzed by using software Image J [13]. Then, the total of leaf area consumed analyzed using non-parametric statistically of the Mann Whitney-U test, to compare at least two non-homogeneous free samples in the same population at P <0.05. The antifeedant category is also determined based on the antifeedant coefficient of Gabrys formulas (the sum of absolute and relative antifeedant percentage) [16]. The absolute and relative antifeedant are referring to the following formula (1) and (2).
\[ \% A = \frac{(C_o - T_o)}{(C_o + T_o)} \times 100\% \]  
\[ \% R = \frac{(C - T)}{(C + T)} \times 100\% \]

\[ [A = \text{The absolute antifeedant percentage }\%], \quad R = \text{The relative antifeedant percentage }\%] \]

The total value of the antifeedant coefficient based on Gabry's formulas \[16\] is referring to the following formula \(3\).

\[ CT = A + R \]

\[ [CT = \text{The Coefficient Total of antifeedant }\%; \quad A = \text{The absolute antifeedant percentage }\%; \quad R = \text{The relative antifeedant percentage }\%].\]

The antifeedant category refers to the division of categories according to Gabry's \[16\] as follows (table 1).

| TC   | Antifeedant Activity Categories       |
|------|---------------------------------------|
| <50  | Weak antifeedant                      |
| 51–100 | Medium antifeedant                    |
| 101–150 | Good antifeedant                     |
| 151–200 | Strong antifeedant                   |

3. Results and Discussion

3.1. The antifeedant test result of fractionations \(L.\) camara leaf extract against 4th instar larvae of \(C.\) pavonana

The choice antifeedant test results showed the three of \(L.\) camara fractionations were deterrent feeding activity on \(C.\) pavonana 4th significantly compared to controls at 2000 ppm-5000 ppm. The best effective minimum concentration, which is 1000 ppm, was showed by ethyl acetate fraction of \(L.\) camara leaf extract treatment (figure 2). The treatment of ethyl acetate fraction at 1000 ppm showed antifeedant activity lower than the effective minimum concentration of \(n\)-hexane (2000 ppm) and ethanolic fraction (2000 ppm) (table 2).

The no-choice antifeedant test results such as choice tests showed a deterrent feeding activity of \(L.\) camara fractionations on \(C.\) pavonana 4th significantly compared to controls at 2000 ppm-5000 ppm. Ethyl acetate and ethanolic fractions were showed the best effective minimum concentration at 1000 ppm (figure 3). The treatment of ethyl acetate and ethanolic fractions at 1000 ppm showed antifeedant activities lower than an effective minimum concentration of \(n\)-hexane at 2000 ppm (table 3).

3.2. The total coefficients and antifeedant categories

The antifeedant coefficient is the accumulation result of absolute and relative antifeedant percentages, and the range of values grouped antifeedant categories from the weakest to the strongest antifeedant.
activity [16]. The lowest concentration 500 ppm of ethanolic fraction L. camara against 4th instar larvae of C. pavonana had a category of week antifeedant activity, that’s the same as n-hexane fraction was also had a category of week antifeedant activity at 500 ppm. But it’s different from the ethyl acetate fraction which had a category of medium antifeedant activity at 500 ppm concentration. The best antifeedant category among the three fractions is shown by ethyl acetate fraction at concentrations of 1000-5000 ppm which had a good antifeedant category (table 4).

3.3. The Phytoconstituents of fractionations L. camara leaf extract

The phytochemical constituents that have been analyzed from fractionations of L. camara leaf extract are shown in table 5.

### Table 2. The antifeedant choice test result of ethanolic, n-hexane, and ethyl acetate fractionations of L. camara against 4th instar larvae of C. pavonana.

| Fractionations | Concentrations (ppm) | Leaf area consumed (cm²) | Mann whitney U-test |
|----------------|----------------------|-------------------------|---------------------|
|                |                      | Control | Treatment |                        |
| Ethanolic      | 500                  | 3.17 ± 1.01 | 2.45 ± 0.77 | n.s |
|                | 1000                 | 5.46 ± 1.18 | 4.29 ± 0.88 | n.s |
|                | 2000                 | 7.41 ± 1.68 | 2.68 ± 0.66 | s* (P<0.05) |
|                | 3000                 | 7.58 ± 1.53 | 2.05 ± 0.51 | s* (P<0.05) |
|                | 4000                 | 10.97 ± 2.21 | 7.02 ± 1.43 | s* (P<0.05) |
|                | 5000                 | 9.85 ± 2.10 | 3.75 ± 0.98 | s* (P<0.05) |
| n-hexane       | 500                  | 4.69 ± 0.96 | 4.36 ± 0.92 | n.s |
|                | 1000                 | 4.24 ± 0.87 | 3.22 ± 0.88 | n.s |
|                | 2000                 | 3.02 ± 0.62 | 1.26 ± 0.26 | s* (P<0.05) |
|                | 3000                 | 5.73 ± 1.20 | 1.97 ± 0.42 | s* (P<0.05) |
|                | 4000                 | 7.40 ± 1.51 | 3.33 ± 0.72 | s* (P<0.05) |
|                | 5000                 | 4.94 ± 1.00 | 1.72 ± 0.63 | s* (P<0.05) |
| Ethyl acetate  | 500                  | 4.29 ± 0.86 | 3.04 ± 0.82 | n.s |
|                | 1000                 | 4.21 ± 1.01 | 0.91 ± 0.38 | s* (P<0.05) |
|                | 2000                 | 4.08 ± 0.83 | 1.09 ± 0.44 | s* (P<0.05) |
|                | 3000                 | 5.13 ± 1.11 | 1.00 ± 0.56 | s* (P<0.05) |
|                | 4000                 | 5.24 ± 1.14 | 1.93 ± 0.55 | s* (P<0.05) |
|                | 5000                 | 4.86 ± 1.00 | 1.01 ± 0.46 | s* (P<0.05) |

n.s = not significant with control P 0.05; s* significant with control (P 0.05)

**Figure 2.** The leaf area consumed of the 3 fractions L. camara against 4th Instar larvae of C. pavonana by choice antifeedant test result (a) Ethanol fraction 1000ppm (b) n-hexane fraction 1000ppm (c) Ethyl acetate Fraction 1000 ppm (C = Control; T = Treatment).
Table 3. The antifeedant no-choice test result of ethanolic, n-hexane, and ethyl acetate fractionations of *L. camara* against 4th instar larvae of *C. pavonana*.

| Fractionations | Concentrations (ppm) | Leaf area consumed (cm$^2$) | Mann Whittney U-test |
|----------------|----------------------|-----------------------------|----------------------|
|                |                      | Control                     | Treatment            |
| Ethanolic      | 500                  | 4.76 ± 0.75                 | 3.93 ± 1.09          | n.s                   |
|                | 1000                 | 11.22 ± 2.44                | 2.26 ± 0.87          | s* (P<0.05)           |
|                | 2000                 | 9.83 ± 2.07                 | 3.25 ± 0.94          | s* (P<0.05)           |
|                | 3000                 | 10.60 ± 2.09                | 4.19 ± 1.48          | s* (P<0.05)           |
|                | 4000                 | 10.77 ± 2.50                | 1.18 ± 0.86          | s* (P<0.05)           |
|                | 5000                 | 14.92 ± 2.97                | 3.69 ± 1.02          | s* (P<0.05)           |
| n-hexane       | 500                  | 10.73 ± 2.05                | 9.22 ± 1.79          | n.s                   |
|                | 1000                 | 12.40 ± 2.50                | 8.44 ± 2.84          | n.s                   |
|                | 2000                 | 7.20 ± 1.63                 | 1.88 ± 0.98          | s* (P<0.05)           |
|                | 3000                 | 11.90 ± 2.28                | 4.80 ± 1.25          | s* (P<0.05)           |
|                | 4000                 | 6.16 ± 2.19                 | 1.57 ± 0.74          | s* (P<0.05)           |
|                | 5000                 | 10.87 ± 2.23                | 5.33 ± 1.25          | s* (P<0.05)           |
| Ethyl acetate  | 500                  | 2.42 ± 1.26                 | 1.08 ± 0.68          | n.s                   |
|                | 1000                 | 7.78 ± 1.70                 | 1.58 ± 1.27          | s* (P<0.05)           |
|                | 2000                 | 4.80 ± 1.06                 | 1.83 ± 1.64          | s* (P<0.05)           |
|                | 3000                 | 5.75 ± 1.69                 | 0.61 ± 0.42          | s* (P<0.05)           |
|                | 4000                 | 6.48 ± 1.94                 | 1.74 ± 1.57          | s* (P<0.05)           |
|                | 5000                 | 8.84 ± 2.04                 | 2.60 ± 2.07          | s* (P<0.05)           |

n.s. = not significant with control P 0.05; s* = significant with control P 0.05

Figure 3. The leaf area consumed of the 3 Fractions *L. camara* against 4th instar larvae of *C. pavonana* by no-choice antifeedant test result (a) control (b) ethanolic fraction 1000 ppm (c) n-hexane fraction 1000 ppm (d) ethyl acetate fraction 1000 ppm (C = Control; T = Treatment).
Table 4. The antifeedant categories of fractionations of leaf extract L. camara against 4th instar larvae of C. pavonana

| Fractionations | Concentrations | Coefficients of antifeedant | Antifeedant category |
|----------------|----------------|-----------------------------|----------------------|
|                |                | R (%) | A (%) | CT |                          |
| Ethanol        | 500            | 12    | 9     | 21 | Weak antifeedant         |
|                | 1000           | 11    | 66    | 77 | Medium antifeedant       |
|                | 2000           | 46    | 50    | 96 | Medium antifeedant       |
|                | 3000           | 57    | 43    | 100| Medium antifeedant       |
|                | 4000           | 21    | 80    | 101| Good antifeedant         |
|                | 5000           | 44    | 60    | 104| Good antifeedant         |
| n-Hexane       | 500            | 3     | 7     | 10 | Weak antifeedant         |
|                | 1000           | 13    | 18    | 31 | Weak antifeedant         |
|                | 2000           | 40    | 58    | 98 | Medium antifeedant       |
|                | 3000           | 48    | 42    | 90 | Medium antifeedant       |
|                | 4000           | 37    | 59    | 96 | Medium antifeedant       |
|                | 5000           | 48    | 34    | 82 | Medium antifeedant       |
| Ethyl acetate  | 500            | 17    | 38    | 55 | Medium antifeedant       |
|                | 1000           | 64    | 66    | 130| Good antifeedant         |
|                | 2000           | 57    | 44    | 101| Good antifeedant         |
|                | 3000           | 67    | 80    | 147| Good antifeedant         |
|                | 4000           | 46    | 57    | 103| Good antifeedant         |
|                | 5000           | 65    | 54    | 119| Good antifeedant         |

Table 5. Phytoconstituents that present (+) or absent (-) in fractionations L. camara leaf extract

| Fractions     | Flavonoids | Alkaloids | Phenolic | Saponins | Steroids | Tannins | Triterpenoids |
|---------------|------------|-----------|----------|----------|----------|---------|--------------|
|               | A | B | C | A | B |       |           |             |             |
| Ethanolic     | - | - | - | + | - | - | + | - | + |
| N-Hexane      | - | - | - | + | - | - | + | + | - |
| Ethyl acetate | - | - | - | + | - | - | + | + | - |

Notes: (+) present, (-) absent

The phytochemical analysis showed its constituted by alkaloids, saponins, and steroids (table 5). A number of secondary metabolites have various bioactivity against insect pests, including antifeedants properties that feeding deterrent substances which are contained in all the major classes of secondary metabolites i.e. terpenoids, alkaloids, and phenolics [17]. Terpenoids, alkaloids, saponins, and steroids were known to have antifeedant activity on insect pests such as P. xylostella and S. litura [7-8]. The antifeedant mode of action was characterized by the response of the sensilla receptor on the larva’s taste organs when tasting the specific (antifeedant) compounds, then initiates the central nervous system to deterrent of feeding activity [11, 17-18].
Biopesticides are currently the best alternative to reduce the application of chemical synthetic insecticides which have been proven to cause resistance and adverse environmental effects [19]. However, antifeedant can reduce the attack of pest insects on host plants [20]. An insect that taste antifeedant will stop feeding until they die of starvation, or become weak to easily eaten by natural enemies, therefore the balance of the food chain is maintained [15, 19-20]. The result of the fractionation screening antifeedant test was found that ethyl acetate fraction is an active antifeedant fraction with category good antifeedant activity. Furthermore, the ethyl acetate fraction is prospective in controlling C. pavonana larvae to reduce the loss of its attack on cabbage products with the benefit of more environmentally.

4. Conclusion
The best effective minimum concentration, which is 1000 ppm, was showed by the treatment of ethyl acetate fraction in choice test and no choice test results, which showed from the lowest of effective minimum concentration among of n-hexane and ethanolic fractions. The best antifeedant category among the three fractions showed by ethyl acetate fraction at concentrations of 1000-5000 ppm which had a good deterrent category. The phytochemical constituents of extract leaf L. camara fractionation are alkaloids, saponins, steroids and triterpenoids that potentially good antifeedant activity against to the 4th instar C. pavonana larvae.

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