Effectiveness of N-Hexane and Ethanol Extract of Giant Calotrope (*Calotropis gigantea* L.) Leaves as Insecticide Against Shallot Pest *Spodoptera exigua* (Hübner)

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ABSTRACT
Shallot (*Allium ascalonicum*) is one of the high-value plants. However, shallot productivity has been decreased due to some disturbing organisms. *Spodoptera exigua* is one of the pests on shallot farming and causes a yield reduction of up to 70%. Farmers use excessive synthetic pesticides to overcome the pest. The use of synthetic pesticides has several negative effects both for the environment and human beings. Therefore, natural insecticides from plants can be an alternative solution. Giant calotropes (*Calotropis gigantea*) is one of potential plant as natural insecticide. The research aimed to find out the most effective concentration and solvent extract of *C. gigantea* to give antifeedant activity and cause toxicity to *S. exigua* second instar larvae. The extract was obtained by gradual maceration of polar (ethanol) and non-polar (n-hexane) solvents. Secondary metabolites of the extracts were tested on thin-layer chromatography (TLC). Ethanol extract exhibits higher toxicity and antifeedant activity than n-hexane extract. The most effective concentration was 3% which caused 91,67±4,41% mortality. Ethanol extract contains a higher group number of secondary metabolites than n-hexane extract.

Keywords: *Giant calotropes, Natural insecticides, Shallots, Spodoptera exigua, TLC.*

1. INTRODUCTION

Shallot (*Allium ascalonicum*) is a high value plant commodities in Indonesia. Pest attacks, mainly insects was caused a high reduction of shallot productivity. *Spodoptera exigua* (Lepidoptera: Noctuidae) is the primary pest attacking shallot farming. *S. exigua* is a cosmopolitan polyphagous insect pest [1,2]. This pest caused up to 70% reduction of shallot yield [3].

Synthetic insecticides have been widely used to reduce the pest population [3]. It has been known that widespread and continuous use of synthetic pesticides has several negative effects both for the environment and human being. Synthetic insecticides lead to insect resistance development and interrupt other beneficial insects [4]. The natural insecticide is one of the alternative solutions to control the pest population. Many plants have been reported to exhibit insecticidal, antibacterial, adan antifungal properties due to their secondary metabolites [5].

Giant calotrope is a wild plant that contains several secondary metabolites [6]. The bioactive compounds of giant calotrope is potential for natural insecticide. Many studies of using this plant extract to control insects have been conducted many times. [7], cited that leaves extract of giant calotrope inhibit the growth of *S. exigua* larvae. However, the optimization, including a comparison of different solvents, has not been known. This research aimed to see the effectiveness of n-hexane and ethanol extract of giant calotrope leaves to cause toxicity and antifeedant activity against second instar larvae of *S. exigua*. This study was also compared the secondary metabolites profile of both extracts corresponding to their insecticide activity.

2. MATERIALS AND METHODS

This research was conducted from August to December 2020 at the Entomology and Biochemistry Laboratories, Faculty of Biology, Universitas Gadjah Mada. The equipments were used in this research included shieves, blender, Erlenmeyer flask, aluminum foil, Beker glass, conical flask, TLC chamber, hairdryer, Silica gel, Whatmann paper, vortex, plastic cup, can, micropipette, analytical scale. The materials were used in this research such as second instar larvae of *S. exigua*, *C. gigantea* leaves, n-hexane, ethanol, ethyl acetate, dragendorf, FeCl3, Vaniline sulphate, quercetin, caffeine, thymol blue, gallic acid, honey, ascorbic acid, sword bean, benzoic, agar, and yeast.

2.1. Sample Collection and Extraction of Calotropis gigantea leaves

Healthy leaves of *C. gigantea* were collected and rinsed before drying. Dry leaves were ground and sifted, resulting in leaves powder. *C. gigantea* leave powder was extracted with a gradual maceration technique. Two hundred grams of leaves powder were soaked in 2000 mL of n-hexane for 48 hours then filtered with Whatmann paper. The filtrate was evaporated to get the pure extract; the residue of leaves powder in this maceration was used for the second solvent (ethanol). Maceration was repeated twice for each solvent [8].

2.2 Artificial Diet Preparation for Spodoptera exigua

The artificial diet of insects was made by following [9] method with some modifications. Two hundred fifty grams of sword beans were soaked for a night then boiled. Boiled beans were drained and ground. The dough was added with 80 gr of yeast, 50 gr of agar, 10 gr of benzoic, and 1200 mL of aquadest, then blended until mixed well. The dough was boiled and cooled to 60ºC. Ten grams of ascorbic acid were added to the dough and mixed. Artificial diet poured in a plastic cup and kept in the refrigerator.

2.3 Insecticidal Activities Test

Insecticidal activities tests were conducted with toxicity and antifeedant test [7, 10] against 2nd instar larvae of *S. exigua*. In this study, the second generation of larvae was used for the test object. Toxicity test was implemented by dripping the extract on an artificial diet with acetone as a carrier. Mortality of larvae was counted after 48 hours. For the antifeedant test, the extract was dripped on shallot leaves (diameter 1,5cm). The feeding area was counted after 24 hours with image digimizer 5.3. For both tests, a commercial insecticide containing emamectin benzoate (Bongkis) was used as a positive control.

2.4 Secondary Metabolites Test

Secondary metabolites were tested with thin layer chromatography used n-hexane and ethyl acetate as solvent (6:4 for ethanol extract and 8:2 for n-hexane extract). Two hundred fifty mg of extract diluted with each solvent for extraction. Silica gel was activated in the oven before being used for the dehumidification process [11]. The compound tested on this research included alkaloid, flavonoid, tannin, and terpenoid.

2.5 Data Analysis

Quantitative data for insecticidal activity analysis was carried out by counting the larvae mortality and measuring the leave area. The quantitative data secondary metabolite was carried out by measuring the racing factor of each spot on TLC. The data were processed using Microsoft Excel and then statistically analyzed using SPSS (Statistical Package for the Social Sciences) v.23, which includes analysis of variance (ANOVA), followed with Tukey-HSD.

3. RESULT AND DISCUSSION

The extraction yield of ethanol solvent shown in Table 1 was higher than those of n-hexane. Thus, it can be inferred that the number of polar compounds in giant calotrope leaves was higher than non-polar compounds. Furthermore, the yield percentage of extract varies corresponding to secondary metabolites containing in plant, which can be affected by maturity and plant location habitat. Many biotic and abiotic factors significantly affect the specific expression of secondary metabolites [12].

Plant secondary metabolite extraction is affected by the type of solvent. The solubility of metabolite compounds is adheres to the like dissolve-like the concept. A polar compound will dissolve in polar solvents and vice versa [13]. Therefore, the different polarities of solvent produces additional extraction yields. Polar extract contains an alkaloid, flavonoid, tannin, saponin, and terpenoid compounds. Meanwhile, nonpolar one dissolves lipopolysaccharides including waxes, color pigment, sterol, and some alkaloid and flavonoid. Thus, the primarily nonpolar extract has a lower extraction yield [14].

Extraction yield both used n-hexane and ethanol in this study was lower than [15, 16]. As mentioned before, secondary metabolites are highly correlated with many factors. Different ages and locations of leaves samples give the lower quantity of extract content. The leaves used in this study were healthy young and old leaves. The previous study only used the old one. According to [17], old leaves have higher photosynthetic capacity supported by higher production of the secondary metabolites.

Table 1. Extraction yield of calotrope C. gigantea leaves by using n-hexane and ethanol extraction

Solvent	Dry weight (g)	Extract weight (g)	Yield (%)	Colour
n-hexane	230	2.8	0.84	Brown yellowish
ethanol	230	10.5	3.23	Dark green

Both n-hexane and ethanol extract had insecticidal activity, causing toxicity and antifeedant against second instar larvae of *S. exigua*. All extracts treatment were statistically different with negative control--toxicity of the extracts is indicated by the mortality of the larvae. The larvae were considered dead if there were no movement after slightly touching. The feeding area of shallot leaves revealed the antifeedant activity of the extracts. Larger areas eaten by larvae showed lower antifeedant activity, vice versa. Statistically, no significant difference between n-hexane, and ethanol extract caused toxicity and antifeedant against *S. exigua* larvae. However, ethanol extract exhibited a higher toxicity effect and had a lower LC_{50} value (as shown in Table 2 and Table 3). On the other hand, various concentrations resulted in different mortality and antifeedant effects. Mortality of *S. exigua* was gradually increased, corresponding with the concentration of the extracts. The feeding area of shallot leaves decreased, corresponding to higher extract concentration (as shown in Table 4).

The mortality of *S. exigua* larvae was caused by the toxic effects of the secondary metabolites in giant calotrope leaf extract. Some groups of secondary metabolites compounds such as flavonoids, alkaloids, tannins, and terpenes are capable of causing disorders of the digestive system, respiration, and nervous system in insects [12]. Mortality results in this study were different from previous research. [6], mentioned that giant calotrope leaf extract can cause the death of 50% of larvae.

(LC_{50}) at a concentration of 2.42%. The extract~~s~~ used in their study was methanol extract. The lower LC_{50} in this study was due to different extraction solvents used. Ethanol, as well as n-hexane used in the extraction, yielded higher secondary metabolite compounds. In addition, the different ages of the plant used can also affect the content of secondary metabolite compounds. As a result, plants of different ages can have different toxicity effect.

The antifeedant activity caused by a tasting disorder in insects. Some phytochemical compounds of the alkaloid group, flavonoids, and terpenes can interfere with the taste cells in insects. There is no ~~a~~ definite mechanism for this effect. Bioactive compounds from plant metabolites are thought to cause stimulation of food-preventing cells or distortion of function of normal nerve cells in receiving phagostimulus compounds [18]. Moreover, non-feeding force activity (antifeedant) can also arise due to the repellent effect of the leaf extract. The repelling effect of secondary metabolites prevents contact between insects and plants. Thus, the eating activity of insects can be inhibited due to this effect [19][20]. Stated that the repellent effect of the giant calotrope flower depends on the concentration of extracts used. The highest concentration (5 mg/cm^2) in their research gave the highest repellent effect. [21], added that a higher concentration of giant calotrope extract increases its antifeedant activity. In addition, in line with our results, extracts with polar solvents (ethanol) had higher repellent activity than extracts with non-polar solvents (chloroform). The higher effect of the polar ones on repelling the *S.exigua* was correlated with the higher amount of secondary metabolites solved in the polar solvent.

The insecticidal activity of *C. gigantea* leaves was affected by secondary metabolites contained in the extract. Although it was not significantly different, ethanol was the most effective one. It corresponded with the higher secondary metabolite groups included in this extract. Ethanolic extract of *C. gigantea* leaves contained alkaloid, tannin, and terpenoid.

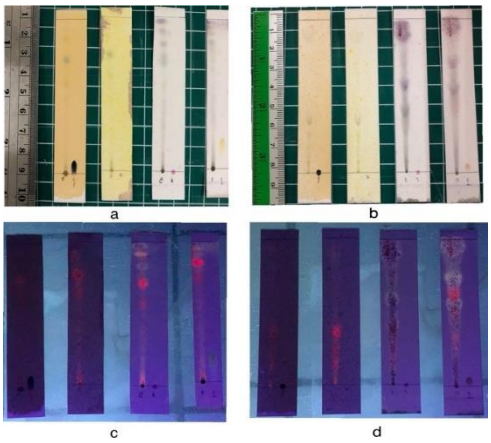

Figure 1 Secondary metabolites profiles of *C. gigantea* leaves on TLC (a)ethanolic extract (b) n-hexane extract (c) ethanolic extract under UV (d) n-hexane extract under UV (tannin test;alkaloid;terpenoid;and flavonoid test, respectively.

On another hand, n-hexane extract only had tannin and terpenoid groups. Spot number of alkaloids and terpenoids in the ethanolic extract was higher than n-hexane extract. In the previous study [15], n-hexane extract of *C. gigantea* leaves only contained terpenoid groups. Since the secondary metabolite is one of the expressions of plant stress adaptation, different plant habitats, as well as other stress factors, result in different secondary metabolite production responses.

Table 2. Lethal Concentration fifty (LC_{50}) of N-hexane and ethanol extract of C. gigantea against second larval instar of S. exigua

Solvent	LC50			Insecticidal activity
	Estimate	Lower Bound	Upper Bound	
n-hexane	0.0934	0.0010	0.3415727	Yes
Ethanol	0.08453	0.0001142	0.2789374	Yes

Table 3. Mortality (%) of S. exigua 2nd larval instar after treated with 0 – 3.0 % calotrope extracts

Concentration (%)	Mortality (%)	
	n-hexane	Ethanol
0.0	13.33 ±1.67^{a*}	13.33 ±1.67^{a*}
0.1	50.00 ±10.00^{ab*}	53.33 ±1.67^{ab*}
0.5	68.33 ± 9.28^{b*}	66.67 ±9.28^{b*}
1.0	70.00 ±11.5^{b*}	78.33 ±14.24^{b*}
1.5	71.67 ±13.64^{b*}	75.00 ±13.23^{b*}
2.0	71.67 ±13.33^{b*}	75.00 ± 2.89^{b*}
2.5	76.67 ±7.26^{b*}	80.00 ± 8.66^{b*}
3.0	83.33 ±1.67^{b*}	**91.67 ± 4.41^{b*}**
Insecticide	81.67 ± 6.01^{b*}	81.67 ± 601^{b*}

Number followed by the same letter within the same columns are not significantly different.

Table 4. Leave damage level of shallot leaves (mm^2) after treated with calotope extracts against 2nd larval instar of S. exigua

Concentration (%)	Feeding Area of Larvae (mm^2)	
	n-hexane	Ethanol
0.0	10.874 ± 0.512^{a*}	10.874 ± 0.512^{a*}
0.1	2.956 ± 0.155^{ab*}	1.698 ± 0.103^{bc*}
0.5	2.712 ± 0.351^{bc*}	0.815 ± 0.212^{c*}
1.0	2.049 ± 0.660^{c*}	0.596 ± 0.369^{c*}
1.5	0.780 ± 0.112^{cd*}	0.812 ± 0.322^{c*}
2.0	0.873 ± 0.057^{cd*}	0.334 ± 0.220^{c*}
2.5	0.423 ± 0.218^{cd*}	0.318 ± 0.251^{c*}
3.0	0.188 ± 0.085^{d*}	**0.170 ± 0.106^{c*}**
Insecticide	0.364 ± 0.040^{cd*}	0.364 ± 0.040^{c*}

Table 5. Secondary metabolites profiles of C. gigantea leaves on TLC

Secondary metabolite groups	Spots		Rf	
	n-hexane	Ethanol	n-hexane	Ethanol
Alkaloid	-	+	-	1) 0.525
				2) 0.638
				3) 0.875
Flavonoid	-	+	-	1) 0.940
Tanin	+	+	1) 0.544	1) 0.738
			2) 0.911	2) 0.938
Terpenoid	+	+	1) 0.316	1) 0.825
			2) 0.886	2) 0.911
				3) 0.975

Terpenoid can be used as insect repellant or known as an antifeedant. In addition, this secondary metabolite group has toxicity and synergistic effect with another toxic chemical [22]. Flavonoid has various insecticide mechanisms against insects. Some of this group interfered with insect reproduction and molting by inhibiting ecdysone hormone [23]. Oxidation mechanism of tannin compound results in radical compounds in the insect gut. Moreover, the compound has a bitter taste, thus exhibiting antifeedant activity [24]. In conclusion, higher content of secondary metabolite in ethanolic extract resulting higher toxicity and antifeedant activity. Further investigation on identifying active ingredients from ethanol extract is of utmost need.

Based on the research that has been done, it can be concluded that different solvent extracts of C. gigantea leaves would be either more or less effective for controlling S. exigua larvae. N-hexane and ethanolic extract of C. gigantea leaves had insecticidal activities, both toxicity and antifeedant activity against second instar larvae of S. exigua. The ethanolic extract exhibited higher insecticidal activity with the value of LC50= 0,08453% than those of N-hexane extract. The higher activity of the ethanolic extract corresponded with higher secondary metabolites content in this extract than those of n-hexane extract.

AUTHORS' CONTRIBUTIONS

EIN carried out the lab experimental and analyzed S. exigua larvae as well as the secondary metabolite, she also drafted the manuscript. LHN and SS were responsible for coordinating the implementation of the research and discussion of the research results. All authors read and approved this final manuscript.

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