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Insecticidal Activity of Leaf Extracts of *Calotropis gigantea* L, *Ageratum conyzoides* L, and *Vitex negundo* L. Against *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae)

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ABSTRACT

Calotropis gigantea L. (Asclepiadaceae), *Ageratum conyzoides* L. (Asteraceae) and *Vitex negundo* L. (Verbenaceae) are weeds, containing secondary metabolites that have insecticidal properties. The aim of this study was to determine the antifeedant activity of *C. gigantea*, *A. conyzoides* and *V. negundo* extracts against *Spodoptera frugiperda* larvae, phytotoxicity and analysis of the phytochemical components of these plant extracts. The antifeedant activity of the extracts was tested using the no choice test and the choice test with the concentrations used for each extract, namely 25, 12.5, 6.25, 3.125, 1.56 μgL^{-1}, and control (aquades), while the phytotoxicity used the spray method with a concentration 2.5%. Identification of compound components was carried out at the Integrated Research and Testing Laboratory (LPPT) of Gadjah Mada University. The results showed that the extracts of *V. negundo, A. conizoydes* and *C. gigantea* had antifeedant activity against *S. frugiperda* larvae. *V. negundo* extract had the highest antifeedant activity compared to other extracts with an antifeedant index value of 98.37. The three types of extracts did not show a phytotoxic effect on plants and contained the same active compounds, namely tannin alkaloids, phenols, flavonoids, saponins and terpenoids. Thus, the plants *C. gigantea, V. negundo* and *A. conizoydes* have potential as vegetable insecticides against *S. frugiperda*.

1. INTRODUCTION

Fall armyworm (FAW) is a polyphagous insect originating from tropical and subtropical regions of the Americas [1] reported to have invaded various types of plants in Africa, Asia and Australia [2]. In Indonesia, FAW has spread to various centers of maize cultivation in West Jawa [3], Lampung [4] Bali [5] and NTT [6].

FAW attacks plants belonging to Poaceae, Asteraceae and Fabaceae, but the greatest damage is attack on maize, rice, sorghum, cotton and sugar cane [7]. Yield loss in maize is 34%, even in the tropics it can reach 100% when attacking young plants [8-10].

The control method used by farmers to reduce FAW attacks is to use chemical insecticides because they work quickly and effectively. But without realizing it has caused various negative impacts such as environmental pollution [11], pest resistance to insecticides [12], poisoning in humans [13, 14] and non-target animals [15]. To reduce the use of insecticides, plant extracts can be used as botanical pesticides [16].

It is known that plants have secondary metabolites that can act as self-defense against herbivorous insects, including alkaloids, saponins, tannins, phenols and terpenoids [17]. Plant secondary metabolites can act as insecticide, antifeedant, repellency, preventing oviposition and growth regulating agent against insects. The advantages of plant extracts as botanical pesticides for pest management are cheap, target specific, less harmful to human health, biodegradable and environmentally friendly [18].

The antifeedant group of compounds represents a different approach to plant protection. Antifeedant acts as a food inhibitor and does not kill insect pests directly, but limits its development potential and acts as a phagodeterrent or phagorepellent [19]. The first antifeedant compound used in agriculture was an organometallic. Isman [20] defines an antifeedant compound as a substance which if tested on insects will stop eating temporarily or permanently depending on the potency of the substance. Thus, antifeedant compounds can be used as botanical insecticides to control pests.

Plant families reported to contain bioactive compounds that have activity against important plant pests include myrtaceae, lauraceae, rutaceae, lamiaceae, asteraceae, apiaceae, cupressaceae, poaceae, zingiberaceae, piperaceae, liliaceae, apocynaceae, solanaceae, caesalpinaceae, sapotaceae, asclepiadaceae, verbenaceae [21, 22]. With this information, it is suspected that even the endemic plant species of Central Sulawesi from the same family have insecticidal properties.

Calotropis gigantea L. (Asclepiadaceae), *Ageratum conyzoides* L. (Asteraceae) and *Vitex negundo* L. (Verbenaceae) plants are found thriving in the drylands of the Palu Valley. The existence of these plants are generally still weeds. *C. gigantea* includes growing in Southeast Asia, the Pacific Island, Australia, South America and Africa. *C. gigantea* is known as a weed that produces white sap from its entire structure and has potential as a medicinal plant and its activity as an insecticide. The compounds contained in the leaf extract of *C. gigantea* consist of alkaloids, steroids, terpenoids, flavonoids, tannins, and phenols [23]. The ethanol extract of

C. gigantea leaves has insecticidal activity and was reported to be effective in controlling *Plutella xylostella* in cabbage [24] and *Tribolium castaneum* [25].

A. conyzoides L. (Asteraceae) is native to South America and is now widespread throughout the tropics, including the invasive weed group. In Africa *A. conyzoides* is widely used as a multi-purpose plant and for pest control. *A. conyzoides* contains flavonoids which are thought to be used as a source of botanical pesticides [26], are antifungal and anti-insect [27, 28]. The extract of *A. conyzoides* can cause the effect of inhibiting egg laying, imago repellent and ovicidal longevity on *Paraeucosmetus pallicornis* [29]. *A. conyzoides* is also capable of causing repellent and feeding deterrent effects on *Tribolium castaneum* and *Sitophilus oryzae* [30]. The insecticidal activity of *A. conyzoides* against *Rhyzopertha dominica* caused the mortality of the pest to reach 88.67% within 24 hours after application [31].

V. negundo L. (Verbenaceae) is distributed in tropical and subtropical areas and some species are also found in temperate climates [32]. The leaves of *V. negundo* contain essential oils composed of sesquiterpenes, terpenoids, ester compounds, alkaloids (vitrisin), flavone glycosides (artemetin and 7-desmethyl artemetin) and non-flavonoid components friedelin, sitosterol, glucosides and hydrocarbon compounds [33]. *V. negundo* has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, insecticidal activity as mosquito repellent against *C. quinquefasciatus* and *A. aegypti*. The plant extract of *V. negundo* can cause mortality in the larvae of the armyworm *Spodoptera exigua* by 55.3%, and the cabbage leaf caterpillar *Plutella xylostella* by 32.3% [34].

Although there have been many studies on the use of plant extracts as pest control on various plants, the use of *C. gigantea*, *V. negundo* and *A. conyzoides* plant extracts for controlling *S. frugiperda* has not been widely reported. The aim of the study was to determine the activity of plant extracts of *C. gigantea, A. conyzoides* and *V. negundo* on the biological activities of FAW, such as antifedants, and phytotoxicity in maize, as well as phytochemical analysis of the active components of these plant extracts to be used as botanical pesticides in controlling FAW.

2. MATERIALS AND METHODS

2.1 Experimental site and meteorological conditions

Plant extraction was carried out at the Laboratory of Pests and Plant Diseases, Faculty of Agriculture and Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Tadulako University, while identification of the chemical content of the material was carried out at the Integrated Research and Testing Laboratory (LPPT) of Gadjah Mada University. The phytotoxicity test of the extract was carried out in a maize plantation in Olobuju Village, Sigi Biromaru District, Sigi Regency at an altitude of 120 m above sea level. The location is 1°0.37"S latitude and 119°56"E longitude. The soil type is inceptisol, with an average daily temperature of 32.4°C, and an average air humidity of 63% [35, 36]. The research was conducted from May 2022 to September 2022.

2.2 FAW insect propagation

The *S. frugiperda* used were insects collected from corn plantations in Bora Village, Sigi Regency. *S. frugiperda* adults were reared in a maintenance box (100 cm x 100 cm x 50 cm) which already contained maize plants aged 21 days after planting as a place for laying eggs fed with 10% honey solution and hung on the box using thread. Eggs that have hatched into larvae are transferred to individual rearing containers using baby corn feed until they become adults. The larvae used in the test are the second generation.

2.3 Preparation of extract

The leaves *of C. gigantea, A. conyzoide*s and *V. negundo* were explored from different places, ground to powder and weighed as much as 1000 grams each, then macerated with 3000 mL of ethanol solvent for 48 hours. The ethanol extract was filtered using a buchner funnel lined with filter paper, then evaporated using a heidolph vacuum rotary evaporator, Weirtheim specifications, Cole-Parmer type 7049-05 waterbath at a temperature of 40-180°C at a speed of 160-280 rpm. The extract obtained was stored in a refrigerator at 10°C.

2.4 Antifeedant assay

Antifeedant test of leaf extracts of *C. gigantea, A. conyzoides* and *V. negundo* against *S. frugiperda* was carried out through a no-choice and no-choice antifeedant test. In the no-choice test, 1 g of the extract was dissolved with 40 ml of distilled water so that the concentrations for each treatment were 25, 12.5, 6.25, 3.125, 1.56 $\mu g l^{-1}$ and the control (aquades). Determination of the concentration value is determined after conducting a preliminary test, namely the range of concentrations that causes the mortality of test larvae of 30-70%.

S. frugiperda larvae used in the third instar that had been starved. for 2 hours prior to the experiment. The feed used in the test was corn leaves in a rectangular shape measuring 3 x 3 cm. In each treatment, 2 pieces of corn leaves were dipped in each concentration for 20 seconds, then air-dried for 45 minutes. In each treatment, 10 larvae were used. Larvae were put individually in petri dishes that had been fed according to the treatment. For the antifeedant choice test, each treatment used 2 pieces of corn leaves (treatment and control).

Larvae were placed individually in each petri dish that had been fed according to the treatment. Each treatment was repeated 5 times. Observations were made every 3, 6 and 9 hours after treatment by calculating the leaf area eaten by the larvae.

2.5 Phytotoxicity test

The phytotoxicity test of each extract was carried out on a semi-field scale before being recommended for field application by planting maize plants in polybags and then spraying them directly using a hand sprayer. The concentration used is the one that shows the high effectiveness of each type of extract in the antifeedant activity test, while the comparison uses chemical insecticides at the recommended concentration. The leaves that have been sprayed are exposed to the sun for 7 hours / day starting at 08.00-15.00.

Phytotoxic symptoms that appear on plants are observed every day for 7 days of leaf exposure. Phytotoxicity symptoms that appeared in plants were observed every day for 7 days of leaf exposure, including: yellow or brownish spots on leaves, black spots, burns on leaf tips, chlorosis, and necrosis.

2.6 Identification of the group of leaf extract compounds of *V. negundo, A. Conozoydes*, and *C. gigantea*

A total of 2 g of extracts from each extract were tested for groups of compounds which included total alkaloids, tannins, phenols, flavonoids, saponins by spectrometry, and terpenoid quantity tests were carried out by layer chromatography thin [37].

2.7 Statistical analysis

Antifeedant activity was calculated based on the formula: inhibition of feeding without choice = (C-T) / C x 100%, inhibition of feeding choice = (C - T)/(C + T) x 100. C = Leaf area eaten in the control, T = the area of the leaf eaten in the treatment. Analysis of variance used ANOVA with SPSS 22.0 program at 95% level and if there was a significant difference, it was continued with Duncan's test at 95% level.

3. RESULTS AND DISCUSSION

3.1 Antifeedant activity

Antifeedant index of *C. gigantea* leaf extract without choice (no-choice antifeedant test) at 3, 6 and 9 hours after application (Table 1) showed significantly different antifeedant activity at each extract concentration. The increase in time after application with a decrease in the concentration of the extract caused a decrease in the antifeedant index with the lowest value above 60%. Antifeedant index of *C. gigantea* leaf extract with choice antifeedant test at 3, 6 and 9 hours, each concentration of *C. gigantea* leaf extract showed significantly different antifeedant activity abilities, and with increasing time after application all extract concentrations tended to experience increase in antifeedant index.

A. conizoydes leaf extract without choice (no-choice) at 3, 6 and 9 hours showed significantly different antifeedant activity against each extract concentration. With the addition of time after application, the decrease in concentration causes a decrease in the antifeedant index until it reaches an index value above 50%. while at high concentrations (25 μg L^{-1}) and medium concentrations (12.5 μg L^{-1}) did not decrease, but at low concentrations 6.25 μgL^{-1}, 3.13 μgL^{-1}, and 1.56 μg L^{-1} tended to decrease in the value of the antifeedant index.

Antifeedant activity of *V. negundo* leaf extract without choice 3, 6 and 9 hours, after application, showed significantly different antifeedant activity against each extract concentration. The decrease in concentration after increasing application time causes a decrease in the value of the antifeedant index, which is above 50%. The value of the antifeedant index at high concentrations (25 μg L^{-1}) did not decrease, while other concentrations tended to decrease even though it was not large.

The activity of no-choice antifeedant and choise antifeedant test extracts of *V. negundo, A. conyzoydes* and *C. gigantea* on feed consumption by *S. frugiperda* larvae overall showed antifeedant activity. In the three extracts, the no-choise antifeedant index at each concentration tends to decrease, while the choice antifeedant index in the three extracts increases over time. At high concentrations, the three extracts with each concentration resulted in lower feed consumption by larvae resulting in an increase in the antifeedant index, while a decrease in the concentration of the treated extracts led to an increase in feed consumption by larvae, resulting in a decrease in the antifeedant index. According to Arivoli and Tennyson [38] and Khasanah et al. [24], a high value of the antifeedant index indicates a decrease in food consumption by the test insects, and vice versa a decrease in the antifeedant index indicates a decrease in antifeedant activity.

The increase and decrease in the antifeedant index in the no-choice antifeedant and the choice antifeedant in each extract was strongly influenced by the concentration and content of the compounds contained in each extract. The leaf extract of *V. negundo* on feed consumption by *S. frugiperda* at all concentrations had a higher antifeedant activity than the extract of *A. conyzoydes* and *C. gigantea* over time, although all three had the same composition of secondary compounds, namely alkaloids, tannins, phenols, flavonoids, saponins and terpenoids (Table 1). This shows that the total content of the group of compounds greatly affects the activity of larvae on feed consumption. The presence of various secondary compounds in food can modify the response to certain stimuli so that it affects the feeding behavior of *S. frugiperda* larvae to refuse feed. Chapman [39] suggested that the increased activity of deterrent sensitive cells was due to suppressed phagostimulant cell activity so that the insect's response to feeding stimuli was dominated by negative input. According to Chanwitheesuk et al. [37], insect feeding behavior is influenced by their response to large amounts of secondary metabolites. In accordance [38, 40], *V. negundo* had the highest antifeedant activity compared to several types of plants tested against *S. litura*.

3.2 Phytotoxicity test

Phytotoxicity is one of the important considerations that can be used to develop a preparation into a new botanical insecticide. Phytotoxicity test of leaf extracts of *V. negundo, A. conizoydes* and *C. gigantea* on maize which were observed daily for 7 days did not identify any phytotoxicity in maize such as necrosis or burning symptoms (Table 2; Figure 1).

The absence of phytotoxicity in the three extracts was strongly influenced by the content of compounds, types of plants and the environment. According to Wieczorek and Wieczorek [41], phytotoxicity is influenced by dose, plant species, and exposure conditions (substrate, temperature, and environment). Plants have developed mechanisms to reduce the toxicity of allelochemicals released by other plants. Detoxification mechanism by using a non-specific enzyme system. Reduction activity is catalyzed by non-specific dehydrogenase and oxidation by P-450-type enzymes [42, 43]. In nature, plant extracts are easily decomposed by sunlight, so they generally have a short persistence in the field. Factors that influence the absence of phytotoxicity during the experiment include plant conditions, environmental conditions, and the nature of the active compounds applied [44, 45]. The absence of phytotoxicity of the leaf extracts of *V. negundo, A. conizoydes* and *C. gigantea* on maize indicates that these three are very suitable candidates for botanical insecticides.

Table 1. Antifeedant activity of leaf extract, *Calotropis gigantea, Ageratum conizoydes*, and *Vitex negundo* on the no-choice and choice antifeedant test against *Spodoptera frugiperda* instar-3 (n = 600) at 3, 6 and 9 hours

Extract	Concentration (μgL^{-1})	Antifeedant Index + SE					
		no-choice			choice		
		3 hours	6 hours	9 hours	3 hours	6 hours	9 hours
	25.00	97.40 ± 2.29^{c}	95.61 ± 1.89^{c}	96.61 ± 1.90^{d}	97.90 ± 0.93^{c}	98.90 ± 0.34^{c}	98.62 ±0.32^{c}
	12.50	96.10 ± 1.79^{c}	86.24 ± 3.51^{bc}	86.298 ± 2.68^{c}	88.08 ± 2.81^{c}	95.09 ± 0.99^{c}	94.40 ± 0.76^{b}
C. gigantea	6.25	75.69 ± 2.87^{b}	76.18 ±3.81^{b}	77.10 ± 3.43^{b}	67.95 ± 3.15^{b}	87.39 ± 1.00^{b}	93.79 ± 0.86^{b}
	3.13	58.55 ± 5.63^{a}	61.78 ± 3.92^{a}	69.05 ± 3.10^{b}	67.79 ± 5.29^{b}	86.87 ± 3.16^{b}	92.30 ± 1.87^{b}
	1.56	57.15 ±6.72^{a}	53.50 ± 4.37^{a}	53.07 ± 2.68^{a}	51.20 ± 6.61^{a}	79.66 ± 6.61^{a}	75.17 ± 1.91^{a}
	25.00	97.49 ± 0.76^{c}	97.49 ± 0.76^{d}	98.18 ± 10.52^{d}	98.07 ± 0.93^{c}	99.18 ± 0.37^{c}	94.46 ±0.38^{c}
	12.50	97.07 ± 0.91^{c}	97.18 ± 0.91^{d}	97.82 ± 0.55^{d}	88.00 ± 32.81^{c}	95.37 ± 1.11^{c}	91.67 ± 0.62^{c}
A.conizoydes	6.25	92.98 ± 1.92^{c}	85.83 ± 1.61^{c}	78.72 ± 2.27^{c}	69.95 ± 3.32^{b}	87.54 ± 1.06^{b}	90.88 ± 0.82^{bc}
	3.13	82.42 ± 71.92^{b}	72.77 ± 92,47^{b}	64.65 ± 2.22^{b}	69.50 ± 75.05^{b}	86.87 ± 2.64^{b}	85.16 ± 2.24b
	1.56	75.19 ±1.25^{a}	59.58 ± 3.77^{a}	50.97 ± 2.19^{a}	54.32 ± 6.94^{a}	80.00 ± 3,52^{a}	75.17 ± 2.41^{a}
	25.00	99.00 ± 0.29^{c}	98.25 ± 0.76^{d}	98.37 ± 0.35^{d}	99.84 ± 0.16^{c}	99.84 ± 0.15^{c}	99.84 ±0.16^{d}
	12.50	97.16 ± 0.56^{c}	93.89 ± 02.14^{cd}	87.21 ± 1.04^{c}	99.72 ± 0.21^{c}	99.24 ± 0.33^{bc}	98.26 ± 0.50^{c}
V. negundo	6.25	95.09 ± 1.03^{bc}	84.12 ± 3.99^{c}	81.50 ± 1.55^{bc}	96.71 ± 0.92^{b}	97.66 ± 0.89^{b}	97.71 ± 0.82^{b}
	3.13	90.98 ± 2.02^{b}	71.60± 4.29^{b}	77.81 ± 2.75^{b}	93.37 ± 1.42^{a}	96.37 ± 0.95^{a}	96.56 ± 0.97^{b}
	1.56	83.10 ± 4.18^{a}	61.35 ± 4.84^{a}	68.57 ± 2.83^{a}	94.32 ± 1.49^{a}	96.81 ± 0.92^{a}	94.80 ± 1.70^{a}

Note: The value (mean AI ± SE) followed by the same letter in the same column for each time was not significantly different according to Duncan ($P < 0.05$)

Table 2. Symptoms of phytotoxicity of maize, one month after spraying with leaf extracts of *Vitex negundo, Ageratum conizoydes* and *Calotropis gigantea* at 7 days of observation

Treatment	Concentration (%)	Relative area of necrotic spots (%) on maize plants at each observation (days)						
		1	2	3	4	5	6	7
Vitex negundo	2.5	0	0	0	0	0	0	0
Ageratum conizoydes	2.5	0	0	0	0	0	0	0
Calotropis gigantea	2.5	0	0	0	0	0	0	0

(a) (b) (c)

Figure 1. Maize grew normally without symptoms of phytotoxicity after the application of *V. negundo, A. conizoydes* and *C. gigantea* extracts; (a) and (b) vegetative phase; (c) generative phase

3.3 Group of leaf extract compounds *V. negundo, A. Conizoydes*, and *C. gigantea*

Based on the identification, the composition of the group of compounds contained in the extracts of *V. negundo, A. conizoydes* and *C. gigantea* consisted of tannin alkaloids, phenols, flavonoids, saponins and terpenoids (Table 3).

The highest total content of the alkaloid group of tannins, phenols, flavonoids, and saponins was *V. negundo* extract, followed by *A. conizoydes* and *C. gigantea* leaf extracts. The same content of *C. gigantea* extract compounds as *A. conizoydes* is toxic and antifeedant against *P. xylostella* [24], has antifeedant properties against *H. armigera* [46], is toxic and antifeedat against *S. exigua* [47], while the compounds in the extract of *V. negundo* are repellent, antifeedant and toxic to *Helicoverpa armigera*. *S. litura* and *Athalia proxima lugens* [48]. The high content of compounds in the *V. negundo* extract, compared to the extracts of *A. conizoydes* and *C. gigantea* (Table 2), is in agreement with [49, 50], that *V. negundo* contains flavonoids, saponins, tannins, alkaloids, glycosides, phenols, terpenoids. According to Arivoli and Tennyson [38], terpenoids, alkaloids, saponins and polyphenols are the most effective compounds in inhibiting eating.

Table 3. Compound Composition of Leaf Extracts of *Calotropis gigantea, Ageratum conizoydes* and *Vitex negundo*

Composition	Value % (w/w)		
	Calotropis gigantea	*Ageratum conizoydes*	*Vitex negundo*
Alkoloid	0.11	0.13	517.36
Tanin	6.06	7.42	16.48
Fenol	2.43	3.21	10.75
Flavonoid	0.53	1.19	2.26
Saponin	0.64	0.60	1.60
Terpenoit	Positif	Positif	Positif

Note: Positive (extract contains terpenoids)

4. CONCLUSION

The extracts of *V. negundo, C. gigantea,* and *A. conizoydes* leaves were antfeedant against *S. frugiperda*. *C. gigantea* leaf extract had the highest antifeedant activity compared to other extracts with an antifeedant index value of 98.25 in the no-choice test and 99.84 in the choice antifeedant test. The three leaf extracts do not cause phytotoxicity in maize, and contain the same active compound composition, namely tannin alkaloids, phenols, flavonoids, saponins and terpenoids, however, *V. negundo* extract has a higher amount than other plant extracts. Thus, the plant extracts *of V. negundo, C. gigantea*, and *A. conizoydes* have potential as botanical insecticides against *S. frugiperda*.

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