Toxicity of *Vitex negundo* L. and *Calotropis gigantea* L. crude extract on fall armyworm *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae)

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***Abstract*—*Vitex negundo* and *Calotropis gigantea* are plants that produce secondary metabolites which are used as defense compounds against insect pests and pathogens. This study aimed to study the toxicity of the extracts of *V. negundo* and *C. gigantea* to *Spodoptera frugiperda*. Toxicity was tested using the leaf dipping method. the concentrations used in each extract were 2.5, 1.25, 0.63. 0.35, 0.13 and control (aquades). In each treatment, ten larvae were used which were placed individually in petri dishes (9 cm diameter). Identification of the compound composition of the leaf extract of *V. negundo* and *C. gigantea* was carried out by the Integrated Research and Testing Laboratory (LPPT) Universitas Gadjah Mada. The toxicity of *V. negundo* extract to *S. frugiperda* based on probit analysis with an estimated LC50 of 0.640% while the toxicity of *C. gigantea* extract has an estimated probit of 0.691%. Extracts of *V. negundo* and *C. gigantea* have the same group of components, namely saponins, alkaloids, flavonoids, tannins, phenols, terpenoids. The composition of extra compounds of *V. negundo* consisted of 32 compounds while that of *C. gigantea* consisted of 31 compounds. The results showed that the extracts of *V. negundo* and *C. gigantea* were potential sources of botanical insecticide compounds in controlling *S. frugiperda*.**

Keywords—*Calotropis gigantea*, extract, *Vitex negundo*, *Spodoptera frugiperda*, toxicity

I. INTRODUCTION

Insecticides, which are typically in the form of alkaloid compounds, saponins, flavonoids, glycosides, steroids, and terpenoids, are one type of bioactive component that can be found in plants. Glycosides, tannins, flavonoids, terpenoids, steroids, diterpenoids, resins, alkaloids, saponins, phenols, essential oils, and cardioactive chemicals are the main categories of bioactive substances [1], [2]. All plant cells typically accumulate bioactive substances as secondary metabolites, however the concentrations vary depending on the location of the plant. The kinds of chemicals that plants possess and how those compounds function have a significant impact on how active those compounds are. *Vitex negundo* and *Calotropis gigantea* are a couple of examples of plants that make bioactive substances that serve as defense mechanisms for herbivorous insects.

The tropical plant species *V. negundo* and *C. gigantea* are common in the Palu valley. Because they are not eaten by insects or other animals, these two plant kinds have only been utilized extensively as hedges. Alkaloids, sugars, glycosides, phenolic and flavonoid compounds, flavonoids, saponins, sterols, acidic and resinous chemicals, non-protein amino acids, and cardenolides are all present in the phytochemical composition of *C. gigantea* plants [3], [4]. In contrast, the main compound content of *V. negundo* is essential oils, glucosides and hydrocarbon compounds [5].

C. gigantea crude extract is toxic and antifeedant on Plutella *xylostella larvae* [6] **,** while *V. negundo* extract is toxic and can cause death of *Spodoptera e*xigua and *P. xylostella* larvae, respectively 32% and 27% [7]. By exploring the possibility of using it as a raw material for botanical insecticides, especially for FAW, in addition to reducing the use of synthetic chemical pesticides, it can also increase the utilization of these plants, which in turn can provide economic value to the community.

This research aimed to study the toxicity of *V. negundo* and *C. gigantea* plant extracts to FAW *S. frugiperda* larvae and determine the composition of the compounds contained in the plant extracts of *C. gigantea* and *V. negundo*.

II. RESEARCH METHOD

A. Study sites

The research was carried out at the Laboratory of Plant Pests, Faculty of Agriculture, University of Tadulako, Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Untad and took place from May 2022 to

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September 2022. Identification of the extract compounds of *Vitex negundo* and *Calotropis gigantea* was carried out at the Integrated Research and Testing Laboratory (LPPT) Gadjah Mada University.

B. Research Implementation

a. Propagation of Spodoptera frugiperda larvae: *S. frugiperda* was obtained from corn plantations in the Sigi Biromaru District, Sigi Regency and propagated in a greenhouse. Propagation consists of seed preparation and maintenance of *S. frugiperda*. Corn seeds were planted in polybag pots (25 x 20 cm in size) with a mixture of soil and manure. Maintenance of corn plants, and watering is done every day. Two polybag pots of corn plants were placed in a rearing box measuring 40 x 100 cm for laying eggs. Then ten adult *S. frugiperda* were put in. Hatched eggs are harvested and maintained in plastic containers (5 x 6 cm) and fed baby corn daily until the larvae become pupae. Pupae are kept in rearing containers until they become imago. After becoming imago transferred to the egg-laying box to produce F2 offspring. The larvae used in this study were third instar larvae

b. Extraction of Vitex negundo and Calotropis gigantea leaves: much as 10 kg each of *V. negundo* and *C. gigantea* leaves were dried in an oven at 40^{o}C for 48 hours, then crushed using a blender to become powder. Each plant powder was soaked in ethanol solvent for 2 x 24 hours. The soaked results were then filtered using a Buchner funnel lined with filter paper. The filter results are then evaporated using a rotary evaporator at low pressure to obtain a crude extract. The extract obtained after evaporation was stored in the refrigerator at 4 ^{o}C for further research.

c. Identification of Vitex negundo and Calotropis gigantea extract compounds: As much as 10 g of extracted leaves of *V. negundo* and *C. gigantea* was used for the analysis of the composition of the compounds to determine the class of compounds, which included total alkaloids, tannins, phenols, flavonoids, saponins and terpenoid quantitative tests. Total alkaloids, tannins, phenols, flavonoids, and saponins were carried out using spectrophotometry, while the terpenoid group was carried out using thin-layer chromatography (TLC) [8]. As much as 2 ml of the treatment and control larvae supernatant was used to test the identification of compounds contained in the bodies of the larvae.

d. Extract toxicity test against Spodoptera frugiferda larvae: The study was arranged in a completely randomized design (CRD). A toxicity test was carried out using the residue method on leaves (feed leaf dipping). The concentrations used were 2.5, 1.25, 0.63, 0.35, and 0.13% and the control (water). Corn leaves were printed in a rectangular shape measuring 3 x 3 cm. Two pieces of corn leaves were dipped in the extract solution for 10 seconds, and the control was dipped in aquadest, then air-dried and put in a petri dish (9 cm in diameter). In each treatment, ten larvae were used individually in Petri dishes according to the predetermined treatment, and three repetitions were carried out. Observations were made every 24, 48 and 72 hours after treatment. After 24 hours, the test larvae that were still alive were transferred to a new container and fed without treatment until the observation was complete. Data were analyzed with Probit [9].

III. RESULTS AND DISCUSSION

A. Toxicity of extracts of Vitex negundo and Calotropis gigantea

The toxicity of *V. negundo* extract to *S. frugiperda* showed an estimated LC50 probit of 0.640% with a 95% confidence interval between 0.430% to 0.944%, while the toxicity of *C. gigantea* extract had an estimated probit of LC50 0.691% with a 95% confidence interval between 0.112% and 0.112% (Table 1).

TABLE 1. TOXICITY OF EXTRACTS OF *VITEX NEGUNDO* AND *CALOTROPIS GIGANTEA* USING AGAINST *SPODOPTERA FRUGIPERDA* LARVAE

Treatment	Volume (ml)	n	Slope (± SE)	LC_{50} (SK95%)	df
Vitex negundo	40	220	0,587(±0,133)	0,64(0,430-,944)	3
Calotropis gigantea	40	220	0,573(±0,059)	0,69(0.112-,463)	3

The toxicity of *V. negundo* and *C. gigantea* extracts showed a toxic effect on *S. frugiperda* larvae. The higher the concentration, the higher the toxicity caused to *S. frugiperda*, even though the leaves consumed were less than the other treatments. The results of [10] showed that high concentrations of *Argemone ochroleuca* extract resulted in high mortality in *S. frugiperda* larvae, even though only a tiny portion of the leaves were consumed. Mortality of *S. frugiperda* larvae increased with increasing exposure time of the extract.

Toxic effects, in general, depend on the dose, time of application and individual characteristics such as sensitivity to the site of action and stage of development [11]. The toxic properties of *V. negundo* extract are strongly influenced by the chemical compounds it contains [12]. The results of research by [13] showed that larval mortality was also caused by reduced larvae-feeding activity. The extracts of *C. gigantea* and *V. negundo* had activity as an antifungal with an inhibitory concentration of 0.21% IC50 through a selected antifeedant test. *V. negundo* is toxic to S. exigua with LC50 0.49% and 0.42% to *P. xylostella. V. negundo* has ovicidal activity against *S. litura* at 23.48 mg/ml [7].

The results of visual observations of the larvae before dying show initial symptoms in the form of black spots on the abdomen, and then the entire body of the larvae becomes black and stiff.

B. Compound composition of V. negundo and C. gigantea

The compound components in the leaf extracts of *V. gigantea* and *C. gigantea* contain the same group of compounds, namely saponins, alkaloids, flavonoids, tannins, phenols, terpenoids (Table 2).

This group of compounds has insecticidal abilities, either singly or in combination, and contribute to their efficacy, such as repellents and inhibition of feeding and physiological efficacy, such as acute toxicity and developmental disorders in various insect species [14].

TABLE 2. COMPOSITION OF GROUP OF COMPOUNDS EXTRACTS VITEX NEGUNDO AND CALOTROPIS GIGANTEA

Compound Composition	Value (%)	
	Vitex negundo	*Calotropis gigantea*
Alkaloids	517.36	0.11
Tannins	16.48	6.06
Phenol	10.75	2.43
Flavonoids	2.26	0.53
Saponin	1.60	0.64
Terpenoid	Positive	Positive

Note: Positive (extract contains terpenoids)

The extracts of *C. gigantea* and *V. negundo* had main activity as antifidants with an IC50 inhibitory concentration of 0.21% through a test with choice (Nasir et al., 2022). *C. gigantea* and *V. negundo* extracts have bioactivity as insecticides, development inhibitors, hormonal disturbances, anti-oviposition and emergence of imago against several types of insects including *S. gregaria, C. maculatus, S. oryzae*, *T. japonica* and *M. domestica* [15], [16]. The study [17] showed that the treatment of *C. gigantea* and *V. negundo* extracts caused the mortality of *Rhyzopertha dominica*.

The composition of the compounds in the *V. negundo* extract identified 32 compounds (Table 3). In comparison, in the *C. gigantea* extract 31 compounds were identified (Table 4), and most of the compounds of the two plants were reported to have various toxic, antimicrobial, anti-inflammatory, antioxidant, antiandrogenic, and antimalerial bioactivities. insectifuge, antifeedant, attractant, deterrent and repellent, anticancer, antidiabetic, and antidermatic.

TABLE 3. COMPOSITION OF *VITEX NEGUNDO* EXTRACT COMPOUNDS

No	Compound	chemical formula	activity
1	Eucalyptol	$C_{10}H_{18}O$	Flammable liquid and vapour, fatal if swallowed and enters the respiratory tract, Causes skin corrosion/irritation, causes allergic skin reactions.
2	1,5,5-Trimethyl-6-methylene-cyclohexene	$C_{10}H_{16}$	Not yet known
3	Caryophyllene	$C_{15}H_{24}$	Harmful, fatal if swallowed and enters the airways and causes an allergic skin reaction
4	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	Antimicrobial
5	Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane], 1-methyl-4-isopropyl-7,8-dihydroxy-, (8S)-	$C_{15}H_{24}O_3$	Antimicrobial
6	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	$C_{15}H_{24}O_2$	Antimicrobial
7	Benzoic acid, 3-hydroxy-	$C_7H_6O_3$	Causes skin irritation and may cause respiratory tract irritation/specific target organ toxicity, single exposure
8	6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	Antibacterial and antioxidant
9	Ppropiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)-	$C_{13}H_{20}O_3$	Anti-cancer
10	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3ß,5Z,7E)-	$C_{27}H_{44}O_3$	Toxic if swallowed, in contact with skin, if inhaled and causes damage to organs through prolonged or repeated exposure
11	1-Naphthalenepropanol, a-ethenyldecahydro-2-hydroxy-a,2,5,5,8a-pentamethyl-, [1R-[1a(R*),2ß,4aß,8aa]]-	$C_{20}H_{36}O_2$	attractant
12	1-Heptatriacotanol	$C_{37}H_{76}O$	Antimicrobial
13	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl] methyl]-, methyl ester	$C_{25}H_{42}O_2$	Antimicrobial, antioxidant and cytotoxic
14	6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate	$C_{25}H_{32}O_8$	Antifungal
15	4,14-Retro-retinol	$C_{20}H_{30}O$	cytotoxic
16	1H-Naphtho[2,1-b]pyran, 3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-, [3R-(3a,4aß,6aa,10aß,10ba)]-	$C_{20}H_{34}O$	Antimicrobial
17	5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol	$C_{20}H_{34}O$	Antimicrobial
18	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	insecticide
19	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1S-(1a,1aa,2a,5ß,5aß,6ß,8aa,9a,10aa)]-	$C_{20}H_{28}O_6$	Not yet known
20	3,3a-Epoxydicyclopenta[a,d]cyclooctan-4ß-ol, 9,10a-dimethyl-6-methylene-3ß-isopropyl-	$C_{20}H_{32}O_2$	Not yet known
21	Phytol	$C_{20}H_{40}O$	Antioxidant, anxiolytic, modulating metabolism,

			cytotoxic, antioxidant, antinociceptive, anti-inflammatory, immune modulating, antimicrobial and antiplasmodial.
22	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	$C_{20}H_{34}O_2$	Enzyme Activators and Inhibitors
23	Dodecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-methanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester, [1aR-(1aa,2a,5ß,5aß,6ß,8aa,9a,10aa)]-	$C_{32}H_{50}O_6$	Toxic
24	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	Antimicrobial and Anti-inflammatory
25	a-D-Glucopyranoside, methyl 2-(acetylamino)-2-deoxy-3-O-(trimethylsilyl)-, cyclic methylboronate	$C_{13}H_{26}BNO_6Si$	
26	Pregn-5-en-20-one, 11-(acetyloxy)-3,14-dihydroxy-12-(2-hydroxy-3-methyl-1-oxobutoxy)-, (3ß,11a,12ß,14ß)-	$C_{28}H_{42}O_8$	Toxic
27	(E)-Labda-8(17),12-diene-15,16-dial	$C_{20}H_{30}O_2$	Pesticides, additives and medical
28	Oxymesterone	$C_{20}H_{30}O_3$	Antimicrobial
29	1-Heptatriacotanol	$C_{37}H_{76}O$	Harmful, reproductive toxic
30	Methyl glycocholate, 3TMS derivative	$C_{36}H_{69}NO_6Si3$	Antioxidant, anti-cancer, anti-inflammatory
31	2-Cyclohexene-1-carboxylic acid, 1,3-dimethyl-2-(3-methyl-7-oxo-1,3-octadienyl)-4-oxo-, methyl ester, (+)-	$C_{19}H_{26}O_4$	Antioxidant
32	4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one, 8-(acetyloxy)-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-3a,6b,8a-trihydroxy-2a-(hydroxymethyl)-1,1,5,7-tetramethyl-, [1ar-(1aa,1bß,1ca,2aa,3aß,6aa,6ba,7a,8ß,8aa)]-	$C_{22}H_{30}O_8$	Flavour

TABEL 4. COMPOSITION OF *CALOTROPIS GIGANTEA* EXTRACT COMPOUNDS

No	Compound	chemical formula	activity
1	Piperidine, 2,3-dimethyl-	$C_7H_{15}N$	Highly Flammable, Harmful if swallowed/acute toxicity, eye corrosion/irritation, respiratory tract irritation, specific target organ toxicity
2	3H-3a,7-Methanoazulene, 2,4,5,6,7,8-hexahydro-1,4,9,9-tetramethyl-, [3aR-(3aa,4ß,7a)]-	$C_{15}H_{24}$	anti-cancer
3	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	$C_{11}H_{18}N_2O_2$	Not yet known
4	Spiro[tricyclo[4.4.0.0(5,9)]decane-10,2'-oxirane], 1-methyl-4-isopropyl-7,8-dihydroxy-, (8S)-	$C_{15}H_{24}O_3$	Antimicrobial
5	4,7-Dimethoxy-5-[prop-1-en-1-yl]-2H-1,3-benzodioxole	$C_{12}H_{14}O_4$	Antimitotic
6	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	Antimicrobial
7	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	$C_{11}H_{18}N_2O_2$	Not yet known
8	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	Not yet known
9	Phthalic acid, isobutyl octadecyl ester	$C_{30}H_{50}O_4$	Not yet known
10	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Antioxidant, Nematicide, Pesticide, Nematicide, Pesticide, flavour, Hemolytic, flavour, Hemolytic, Alphareductase inhibitor
11	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Toxic, skin corrosion/irritation, causes severe eye irritation, causes respiratory irritation, Hazardous to the aquatic environment, long-term hazard
12	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{35}H_{68}O_5$	Not yet known
13	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	α-glucosidase inhibitors
14	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	Not yet known
15	Phytol	$C_{20}H_{40}O$	Antioxidant, anxiolytic, metabolic modulation, cytotoxic, antioxidant, antinociceptive, anti-inflammatory, immune modulating, antimicrobial and antiplasmodial.
16	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	antimicrobial
17	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	Cytotoxic, antioxidant
18	9,12,15-Octadecatrienoic acid, 2-phenyl-1,3-dioxan-5-yl ester	$C_{28}H_{40}O_4$	Anti-viral and anti-obesity
19	5,8,11-Eicosatriynoic acid, tert-butyldimethylsilyl ester	$C_{26}H_{42}O_2Si$	aromatic

20	Diisooctyl phthalate	$C_{24}H_{38}O_4$	Toxic
21	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	Antimicrobials and anti-virals
22	Methyl glycocholate, 3TMS derivative	$C_{36}H_{69}NO_6Si_3$	antioxidants
23	2,4,6-Decatrienoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a-dihydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-11-oxo-1H-2,8a-ethanocyclopenta[a]cyclopropa[e]cyclodecen-6-yl ester, [1aR-(1aa,2a,5ß,5aß,6ß,8aa,9a,10aa)]-	$C_{30}H_{40}O_6$	Anticancer
24	8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3ß-methoxy-4,4-dimethyl-	$C_{24}H_{36}O_6$	Not yet known
25	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	anti-inflammatory, anticancer antimicrobial, antiasthma, diuretic
26	Ergosta-5,22-dien-3-ol, acetate, (3ß,22E)-	$C_{30}H_{48}O_2$	antituberculosis
27	8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17-acetoxy-3ß-methoxy-4,4-dimethyl-	$C_{24}H_{36}O_6$	Not yet known
28	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	Not yet known
29	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	$C_{12}H_{38}O_5Si_6$	Antimicrobial and antiseptic
30	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	$C_{27}H_{42}O_4$	Antibacterial, antitumor and tyrosine phosphatase 1B (PTP 1B) inhibitor
31	Glycine, N-[(3a,5ß)-24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester	$C_{30}H_{53}NO_4Si$	antibacterial

IV. CONCLUSION

The amount, type, and function of the bioactive chemicals found in the leaf extracts of *V. negundo* and *C. gigantea* all had an impact on how toxic they were to *S. frugiperda.* The chemical make-up of the *C. gigantea* and *V. negundo* extracts has the potential to serve as a source of botanical insecticides.

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