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Effectiveness extract of *Crataeva nurvala* leaves as insecticide against *Spodoptera litura*

Hastini Ma'rufah*, L. Hartanto Nugroho, Sukirno Sukirno

Department of Tropical Biology, Faculty of Biology, Gadjah Mada University, Yogyakarta 55281, Indonesia

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Abstract

Spodoptera litura Fabricius, an insect pest, is known to be highly detrimental to farmers for having a variety of host plants. The application of synthetic insecticides to eradicate pests has been proven to bring many negative impacts, especially regarding the cases of resistance and the presence of residues that are harmful to the environment. This study aims to study the effectiveness of potential bioactive compounds of Tigarun leaves (*Crataeva nurvala* Buch. Ham) as the biochemical insecticides of *S. litura*. Tigarun plant, widely used as traditional medicine, contains the potential bioactive compounds for bioinsecticides. The extraction process was carried out by maceration using methanol and ethyl acetate solvents, which were then identified for compound content through GCMS analysis. The bioassay method was performed using the test parameters of mortality and eating power of *S. litura* instar II larvae. The crude extracts from the two solvents obtained showed their effectiveness as bioinsecticides against *S. litura* instar II larvae. The highest efficacy occurred in the ethyl acetate extract using the contact poison method with the lowest LC50 value of 0.11. Both extracts were also able to reduce the appetite and provide sublethal effects on *S. litura* larvae. GCMS analysis indicated the presence of several compounds as insecticides in both Tigarun leaf extracts such as 1,2,3-Propanetriol (CAS) Glycerol; Tetradecanoic acid (CAS) Myristic acid; 9-Hexadecenoic acid; n-Hexadecanoic acid; oleic acid; Heneicosane; and Neophytadiene and several other compounds. This study recommends Tigarun leaf extract (*C. nurvala*) ethyl acetate with the contact method as a natural insecticide against *S. litura*.

Keywords: Bioinsecticide, GCMS, *Crateava nurvala, Spodoptera litura*

1. Introduction

Armyworm (*Spodoptera litura* F.) is one of the important pests that must be entirely controlled as the attack of *S. litura* larvae can cause damage and make farmers to suffer losses. The wide host range has potentially made *S. litura* as a major pest in many types of plants such as plantations, fruits, vegetables, and food crops. The hosts of armyworms include tobacco, cabbage, sweet potatoes, potatoes, and soybeans [1,2]. As revealed from the data of the Ministry of Agriculture [3], *S. litura* is considered a dangerous pest for being capable of attacking more than 80 plant species. In Europe and Africa, the attacks of *S. litura* have resulted in losses of 8.3 - 20.6 million tons per year with an economic loss value of between US $ 2.5 and 6.2 billion per year. *S. litura* attacks by eating leaves until only a thin epidermis remains on the surface and leaf veins (Fig. 1). This, as a consequence, results in the loss or decrease of farmers' plantation yields [4].

Currently, the use of synthetic insecticides is increasingly used by farmers to control pests. Synthetic insecticides, nevertheless, can bring a negative impact on both environment and human health. The residue left by the insecticides after application will still remain in the soil for years, thus affecting soil microorganisms [5]. WHO confirmed that pesticide/insecticide poisoning kills more than two hundred thousand people each year. In addition, the World Resource Institute reported that more than 500 insects have become resistant to insecticides [6]. The use of insecticides should be the last resort in accordance with a sustainable Integrated Pest Management (IPM) system [7]. Therefore, to support the reduction of chemical pesticides against *S. litura* pests, it is necessary to address it with knowledge related to *S. litura* biology, the level of damage and pest control techniques. A safer control technique is to substitute the use of synthetic insecticides, considering many negative impacts on the environment. In addition to environmental problems, the use of artificial insecticides can cause resistance in *S. litura* pests. This resistance then causes insecticide applications to no longer have an effect on pest mortality. In this case, botanical insecticides or biopesticides can be an alternative to be used.

Tigarun (*Crataeva nurvala* Buch Ham) is tree plants, living in terrestrial ecosystems, and having plant organs such as roots, stems, leaves, flowers, and fruits (Fig. 2). The Banjar

* Corresponding author. Tel: +6287846948010
Email: hastinimarufah@mail.ugm.ac.id
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community recognizes this plant as Tigarun or Tigaron, while in Indonesian this plant is called as Sempal Wadak. Among Banjar community, Tigarun - generally the flower part – is used as a food ingredient. Jaruk tigarun is one of the traditional fermented foods from South Kalimantan, which is made by soaking Tigarun flowers in boiled water for several days. The flower part and the tip of the Tigarun plant stem are taken to be processed into Jaruk or fermented Tigarun. The Jaruk Tigarun is a complementary food for side dishes that can increase appetite [8]. Meanwhile, the Indian community uses the roots and stems of the Tigarun plant as a traditional medicine that can cure several diseases. Unlike other organs above, the leaf part of this plant has not been optimally consumed or utilized.

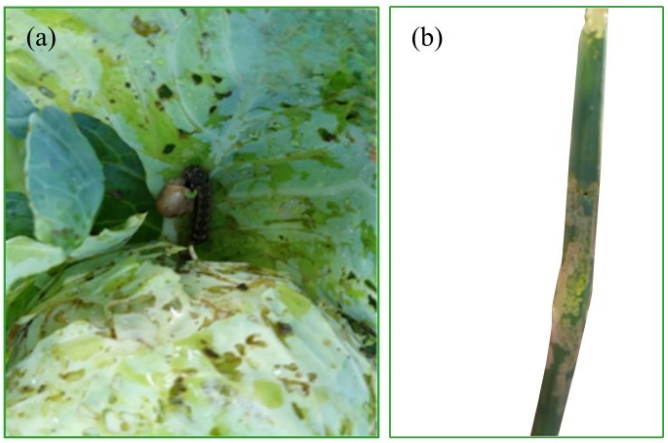


Fig. 1. Damage caused by *Spodoptera litura* on (a) cabbage (*Brassica oleracea* L.) and (b) leek (*Allium fistulosum*) plants

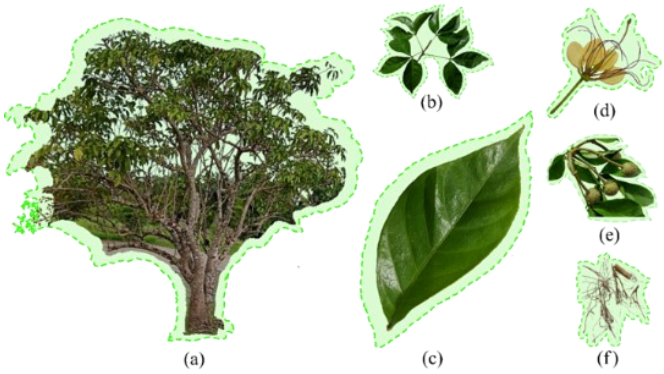


Fig. 2. Tigarun (*Crataeva nurvala* Buch Ham). (a) A tree-like plant, (b) three leaves on one petiole, (c) leaves, (d) flowers, (e) fruit, and (f) roots

Along with the development of research, the parts known to contain various secondary metabolites include alkaloids, flavonoids, phenols, hydroquinones, tannins, and essential oils. The secondary metabolites are the potential compounds to be used as herbicides or insecticides. Secondary metabolite compounds produced by plants can act as the repellents or inhibitors of eating, inhibitors of development, inhibitors of egg laying and as chemicals killing insects quickly [9].

The use of *C. nurvala* as bioinsecticides has been the root organs previously used to combat *N. depunctalis* pests [10], yet since *C. nurvala* commonly is harvested every flowering season, it finds difficult to take the root part of the tree. Unlike the leaf organ, when used for bioinsecticides, *C. nurvala* does not cause death to plants. Therefore, the author is interested in utilizing the leaves of *C. nurvala* to explore its potential as a bioinsecticide for armyworms.

The potential use of *C. nurvala* as a botanical pesticide is in consideration to the presence of phenol contained in it that can act as an antifeedant to inhibit insect development. Several types of phenol are also known to attract the natural enemies of insect pests. *C. nurvala* leaves were reported by [3] to show the presence of phenolic compounds in a study using ethanol extract. In addition, several studies have shown the presence of saponins in *C. nurvala* leaves, increasing the potential of this plant to be used as a natural insecticide. Saponins can affect the development of insect pests from larvae to pupae. Other metabolite compounds such as alkaloids with chemical structures in the form of Lycopsamine and Echinatin have been developed into natural pesticides obtained from plants. Both compounds are toxic to Lepidoptera insects. Therefore, it is likely that *C. nurvala* leaf extract can bring a negative impact on the death, development, and inhibition of *S. litura* pests.

In relation to the use of plant extracts as bioinsecticides, it is necessary to select the best solvent to maximize the extraction of active substances that are potential to have toxic effects on pests. The selection of solvents used, nevertheless, must be based on the type of compound being extracted. Considering no previous research on the use of solvents [11] and the absence of previous research about the use of Tigarun leaf solvents as the bioinsecticide of *S. litura* caterpillars, this study is expected to provide suitable solvents information for extracting metabolic compounds from Tigarun leaves based on their polarity.

This research mainly aims to test the effectiveness of the leaves as the controller of the pest. To maximize the use of *C. nurvala* leaves as an *S. litura* insecticide, this study was equipped with the identification of the bioactive compounds of the type of solvent used through GCMS (Gas Chromatography Mass Spectrometry) analysis. The identification of compounds from solvents will be useful for identifying any specific compounds that can act as bioinsecticides.

2. Materials and Methods

2.1. Collection of plant materials and extraction

Crataeva nurvala leaves were obtained from Tunggul Irang, Martapura, South Kalimantan. The selected *C. nurvala* leaves were those that were not wilted and visually free of parasites. The collected Tigarun leaves were cleaned using running water. The simplicia was air-dried indoors until being dried for some time to reduce its water content; by so doing, it could be stored longer and was uneasily damaged. Then, the simplicia was ground into powder.

Tigarun leaf extraction was carried out through the maceration method using the graded solvents started from the semi-polar solvent ethyl acetate to the polar solvent methanol. The simplicia powder was soaked using 2 solvents successively, semi-polar (ethyl acetate), and polar (methanol) with a ratio of simplicia powder: solvent 1: 4 w / v (75 grams / 300 ml). The mixture was then shaken until the powder was completely soaked and homogeneous and shaken every 24 hours. The macerate after 48 hours was filtered using Whatman

No. 1 filter paper. Subsequently, the maceration residue was re-macerated with the similar volumes of solvent and stirring time. It was continued with the evaporation of the macerate obtained using a rotary evaporator at a temperature of 50°C.

2.2. Larvae collection and Insect rearing

S. litura samples obtained from the field were kept in the Entomology Laboratory of the Faculty of Biology UGM with artificial feed. Artificial feed used 250 grams of sword beans porridge (soaked for 24 hours, and boiled for 1 hour) added with 80 grams of yeast, 10 grams of benzoic acid, 50 grams of agar, and 1200 ml of distilled water. The mixture was boiled for one hour while stirring and cooled at a temperature of 60O°C to add 20 grams of ascorbic acid. The feed was put in a mould or plastic cup and stored at a temperature of -4°C.

The process of transferring larvae to different cups was carried out when the larvae entered the instar 2 phase. If the size of the larvae started to grow (entering the instar 4-5 phase), it could be transferred to a different cup with a limit of 4-6 larvae per cup until reaching the pupation phase. The pupae formed were then transferred into a glass jar (30-40 pupae), the base of which has been lined with 2 layers of tissue and has been given with opaque paper (zig-zag folds) as a place to lay eggs after being imago and being reproduced. Nutrient supply in the form of honey was diluted with distilled water at a ratio of 1:10. The honey solution was then dripped onto cotton that was tied with rubber on the upper surface of the glass jar, which was kept moist. When the imago produced and laid the eggs on the paper given, the eggs were then taken by cutting the paper and stored in a cup containing artificial feed. Furthermore, the cut results were glued to tissue paper and stored on the upper surface of the cup filled with artificial feed. After the larvae hatched approximately after 2-3 days, the larvae were transferred to another container. Checking was carried out periodically by concerning with the condition of the feed, and the number of larvae in each cup and by checking the temperature and humidity of the room during the rearing process.

2.3. Insecticidal and antifeedant bioassay

1 ml stock solution was prepared for each extract. The methanol extract was diluted with 0.01% tween 80, and the ethyl acetate extract used 0.2% tween 80. The final concentrations were then prepared from 0.1%, 0.3%, 0.5%, 5%, to 10% stock solutions using distilled water. Insecticide activity test was carried out through toxicity test with the parameters of mortality and decreased appetite effects on instar 2 larvae of *S. litura*. The second generation larvae were used as the test objects with 20 larvae for each test unit. The test used a completely randomized design with 5 replications for each test concentration. Meanwhile, the toxicity test was carried out by dipping the larvae into the extract with 5 concentration variations as treatments. Larval mortality was calculated after 3 days and after 7 days. The caterpillar's appetite was measured by calculating the decrease in feed weight as an antifeedant activity. For positive control, commercial insecticide, i.e. Marshal 200 EC (Emulsifiable Concentrate), an insecticide with active ingredient carbosulfan was used. While, for negative control, crude extract solvent, i.e. tween 0.01% for methanol extract and tween 0.2% for ethyl acetate extract was used.

2.4. Statistical analysis

Quantitative data were obtained from the analysis of observation data on larval mortality and the weight of feed consumed by larvae by means of Analysis of Variance (ANOVA). If differences between treatments were obtained, it then continued with the Duncan test at a significance level of 5%. The percentage of larval death/mortality was calculated using the following formula [12]:

$$\text{Mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of test larvae}} \text{ x } 100\% \quad (1)$$

2.5. GCMS analysis

Crude extracts from methanol and ethyl acetate solvents were put into microtubes containing 1.5 ml of ethanol each as much as 0.5 ml. Furthermore, the mixture was homogenized using a vortex mixer for 1 minute and centrifuged for 3 minutes at a speed of 9000 rpm. The supernatant formed can be tested for 60 minutes on the GCMS QP2010S Shimadzu using Agilent DB-5MS. (injector temperature 300°C, detector 250°C, and column 325°C). Helium gas to be used as a carrier gas used a constant flow rate of 1 ml/min and ionization used El 70 ev.

3. Results and Discussion

In this study, *C. nurvala* leaves were soaked in different solvents, namely methanol (polar) and ethyl acetate (semi-polar) with the yield results as presented in Table 1. It was found that the methanol extract had a higher concentration compared to the ethyl acetate extract. Here, the selection of solvents was based on the type of polarity solvent. Although using the same extraction method and plant parts, the two extracts were analyzed using different solvent polarities, thus making the composition of the active ingredients contained also different. Soaking with maceration techniques using different solvents can allow for the extraction of more varied compounds. Contact between the solvent and the simplicia causes the active ingredients to diffuse perfectly and be attracted based on the polarity of the compound. Methanol solvents will attract polar compounds in *C. nurvala* leaves. While, ethyl acetate solvents had lower polarity (semi-polar), so that the compounds that could be withdrawn by this solvent were more diverse. With the comparison of effectiveness through bioassay tests and comparison of compound content through GCMS, it was found that different solvents attracted different compounds, and affected or increased effectiveness as bioinsecticides specifically, as well as the qualitative content of leaf extracts in general.

This experiment showed that the amount of polar active substances in the leaves were more dominant compared to the semi-polar active substances. The dissolved active substances adjusted to the type of solvent polarity used. Active substances with high polarity will be attracted to polar solvents and vice versa, active compounds with low polarity will be attracted to non-polar solvents [13]. The extraction in this study was higher

than that of previous studies, which produced a yield from dry simplicia by methanol solvents [14]. The quality of different yield results can occur because it is determined by geographical conditions, season and flowering time, development stage, organs used, to drying and storage of simplicia powder. Environmental factors such as the presence of criticism, plant nutrition, and different soil conditions affect the synthesis of various compounds contained in plants, thereby influencing differences in extraction results [15].

Table 1. The yield of *Crataeva nurvala* leaf extract using methanol and ethyl acetate solvent

Solvent	Dry weight (g)	Extraction Results		Color
		Weight (g)	Yield (%)	
Methanol	75	8.99	11.99	Dark green
Ethyl Acetate		1.15	1.54	Dark green

3.1. Insecticidal activity

The treatment of methanol and ethyl acetate extract concentrations showed significantly different results. The percentage of mortality increased along with the increase of the concentration of the extract. Although the comparison between mortality at concentrations of 0.1%, 0.3%, and 0.5% was not significantly different, the three concentrations were significantly different when compared to concentrations of 5% and 10%. The highest larval mortality on day 3 occurred in 10% ethyl acetate extract with a mortality percentage of 97% exceeding the positive control (91%), followed by 10% methanol extract with a percentage of 84%. While, the lowest mortality percentage occurred in 1% methanol extract, which was insignificantly different from 0.3%, but the results were significantly different from the negative control (0%), only causing the mortality of 12.50%.

Mortality on the third day showed that ethyl acetate extract with the same concentration was able to kill more than methanol extract. A concentration of 0.1% in ethyl acetate extract was able to kill >50% of caterpillars, reaching 63%, while at the same concentration (0.1%), the mortality of methanol extract was only 29% (significantly different). This also occurred at other higher concentrations as depicted in Table 2. The average mortality on day 7 was also analysed and showed no significant difference compared to day 3 for each concentration. This indicated that the length of application time does not affect mortality, and the highest mortality occurs at the beginning of treatment.

The highest mortality on day 7 was not much different from day 3. 10% ethyl acetate extract with a mortality percentage reached 98%, not significantly different from 10% methanol extract with a mortality of 85%. Over time, the mortality pattern from days 3 and 7 showed no effect of delaying caterpillar death. The toxic substances contained in the Tigarun leaf extract (*C. nurvala*) apparently showed a rapid death effect as it could reach mortality of >80% in a short time.

Probit analysis (Table 3) obtained from mortality data was carried out to determine LC50. The greatest extract effectiveness was found in the use of ethanol solvent with the lowest LC50 value of 0.11% for 3 days after treatment. Whereas, the concentration required to achieve 50% mortality of larvae until the seventh day of ethyl acetate extract was 0.22%. Based on this, it can be concluded that both types of extracts have insecticidal activity and ethyl acetate extract is more effective than methanol extract.

Table 2. The effectiveness of Tigarun leaf extract (*Crataeva nurvala*) methanol and ethyl acetate extracts on the percentage of mortality of *Spodoptera litura* larvae after 3 and 7 days of treatment

Treatment		Mortality (%mean±SE)	
Solvent	Concentration (%)	Day 3	Day 7
Methanol	0	12.50±1.44^{a}	13.75±1.25^{a}
	0.1	29.00±5.79^{bc}	33.00±6.04^{bc}
	0.3	30.00±6.71^{bc}	30.00±6.71^{b}
	0.5	42.00±6.04^{c}	46.00±7.48^{c}
	5	62.00±6.44^{d}	66.00±4.85^{d}
	10	84.00±4.85^{fg}	85.00±4.47^{fg}
Ethyl Acetate	0	16.00±4.00^{ab}	19.00±3.32^{ab}
	0.1	63.00±5.39^{de}	70.00±7.58^{de}
	0.3	63.00±6.44^{de}	69.00±4.58^{de}
	0.5	64.00±3.32^{de}	69.00±2.45^{de}
	5	79.00±7.48^{ef}	82.00±4.64^{ef}
	10	97.00±2.00^{g}	98.00±1.22^{g}
Carbosulfan		91.00±2.92^{fg}	91.00±2.92^{g}

Note: numbers in one column followed by the same notation indicate no significant difference with a significance of $p<0.05$. SE=Standard Error

Overall, the effectiveness of ethyl acetate extract showed higher mortality than that of methanol extract. This is in line with results of research by [16] using *Moringa oleifera* leaf extract. The study showed that methanol extract had a toxic effect on *S. litura* larvae, which was significantly different from ethyl acetate extract. However, the use of methanol and ethyl acetate extracts of *M. Oleifera* leaves did not differ much in the duration of *S. litura* larval development. The study using *Annona Squamosa* seed extract [17] showed that non-polar solvents (N-hexan) had better results than polar solvents (ethanol) on the *S. litura* mortality value. Yet, in general, the two polar and nonpolar solvents showed positive results as bioinsecticides from *S. litura*. The difference in effectiveness of different solvents was likely due to the influence of substances absorbed by the two solvents from the leaves through the maceration process.

Table 3. Lethal Concentration (LC) of methanol and ethyl acetate extracts of Tigarun leaves (*Crataeva nurvala*) on *Spodoptera litura* larvae after 3 and 7 days of treatment

Treatment	*Lethal Concentration* (%)			
	LC_{50}	Lower bond	Upper bond	Insecticidal activity
Me-3	3.44	2	5.32	Yes
Me-7	3.01	1.28	5.2	Yes
EA-3	0.11	N/A	N/A	Yes
EA-7	0.22	N/A	N/A	Yes

Note: Me-3=Methanol extract treatment day 3; Me-7=Methanol extract treatment day 7; EA-3=Ethyl acetate extract treatment day 3; and EA-7=Ethyl acetate extract treatment day 7. N/A=not available

The ability of insecticides to affect the eating power of a pest is one of the useful features for controlling plant damage, in addition to toxic substances that cause mortality effects. The lower the larval eating power, the better the ability of Tigarun leaf extract (*C. nurvala*) to inhibit the feeding activity of *S. litura larvae*. There was a significant difference between the extract treatment and the negative control, except for the methanol extract with the low concentrations of 0.1% and 0.3% (Table 4). Meanwhile, several treatments with high concentrations also caused high larval mortality, so that the feeding power could not be measured because the larval density was low (data were not available for statistical analysis). The density of *S. litura* larvae based on [18] could affect feeding activity. High population density causes competition in feeding to be high, so that feeding activity also increases.

Table 4. The effect of Tigarun leaf extract (*Crataeva nurvala*) methanol extract and ethyl acetate on the feeding ability of *Spodoptera litura* larvae after 7 days of treatment

Concentration	Edibility±SE (grams)			
	n	Methanol	n	Ethyl Acetat
0%	5	2.02 ± 0.18^{f}	5	1.57 ± 0.14^{e}
0.1%	5	1.23 ± 0.10^{de}	5	0.45 ± 0.12^{a}
0.3%	5	1.20 ± 0.11^{cde}	5	0.52 ± 0.06^{a}
0.5%	5	1.01 ± 0.16^{bcd}	5	0.47 ± 0.04^{a}
5%	5	0.82 ± 0.10^{abc}	2	0.66 ± 0.02^{ab}
10%	4	0.48 ± 0.10^{a}	N/A	N/A
Carbosulfan			N/A	

Note: numbers in one column followed by the same notation indicate no significant difference with significance $p<0.05$. n=replication (20 larvae in each replicate); N/A=not available (data not available); SE=Standard Error

Flavonoids and tannins are the inhibitors of the α-amylase enzyme. The α-amylase enzyme plays a role in the breakdown of starch. The decrease in the activity of this enzyme by the presence of inhibitors can cause a decrease in energy production for insect growth [19, 20, 21]. As quoted from the research of [22] regarding the secondary metabolite content of Tigarun leaves (*C. nurvala*), flavonoid and tannin compounds showed positive results in the compound detection test in ethanol extract.

The secondary metabolite content of leaf *C. nurvala* shows that the potential of this plant leaves becomes a very high vegetable pesticide. Secondary metabolites in addition to having a negative impact on the survival also show the effect at the stage of exposed larvae development, for example causing different morphological disorders at the developmental stages such as the lack of larvae and cocoons, as well as the transitional stages of larvae [23]. This form of abnormality can be associated with an imbalance of ecdysteroids as reported earlier by many studies. Metamorphosis abnormalities in the larvae of *S. litura* and pupae have been reported due to various plant extracts [24, 25].

3.2. GCMS Characterization of chemicals C. nurvala extract

The identification of *C. nurvala* extract compounds with two different types of solvents aimed to determine potential compounds as biopesticides. *C. nurvala* leaf extracts derived from methanol and ethyl acetate solvents were identified using GCMS. The peaks formed in the methanol extract showed that there were 51 compounds (Fig. 3), while the ethyl acetate extract had more peaks, 53 compounds (Fig. 4). Although using the same extraction method and plant parts, the two extracts analyzed used different solvent polarities, so the composition of the active ingredients contained was also different. The quality of the active ingredient extract depends on the solvent used.

The GC analysis (Table 5) based on the chromatogram of methanol extract of *C. nurvala* leaves showed the first peak at a retention time of 3.32 minutes with a peak area of 0.82%, while the first peak of ethyl acetate extract appeared at 4.12 minutes and a peak area percentage of 2.02%. Methanol extract produced the highest peak with the retention time of 19.74% at 7.5 minutes, while ethyl acetate extract was 17.23% at 57.93 minutes. The smallest peak area in each of the methanol and ethyl acetate extracts was 0.19% at 33.36 minutes and 0.2% at 53.21 minutes. There 66 compounds identified from the two solvents of *C. nurvala* leaf extract (Table 3) with a Similarity Index (SI) or selected similarity above 80%.

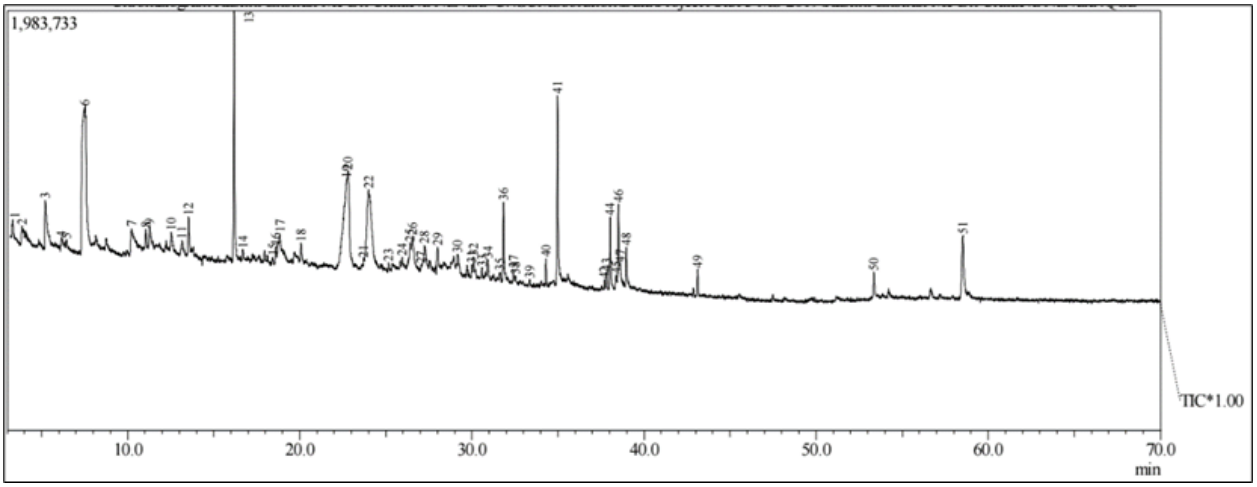


Fig. 3. Chromatogram of GC analysis of methanol extract of Tigarun leaves (*Crataeva nurvala*)

Methanol extracts at peak 2 and 3 were Propanal, 2,3-dihydroxy-Glycerose with a percentage of peak area or concentration of 0.08% and 2.04% respectively. The peaks that were different from the results of the identification of the same compound in the ethyl acetate extract included Ethylbenzene or Benzene, 1,3-dimethyl- at peak 2 and 3; Farnesyl acetone at peak 21 and 35; 12,15-Octadecadienoic acid (Z,Z) at peak 25 and 28. Meanwhile, HEPTADECENE-(8)-CARBONIC ACID-(1) was identified at peak 30 and 32; and Tetratetracontane was found at peak 49 and 53 with concentrations or peak areas as shown in Table 3. The compounds that appear in different peaks according to [26] occur as the active ingredients are not in a single form, even though they have the same compound formula and molecular weight.

Some compounds in the methanol extract of *C. nurvala* leaves were also seen overlapped with compounds contained in the ethyl acetate extract, including 1,2,3-Propanetriol (CAS) Glycerol; 1,2,3-Propanetriol, 1-acetate; Tetradecanoic acid (CAS) Myristic acid; 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6; NEOPHYTADIENE; PHYTOL; Hexadecanoic acid (methyl ester); 6-Octadecenoic acid; Stigmast-5-en-3-ol, (3.beta.,24S)- (CAS) Clionasterol. Some of the compounds in the ethyl acetate extract also appeared at two different peaks.

GCMS results of methanol extract of Tigarun leaves (*C. nurvala*), 1,2,3-Propanetriol or Glycerol (Fig. 5(a)) are the compound with the highest concentration of 19.74%. The high polarity in glycerol enables it to dissolve any inorganic salts, enzymes, acids, bases, and even organic compounds that are insoluble in water [27]. This is what causes the methanol solvent to attract glycerol compounds in quite large amounts. Glycerol also appears in the semi-polar ethyl acetate extract but in low concentrations (1.07%).

Levoglucosan (1,6-Anhydro-.beta.-D-glucopyranose) is another compound with the highest concentration after glycerol, namely (10.87%) in the methanol extract. Levoglucosan (Fig. 5(b)) is an anhydrous sugar formed from the cellulose pyrolysis process [28]. In addition, 8.91% 2-Furancarboxaldehyde, 5-(hydroxymethyl)- was identified in the methanol extract. The compound Furancarboxaldehyde, 5-(hydroxymethyl)- (Fig. 5(c)) has antimicrobial and antioxidant effects, and is widely used as a preservative [29].

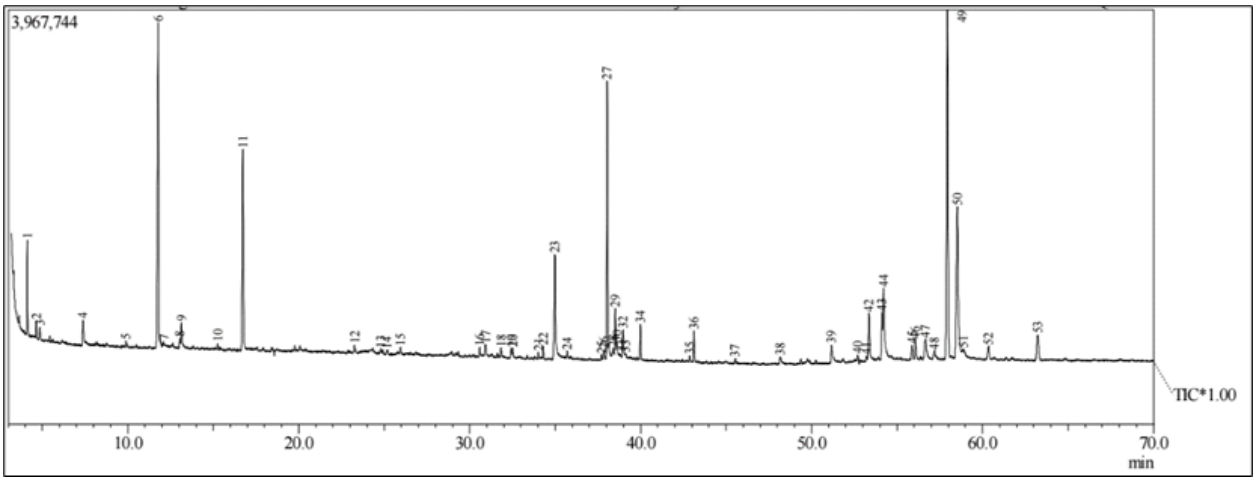


Fig. 4. Chromatogram of GC analysis of ethyl acetate extract of Tigarun leaves (*Crataeva nurvala*)

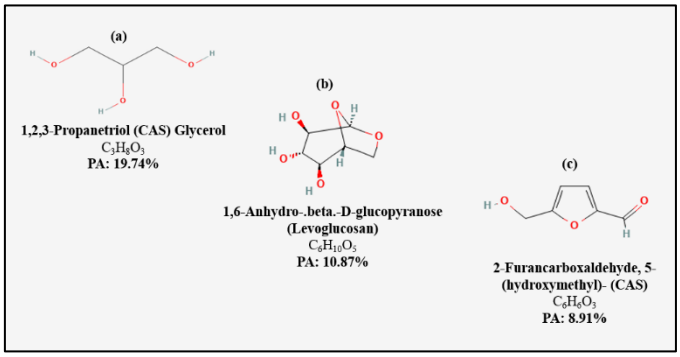


Fig. 5. The structure of dominant compounds found in methanol extract of Tigarun leaves (*Crataeva nurvala*). PA: Percentage of peak area

The dominant compounds found in the ethyl acetate extract of Tigarun leaves (*C. nurvala*) with the highest concentrations included Tetratetracontane; 1,2,3-Propanetriol, 1-acetate; Stigmast-5-en-3-ol, (3.beta.,24S)- Clionasterol; and Phytol (Fig. 6). Tetratetracontane became the first large component identified with a concentration of 17.23%. This compound is a long chain alkane (Fig. 9(a)) with antibacterial activity [30]. This was followed by the second most abundant compound, namely 1,2,3-Propanetriol, 1-acetate (Fig. 6(b)) which appeared at two different peaks: peaks 6 and 11 with the concentrations of each peak of 14.62% and 8.44% respectively. These compounds have the potential as antimicrobials, anti-inflammatory, and anticancer [31].

The compound stigmast-5-en-3-ol was found in a fairly large amount in the ethyl acetate extract with a concentration of 10.68%, which is a phytosterol commonly found in many plants (Fig. 6(c)). This compound is reported to have antidiabetic activity and has the potential to provide antiproliferative effects [32, 33]. Meanwhile, the Phytol compound (10.14%) as the highest compound in the ethyl acetate extract was an acyclic diterpene (Fig. 6(d)) with anticancer, antidiuretic, nematicidal, hepatoprotective, hypocholesterolemic, anticoronary, antiandrogenic, antimicrobial, antioxidant, antiarthritic, anti-inflammatory, antidiabetic, and immunostimulant properties [34].

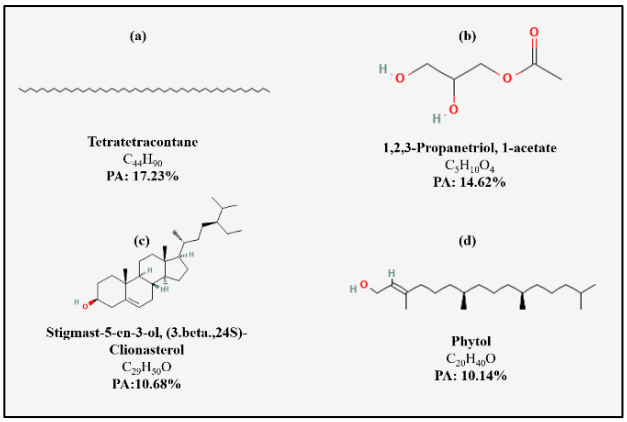


Fig. 6. The structure of the dominant compound found in the ethyl acetate extract of Tigarun leaves (*Crataeva nurvala*). PA: Percentage of peak area

3.3. *Potential of C. nurvala extract compound as a bioinsecticide*

The results of GCMS identification in this study showed that ethyl acetate extract contained more compound variations than methanol extract. Several typical compounds found only in ethyl acetate are assumed to play a role in its effectiveness, making it better than methanol extract (Table 6). Phytochemical characterization in the study [35] using the GCMS technique on *Ageratum conyzoides* plants revealed 8 bioactive compounds that were the same as this study, including in the ethyl acetate extract Hexahydrofarnesyl acetone peak 20 (0.36%); Octadecyl acetate peak 46 (0.93%); 1,2-Benzenedicarboxylic acid, dioctyl ester peak 37 (0.23%); Heneicosane peak 43 (1.78%); and n-Hexadecanoic acid peak 23 (4.54%). Meanwhile, the same compounds in the methanol extract were NEOPHYTADIENE peak 37 (0.33%) also appearing in the ethyl acetate extract peak 19 (0.35%) and 34 (1.06%); Hexadecanoic acid, methyl ester peak 40 which also appeared in the ethyl acetate extract peak 22 (0.36%); and Octadecanoic acid peak 48 (1.16%). The extract of *A. conyzoides* with chloroform solvent (soxlet method) based on the study had the highest toxicity to *S. litura* larvae at a dose of 1000 ppm in the second instar (98.23%) compared to other doses [35].

Table 5. Compounds from Identification of Methanol and Ethyl Acetate Extracts of Tigarun (*Crataeva nurvala*) Leaves

Compound Name	Formula	Molecular Weight	Methanol			Ethyl Acetate		
			Peak	RT	% Area	Peak	RT	% Area
1,3-Butanediol	C4H10O2	90	1	3.32	0.82		-	
Propanal, 2,3-dihydroxy-Glycerose	C3H6O3	90	2	3.86	0.88		-	
			3	5.21	2.14			
4-Octen-3-one	C8H14O	126	4	6.13	1.04		-	
1-isothiocyanato-2-methylpropane	C5H9NS	115	5	6.43	0.27		-	
1,2,3-Propanetriol (CAS) Glycerol	C3H8O3	92	6	7.51	19.74	4	7.38	1.07
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C6H8O4	144	12	13.54	1.29		-	
2-Furancarboxaldehyde, 5-(hydroxymethyl)- (CAS)	C6H6O3	126	13	16.18	8.91		-	
1,2,3-Propanetriol, 1-acetate	C5H10O4	134	14	16.69	0.3	6	11.78	14.62
						11	16.71	8.44
1H-Indole (CAS) Indole or 1-azaindene	C8H7N	117	15	18.42	0.24		-	
Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)- (CAS) Nicotine	C10H14N2	162	18	20.06	0.85		-	
Guanosine (CAS) Guo	C10H13N505	283	20	22.79	6.36		-	
1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan)	C6H10O5	162	22	23.99	10.8		-	
Ethanone, 1-(4-hydroxy-3-methoxyphenyl)- (CAS) Acetovanillone	C9H10O3	166	25	26.40	0.76		-	
alpha.-L-Galactopyranoside, methyl 6-deoxy- (CAS) Methyl .alpha.-L-fucopyranoside	C7H14O5	178	28	27.25	1.6		-	
CYCLOBUTENE-3,4-DIOL, TETRAMETHYL-	C8H14O2	142	30	29.17	0.59		-	
Tetradecanoic acid (CAS) Myristic acid	C14H28O2	228	33	30.57	0.28	16	30.57	0.33
			41	34.98	6.23			
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6.beta.-hydroxy-4,4,7a.beta	C11H16O3	196	34	30.93	0.7	17	30.91	0.49
NEOPHYTADIENE or 2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECANE	C20H38	278	37	32.41	0.33	19	32.42	0.35
						34	39.98	1.06

Table 5 continued. Compounds from Identification of Methanol and Ethyl Acetate Extracts of Tigarun (*Crataeva nurvala*) Leaves

Compound Name	Formula	Molecular Weight	Methanol			Ethyl Acetate		
			Peak	RT	% Area	Peak	RT	% Area
PHYTOL or 3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	39	33.35	0.19	27	38.04	10.14
			44	38.04	2.58	31	38.78	0.28
Hexadecanoic acid, methyl ester	C17H34O2	270	40	34.30	0.67	22	34.29	0.36
9,12-Hexadecadienoic acid, methyl ester	C17H30O2	266	42	37.7	0.32		-	
6-Octadecenoic acid, methyl ester, (Z)-	C19H36O2	296	43	37.8	0.55	26	37.84	0.39
11,14-Eicosadienoic acid, methyl ester	C21H38O2	322	45	38.3	0.52		-	
9-Hexadecenoic acid	C16H30O2	254	46	38.5	2.85		-	
Octadecanoic acid	C18H36O2	284	48	38.9	1.16		-	
Dioctyl adipate	C22H42O4	370	49	43.1	0.74		-	
Stigmast-5-en-3-ol, (3.beta.,24S)- (CAS) Clionasterol	C29H50O	414	51	58.5	3.58	50	58.52	10.6
2-Pentanone, 4-hydroxy-4-methyl-	C6H1202	116		-		1	4.11	2.02
Benzene, 1,3-dimethyl-	C8H10	106		-		2	4.64	0.39
						3	4.84	0.45
1,8-CINEOLE /Eucalyptol	C10H18O	154		-		5	9.88	0.26
1,3-Diacetoxypropane	C7H12O4	160		-		8	13.0	0.31
1,2-Ethanediol, diacetate	C6H10O4	146		-		9	13.1	0.9
Ethanol, 2-(diethylamino)-, N-oxide	C6H15NO2	133		-		10	15.2	0.21
Hexanoic acid, hydroxy-, methyl ester	C7H14O3	146		-		12	23.2	0.27
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	C11H16O2	180		-		14	25.1	0.24
Hexahydrofarnesyl acetone	C18H36O	268		-		20	32.5	0.36
Farnesyl Acetone	C18H30O	262		-		21	34.0	0.21
						35	42.8	0.23
Hexadecanoic acid	C16H32O2	256		-		23	34.9	4.54
2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl	C15H26O	222		-		24	35.7	0.28
12,15-Octadecadienoic acid (Z,Z)-, methyl ester. 11-Octadecenoic acid, methyl ester	C19H34O2	294		-		25	37.7	0.2
						28	38.3	0.69
9,12-Octadecadienoyl chloride, (Z,Z)-	C18H31ClO	298		-		29	38.5	2.16
HEPTADECENE-(8)-CARBONIC ACID-(1), Oleic acid	C18H34O2	282		-		30	38.6	0.64
						32	38.9	1.2
Dioctyl hexanedioate	C22H44O4	370		-		36	43.1	0.96
1,2-Benzenedicarboxylic acid, dioctyl ester	C24H38O4	390		-		37	45.5	0.23
n-Hentriacontane	C31H64O	452		-		38	48.1	0.36
Cyclo Octacosane	C28H56	392		-		39	51.1	0.92
Heptadecyl acetate	C19H38O2	298		-		40	52.6	0.22
Tetradecanal	C14H28O	212		-		41	53.2	0.2
2H-1-Benzopyran-6-ol, 3,4-dihydro-2,7,8-trimethyl-2-(4,8,12-trimethyl tetradecyl)-	C28H48O2	416		-		42	53.3	2.01
Heneicosane	C21H44	296		-		43	54.1	1.78
1-Hexacosene	C26H52	364		-		44	54.2	4.06
Octadecane	C18H38	254		-		45	55.8	0.73

Table 5 continued. Compounds from Identification of Methanol and Ethyl Acetate Extracts of Tigarun (*Crataeva nurvala*) Leaves

Compound Name	Formula	Molecular Weight	Methanol			Ethyl Acetate		
			Peak	RT	% Area	Peak	RT	% Area
Octadecyl acetate	C20H40O2	312		-		46	56.0	0.93
Ergost-5-en-3-ol, (3beta,24R)-	C28H48O	400		-		47	56.6	1.53
Tetratetracontane	C44H90	619		-		49	57.9	17.2
						53	63.2	1.89
n-Pentacosane	C25H52	352		-		52	60.3	0.91

Note: % Area= Percentage of area. RT=Retention Time

The compound HEPTADECENE-(8)-CARBONIC ACID-(1) or Oleic acid appearing in peak 30 (0.64%) and at peak 32 (1.2%) and 12,15-Octadecadienoic acid (Z,Z)-, methyl ester at peak 25 (0.2%) and 28 (0.69%) in ethyl acetate extract, 6-Octadecenoic acid, methyl ester, (Z) in methanol extract appearing at peak 43 (0.55%) and ethyl acetate at peak 26 (0.39%) based on [36] has biological activity as a pesticide. This indicates that ethyl acetate extract contains more potential compounds as insecticides than methanol extract.

Other identified compounds also showed growth inhibitory activity based on research in a trial on *S. litura* and *Helicoverpa armigera* larvae. These compounds included in the methanol extract, i.e. 1,2,3- Propanetriol (19.74%) at peak 6; Hexadecenoic acid at peak 46 (2.85%); in the ethyl acetate extract Octadecadienoic acid at peak 25 (0.2%) and 28 (0.69%); and Tetradecanoic acid at peak 16 (0.33%) that also appeared in the methanol extract at peak 33 (0.28%) and peak 41 (6.23%) [37]. In addition to potential compounds having bioinsecticide activity, several compounds have also been identified in several plants that have the potential as natural pesticides. The insecticidal properties of *Citrus hystrix* leaf essential oil against *S. litura*, have been proven to effectively kill and affect the larvae development. One of the essential oils contained in the study was 1,8-CINEOLE or Eucalyptol. 1,8-CINEOLE was identified in the ethyl acetate extract with a peak area percentage of 0.26% [38].

The compound 4-Octen-3-one (1.04%) in the methanol extract appearing at peak 4 is part of the essential oil compound isolated from *Teucrium polium* L., which in the study of [39] showed that *T. polium* L. had biopesticide properties against Tribolium castaneum with a mortality rate of 97%. In addition, research on the identification of insecticidal compounds in methanol extract of *Terminalia arjuna* tree bark by [40] reported that 4H-Pyran-4-one, which appeared at peak 12 (1.29%), along with other compounds found in this study, i.e. Tetradecanoic acid, Hexadecanoic acid, n-Hexadecanoic acid, and Octadecanoic acid have insecticidal activity based on the NIST library. Tetradecanoic acid or Myristic acid with a fairly high content (16.3%) was also identified using GCMS in vegetable oil such as palm kernel oil (PKO) [41].

Terpenoids are the compounds with a bitter taste (bitter) which functions as anti feeds against insects. GCMS analysis of both Tigarun leaf extracts (*C. nurvala*) showed that there were several types of compounds included in the terpene group. At least one terpene compound from the methanol extract was identified in this study, namely, Phytol (diterpene group). While in the ethyl acetate extract, Phytol, 1,8-CINEOLE, Eucalyptol (Monoterpene) were found; Farnesyl Acetone; and Hexahydrofarnesyl acetone.

Hexahydrofarnesyl acetone which appeared at peak 20 with a concentration (0.36%), is a sesquiterpenoid compound that has broad biological activities, such as antimicrobial, allelopathic, cytotoxic, and antifeedant activities [34]. Terpenoids are absorbed by the middle digestive tract, which functions as a place for enzymatic destruction of food. The entry of these compounds disrupts the secretion of digestive enzymes, the absence of digestive enzymes will disrupt digestive metabolism [42]. If this takes place continuously, it will cause *S. litura* larvae to die due to the lack of nutrients for their survival.

Table 6. Potential bioactive compounds as biopesticides in Tigarun leaves (Crataeva nurvala) methanol and ethyl acetate extracts

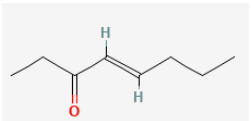
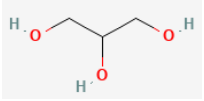
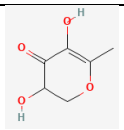
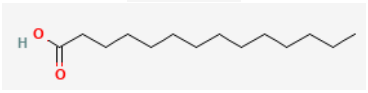
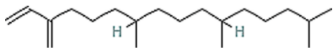
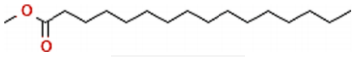
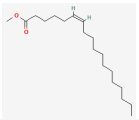
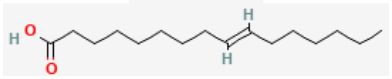
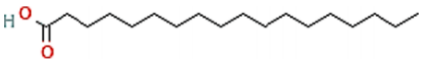
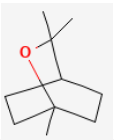
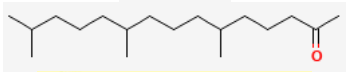
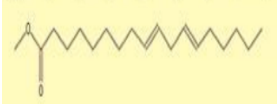
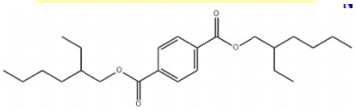
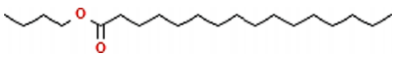
Compound Name	Structure	Concentration (%)	
		Methanol	Ethyl Acetate
4-Octen-3-one		1.04	-
1,2,3-Propanetriol (CAS) Glycerol		19.74	1.07

Table 6 continued. Potential bioactive compounds as biopesticides in Tigarun leaves (Crataeva nurvala) methanol and ethyl acetate extracts

Compound Name	Structure	Concentration (%)	
		Methanol	Ethyl Acetate
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl		1.29	-
Tetradecanoic acid (CAS) Myristic acid		0.28; 6.23	0.33
NEOPHYTADIENE		0.33	0.35; 1.06
Hexadecanoic acid, methyl ester		0.67	0.36
6-Octadecenoic acid, methyl ester, (Z)-		0.55	0.39
9-Hexadecenoic acid		2.85	-
Octadecanoic acid		1.16	-
1,8-CINEOLE atau Eucalyptol		-	0.26
Hexahydrofarnesyl acetone		-	0.36
n-Hexadecanoic acid		-	4.54
12,15-Octadecadienoic acid (Z,Z)-, methyl ester. 11-Octadecenoic acid, methyl ester		-	0.2; 0.69
HEPTADECENE-(8)-CARBONIC ACID-(1), Oleic acid		-	0.64; 1.2
1,2-Benzenedicarboxylic acid, dioctyl ester		-	0.23
Heneicosane		-	1.78
Octadecyl acetate		-	0.93

4. Conclusion

In this study, ethyl acetate solvent was found to have the highest effectiveness in causing mortality with LC50 reaching 11% on the third day, decrease eating ability, and decrease normal weight of caterpillars compared to methanol solvent (LC50 3.44%). The best concentration used for both extracts was 10%. In methanol extract, 10% extract caused mortality reaching 84% on the third day, and 85% on the seventh day. While the concentration of 10% ethyl acetate extract killed caterpillars on the 3rd and 7th days as much as 97% and 98% respectively. Ethyl acetate extract contains more potential compounds (14 compounds) as natural insecticides compared to methanol extract (9 compounds).

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