RESEARCH ARTICLE

Larvicidal Activity of Ethanol Extract of *Cinnamomum sintoc* Blume Bark against *Aedes aegypti*: Role of the Extract as an Acetylcholinesterase Enzyme Inhibitor

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ABSTRACT

The display ponders evaluated the larvicidal exercises of ethanol bark extricates of *Cinnamomum sintoc* BI against the third instar hatchlings of *Aedes aegypti*. For superior understanding, this show ponders moreover examined the instrument of plant extricates against the hatchlings through in silico examination. Larvicidal exercises of plant extricate were considered within the extent of 0.01 to 0.1% and two control bunch with aquades and temephos within the research facility bioassays against early third instar hatchlings of *A. aegypti*. The mortality was checked and subjected to probit investigation to decide the deadly concentrations (LC50 and LC90) to murder 50 and 90% of the treated hatchlings of the particular species. Also, to examine the instrument, four dynamic compounds within the plant extricate with the most noteworthy substance analyzed in silico with the acetylcholinesterase protein in *A. aegypti*. The appears about appear expanding the concentration will increment the passing of the hatchlings and the viability of the extricate is found to be superior to temephos. The comes about of in silico investigation appeared that the two dynamic compounds in plant extricates, specifically α-copaene and γ-muurolene have a component of activity comparable to temephos. Both of these compounds work as inhibitors of acetylcholinesterase chemicals. Our information recommends that the bark ethanol extricate of *C. sintoc* BI have the potential to be utilized as a larvicidal operator for the control of the *A. aegypti*.

Keywords: *Aedes aegypti*, *Cinnamomum sintoc* BI, Larvicide

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INTRODUCTION

Indonesia is one of the endemic areas for dengue virus infection. The disease is transmitted by the *Aedes* sp mosquito.[1-2] About 97% of provinces in Indonesia are endemic areas for this disease.[3] This disease is still one of the health problems in Indonesia due to the increase in the number of cases and deaths every year. Data from the Indonesian Ministry of Health states that in 2019 dengue cases increased by 65,602 compared to 2018. Meanwhile, the number of deaths due to this disease also increased from 467 in 2018 to 919 in 2019.[4]

As mentioned in the previous paragraph, dengue virus infection is transmitted through the *Aedes* sp. mosquito.[5] The development of this mosquito is influenced by many factors including human population density and humidity. A humid area like rivers, rice fields, and swamps are very good places for mosquito breeding. In addition, human density as a source of food source is an attraction for mosquitoes to survive.[6,7] To inhibit this development, an agent that can kill mosquito larvae (larvicide) is used. The using of this larvicide agent prevents the mosquitoes from developing into adult form.[8]

One of the most widely used chemical larvicidal agents in Indonesia is temephos. Temephos has been used in Indonesia since 1980. The use of temephos is known to have several negative impacts. Long-term use of temephos is known to increase resistance, resulting in decreased of effectiveness.[9] In addition, temephos also cause other environmental

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problems because the difficulty of decomposition, resulting in environmental pollution. Therefore, it is necessary to develop new, more potent larvicides with better environmental impact.[10]

One potential larvicide with better effects on the environment is the use of plant-based larvicides. Several studies have revealed this.[11] Research by Awalludin *et al.*[8] states that ethanol extract of *Morinda citrifolia* leaves. L has the potential as an *Aedes* sp larvicide with LC_{50} and LC99 of 1040 ppm and 2439 ppm, respectively. Research by Botelho *et al.*[12] also found that *Ocimum basilicum* var. minimum (L.) Alef. can be a larvicide against *Aedes* sp with LC_{50} of 69.91 (µg/mL) and LC90 of 200.62 (µg/mL). Some of these results suggest that the potential of these plants as larvicides is due to the phytochemical compounds that can act as inhibitors of the enzyme acetylcholinesterase in larvae.[13]

Cinnamomum sintoc Bl is a plant that grows in the South Kalimantan region. This plant contains phytochemical compounds and has the potential to be utilized as antioxidant, antifungal, and antibacterial. These phytochemical compounds also have the potential to be utilized as larvicides.[14-15] Until now, research on the larvicidal activity of *C. sintoc* has not been widely revealed. To prove this, it is necessary to conduct larvicidal activity research on *C. sintoc* BI. In addition, to find out the mechanism of these plants as larvicides, an in-silico study will also be carried out on this plant to inhibit the growth of *Aedes aegypti* larvae.

MATERIAL AND METHODS

Plant Material and Extraction

New bark of *C. sintoc* Bl were assembled from Loksado, South Hulu Sungai, South Kalimantan, Indonesia amid the stormy season in 8th September 2022. Sometime recently extraction, the plant's materials were cleaned and washed beneath the running tap water, at that point amplified by ground in one layer, and cleared out dry within the shade interior the restrain at room temperature, absent from sunlight. After this period, the dried plant ought to be pounded by a processor to obtain a powder shape and after that protected in a holder absent from light, warmth, and dampness for afterward utilization.

A powdered test of *C. sintoc* Bl barks put in a maceration holder. The 70% ethanol was included until the simplicial was totally submerged. At that point cleared out for 24 hours, mixing once in a while. At that point sifted and isolated the dregs and filtrate. The residue was macerated once more employing a modern ethanol channel. This was done for 3 continuous days and the ethanol was supplanted 3 times each 24 hours.

Preparation of *Aedes aegypti* Larvae

The eggs of *A. aegypti* were obtained from Parasitology Laboratories, Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, Indonesia. The eggs were placed in to covered trays containing 750 mL of filtered water. After that, eggs are colonized until they hatch and become larvae. Egg hatching is done by adjusting the room temperature in the range of 26 to 27°C with a room temperature regulator. The eggs will hatch in about 1 to 2 days and become first instar larvae. Furthermore, the larvae were fed on a chicken liver-based diet until they reached the third instar stage, determined by the head/body size ratio.

Larvicidal Activity Analysis

The larvicidal effect was assessed by using the procedure as described earlier by Tarmadi *et al.*[16] with some modifications. The analysis was divided into 7 groups of treatment. Group 1 served as a negative control (K-) which consists of equates, while group 2 served as a positive control (K+) which consists of 1% temephos (Abate ®). Group 3, 4, 5, 6, and 7 served as an experimental group (P1, P2, P3, P4, and P5) which consists of plant extracts with concentrations 0.001, 0.005, 0.01, 0.05, and 0.1%, respectively. Twenty-five third-instar of *A. aegypti* larva were subjected to 150 mL aquades of each treatment. Four replications were prepared and the numbers of dead larvae were counted at 24-hour exposure.

In-silico Analysis

The target protein used was the acetylcholinesterase enzyme in *A. aegypti* obtained from AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/) with the uniprot code A0A650ATI0. Then, the acetylcholinesterase protein structure was saved in pdb format. Meanwhile, four active compounds found in *C. sintoc* Bl with the highest content were selected as ligands.[17] The compounds include linalool, α-copaene, tetradecanal, and γ-muurolene. The compounds were then entered into the Pubchem chemical compound structure database (https://pubchem.ncbi.nlm.nih.gov/)[18] and obtained the code for each ligand, such as, linalool (CID: 6549), α-copaene (CID: 19725), tetradecanal (CID: 31291), and γ-muurolene (CID: 6432308). Furthermore, temephos (CID: 5392) was used as a control. Then, all the ligands were saved in pdb format.

After that, docking was done with peptide and ligand using SeamDock (https://bioserv.rpbs.univ-paris-diderot.fr/services/SeamDock/). The docking results include affinity (Kcal/mol) and interaction between ligand and protein, namely hydrogen bonding and hydrophobic bonding.[19]

Statistical Analysis

The results were presented as mean ± SD, and to perform statistical analysis, MS Office Excel 2019 was used. All the data were then analyzed by One Way ANOVA test to determine the statistical differences between the control group and the five treatment groups and followed by Mann-Whitney test at a significance level of $p < 0.05$. The average larval mortality data were subjected to probit analysis for calculating LC50 and LC90 (lethal concentration) values and their 95% confidence limits were estimated by fitting a probit regression model to the observed relationship between the percentage of mortality of larvae and logarithmic concentration of the substance. All this statistical analysis were performed using the SPSS (Statistical Package Social Science) software version 13.0 for Windows 11.

RESULTS

The results of this present study shows that an increase in the concentration of the extracts led to an increase in larval mortality within 24 hours. This can be seen in Figure 1.

The results of measurable investigation showed that there were noteworthy contrasts within the mortality of *A. aegypti* hatchlings in different treatment bunches with bunches K- and K+ ($p = 0.014$; $p < 0.05$). The comes about too appeared that there were noteworthy contrasts between bunches P1, P2, P3, P4, P5 with K+. This implies that the concentration used in this consideration isn't comparable to P2 but is successful as a larvicide. In expansion, the comes about of probit examination showed LC_{50} and LC_{90} values of 0.043 and 0.090%, individually.

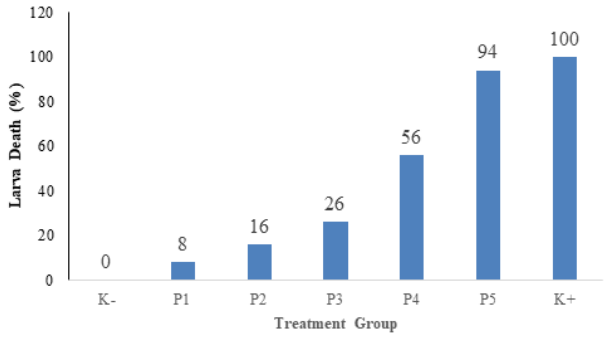

Figure 1: Larval mortality in various treatment groups

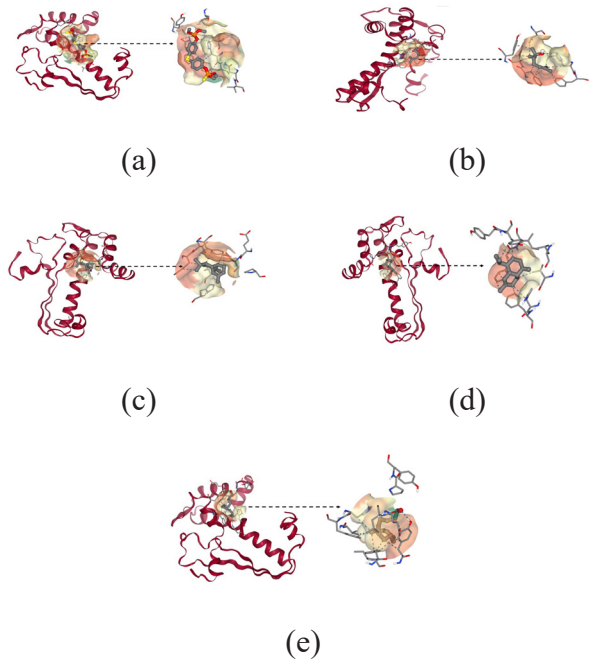

Figure 2: Interaction of bioactive compounds of sintok stem bark with acetylcholinesterase (a) temephos (b) linalool (c) α-copaene (d) γ-muurolene and (e) tetradecanal

The comes about of atomic docking investigation appeared an interaction between the larval acetylcholinesterase protein and different dynamic compounds of *C. sintoc* Bl. The interaction that happens can be seen in Figure 2.

The results of molecular docking analysis tests including affinity, hydrogen bonding, and hydrophobic bonding between the active compounds of *C. sintoc* Bl and the larval acetylcholinesterase enzyme are presented in Table 1.

DISCUSSION

The results showed that ethanol extract of *C. sintoc* Bl stem bark has a better larvicidal effect than temephos. Temephos is one of the insecticides of choice in controlling vectors of dengue virus infection. Temephos is a class of organophosphates or organic phosphates.[20] The larvicidal effect of temephos is through the inhibition of the enzyme acetylcholinesterase.[21] This inhibition is because temephos interacts with the acetylcholinesterase enzyme at residues Phe35 and His104 through hydrophobic bonds. In addition, temephos also works through hydrogen bonds at residues Phe35 and Leu64 in the acetylcholinesterase enzyme.

The results showed that the affinity of α-copaene and γ-muurolene with the acetylcholinesterase enzyme was higher than temephos. This is based on the affinity value of α-copaene and γ-muurolene with the acetylcholinesterase enzyme. The affinity value is the number of atoms or molecules bound to a protein or receptor residue. The more negative the affinity value, the more residues are bound to the protein or receptor.[22,23]

The α-copaene and γ-muurolene are active components that have a more negative affinity than temephos. These compounds can cause digestive disorders and inhibit the work of the cholinesterase enzyme in larvae. The γ-murolene is a compound that works by binding to the same amino acid residues as termephos, namely residues Phe35 and His104, while α-copaene works on residues Phe35 and Leu64. Thus, the compounds γ-muurolene and α-copaene also have the ability to inhibit acetylcholinesterase.

Table 1: Interaction between active compounds and amino acid residues on acetylcholinesterase

Ligand	Molecular weight (g/mol)	Binding affinity (Kkal/mol)	Hydrophobic bond	Hydrogen bond
temephos	466.5	-5.2	Phe35; His104	Phe35; Leu64
linalool	154.25	-4.8	Phe35; Tyr39; Leu64; Val100; His104; Phe104	His104
α-copaene	204.35	-6.3	Phe35; Tyr39; Leu64; Val100	
γ-muurolene	204.35	-6.9	Phe35; Tyr39; Val100; His104; Phe105	
tetradecanal	212.37	-4.7	Phe35; Tyr39; Leu64; Val100; His104; Phe135	Tyr39; Glu63

Cholinesterase enzyme plays an important role in neuromuscular processes. It is a serine hydrolyzing enzyme and catalyzes the breakdown of acetylcholine into acetate and choline. This process will form a substrate enzyme complex followed by acylation of the hydroxyl group of the amino acid serine and deacylation. Choline, which functions to conduct nerve impulses, will be reduced. If there is an accumulation of acetylcholine, various abnormalities in the body's work will occur. The mechanism of acetylcholine inhibition is initiated by the introduction of α-copaene and γ-muurolene which bind to amino acid residues on the active site of the acetylcholinesterase enzyme. The binding causes inhibition of this enzyme so that acetylcholine is not hydrolyzed into acetate and choline. As a result, acetylcholinesterase does not function and there is a buildup of acetylcholine at the nerve endings, so the nerves in the body continuously send commands to certain muscles. This causes the muscles to contract uncontrollably, leading to the death of the larvae.[24]

CONCLUSION

In conclusion, our findings showed that ethanol bark extract of *C. sintoc* BI can be developed as a larvicide. This plant extract works through a mechanism similar to temephos, namely as an inhibitor cholinesterase enzyme. Further research may be needed to determine whether the active compounds in this plant extract actually have larvicidal effects. Furthermore, the toxic effect of the plants on animals and humans should also be checked, only then they can be deemed safe for human use.

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