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Larvicidal Activity of Ketapang Leaf Fraction (*Terminalia catappa* L) on *Aedes aegypti* Instar III

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Abstract

BACKGROUND: Mosquito control is essential in preventing mosquito-borne diseases. Natural substances originated from plants possessed the great potential of insecticidal properties, the resistance occurs at a slower rate compared to the synthetics, with less toxicity to other living creatures.

AIM: This study aimed to identify the fraction of ketapang leaf (Terminalia catappa L) with larvicidal activity on Aedes aegypti instar III larvae, the concentration of ketapang active leaf fraction comparable to temephos, and Lethal Concentration 50 (LC50).

METHODS: This study was experimental in vitro. The experiment was performed with five different concentrations of ketapang leaf water-ethanol, i.e. 1200 ppm, 1400 ppm, 1600 ppm, 1800 ppm, 2000 ppm. Observation of morphological damage of mosquito larvae was conducted with the microscope and ImageJ application. Data analysis was performed using One Way ANOVA. LC50 was determined with probit analysis.

RESULTS: Phytochemical screening revealed a water-ethanol fraction of ketapang leaf contained tannin, saponin, and flavonoid compounds. The water-ethanol fraction with 1800 ppm concentration possessed significant larvicidal effect comparable to temephos. Probit test revealed an LC50 value of 1563.082 µg/ml.

CONCLUSION: Water-ethanol fraction of ketapang leaf possessed high biolarvicidal activity against Ae. aegypti larvae instar III comparable to temephos.

Introduction

Aedes aegypti mosquitoes are responsible for the epidemics of dengue in populations, with several affecting factors comprising climatic conditions (tropics and subtropics), population growth and travels. In recent decades, the incidence has rapidly arisen around the world. It is estimated there are 390 million dengue infections per year (95% credible interval 284 – 528 million), with clinical manifestations of 96 million (67 – 136 million). In another study, 3.9 billion people, in 128 countries, are at risk of dengue infection with an estimated 2.5% annual case fatality [1].

Mosquito control is essential in preventing mosquito-borne diseases. Mosquitocidal factors that interrupt vector ecology are ovicidal, larvicidal, pupicidal, and adulticidal and include organochlorides, organophosphates, and synthetic pyrethroids [2]. One dominant practice in mosquito control is the use of synthetic insecticides such as organochlorine and organophosphate compounds. However, continuous and repeated use of synthetic insecticides can lead to environmental pollution, the death of various species of living creatures, and it is possible to raise resistances of various species of mosquitoes as diseases vector. Interest in alternative methods of mosquito control with less environmental damage has commenced. Natural substances originated from

plants possessed the great potential of insecticidal properties, the resistance occurs at a slower rate compared to the synthetics, with less toxicity to other living creatures [3], [4].

Abundant prior studies had proven the potential of natural sources as an alternative in insecticides. Hirota _et al._ performed a study to investigate the larvicide activities of _Smilax larvata Griseb._ (Smilacaceae) extracts against _Ae. aegypti_ larvae. The crude ethanolic extract presented larvicidal effect on instar III _Ae. aegypti_ larvae [5]. A study by Ashwini _et al._ discovered _Acalypha indica_ leaf extract possessed larvicidal activities against dengue vector _Aedes albopictus_. Instar III larvae of _Ae. albopictus_ were exposed to 1000, 2000, 3000, 4000 and 5000 ppm concentrations of petroleum ether, chloroform, ethyl acetate, n-butanol, ethanol and aqueous extracts of _A. indica_ [6].

El-Akhal _et al._ revealed the properties of larvicidal activity of essential oils of _Thymus vulgaris_ and _Origanum majorana_ family of Lamiaceae against the larvae of the malaria vector _Anopheles labranchiae_ [7]. Ramar _et al._ found the efficacy of essential oils (EOs) as anti-mosquito agents and its adulticidal prospective of the essential oils against _Culex quinquefasciatus_ [8].

These studies further develop natural sources as an alternative to chemicals in insecticide. Ketapang possesses the properties of antimicrobial, antioxidative, anti-inflammatory, hepatoprotective, antidiabetic, anticarcinogenic, antimalaria, and antinociceptive [9], [10], [11], [12], [13], [14], [15], [16], [17]. Natural products isolated from ketapang comprising of triterpenoids (ursolic acid, Asiatic acid), squalene but no caffeine, flavonoids (isovitexin, vitexin, and rutin), gallic acid, hydrolysed tannins such as punicalagin anomers as a major component, punicalin, terflavins A and B, tergallagin, tercatain, chebulagic acid, geranin, granato B, and corilagin [12], [18].

This study aimed to identify the fraction of ketapang leaf (_Terminalia catappa L_) with larvicidal activity on _Aedes aegypti_ instar III larvae, the concentration of ketapang active leaf fraction comparable to temephos, and Lethal Concentration 50 (LC50).

Material and Methods

This study was experimental in vitro performed from February to March 2018. Ketapang leaf extraction and fractionation were conducted at Biomolecular Laboratory, Faculty of Medicine, Sriwijaya University, Palembang. Ketapang leaf was obtained from Cibanteng Village, Ciampea District, Bogor, Indonesia. The dried leaves were mashed with a blender and sieved to obtain the fine powder. The fine powder was macerated with 96% ethanol for 2 x 24 h. The macerate was evaporated using a water bath for 2 h with a temperature of ± 80°C until thickened and pasty mass was formed. The fractionation process was carried out by liquid-liquid fractionation method in which the active fraction was partitioned in the separation funnel.

The efficacy test of ketapang leaf fraction against _Ae. aegypti_ larvae were performed at Entomology Laboratory of Lokalitbang P2B2, Baturaja, Ogan Komering Ulu Regency, South Sumatra Province. The efficacy test was performed with a preliminary test on _Ae. aegypti_ instar III larvae with three ketapang leaf fractions using n-hexane, ethyl acetate and water-ethanol at 2000 ppm concentration and observed for 24 h. The preliminary test exhibited that water-ethanol fraction possessed the highest larvicidal activity on _Ae. aegypti_ instar III larvae with larvae death of 96.67% at 2000 ppm concentration. The water-ethanol fraction was selected to undergo further investigation with five concentrations. After obtaining the fraction concentration in the preliminary test, the experimental group were treated with five different concentrations of water-ethanol ketapang leaf fraction, i.e. 1200 ppm, 1400 ppm, 1600 ppm, 1800 ppm, 2000 ppm. Temephos (Abate®, BASF, Ludwigshafen, Germany) 1000 ppm dose was used as a positive control and aqua destilata as the negative control. In each experimental glass, 30 _Ae. aegypti_ larvae instar III and 100 ml of water was inserted. To obtain the optimal concentration to kill the larvae, the process was repeated three times.

Observation of morphological damage of mosquito larvae before and after treatment was performed under an Axioplan (Zeiss) microscope and images were captured with the digital camera AxioCam HRC (Zeiss) and ImageJ application. Data analysis was conducted using One Way ANOVA with SPSS 21.0 software (SPSS Inc., Chicago, USA), followed by Games Howell post hoc test. LC50 was determined using probit analysis.

Results

Phytochemical screening of ketapang leaf on n-hexane fraction revealed the contents of steroid, terpenoid, saponin, and flavonoid compounds, while ethyl acetate and water-ethanol fraction contained tannin, saponin, and flavonoid compounds. The preliminary test exhibited that water-ethanol fraction possessed the highest larvicidal activity on _Ae. aegypti_ instar III larvae with larvae death of 96.67% at 2000 ppm concentration. The water-ethanol fraction was selected to undergo further investigation with five concentrations. Figure 1 exhibited the death of _Ae._

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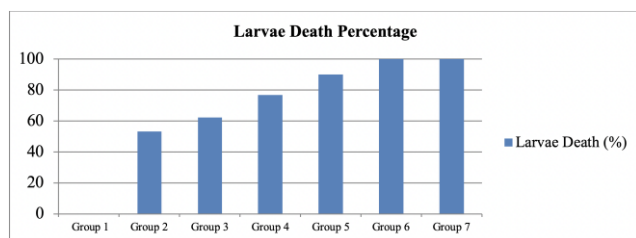*aegypti* larvae were at its highest (100%) at 2000 ppm ketapang leaf concentration.

Figure 1: Percentage of Ae. aegypti instar III larvae deaths 24 h after administration of water-ethanol fraction of ketapang leaf (T. catappa L); Group 1: aqua destilata; Group 2: ketapang leaf 1200 ppm; Group 3: ketapang leaf 1400 ppm; Group 4: ketapang leaf 1600 ppm; Group 5: ketapang leaf 1800 ppm; Group 6: ketapang leaf 2000 ppm; Group 7: temephos

The average percentage of morphological damage of instar III larvae such as head, piston, abdomen, siphon, and anal papillae from various ketapang leaf concentrations and controls were presented in Table 2.

Table 2: Percentage of morphological damage to Ae. aegypti instar III larvae after administration of water-ethanol fraction of ketapang leaf (T. catappa L)

Group	Mean percentage (%) of *Ae. aegypti* larvae damage				
	Head	Pectoral	Abdomen	Siphon	Anal papillae
1	1.64	1.39	1.69	1.67	1.69
2	69.56	60.36	50.37	54.57	30.54
3	67.19	56.73	48.56	53.67	30.53
4	63.20	55.98	45.83	51.94	28.18
5	59.58	44.98	31.51	49.29	21.35
6	59.76	43.16	29.87	47.53	21.88
7	70.64	65.39	55.69	55.67	36.69

Group 1: aqua destilata; Group 2: ketapang leaf 1200 ppm; Group 3: ketapang leaf 1400 ppm; Group 4: ketapang leaf 1600 ppm; Group 5: ketapang leaf 1800 ppm; Group 6: ketapang leaf 2000 ppm; Group 7: temephos.

Table 2 showed the highest occurrence of morphological damage of the larvae was at 1200 ppm concentration aside from the positive control and was the lowest at 2000 ppm concentration.

Table 3: Lethal Concentration (LC50) from a water-ethanol fraction of ketapang leaf

LC50 (µg/ml)	1563.082

There were significant differences in regards to the number of *Ae. aegypti* instar III larvae death between different concentrations of a water-ethanol fraction of ketapang leaf (p < 0.05), followed by Games-Howell post-hoc test with a p-value at 1800 ppm concentration 0.134 (p > 0.05). Ketapang leaf fraction larvicidal activity at 1800 ppm concentration was comparable to temephos. Probit test exhibited Lethal Concentration (LC50) from a water-ethanol fraction of ketapang leaf at 1563.082 µg/ml.

Discussion

To protect themselves from herbivores, plants possess the evolving secondary metabolites that transform into active toxic ingredients, which insects feed on them. It is potential for insects to be exposed to these toxic secondary metabolites and affected physiologically with various impacts on targets ranging from proteins (enzymes, receptors, signalling molecules, ion-channels and structural proteins), nucleic acids, biomembranes, and other molecular components. It is potential for the receptor sites to be affected, including the alteration of neurotransmitter synthesis, storage, release, binding, and re-uptake, also other metabolic processes such as receptor functionality, enzymes in signal transduction pathway, etc. [19], [20], [21].

Secondary metabolites affected the insect physiology through several mechanisms include essential oils through inhibition of acetylcholinesterase (AChE), disruption of morphogenesis and alteration in the behaviour and memory of cholinergic system, thymol through gamma-aminobutyric acid (GABA) gated chloride channel and octopamine receptors, pyrethrin through sodium and potassium ion exchange disruption, and rotenone through inhibition of cellular respiration, ryanodine through the blockage of calcium channels, sabadilla through nerve cell membrane action, azadirachtin through hormonal balance disruption and mitotic poisoning. Out of several mechanisms, AChE inhibition holds a pivotal role in attenuating neurotransmitter through the synaptic pathway. Altered AChE is the mechanism of insect pests resistance and AChE has been reported to be organophosphorus and carbamate resistant [19], [20], [21].

Phytochemical screening of ketapang leaf revealed water-ethanol fraction contained tannin, saponin, and flavonoid compounds. Tannins can interfere with an insect's ability to digest food and absorb protein, through binding to proteins essential for growth [22]. High cytoplasmic vacuolation, absence of cytoplasmic limits, apical vesicle formation with the release of cytoplasmic contents of the cells, increased intercellular space and detached cells from the basement membrane, are major toxicities caused by tannins in mesenteron cells of *Ae. aegypti* instar III. The mechanisms resemble the processes of insects encountering toxic substances. When tannic acid is utilised against Diptera larvae, histopathological processes initially occurs in the anterior region of the midgut, progressing to the median and posterior regions [23].

On the other hand, saponins exerted membrane-permeabilising and haemolytic properties. Saponins are freely soluble and can be extracted in both aqueous and organic solvents. Saponins attack the cuticle membrane of the larvae, disturbing the membrane, which leads to larval death [24]. Saponins

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increase mortality levels, lower food intake, causing weight reduction, retardation in development, disturbances in development and decreased reproduction in pest insects. The suggested mechanisms underlying these are that saponins act as a repellent on food, or to cause digestive problems due to moulting defects or toxic effects on cells [25]. Flavonoid compounds also possess promising larvicidal potential. Mechanism of action of the compound on mosquito larvae are not yet established, but previous studies demonstrated that chemicals interfered with the mitochondria at the proton sites [26].

The LC50 value of ketapang leaf water-ethanol fraction to the death of *Ae. aegypti* instar III larvae were at 1563.082 µg/ml. LC50 value of the water-ethanol fraction of ketapang leaf was categorised as having an effective larvicidal power, due to its value was still below the WHO standard of concentration value [27].

In conclusion, phytochemical screening revealed a water-ethanol fraction of ketapang leaf contained tannin, saponin, and flavonoid compounds. The water-ethanol fraction of ketapang leaf possessed high biolarvicidal activity against *Ae. aegypti* larvae instar III. The water-ethanol fraction with 1800 ppm concentration possessed significant larvicidal effect comparable to temephos.

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