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Research Article

Larvicide activity of ethanol extracts from Zodia (*Evodia suaveolens S.*) leaves and Cananga (*Cananga odorata*) flowers on *Aedes aegypti* larvae

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ABSTRACT

Controlling the *Aedes aegypti* population in Dengue management programs is often carried out using high doses of synthetic insecticides, such as temephos, that may lead to resistance. An alternative of natural ingredients, such as Zodia (*Evodia suaveolens* S.) leaves, which contain evodiamine, and Cananga (*Cananga odorata*) flower, which contains linalool, appears to be a promising larvicide. This study aims to determine the effectivity of ethanol extracts from Zodia leaves and Cananga flowers on the mortality of *Aedes aegypti* larvae. This experimental study used *Aedes aegypti* larvae at stage III-IV. Each ingredient was extracted using the maceration method and then prepared at 2.5% and 5% concentrations. PEG 400 was also added as a diluent. Observations are performed every 6 hours for 24 hours. Data analysis using Kruskal Wallis test and Dunn's test. Within 24 hours, each study group had 100% larval mortality. The Kruskal-Wallis test resulting a p-value of <0.05. From Dunn's test, comparisons between each study group and the negative and positive control groups yielded p-values <0.05 and 1.000, respectively. Thus, it can be said that ethanol extracts from Zodia leaves and Cananga flowers are as effective as temephos as larvicide on *Aedes aegypti* larvae

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INTRODUCTION

As the most prevalent and rapidly spreading mosquito-borne viral disease, Dengue fever, or Dengue hemorrhagic fever, remains a global health concern, particularly in Southeast Asia (Yang et al., 2021). Temephos is one of the most preferred chemical interventions in dengue control programs due to its low cost, ease of use, and significant efficacy (Martínez-Mercado et al., 2022). However, a new challenge has emerged. There have been several reports of *Aedes aegypti* resistance to temephos. For example, a study conducted by Palomino et al., (2022) in Peru reported resistance in *Aedes aegypti* despite the cessation of temephos use for up to 3 years. Resistance was also reported in a literature review by Saeung et al. (2020), who found *Aedes aegypti* resistance in 73 of 206 sampling sites in Thailand and 25 of 56 sampling sites in Malaysia, Singapore, Myanmar, Laos, and Cambodia. That is why, nowadays, traditional medicine is increasingly being examined by the world for further research, including the use of natural-based ingredients. If the material is proven safe and effective, the WHO (World Health Organization) recommends it be included in the national health system (Akbar, 2020).

Zodia (*Evodia suaveolens S*) is an Indonesian flora traditionally used as a medicinal plant. It is often used as a repellent, especially in the Papua region, by rubbing the leaves against the skin (Iriani & Yanuastri, 2020). In another study by Yabansabra et al. (2023), it was shown that Zodia leaf oil at a concentration of 75% has a protective effect of up to 93.33% after 3 hours of application. The same situation was also reported by Setiyadi et al., (2020), in their study on the larvicidal test of Zodia leaf extract with hexane solvent against *Aedes aegypti* larvae; the LC50 and LC90 at 24 hours were 0.443 ppm and 0.788 ppm. It is known that Zodia leaves have about 23 types of active metabolites, and the most important one is evodiamine or evodone (Iriani & Yanuastri, 2020). This compound is a quinazolinocarboline alkaloid, which shows apparent insecticidal activity, such as feeding cessation, muscular asthenia, and dehydration atrophy of the body (Liu et al., 2022).

In addition to Zodia, another plant that is thought to have larvicidal activity is Cananga, also known as Ylang ylang (*Cananga odorata*) flower, although its use is more common in the cosmetic industry (Chakira et al., 2022; Syed Ismail et al., 2020). The main constituent of this flower is linalool, a kind of acyclic monoterpene tertiary alcohol, about 21.78% (Syed Ismail et al., 2020). This compound inhibits four types of enzymes, i.e. acetylcholinesterase (AChE), monooxygenases (MO), α-esterase, and β-esterase (Huang et al., 2020). Several studies have been conducted to support these findings. The study by Puspita et al., (2022) found that essential oil from Cananga flowers could provide up to 96.3% protection against *Aedes aegypti* in the first 1 hour of application and reduced to 75% after 6 hours, which is undoubtedly a good level of protection. Chaiphongpachara and Laojun, (2020), in their study comparing commercial Cananga essential oil from India and Thailand against *Aedes aegypti* larvae, also stated that both were equally effective.

Many studies have explored the benefits of Zodia leaves and Cananga flowers against *Aedes aegypti*. However, the form used is a repellant and only focuses on their essential oils. Therefore, this study will further investigate the utilization of both as larvicidal agents, with 96% ethanol extract-based materials, which are expected to elicit other secondary metabolites that strengthen the larvicidal effect.

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METHODS

This experimental laboratory study has a post-test-only controlled group design. Each plant-based material was extracted using the maceration method using ethanol at a concentration of 96%. First, they were dried under the sun for about a week and then crushed into powder. The powder was then weighed, and ethanol was added. The mixture is stirred momentarily and left to stand all day. This process was repeated for 7 days with daily stirring. The macerate was then concentrated using a rotary evaporator and water bath to form a thick extract. Each extract produced was then diluted with distilled water and PEG 400 and prepared in two concentrations: 2,5% and 5% (Sutrisna, 2016).

For the larvicide test, we prepared 6 study groups, consisting of a positive control group using temephos, a negative control group using distilled water and PEG, four treatment groups using Zodia leaves and Cananga flower extract at a concentration of 2.5% and 5%, respectively. About 25 larvae of *Aedes aegypti* at stage III-IV were exposed to each group (World Health Organization, 2005). Observation was performed every 6 hours for 24 hours. Larvae that appeared to drown or did not move when a needle or stick probed them were considered dead and then counted. The procedure in each group was repeated 4 times. All the data obtained was analyzed using the Kruskal Wallis and Dunn's tests.

This study has passed the ethical feasibility test conducted by the Faculty of Medicine Ethics Committee of Universitas Muhammadiyah Surakarta with letter number 5093/A.1/KEPK-FKUMS/XI/2023. All of the study process was conducted for about four months. The extraction process was performed at the Pharmacology Laboratory of Universitas Muhammadiyah Surakarta, while the larvicide test was performed at the Parasitology Laboratory of Universitas Muhammadiyah Surakarta.

RESULTS

From the extraction process of 1000 grams of Zodia leaves and Cananga flowers, the yield obtained was 11.3% and 13.7%, respectively. Larval mortality data at each 6-hour observation period are shown in Table 1 as follows.

Table 1. Larval mortality at each 6-hour observation period

Study group	The average number of larval deaths (heads)			
	6 hours	12 hours	18 hours	24 hours
Positive control	25	25	25	25
Negative control	0	0	0	0
2.5% of Zodia leaf extract	18	25	25	25
5% of Zodia leaf extract	25	25	25	25
2.5% of Cananga flower extract	20	25	25	25
5% of Cananga flower extract	24	25	25	25

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Table 2. The Dunn's test result

Groups	Positive control	Negative control	2.5% of Zodia leaf extract	5% of Zodia leaf extract	2.5% of Cananga flower extract	5% of Cananga flower extract
Positive control		0.025*	1.000	1.000	1.000	1.000
Negative control	0.025*		0.025*	0.025*	0.025*	0.025*
2.5% of Zodia leaf extract	1.000	0.025*		1.000	1.000	1.000
5% of Zodia leaf extract	1.000	0.025*	1.000		1.000	1.000
2.5% of Cananga flower extract	1.000	0.025*	1.000	1.000		1.000
5% of Cananga flower extract	1.000	0.025*	1.000	1.000	1.000	

* Significantly different

The larval mortality data were then previously analyzed using the Saphiro-Wilk test and the Levene test to see the data distribution. The results of both tests showed that the data were not normally distributed or homogeneous. Therefore, the data in this study did not qualify for further analysis using parametric tests, so the Kruskal-Wallis nonparametric test was used. The Kruskal-Wallis test is a test of the difference between 2 data groups. The p-value generated in this analysis is <0.05, meaning that at least two groups have significantly different data. Post-hoc Dunn's test was conducted as a further test to find which data in which groups were significantly different. Dunn's test results are presented in Table 2.

DISCUSSION

As shown in Table 1, the extracts exposed to *Aedes aegypti* larvae, both from Zodia leaves and Cananga flowers, both at 2.5% and 5% concentrations, were able to kill *Aedes aegypti* larvae from the first 6 hours of observation. The mortality rate of *Aedes aegypti* larvae in each treatment group in the first 6 hours was varied, with the most consecutive order being the 5% of the Zodia leaf extract group, 5% of the Cananga flower extract group, 2.5% of Cananga flower extract group, and 2.5% of Zodia leaf extract group. This finding is consistent with a study conducted by Yabansabra et al. (2023), who formulated Zodia oil as a repellent. When the concentration of the tested material he used increased, the protective effect also became more substantial. As the concentration of the extract rises, the active ingredients contained also increase, thereby improving the chance of exposure to the tested sample, resulting in a higher expected effect. From Table 1, we can also see that the mortality rate of *Aedes aegypti* larvae reached 100% after 12 hours of observation. This indicates that the longer the exposure, the more active ingredients enter the larval body, causing increased body damage that leads to death. This is in line with a study by Sarma et al. (2019), who observed the ovicidal, larvicidal, and adulticidal activity of *Citrus aurantifolia* against *Aedes aegypti*,

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with observations made at 24, 48, and 72 hours. She stated that as the duration of exposure lengthened, the effects were also more significant.

Data in Table 2, the results from the Dunn's test on larval mortality for 24 hours, show that all treatment groups, when compared with the positive control group, resulted in a p-value of 1.000, while when compared with the negative control group, resulted in a p-value of <0.05. Thus, it can be said that Zodia leaves and Cananga flowers are proven to have larvicidal effects against *Aedes aegypti* larvae. Not only that, but the larvicidal effect is also superior to that of temephos. The addition of PEG, commonly referred to as PEGylation, is intended to increase the solubility of plant extracts in water so that the active ingredients are evenly distributed and may increase their toxic effect on larvae. On the other hand, its non-toxic effects will not affect larval mortality, so the larval mortality in this study was caused solely by the active ingredient of the plant extract, not by PEG (Dewi et al., 2023).

The larvicidal effect shown by ethanol extracts of Zodia leaves and Cananga flowers is due to the presence of metabolites extracted during the maceration process, such as flavonoids, alkaloids, tannins, and saponins, in addition to the main content of Zodia, evodiamine, and the main content of Cananga flowers, linalool (Chaiphongpachara & Laojun, 2020; Iriani & Yanuastri, 2020; Puspita et al., 2022; Setiyadi et al., 2020; Syed Ismail et al., 2020; Yabansabra et al., 2023). Flavonoids are a group of plant phenolic compounds widely distributed and most abundant in all parts of plants. Flavonoids penetrate the larval body through the siphon and work as respiratory toxins. Larvae with a damaged respiratory system will compensate for this damage by positioning their bodies parallel to the water surface to facilitate oxygen uptake (Zuharah et al., 2021). Alkaloids are relatively temephos-like metabolites that act as neurotoxins. Alkaloids affect the sodium channels in larval nerve cell membranes, inhibiting the transmission of nerve impulses and triggering seizures or paralysis (de Souza Wuillda et al., 2019). Tannins are digestive toxins that reduce the activity of protease enzymes in the formation of amino acids. At the same time, saponin is a combination of alkaloids and tannins because it works as a neurotoxin and a digestive toxin (Redo et al., 2019).

Other than these metabolites, this study highlights evodiamine and linalool. Evodiamine is a type of alkaloid, so its mechanism of action resembles that of alkaloids, namely as a neurotoxin. It is believed that Ryanodine receptors (RyR) are the main target of evodiamine (Liu et al., 2024). Ryanodine receptors (RyR) are located in the sarcoplasmic/endoplasmic reticulum membrane and are responsible for calcium release from intracellular stores during excitation-contraction coupling in cardiac and skeletal muscle. RyR is the largest ion channel known and exists in three mammalian isoforms (RyR 1-3), all of which are homotetrameric proteins that interact and are regulated by phosphorylation, redox modifications, and various small proteins and ions. RyR channels release calcium from internal stores during muscle excitation-contraction coupling. Mutations in these channels underlie disorders such as catecholaminergic polymorphic ventricular tachycardia (Lanner et al., 2010).

In contrast to linalool, there is an acyclic monoterpene tertiary alcohol, whose primary mechanism of action is to affect acetylcholinesterase (AChE), monooxygenases (MO), α-esterase and β-esterase enzymes. Insects can use the nerve-conducting enzyme AChE to reduce the sensitivity of pesticide target sites. AChE can rapidly hydrolyze the neurotransmitter acetylcholine (ACh) at the

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synaptic cleft, thereby interfering with the conduction of nerve stimuli. The activity of AChE is inhibited to a certain extent and can directly cause paralysis and death of insects. The MO of Ae. aegypti plays an important detoxification function and is implicated in enhancing insecticide resistance. Esterases are involved in the detoxification process of insecticides, converting them into less toxic metabolites. Therefore, esterase can be used as a marker to estimate the toxic effects on various target insects (Huang et al., 2020).

CONCLUSION

Ethanolic extracts of Zodia leaves and Cananga flowers, both at 2.5% and 5% concentrations, effectively kill *Aedes aegypti* larvae. However, this study could not determine the LC50 or LC90 because the concentration variations have a narrow range. Therefore, it is suggested that a similar study with more concentration variations and a more extended range be conducted to determine the LC50 and LC90.

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