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LARVACIDAL ACTIVITY OF *ILLICIUM VERUM* ETHANOLIC EXTRACT AGAINST *AEDES AEGYPTI*

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ABSTRACT

The long-term use of chemical larvicides poses environmental risks and has led to resistance in Aedes aegypti mosquito populations, reducing their effectiveness. Temephos, a commonly used synthetic larvicide, has shown resistance development in various regions. Therefore, exploring plant-based alternatives is essential to mitigate these issues. Illicium verum (star anise) contains secondary metabolites such as flavonoids, saponins, tannins, and alkaloids, which exhibit larvicidal properties. This study aimed to evaluate the effectiveness of Illicium verum ethanolic extract as a natural larvicide against Aedes aegypti.The study employed an experimental design with a post-test-only control group. Larvicidal activity was tested using five groups with four repetitions, analyzed using the Kruskal-Wallis test, post-hoc Mann-Whitney test, and probit analysis at a 95% confidence interval.Results showed no significant difference between the tested concentrations (0.25%, 0.125%) and 1% temephos (p>0.05). Probit analysis determined the LC50 value as 0.476% and LC90 as 2.42%. At the highest concentration, LT50 and LT90 values were 0.037 and 0.269 hours, respectively. The findings indicate that Illicium verum ethanolic extract outperforms 1% temephos, making it a promising eco-friendly alternative to synthetic larvicides.

Keywords: Larvicidal activity, *Illicium verum* ethanolic extract, *Aedes aegypti*

ABSTRAK

Penggunaan larvasida kimia dalam jangka panjang menimbulkan risiko lingkungan dan menyebabkan resistensi pada populasi nyamuk Aedes aegypti, sehingga efektivitasnya berkurang. Temephos, salah satu larvasida sintetis yang umum digunakan, telah menunjukkan perkembangan resistensi di berbagai wilayah. Oleh karena itu, pengembangan larvasida berbasis tanaman menjadi solusi alternatif yang lebih ramah lingkungan. Illicium verum (bunga lawang) mengandung metabolit sekunder seperti flavonoid, saponin, tanin, dan alkaloid yang memiliki sifat larvasida. Penelitian ini bertujuan untuk mengevaluasi efektivitas ekstrak etanolik Illicium verum sebagai larvasida alami terhadap Aedes aegypti.Penelitian ini menggunakan metode eksperimen dengan desain post-test-only control group. Aktivitas larvasida diuji pada lima kelompok dengan empat kali pengulangan, kemudian dianalisis menggunakan uji Kruskal-Wallis, uji post-hoc Mann-Whitney, dan analisis probit dengan tingkat kepercayaan 95%. Hasil penelitian menunjukkan tidak ada perbedaan signifikan antara konsentrasi yang diuji (0,25%, 0,125%) dan 1% temephos (p>0,05). Analisis probit menunjukkan nilai LC50 sebesar 0,476% dan LC90 sebesar 2,42%. Pada konsentrasi tertinggi, nilai LT50 dan LT90 masing-masing adalah 0,037 dan 0,269 jam. Temuan ini menunjukkan bahwa ekstrak etanolik Illicium verum lebih efektif dibandingkan 1% temephos, menjadikannya alternatif larvasida alami yang ramah lingkungan.

Kata kunci: Aktivitas larvasida, Ekstrak etanol *Illicium verum*, Nyamuk *Aedes aegypti*

INTRODUCTION

The dengue virus, which is transmitted by the *Aedes aegypti* mosquito, continues to be a significant vector-borne illness that poses a critical threat to public health in tropical and subtropical areas, resulting in significant mortality and morbidity (Cui et al., 2018). As of December 2023, the World Health Organization documented a tenfold increase in the number of dengue cases worldwide, from 500,000 to 5.2 million, over the period of the previous decade. This represents a significant rise in the incidence of dengue worldwide (WHO, 2023). Since its first documented case occurred in 1968, the prevalence of dengue in Indonesia, a tropical region, has steadily risen. Nonetheless, a fluctuating pattern has existed between 2020 and 2022, which the COVID-19 pandemic situation may impact by ostensibly disrupting the dengue incidence reporting and diagnosis system. In 2022, the incidence of dengue rose from 103,781 cases in 2020 to 143,266 cases, according to data from the Ministry of Health of the Republic of Indonesia (Kemenkes RI, 2023b).

Consequently, in an effort to decrease dengue incidence, diverse strategies are being implemented to regulate the population of *Aedes aegypti*, the main vector of the dengue virus. Pesticides are regarded as a critical element in numerous vector-borne disease control programs, according to the World Health Organization (World Health Organization, 2006). Larviciding refers to the practice of eliminating immature mosquitoes by using insecticidal substances known as larvicide. Mosquitoes typically remain in the larval stage throughout the majority of their life cycle, rendering them vulnerable to both predation and control efforts. Amidst this phase, the larvae are confined to well-defined aquatic boundaries, exhibiting minimal mobility and dispersal capacity, and are readily observable (Veer & Gopalakrishnan, 2016). As a conventional method of eliminating mosquito larvae, temephos, a general synthetic organophosphate insecticide, is utilized globally. Unfortunately, the implementation of temephos promoted the growth of resistance within mosquito populations, thereby diminishing its effectiveness in eliminating the larval stage (Veer & Gopalakrishnan, 2016).

Reportedly, temephos resistance has occurred widely. In the African continent, Cape Verde showed resistance to temephos. In America, temephos resistance was observed in *Aedes aegypti* populations of the Caribbean, Cuba, Trinidad, Santo Domingo, Dominican Republic, and other Latin-American countries. The highest resistant ratio value to temephos was in Havana City, Panama, Costa Rica, Peru, Jamaica, and Venezuela. Meanwhile, in the Asia continent, such as Thailand, some areas in the north, south, central, and eastern parts of Thailand demonstrated the resistance of temephos to all strains of *Aedes aegypti* larvae except strains from the Nakhon Ratchasima region. In Sri Lanka, the resistance ratios (RR50) of temephos varied between 0.69 and 3.93. In India, some studies showed that only one *Aedes aegypti* population had high levels of resistance to temephos. In China, temephos resistance was registered both in adult and larvae populations in Guanzhou (Asgarian et al., 2023). Temephos resistance in Indonesia was reported to occur in several regions, such as in Bengkulu (Triana et al., 2021). Another organophosphate insecticide resistance, malathion, was found in Jakarta and South Denpasar (Asgarian et al., 2023).

A viable approach to manage these vectors involves utilizing plants as organic insecticides to mitigate resistance and reduce the negative consequences associated with chemical pesticides, such as health issues for humans and animals, as well as their adverse impacts on the environment and ecological balance (Veer & Gopalakrishnan, 2016). Various innovative concepts have emerged in response to the urgent requirement for a more environmentally friendly and enduring strategy (Polyxeni et al., 2016). Hence, it is necessary to implement the use of environmentally safe natural insecticides to address community requirements (Ahmed et al., 2022; Polyxeni et al., 2016).

Illicium verum, or star anise fruit, is the indigenous spice of tropical and subtropical areas. This spice consists of star-shaped, reddish-brown fruits with six to eight carpels arranged in a whorl pattern; each carpal is boat-shaped and contains a seed. The fruits are commonly used as a spice for many culinary uses, for incense, and also for traditional medicine (Mishra, 2020). *Illicium verum* is classified as a spice derived medical plant that can easily be gathered since its growth is extensively

spread across various regions of the world, such as South Eastern North America, the West Indies, and Eastern and South Eastern Asia. As a consequence of this specific factor, this plant frequently maintained an opulent global distribution and functioned as an inherent reservoir of cost-effective raw materials (Mishra, 2020; Wijesekera, 2017). *Illicium verum* has been discovered to exhibit insecticidal activity as a result of its phytochemical properties, particularly Anethole, which is the primary component of its essential oil (Choi et al., 2022), It also contains other properties such as Flavonoid, Alkaloid, Saponins, and Tannins, which have insecticidal effects (Patil et al., 2014). These properties have been found to be effective against larvae of Aedes aegypti (Mishra, 2020).

The botanical plant *Illicium verum* is being considered as a new alternative to chemical pesticides due to its non-toxicity to non-target organisms and its lack of harm to the environment. Additionally, it is relatively safe, affordable, and obtainable easily. The efficacy of *Illicium verum* essential oil against specific insects has been established in studies conducted by Freitas et al. (2021); Matos et al. (2020); Pandiyan et al. (2019); and Soonwera et al. (2021). Currently, there is no available data on the utilization of *Illicium verum* in alternative forms, such as ethanol extract, for the purpose of controlling *Aedes aegypti* mosquitos in their aquatic stage, as a larvae. Considering the information provided, the current study is to assess the effectiveness of the larvicidal properties of the *Illicium verum* ethanol extract as a means of controlling *Aedes aegypti* larvae with the goal of identifying a suitable botanical agent for larval management.

METHOD

Participant characteristics and research design

This study employed an experimental method with a post-test-only control group design, using a completely randomized design (CRD) for the sampling technique. The research was conducted between October and November 2023. The plant material, dried star anise fruit (Illicium verum), was purchased from a local market in Bandar Lampung, Indonesia. The Aedes aegypti larvae (Instar III) were obtained in egg form on dried filter paper from Litbang P2B2 (Research and Development Center for Eradicating Animal-Based Diseases) in Ciamis, Indonesia.

This study received approval from The Health Research Ethics Committee, Faculty of Medicine, Universitas Lampung (No.3436/UN26.18/PP.05.02.00/2023) and was conducted in accordance with ethical, legal, and regulatory guidelines set by the committee.

Sampling procedures

The botanical identification and authentication of Illicium verum were conducted at the Botany Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Lampung. The dried fruit was cleaned of dust and contaminants, ground into a fine powder using a grinder and blender, and prepared for extraction.

The 96% ethanol extraction process was performed at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Lampung. The powdered plant material was macerated with 96% ethanol for 72 hours, followed by filtration and solvent evaporation using a rotary vacuum evaporator and a water bath at 40°C for 48 hours to remove excess solvent (Mustofa et al., 2024). A phytochemical test was conducted to identify the bioactive compounds present in the ethanol extract of Illicium verum.

Sample size, power, and precision

The efficacy test followed the WHO Guidelines for Laboratory and Field Testing of Mosquito Larvicides (WHO/CDS/WHOPES, 2005). The laboratory-reared Instar III larvae were obtained by immersing mosquito eggs in mineral water inside a container. Fish pellet powder was added to

synchronize and promote hatching, ensuring a homogeneous population of third instar larvae within five to seven days.

A preliminary test was conducted using four ethanol extract concentrations (0.25%, 0.50%, 0.75%, and 1%), each tested in batches of 25 larvae per cup containing 200 ml of water. The larvae were exposed for 12 hours, and the test was replicated four times on different days. Since 100% mortality was observed across all concentrations, diluted solutions (0%, 0.0625%, 0.125%, and 0.25%) were prepared using distilled water.

For the final test, test cups with 200 ml of water were used, each containing 25 third instar larvae. Temephos 1% served as the positive control. Larval mortality was observed at 1.5 h, 3 h, 6 h, 12 h, 24 h, and 48 h, and the percentage mortality was recorded.

Measures and covariates
The primary outcome measure was larval mortality at different exposure times. The main covariates included:

- Ethanol extract concentrations (0%, 0.0625%, 0.125%, and 0.25%)
- Observation time points (1.5 h, 3 h, 6 h, 12 h, 24 h, 48 h)
- Positive control (1% temephos)

To ensure reliability, the larvicidal test was repeated four times for each concentration on four different days, following WHO standard protocols (WHO/CDS/WHOPES, 2005).

Data analysis
Mortality data were analyzed using probit analysis, generating LC50 and LC90 values with a 95% confidence interval (WHO/CDS/WHOPES, 2005). The statistical analysis ensured robust assessment of the extract's efficacy as a botanical larvicide.

RESULTS AND DISCUSSION

Results

1. Larvicidal Test

Samples of *Aedes aegypti* eggs on dried filter paper were reared until they reached instar III larvae. The method of producing a ethanolic extract from *Illicium verum* was maceration, obtaining 63 grams of concentrated solution with 100 % ethanol content. Then, the distribution of the treatment solution was carried out in 4 concentration levels, namely 0%; 0.,0625%; 0,125% and 0,25%, with 1% temephos solution as a positive control. Exposure of *Illicium verum* ethanolic extract to instar III larvae was observed at 1,5 h; 3 h; 6; 12 h; 24 h and 48 h. Tha average percentage of dead *Aedes aegypti* larvae at various concentrations of star anise (*Illicium verum*) ethanol extract in 48 hours were recorded in Table 1 below.

Table 1
Average Percentage of Dead Aedes aegypti Larvae at Various Concentrations of Star Anise (Illicium verum) Ethanol Extract in 48 hours.

Concentration (%)	Average Mortality Percentage of *Aedes aegypti* Larvae Post-Exposure					
	1,5 h	3 h	6 h	12 h	24 h	48 h
0	0	0	0	0	0	0
0,0625	1	2	4	8	8	11
0,125	77	84	97	100	100	100
0,25	100	100	100	100	100	100
1% Temephos	74	85	99	100	100	100

Figure 1 shows that the mean percentage of *Aedes aegypti* larval mortality is unchanged at a concentration of 0% with a percentage value of 0 and 0,25% with a maximum percentage value of 100. Constant increase was obtained from other concentrations of 0,0625%; 0,125%, and 1% temephos. It was then seen that the concentration of 0,25% reached a maximum larval mortality percentage faster than 1% temephos as a positive control.

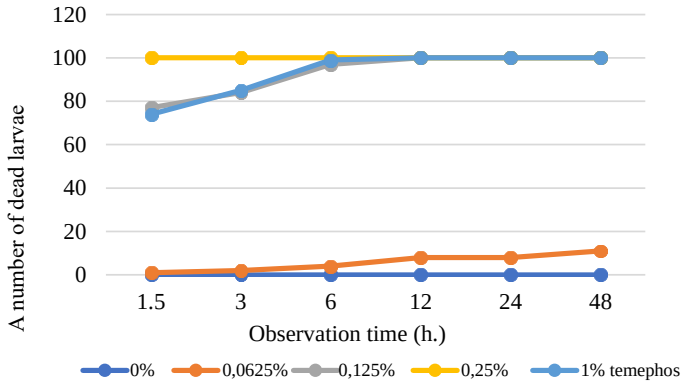

Figure 1. Graphic of Average Mortality Percentage of *Aedes aegypti* Larvae

The ethanol extract then went through phytochemical tests with qualitative results. The results of phytochemical tests are in the qualitative value of the presence of strong positive content in saponin compounds, terpenoids, tannins, flavonoids, and phenolics, as well as positive content in alkaloid compounds in samples of *Illicium verum* ethanolic extract detailed in Table 2 below.

Table 2
Phytochemical Test Results of Illicium verum Ethanol Extract.

Phytochemical qualitative test	Test Results	Interpretation
Saponins	+++	Strong Positive
Steroids	-	Negative
Terpenoid	+++	Strong Positive
Tannins	+++	Strong Positive
Alkaloid	++	Positive
Flavonoid	+++	Strong Positive
Phenolic	+++	Strong Positive

Kruskal-Wallis statistical tests were carried out; the results of the Kruskal-Wallis test were p = 0,00 which showed there was a significant difference in the average mortality of *Aedes aegypti* larvae in the administration of various concentrations of Star Anise (*Illicium verum*) Ethanol Extract with a confidence interval of 95%.

Then, the statistical test continued with the Mann-Whitney Post-hoc test shown in Table 3. The test results obtained that concentrations of 0,125%; 0,25% and 1% temephos, each of which had no significant difference with a value of p>0,05 with a confidence interval of 95%. Concentrations of 0,125% and 0,25% showed no difference in efficacy as a larvicide agent when compared to 1% temephos as a positive control. At those concentrations, the ethanolic extract of *Illicium verum* was as effective as the positive control, 1% temephos, as a botanical larvicide against *Aedes aegypti* larvae, as demonstrated by this result.

Table 3
p-value of Mann Whitney post-hoc test results on each treatment

(Conc.) %	0	0,0625	0,125	0,25	1% temephos
0					
0,0625	0,002*				
0,125	0,002*	0,004*			
0,25	0,001*	0,002*	0,059		
1% temephos	0,002*	0,004*	0,932	0,059	

confidence interval: 95%

2. Lethal Concentration 50% & Lethal Concentration 90% (LC50 & LC90)

The results of the probit test to obtain LC50 and LC90 values, within a 95% confidence interval, obtained a decrease in LC value along with the increase in the length of exposure shown in Table 4. The maximum observation time of 48 hours yielded LC50 values of 0.080% and LC90 values of 0.098%.

Table 4
LC50 and LC90 Values of Aedes aegypti Larvae at Various Observation Times.

Time (hours)	LC_{50} (%)	LC_{90} (%)
1,5	0,110	0,136
3	0,105	0,131
6	0,092	0,122
12	0,083	0,100
24	0,083	0,100
48	0,080	0,098

confidence interval: 95%

3. Lethal Time 50% & Lethal Time 90% (LT50 & LT90)

Probit test results to obtain LT50 and LT90 values, within a 95% confidence interval, found a decrease in the time needed to kill mosquito populations along with the increase in concentrations described as shown in Table 5. LT50 values of 0,037 hours and LT90 values of 0,269 hours were obtained at a maximum concentration of 0.25%. Those value compared and indicated that ethanolic extract of *Illicium verum* was more potent than 1% temephos.

Table 5
LT50 and LT90 Values of Aedes aegypti Larvae at Various Concentrations.

Concentration	LT_{50} (h.)	LT_{90} (h.)
0%	1996.865	14357.953
0.0625%	185.641	1334.803
0,125%	0.449	3.228
0.25%	0.037	0.269
1% temephos	0.458	3.294

confidence interval: 95%

Discussion

1. Larvicidal Test

The powdered sample of *Illicium verum* (500 g) extracted from maceration with 96% ethanol (5 L) generated 63 g of condensed extract, obtaining as much as a 12,6% yield. The yield exceeded 10% and was in line with the Indonesian Ministry of Health in Farmakope Herbal Indonesia 2nd Edition (Kemenkes RI, 2023), that the requirement for the yield of the thick extract is that the value is not less than 10%. Then, it was divided by dilution into four concentrations below 1% (0%; 0,0625%;

0,125% and 0,25%) and carefully processed for evaluation for larvicidal activities against reared Instar III Aedes aegypti larvae by exposing and recording the mortality rate for 1.5 h, 3 h, 6 h, 12 h, 24 h, and 48 h. This protocol for larvicidal activities is based on Dey et al. (2020) and refers to (the WHO/CDS/WHOPES (2005) guideline, which states for laboratory testing of mosquito larvicide, laboratory-reared mosquito larvae are exposed for 24 h to 48 h in water treated with larvicide at various concentration and mortality is recorded.

In our study, the larvicidal activity showed varying degrees of mortality against Instar III *Aedes aegypti* larvae and the result is listed in Tables 1 and 2. The tables showed larvae mortality in every concentration except at 0% as a negative control. While 0.0625% and 0.125% showed continuously increased mortality rates of Instar III *Aedes aegypti* larvae, 0%, and 0.25% concentrations showed constant mortality rates from the first mortality recording. 100% mortality rate The larvicidal activity of *Illicium verum* ethanol extract was found at 0.25% and 0.125%, each of which caused 77% and 100% mortality rate on the first mortality rate recording time (1.5 h). Both showed faster larvicidal activity compared to temephos 1% as a positive control, which showed 74 % on the same time recording. Our result showed the potential larvicide efficacy of *Illicium verum* ethanol extract against the larvae of *Aedes aegypti*, at concentrations of 0.25% and 0.125%, showed faster larvicidal properties compared to positive control; this might be due to some important compounds present in the extract. A paper by Soonwera et al. (2021) reported that 2.5% *Illicium verum* essential oil strengthened by a combination of 2.5% trans-anethole, *Illicium verum* principal constituent, was much more effective than 1% temephos against *Aedes aegypti* larvae. Furthermore, Voris et al. (2018) observed excellent activity of the essential oil of *Illicium verum* (EOIV) against the third instar of *Aedes aegypti*. It is observed that the percentage of mortality is directly proportional to the concentration of EOIV. Its result is related to EOIV's chemical composition. Another study conducted by Chaiyasit et al. (2006) showed 11.76 µg/mg essential oil of *Illicium verum* as adulticidal activity against *Aedes aegypti*, it was resulting 87±7.07 % mortality rate on laboratory strain. While Pandiyan et al. (2019) reported that their study showed the essential oil *Illicium verum* as an individual essential oil showed 22.33±2.96% mortality on concentration 32.66 mgL-1 for larvicidal activity against *Aedes aegypti* larvae.

According to WHO/CDS/WHOPES (2005), only concentrations giving values between 10% and 95% mortality should be chosen. Meanwhile, the Indonesian Department of Agriculture Pesticides (2012) stated effective larvicidal agents showed the capability of its substance to kill 90-100% of sample larvae. Hence, from the result of this study for 48 h observation, *Illicium verum* ethanol extract at concentrations of 0.125% and 0.25% showed potential larvicide efficacy against the larvae of *Aedes aegypti*.

The result of the qualitative phytochemical test on the ethanol extract of *Illicium verum* showed chemical constituent identification, which is listed in Table 3. In the case of the ethanol solvent of *Illicium verum* drawn, a total of six components from seven components tested were present, i.e. Flavonoids, Saponins, Tannins, Alkaloids, Terpenoids, and Phenolics. Flavonoids act as the inhibitors of the Noppera-bo (AeNobo) protein, which plays a role in the biosynthesis of the ecdysone hormone, which is required for molting and metamorphosing for the life cycle of insects (Inaba et al., 2022; Palma-Tenango et al., 2017). Saponins have deterrent activity by provoking insect molting defects, causing cellular toxicity, and have been observed to reduce food intake, indigestion, weight reduction, developmental delay, decreased reproduction rate, and cause mortality (Singh & Kaur, 2018). Tannins can reduce growth and reproductive capacity of certain insects, act as toxins in insects' physiology (Rathod & Roy, 2015; Schultz, 1989), other study by Ahmed et al. (2022), some insect die by tannins as a result of its antifeedant or deterrents for feeding. Alkaloids play a role as effective insect controlers in nature and act as larvicidal and antifeedant parasiticidal activity (Ahmed et al., 2022). A study conducted by Andrade-Ochoa et al. (2018), Terpenoid and phenolic have larvicide

mechanisms that are not yet clearly known, allegedly chemical structure of terpenoids has lipophilic molecular properties that make terpenoids easily pass through the cuticle of larvae. Furthermore, through docking studies, terpenoids targeting sterol carrier protein-2 play a role in cholesterol and fatty acid metabolism. While phenol compounds have larvicide properties, it might be due to its inhibitory effect of the enzyme acetylcholinesterase, causing a neurotoxic effect similar to organophosphorus and carbamate insecticides (Andrade-Ochoa et al., 2018). Study conducted by Dey et al. (2020), the aqueous extract of *Piper longum* which contains tannin, saponin, alkaloids, flavonoids, terpenoids etc., caused histopathological changes in the larval midgut including deformation, swelling, and elongation of epithelial cells.

In the present study, the mortality of larvae might be due to mechanisms of botanical components of *Illicium verum*. It explains the death phenomenon of groups of larvae which exposed to ethanol extract of *Illicium verum* ensued seizure, were observed incapable of swimming to the water's surface to breathe, and died as evidenced by the immobility of larvae lying at the bottom of the plastic glass even though it had been stimulated by the vibration of glass containers and poked by needles in the larvae's body parts. This aligns with the guidelines outlined in WHO/CDS/WHOPES (2005), which define moribund larvae as those unable to swim to the surface or exhibit a diving reaction when the media is disturbed and dead larvae as those unresponsive when stimulated with a needle in the siphon or cervical region.

During the Kruskal-Wallis analysis, a significant difference in the average mortality of *Aedes aegypti* larvae was seen when different doses of *Illicium verum* ethanolic extract were used, with a p-value of 0.000, indicating statistical significance ($p < 0,05$). The Mann-Whitney Post-hoc test revealed significant differences between the 0% and 0,0625% concentration treatment groups and the overall treatment group. The 0,125% concentration treatment group did not show a significant difference compared to the 0.25% concentration treatment group and 1% temephos as the positive control group. Similarly, the 0.25% treatment group did not differ significantly from 1% temephos. The results indicate no significant difference between the 0.125% and 0.25% concentration test groups with 1% temephos and the 0.25% concentration test group with 1% temephos in Table 4. Based of 95% CI at mortality rate, it was apparent that both 0.125% and 0.25% concentrations exhibit similar larvicide effects as 1% temephos, which is used as a positive control. Both concentrations show potential efficacy as botanical larvicides. This is similar to the results of research by Soonwera et al. (2021) found that the *Illicium verum* ethanolic extract shows potential as a mosquito vector controller, identical to temephos 1%, using plant-based components that are safer for the environment and humans.

2. Lethal Concentration 50% & Lethal Concentration 90% (LC50 & LC90)

Regarding larvicidal efficacy results, based on the probit test, the values of LC50 and LC90 were presented to be decreased with increased exposure duration, as seen in Table 5 This implies that as the exposure time increases, the concentration required to cause 50% mortality in the larval population decreases. (Dias & Moraes, 2014). According to Hu et al. (2015), a long period of pesticide exposure was found to increase abnormality, defect, and death in exposed organisms. Additionally, LC50 and LC90 values are helpful in comparing the toxicities of different concentrations containing the same active ingredient; the lower its value, the greater its toxicity to organism (Hock, 2006).

As the WHO claimed the potency of a chemical against a particular species of mosquito larvae must be compared with other insecticides, its guidelines lack standard criteria for assessing natural product larvicidal activity (WHO/CDS/WHOPES, 2005). Several authors have proposed individual criteria to characterize the potency of mosquito larvacides made using natural products (Chantraine et al., 1998; Magalhães et al., 2010; Massebo et al., 2009). Komalamisra et al. (2005) classified compounds

with LC50<0.005% as active, 0.005%<LC50<0.01% as moderately active, and 0.01%<LC50<0.075% as effective, and LC50>0.075% as inactive (Komalamisra et al., 2005). While this study obtained the LC50 values were 0,080% and the LC90 values were 0,098% at the end of the research observation time (48 h), marked LC50 value of this study classified as inactive. On the other hand, Dias et al. (2015) emphasized those classifications must be closely associated with exposure period and larval origin, both of which can affect LC50 values that are set for criteria (Dias et al., 2015).
These results are comparable to the reports by another researcher. A study by Pandiyan et al. (2019) reported larvicide effects on Aedes aegypti larvae using a combination of essential oils derived from *Syzygium aromaticum, Illicium verum* and *Trachyspermum ammi*. The combined essential oil of *Illicium verum* and *Trachyspermum ammi* showed LC50 at 27,67×10-4% and LC90 at 39,50×10-4%, while the essential oil combination of *Illicium verum* and *Syzygium aromaticum* displayed LC50 at 49,07×10-4% and LC90 at 75,50×10-4%. Another study conducted by Soonwera et al. (2021) showed the Essential oil of *Illicium verum* combined with trans-anethol, which is the main constituent of *Illicium verum,* resulting in a range of 2.4%-3.4% LC50 value.

The difference in the results of the present study compared to the other two studies above is mainly due to differences in preparations and concentration. It was found that the preparation of ethanolic extract of *Illicium verum* has different larvicide activity when compared to essential oil preparations. The preparation of *Illicium verum* ethanolic extract in this study has lower larvicide activity when compared to the essential oils combination of *Illicium verum* with *Trachyspermum ammi* and *Syzygium aromaticum*. The present study result is higher when compared to *Illicium verum* essential oil strengthened by a combination of trans-anethole. Similar to this study by Choi et al. (2022) conducted to screen the insecticidal activity of ethanolic extract from *Illicium verum* against Plodia interpunctella larvae, the reason is because ethanolic extract appeals to be more convenient to extract agents, which can be an excellent natural substance to substitute for synthetic chemicals outstanding its activity (Choi et al., 2022). On the other hand, *Illicium verum* essential oil has a low production yield of only about 2.0%, and it also needs some specific equipment (Balti et al., 2018). Another study by Kolar et al. (2018) presented the ethanolic extract exhibiting the highest phenolics and flavonoid contents in comparison to the aqueous extracts; it is possibly might be due to its chemical structures. A paper by Salih et al. (2021) stated the optimal solvent for extraction depends on the part of the plant material and the compounds to be isolated. Their study was conducted to optimize the method by comparing the organic solvent from its capacity to extract bioactive compounds of leaf and seed extract of Juniperus procera from its estimation value of total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC). The result revealed that ethanol recorded the highest TFC (5.9 mg) in leaf extract among other solvents. It concluded that ethanol was the best solvent for both TPC (2.6 mg) and TFC (1.6 mg) recovery in comparison to another solvent (Salih et al., 2021). Furthermore Jamaleddine et al. (2022) stated ethanol or ethanol:water extract drew the highest value of polyphenol or flavonoid contents compared to other green solvents (ethyl acetate and ethanol:ethyl acetate).

3. Lethal Time 50% & Lethal Time 90% (LT50 & LT90)

After log10 data transformation on probit analysis, LT50 and LT90 values were determined in the current study. The values dropped as *Illicium verum* ethanolic extract content increased, as indicated in Table 6. It means increased concentrations of *Illicium verum* ethanolic extract led to quicker larval death. Soonwera et al. (2021) stated that a probit analysis was utilized to determine the time required for a treatment to achieve 50% and 90% insect mortality (LT50 and LT90). The LT value in insect mortality tests indicates that the higher the concentration, the less time it takes to kill the larvae population (Bahuwa et al., 2022). In this present study, this occurrence might be due to the increasing number of larvicide agent compounds in *Illicium verum* that enter the body of *Aedes aegypti* instar

III larvae. Larvicidal compounds exert their effects via absorption through the respiratory tract, salivary glands, or gastrointestinal tract (Cantrell et al., 2010). Substances that enter the larva have the potential to either localize at the site of action or induce systemic effects via diffusion across various tissues (Souza et al., 2012). A study by Koraag (2020), which conducted larvicide tests using Etlingera elatior, which was observed within observation times of 1 hour, 3 hours, 6 hours, 9 hours, and 24 hours, then probit tests were carried out to determine LT50 and LT90, results were obtained to decrease the time needed to kill *Aedes aegypti* larvae Along with the increase in the concentration of *Etlingera elatior*. The decrease in LT50 and LT90 values in the study was similar to the results of the LT50 and LT90 tests in this study. This is thought to be due to the similarity in the content of compounds in *Etlingera elatior* and *Illicium verum.*

The present probit test showed that at the highest concentration (0.25%), the LT50 values were 0,037 h, and the LT90 values were 0,269 h, as shown in Table 6. These values indicate the lesser time that *Illicium verum* ethanolic extract at 0,25% concentration takes to kill 50% and 90% of the mortality of the population compared to the 1% temephos, which achieved the LT50 values were 0,458 h, and the LT90 was 3.294 h. A similar study conducted by Soonwera et al. (2021), proposed the essential oil of *Illicium verum* combined with trans-anethole, which is the main constituent of *Illicium verum* , resulting in a range of 2.9 h-3.1 h LC50 value, which is more effective than 1% temephos.

LIMITATION OF THE STUDY

This study was conducted under controlled laboratory conditions, which may not fully represent natural *Aedes aegypti* breeding environments. While phytochemical tests identified bioactive compounds, the results were qualitative, limiting insight into their specific contributions to larvicidal activity. Additionally, the study focused on acute effects within 48 hours, without assessing long-term efficacy or environmental impact. Future research should include quantitative phytochemical analysis, field trials, and toxicity assessments to ensure the practical applicability and safety of *Illicium verum* as a botanical larvicide.

CONCLUSIONS AND SUGGESTIONS

The outcome of this study showed ethanolic extract of *Illicium verum* exhibited more efficacy as a botanical larvicide against *Aedes aegypti* larvae compared to 1% temephos. This natural product can be expected to be significantly safer for humans and the environment. Additionally, it presents an alternative innovation that can effectively combat the emergence of synthetic larvicide resistance, justifying its continued development into commercial insecticide products.

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Conflict of Interest Statement

The authors declare that they have no conflicts of interest related to this study. No financial, professional, or personal relationships that could inappropriately influence or bias the research, data analysis, or manuscript preparation exist. Additionally, there has been no funding, sponsorship, or consultancy agreement within the last three years that could be perceived as affecting the integrity of this work. This declaration ensures transparency and allows readers to assess the findings without concerns of potential bias.

REFERENCES

Ahmed, N., Alam, M., Saeed, M., Ullah, H., Iqbal, T., Awadh Al-Mutairi, K., Shahjeer, K., Ullah, R., Ahmed, S., Abd Aleem Hassan Ahmed, N., Fathy Khater, H., & Salman, M. (2022). Botanical Insecticides Are a Non-Toxic Alternative to Conventional Pesticides in the Control of Insects and Pests. *Global Decline of Insects*. https://doi.org/10.5772/intechopen.100416

Andrade-Ochoa, S., Correa-Basurto, J., Rodríguez-Valdez, L. M., Sánchez-Torres, L. E., Nogueda-Torres, B., & Nevárez-Moorillón, G. V. (2018). In vitro and in silico studies of terpenes, terpenoids and related compounds with larvicidal and pupaecidal activity against Culex quinquefasciatus Say (Diptera: Culicidae). *Chemistry Central Journal*, *12*(1). https://doi.org/10.1186/s13065-018-0425-2

Asgarian, T. S., Vatandoost, H., Hanafi-Bojd, A. A., & Nikpoor, F. (2023). Worldwide Status of Insecticide Resistance of Aedes aegypti and Ae. albopictus, Vectors of Arboviruses of Chikungunya, Dengue, Zika and Yellow Fever. *Journal of Arthropod-Borne Diseases*, *17*(1), 1–27. https://doi.org/10.18502/jad.v17i1.13198

Bahuwa, I. C., Lamondo, D., & Katili, A. S. (2022). Short Communication: Papaya (Carica papaya) seed extract test againts Spodoptera litura mortality. *Cell Biology and Development*, *6*(1). https://doi.org/10.13057/cellbioldev/v060101

Balti, M. A., Hadrich, B., & Kriaa, K. (2018). Lab-scale extraction of essential oils from Tunisian lemongrass (Cymbopogon flexuosus). *Chemical Engineering and Processing: Process Intensification*, *124*, 164–173. https://doi.org/10.1016/j.cep.2017.12.012

Cantrell, C. L., Pridgeon, J. W., Fronczek, F. R., & Becnel, J. J. (2010). Structure-activity relationship studies on derivatives of eudesmanolides from Inula helenium as toxicants against Aedes aegypti larvae and adults. *Chemistry and Biodiversity*, *7*(7), 1681–1697. https://doi.org/10.1002/cbdv.201000031

Chantraine, J. M., Laurent, D., Ballivian, C., Saavedra, G., Ibañez, R., & Vilaseca, L. A. (1998). Insecticidal activity of essential oils on Aedes aegypti larvae. *Phytotherapy Research*, *12*(5), 350–354. https://doi.org/10.1002/(SICI)1099-1573(199808)12:5<350::AID-PTR311>3.0.CO;2-7

Choi, I., Kim, S., Lee, J. S., Chang, Y., Na, J. H., & Han, J. (2022). Analysis of the insect-repelling mechanism of star anise extract and its major active compounds against Plodia interpunctella. *Food Science and Biotechnology*, *31*(4), 451–462. https://doi.org/10.1007/s10068-022-01053-8

Cui, Y. C., Wu, Q., Teh, S. W., Peli, A., Bu, G., Qiu, Y. S., Benelli, G., & Kumar, S. S. (2018). Bone breaking infections – A focus on bacterial and mosquito-borne viral infections. *Microbial Pathogenesis*, *122*, 130–136. https://doi.org/10.1016/j.micpath.2018.06.021

Dey, P., Goyary, D., Chattopadhyay, P., Kishor, S., Karmakar, S., & Verma, A. (2020). Evaluation of larvicidal activity of Piper longum leaf against the dengue vector, Aedes aegypti, malarial vector, Anopheles stephensi and filariasis vector, Culex quinquefasciatus. *South African Journal of Botany*, *132*, 482–490. https://doi.org/10.1016/j.sajb.2020.06.016

Dias, C. N., Alves, L. P. L., Rodrigues, K. A. D. F., Brito, M. C. A., Rosa, C. D. S., Amaral, F. M. M. Do, Monteiro, O. D. S., Andrade, E. H. D. A., Maia, J. G. S., & Moraes, D. F. C. (2015). Chemical composition and larvicidal activity of essential oils extracted from Brazilian legal AMAZOn plants against Aedes aegypti L. (Diptera: Culicidae). *Evidence-Based Complementary and Alternative Medicine*, *2015*. https://doi.org/10.1155/2015/490765

Dias, C. N., & Moraes, D. F. C. (2014). Essential oils and their compounds as Aedes aegypti L. (Diptera: Culicidae) larvicides: Review. *Parasitology Research*, *113*(2), 565–592. https://doi.org/10.1007/s00436-013-3687-6

Freitas, J. P., De Jesus, I. L. R., De Oliveira Chaves, J. K., Gijsen, I. S., Campos, D. R., Baptista, D. P., Ferreira,

T. P., Alves, M. C. C., & Coumendouros, K. (2021). Efficacy and residual effect of lilicium verum (star anise) and Pelargonium graveoiens (rose geranium) essential oil on cat fleas Ctenocephaiides feiis feiis. *Revista Brasileira de Parasitologia Veterinaria*, *30*(4). https://doi.org/10.1590/S1984-29612021088

Hock, W. K. (2006). Toxicity of pesticides. In *Pesticide Safety Fact Sheet* (pp. 233–247). The Pennsylvania State University. https://doi.org/10.1080/04345546009415725

Hu, R., Huang, X., Huang, J., Li, Y., Zhang, C., Yin, Y., Chen, Z., Jin, Y., Cai, J., & Cui, F. (2015). Long- and short-term health effects of pesticide exposure: A cohort study from China. *PLoS ONE*, *10*(6). https://doi.org/10.1371/journal.pone.0128766

Inaba, K., Ebihara, K., Senda, M., Yoshino, R., Sakuma, C., Koiwai, K., Takaya, D., Watanabe, C., Watanabe, A., Kawashima, Y., Fukuzawa, K., Imamura, R., Kojima, H., Okabe, T., Uemura, N., Kasai, S., Kanuka, H., Nishimura, T., Watanabe, K., … Niwa, R. (2022). Molecular action of larvicidal flavonoids on ecdysteroidogenic glutathione S-transferase Noppera-bo in Aedes aegypti. *BMC Biology*, *20*(1). https://doi.org/10.1186/s12915-022-01233-2

Jamaleddine, A., De Caro, P., Bouajila, J., Evon, P., Haddad, J. G., El-Kalamouni, C., Hijazi, A., & Merah, O. (2022). In Vitro Bioactivities of Extracts from Tomato Pomace. *Frontiers in Bioscience - Landmark*, *27*(9). https://doi.org/10.31083/j.fbl2709259

Kemenkes RI. (2023a). Farmakope Herbal Indonesia edisi II. In *Pills and the Public Purse*.

Kemenkes RI. (2023b). Laporan Tahunan 2022 Demam Berdarah Dengue. In *kemenkes RI*.

Kolar, F. R., Gogi, C. L., Khudavand, M. M., Choudhari, M. S., & Patil, S. B. (2018). Phytochemical and antioxidant properties of some Cassia species. *Natural Product Research*, *32*(11), 1324–1328. https://doi.org/10.1080/14786419.2017.1342085

Komalamisra, N., Trongtokit, Y., Rongsriyam, Y., & Apiwathnasorn, C. (2005). Screening for larvicidal activity in some Thai plants against four mosquito vector species. *Southeast Asian Journal of Tropical Medicine and Public Health*, *36*(6), 1412–1422.

Koraag, M. E. (2020). Lethal Time Ekstrak Bunga Kecombrang (Etlingera elatior) Terhadap Larva Aedes aegypti. *Seminar Nasional Biologi*, 300–309. http://103.76.50.195/semnasbio/article/view/15307

Magalhães, L. A. M. I., Da Paz Lima, M., Marques, M. O. M., Facanali, R., Da Silva Pinto, A. C., & Tadei, W. P. (2010). Chemical composition and larvicidal activity against Aedes aegypti larvae of essential oils from four Guarea species. *Molecules*, *15*(8), 5734–5741. https://doi.org/10.3390/molecules15085734

Massebo, F., Tadesse, M., Bekele, T., Balkew, M., & Gebre-Michael, T. (2009). Evaluation on larvicidal effects of essential oils of some local plants against Anopheles arabiensis Patton and Aedes aegypti Linnaeus (Diptera, Culicidae) in Ethiopia. *African Journal of Biotechnology*, *8*(17), 4183–4188.

Matos, L. F., da Cruz Lima, E., de Andrade Dutra, K., Navarro, D. M. do A. F., Alves, J. L. R., & Silva, G. N. (2020). Chemical composition and insecticidal effect of essential oils from Illicium verum and Eugenia caryophyllus on Callosobruchus maculatus in cowpea. *Industrial Crops and Products*, *145*, 112088.

Mishra, N. (2020). Ethnopharmacological Investigation of Indian Spices. In *Advances in Medical Diagnosis, Treatment, and Care*. IGI Global, Medical Information Science Reference.

Mustofa, S., Adjeng, A. N. T., Kurniawaty, E., Ramadhita, L., & Tamara, T. (2024). Influence of Rhizophora apiculata barks extract on Cholesterol, Triglyceride, LDL, and HDL Levels of Rattus norvegicus (Sprague Dawley) fed high-cholesterol diet. *Research Journal of Pharmacy and Technology*, *17*(1), 396–400.

Palma-Tenango, M., Soto-Hernández, M., & Aguirre-Hernández, E. (2017). Flavonoids in Agriculture. In *Flavonoids - From Biosynthesis to Human Health*. InTech. https://doi.org/10.5772/intechopen.68626

Pandiyan, G. N., Mathew, N., & Munusamy, S. (2019). Larvicidal activity of selected essential oil in synergized combinations against Aedes aegypti. *Ecotoxicology and Environmental Safety*, *174*, 549–556. https://doi.org/10.1016/j.ecoenv.2019.03.019

Patil, B. R., Thribhuvan, K. ., Manukumar, H. ., & Madhu, C. . (2014). Phytochemical, nutritional and mineral constituents of illicium verum hook (star anise). *Manukumar et Al. World Journal of Pharmaceutical Research*, *3*(2), 2888–2896. www.wjpr.net

Polyxeni, N., Sotirios, M., Chrysanthi, K., Panagiotis, S., & Luc, H. (2016). Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. *Frontiers in Public Health*, *4*.

Rathod, A., & Roy, S. (2015). Nutritional Ecology of Insect. *Agrobios*, 87–89.

Salih, A. M., Al-Qurainy, F., Nadeem, M., Tarroum, M., Khan, S., Shaikhaldein, H. O., Al-Hashimi, A., Alfagham, A., & Alkahtani, J. (2021). Optimization method for phenolic compounds extraction from medicinal plant (Juniperus procera) and phytochemicals screening. *Molecules*, *26*(24).

https://doi.org/10.3390/molecules26247454
Schultz, J. C. (1989). Tannin-Insect Interactions. *Chemistry and Significance of Condensed Tannins*, 417–433. https://doi.org/10.1007/978-1-4684-7511-1_26
Singh, B., & Kaur, A. (2018). Control of insect pests in crop plants and stored food grains using plant saponins: A review. *Lwt*, *87*, 93–101. https://doi.org/10.1016/j.lwt.2017.08.077
Soonwera, D. M., Moungthipmalai, T., Aungtikun, J., & Sittichok, D. S. (2021). Combinations of Plant Essential Oils and Their Major Compositions Inducing Mortality and Morphological Abnormality of Aedes Aegypti and Aedes Albopictus. *SSRN Electronic Journal*. https://doi.org/10.2139/ssrn.3990200
Souza, T. M., Cunha, A. P., Farias, D. F., Machado, L. K., Morais, S. M., Ricardo, N. M. P. S., & Carvalho, A. F. (2012). Insecticidal activity against Aedes aegypti of m-pentadecadienyl-phenol isolated from Myracrodruon urundeuva seeds. *Pest Management Science*, *68*(10), 1380–1384. https://doi.org/10.1002/ps.3316
Triana, D., Siregar, F. N., Utami, E. P. W. T., Suteky, T., & Wicaksono, S. (2021). Entomological Parameters and Characterization of Insecticide Resistance in Dengue Vector Aedes Aegypti Larvae From Bengkulu City, Indonesia. *Malaysian Journal of Public Health Medicine*, *21*(1), 96–102. https://doi.org/10.37268/MJPHM/VOL.21/NO.1/ART.467
Veer, V., & Gopalakrishnan, R. (2016). Herbal insecticides, repellents and biomedicines: Effectiveness and commercialization. In *Herbal Insecticides, Repellents and Biomedicines: Effectiveness and Commercialization*. https://doi.org/10.1007/978-81-322-2704-5
Voris, D. G. da R., Dias, L. dos S., Lima, J. A., Lima, K. dos S. C., Lima, J. B. P., & Lima, A. L. dos S. (2018). Evaluation of larvicidal, adulticidal, and anticholinesterase activities of essential oils of Illicium verum Hook. f., Pimenta dioica (L.) Merr., and Myristica fragrans Houtt. against Zika virus vectors. *Environmental Science and Pollution Research*, *25*(23), 22541–22551. https://doi.org/10.1007/s11356-018-2362-y
WHO/CDS/WHOPES. (2005). Guidelines for laboratory and field testing of mosquito larvicides. *World Health Organization*, *10*, 1–41. http://whqlibdoc.who.int/hq/2005/WHO_CDS_WHOPES_GCDPP_2005.13.pdf?ua=1
WHO. (2023). *Dengue and severe dengue*. Who. http://www.who.int/mediacentre/factsheets/fs117/en/%0Ahttps://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue%0Ahttps://www.who.int/health-topics/dengue-and-severe-dengue#tab=tab_1
Wijesekera, R. O. B. (2017). The medicinal plant industry. In *The Medicinal Plant Industry*. https://doi.org/10.1201/9780203736395
World Health Organization. (2006). Pesticides and their application for the control of vectors adn pests of public health importance. In *World Health Organization* (Vol. 1, Issue 6). http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.1_eng.pdf