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ACETYLCHOLINESTERASE INHIBITION-BASED LARVICIDAL ACTIVITY OF CARICA PAPAYA LEAF AND DURIO ZIBETHINUS PEEL EXTRACTS AGAINST AEDES AEGYPTI: A PROBIT AND GC–MS APPROACH

AKTIVITAS LARVASIDA BERBASIS INHIBISI ASETILKOLINESTERASE DARI EKSTRAK DAUN CARICA PAPAYA DAN KULIT DURIO ZIBETHINUS TERHADAP AEDES AEGYPTI: PENDEKATAN PROBIT DAN GC–MS

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ABSTRACT
This study investigated the biolarvicidal potential of Carica papaya Linn leaf extract and Durio zibethinus Murr peel extract against Aedes aegypti mosquito larvae, the primary vector of dengue fever. Extracts were prepared via maceration with 70% ethanol. Toxicity assessments were conducted on third-instar larvae, evaluating individual and combined extract effects across various concentrations. Larval mortality was recorded at 24 and 48 hours, and the LC50 value was determined using Probit analysis. Bioactive compounds were identified through Gas Chromatography-Mass Spectrometry (GC-MS). Results indicated that a 50%:50% combination of papaya leaf and durian peel extracts, as well as a 25% papaya:75% durian ratio, achieved a notable 70% larval mortality. While these combinations demonstrated high efficacy, further statistical analysis, such as Abbott's formula, is required to confirm synergistic interactions definitively. The calculated LC50 of 131.95 ppm classified the extracts as toxic to A. aegypti larvae. GC-MS analysis of papaya leaf extract revealed neurotoxic and hormonal-disrupting compounds, including Prilocaine and Lidocaine. The study concludes that papaya leaf extract possesses superior larvicidal properties compared to durian peel extract, likely attributable to its significant alkaloid content, which inhibits acetylcholinesterase enzyme activity.
Keywords: larvasida, carica papaya, durio zibethinus, aedes aegypti, inhibisi asetilkolinesterase, analisis probit, gc-ms, lc50, senyawa bioaktif, toksisitas larva

ABSTRAK
Penelitian ini mengkaji potensi biolarvasida dari ekstrak daun Carica papaya Linn dan ekstrak kulit Durio zibethinus Murr terhadap larva nyamuk Aedes aegypti, sebagai vektor utama penyakit demam berdarah. Ekstrak disiapkan melalui metode maserasi menggunakan etanol 70%. Uji toksisitas dilakukan pada larva instar ketiga dengan mengevaluasi efek ekstrak secara individu maupun kombinasi pada berbagai konsentrasi. Mortalitas larva diamati pada 24 dan 48 jam, serta nilai LC50 ditentukan menggunakan analisis probit. Senyawa bioaktif diidentifikasi menggunakan Gas Chromatography-Mass Spectrometry (GC-MS). Hasil penelitian menunjukkan bahwa kombinasi 50%:50% ekstrak daun pepaya dan kulit durian, serta rasio 25% pepaya:75% durian, menghasilkan mortalitas larva sebesar 70%. Meskipun kombinasi ini menunjukkan efektivitas tinggi, analisis statistik lanjutan seperti rumus Abbott diperlukan untuk memastikan adanya interaksi sinergis secara definitif. Nilai LC50 sebesar 131,95 ppm mengklasifikasikan ekstrak sebagai bersifat toksik terhadap larva A. aegypti. Analisis GC-MS pada ekstrak daun pepaya mengungkap adanya senyawa bersifat neurotoksik dan pengganggu hormon, termasuk Prilocaine dan Lidocaine. Penelitian ini menyimpulkan bahwa ekstrak daun pepaya memiliki aktivitas larvasida yang lebih unggul dibandingkan ekstrak kulit durian, yang kemungkinan disebabkan oleh kandungan alkaloidnya yang signifikan dalam menghambat aktivitas enzim asetilkolinesterase.
Kata kunci: larvasida, carica papaya, durio zibethinus, aedes aegypti, inhibisi asetilkolinesterase, analisis probit, gc-ms, lc50, senyawa bioaktif, toksisitas larva

1. INTRODUCTION

Control of disease vectors, especially the Aedes aegypti mosquito, is a a major public health challenge in tropical areas. Diseases transmitted by these mosquitoes, such as dengue fever, chikungunya, and Zika, continue to be major health problems with high incidence rates in various regions, indicating the need for effective and sustainable strategies in vector control (Hidayat et al., 2023; Rahaman et al., 2024). One of the main methods of vector control is the use of synthetic insecticides, which have been proven to suppress mosquito populations. However, excessive and uncontrolled use of these insecticides has led to increasing insect resistance to these insecticides and has had negative impacts on the environment and non-target organisms (Rahaman et al., 2024; Vincent and Marin, 2024). This has made the focus of research begin to shift to more environmentally friendly alternatives, including natural larvicides based on plant extracts.

A promising alternative approach is the use of biolarvicides based on natural ingredients, which come from plant extracts containing bioactive compounds. These compounds can inhibit the development of mosquito larvae without causing negative impacts on the environment (Hegazy and Baz, 2023; Ranasinghe et al., 2024). Research shows that bioactive compounds, such as flavonoids, saponins and tannins found in various plants, have great potential as natural larvicides. For example, papaya leaf extract (Carica papaya Linn) is known to contain compounds that have toxic effects on larvae Aedes aegypti (Sreeshivani et al., 2024). In addition, durian skin (Durio zibethinus Murr) is also known to contain bioactive compounds that play a role in inhibiting the growth of mosquito larvae (Ribeiro et al., 2024).

Although previous research has indicated the larvicidal potential of individual plant extracts, there are still weaknesses in understanding the effectiveness of combinations of extracts that can increase larvicidal activity through synergistic mechanisms (Rahaman et al., 2024). This gap indicates the need for further exploration to understand how certain combinations of extracts can provide a synergistic effect in controlling mosquito larvae. Several studies suggest that interactions between bioactive compounds in plant extracts can influence the mechanism of larvicide action, for example, through metabolic disturbances and inhibition of essential enzymes, which can increase the effectiveness of toxicity against larvae (Ranasinghe et al., 2024; et al., 2023). Therefore, a combination of appropriate extracts from various plants could be an effective strategy to address mosquito larvae populations and reduce reliance on potentially harmful synthetic insecticides.

In this context, further research needs to be carried out to explore the interactions between different plant extracts and to identify the most effective and safe combinations. In addition, emphasis must be placed on the need for a sustainable approach to mosquito control, including studies of the long-term potential of biolarvicides, in order to minimize environmental impacts and maintain sustainability in controlling vector-borne diseases (Rahaman et al., 2024; Muflikhah, 2023).

This study aims to evaluate the toxicity of papaya leaf extract (Carica papaya) and durian skin (Durio zibethinus) on larval mortality Aedes aegypti through single and combined effects analysis. In addition, this research will also identify bioactive compounds that play a role in larvicidal activity and explore the synergistic or antagonistic potential of the combination of extracts used. To achieve these objectives, the methodology employed involved the extraction of plant materials using 70% ethanol via maceration. Subsequently, toxicity tests were conducted on third-instar larvae, observing mortality rates over 24 and 48 hours, and determining LC50 values through Probit analysis. Bioactive compounds were identified using Gas Chromatography-Mass Spectrometry (GC-MS).

The novelty of this study lies in its comprehensive investigation into the synergistic effects of combining papaya leaf and durian peel extracts, addressing a critical gap in understanding how such combinations can enhance larvicidal activity. While individual plant extracts have been studied, the exploration of specific ratios and their combined efficacy, particularly in identifying optimal synergistic interactions for Aedes aegypti control, represents a significant advancement. This research provides deeper insight into the mechanisms of action of bioactive compounds in environmentally based vector control and contributes to the development of alternative mosquito control that is more sustainable and environmentally friendly

2. METHODS

This research used various laboratory equipment such as: blender, digital balance, knife, filter, distillation tool and rotary evaporator, Erlenmeyer glass, measuring cup, micropipette, petri dish, beaker glass, stereo microscope, camera, pH meter, thermometer and Agilent 5977C GC/MSD Gas Chromatography-Mass Spectrometry (GC-MS).

The materials used in this research were durian skin, papaya leaves, 70% ethanol, distilled water. Temephos, larva Aedes aegypti instar III.

2.1. Extract Making Process

Extraction of durian skin and papaya leaves was carried out using the maceration method using 70% ethanol as a solvent. The process includes washing, drying, grinding, soaking for 72 hours, filtering, and evaporation to obtain a concentrated extract. The extract was stored at 4°C to maintain the stability of the active compound before testing.

2.2. Larvicide Effectiveness Testing

Larvicidal efficacy tests were conducted against third-instar Aedes aegypti larvae using seven treatment groups. Each test solution was prepared in a final volume of 100 mL in individual Petri dishes or beakers. The treatment groups included various concentrations of single and combined extracts of durian peel and papaya leaf, alongside control groups. A Negative Control group consisted of larvae in distilled water only (0 ppm). A Positive Control group utilized larvae exposed to a standard insecticide solution of Temephos at a concentration of 25 ppm. While Temephos was included as a positive control in the experimental design, its corresponding mortality data are not presented in the results section of this manuscript.

The range of total extract concentrations tested for LC50 determination was 0, 25, 50, 100, and 200 ppm. For combination testing, the extract ratios (e.g., 50%:50% as presented in Table 1) refer to the proportion of each extract within a given total ppm concentration. It is important to note that the mortality percentages presented in Table 1 correspond to a specific total extract concentration from the tested range (0, 25, 50, 100, 200 ppm). However, the specific total concentration at which the data in Table 1 were obtained is not explicitly stated in the provided manuscript and represents a critical piece of missing information for full reproducibility and interpretation. Larval mortality was observed and recorded after 24 and 48 hours of exposure. Data were subsequently analyzed to evaluate the larvicidal effectiveness of the extracts.

2.3. Bioactive Compound Content Analysis (GC-MS)

GC-MS analysis was employed to identify the bioactive compounds present in both durian peel and papaya leaf extracts that potentially contribute to their larvicidal activity. The procedure involved sample preparation, chromatographic separation of compounds based on their volatility, and subsequent identification using mass spectra. This analysis aimed to provide detailed information regarding the active components influencing the efficacy of the natural biolarvicides against Aedes aegypti. For enhanced reproducibility, specific operational conditions of the GC-MS, such as the column type, temperature program, and carrier gas flow rate, were not detailed in the provided manuscript. These parameters are crucial for replicating the analytical process and should be included in future iterations.

2.4. Research Replication and Validation

Research Replication and Validation To ensure the validity of the results, each treatment was carried out in three replications (n = 3). Each replication used 10 Aedes aegypti instar III larvae, resulting in a total of 30 larvae for each treatment group (10 larvae/replication x 3 replications). This approach ensures that the results obtained can be retested and analyzed with a high level of confidence.

2.5. Larvae Source and Maintenance

Information regarding the source and maintenance of the Aedes aegypti larvae used in this study is currently absent from the manuscript. Details such as whether the larvae were obtained from a standard laboratory colony or collected from the wild, along with their rearing conditions (e.g., temperature, humidity, feeding regimen), are essential for understanding the experimental context and ensuring reproducibility. This information should be provided.

3. RESULTS AND DISCUSSIONS

The use of plant extracts as natural ingredient-based biolarvicides has been widely studied because of their secondary metabolite content which is toxic to insects. In this study, papaya leaf extract (Carica papaya L.) and durian peel (Durio zibethinus) were tested to assess their effectiveness in causing the death of Aedes aegypti larvae. Based on the research results, larval mortality varied depending on the concentration and combination of extracts given.

Table 1. Mortality of Aedes aegypti Larvae at Various Extract Concentrations

Durian Concentration (%)	Papaya Concentration (%)	Life	Dead	Mortality (%)
50	50	3	7	70.0
25	75	6	4	40.0
75	25	3	7	70.0
0	100	5	5	50.0
100	0	9	1	10.0

Table 1 shows that papaya leaf extract is more effective than durian peel extract in killing Aedes aegypti larvae. At a concentration of 100% durian peel extract, only 10% of larvae died, while at a concentration of 100% papaya leaf extract, the death rate reached 50%. The combination of papaya leaf extract and durian peel in a ratio of 50:50% produced the highest mortality (70%), which, alongside the 75% durian and 25% papaya combination also yielding 70% mortality, suggests promising efficacy. However, a formal statistical analysis (e.g., using Abbott's formula) is required to quantitatively assess and confirm any synergistic, additive, or antagonistic interactions between the two extracts.

The research results show that papaya (Carica papaya) leaf extract contains flavonoids, alkaloids and saponins which can contribute to its insecticidal activity. The active compounds in papaya leaf extract are known to affect the nervous system of larvae, which has the potential to disrupt the feeding process and increase the risk of starvation and death (Sari et al., 2022).

Research conducted by Sari et al. (Sari et al., 2022) confirmed that bioactive compounds in plants, including flavonoids, can exert toxic effects on larvae by damaging the digestive and respiratory systems, which in turn affects their metabolism. And according to Rochmat et al. (2017), active compounds in plant extracts can inhibit taste receptors in larvae, causing eating disorders and even starvation and death

These results are in line with previous research which reported that the flavonoids, alkaloids and saponins in papaya leaves have higher insecticidal activity than the compounds found in durian peel (Ramadhani et al., 2023; Maulana et al., 2022).

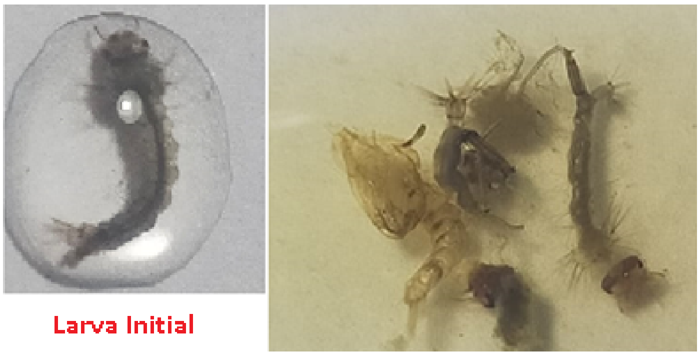

Figure 1. Changes in larval morphology Untreatment and Treatment

The combination of these internal and external factors plays an important role in maximizing the potential of papaya leaf extract as a biolarvicide against Aedes aegypti. The effectiveness of larvicide depends on the quality and concentration of the extract, along with the intrinsic properties of the compounds present in it, which have the potential to be used in controlling larval-based disease vectors (Sari et al., 2022; Hidayat et al., 2023).

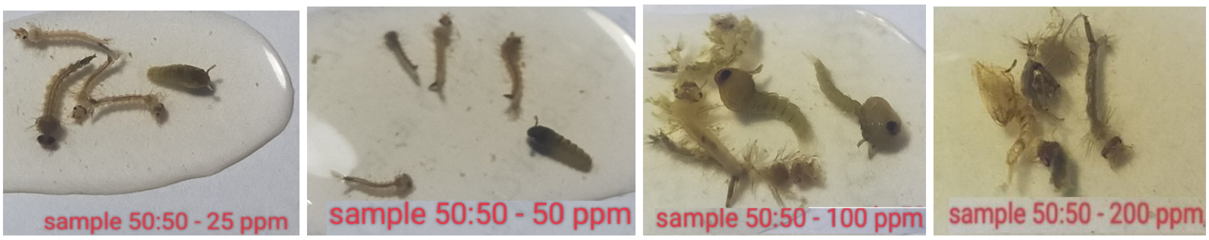

Figure 2. Changes in Physical Shape of Larvae at Various Extract Concentrations

Figures 1 and 2 show changes in larval morphology after exposure to the extract. At a concentration of 50 ppm, the larvae began to show head swelling, while at a concentration of 100 ppm, the larval body ruptured. This shows that the compounds in papaya leaf extract and durian peel work as stomach and contact poisons, causing digestive disorders and cellular lysis.

The high alkaloid content in papaya leaves (16.5%) plays a role in causing paralysis in larvae through inhibiting the enzyme acetylcholinesterase, which plays a role in nervous system function (Alzanando et al., 2022). On the other hand, the alkaloid content in durian skin is relatively low (1.5%) so its effectiveness as a biolarvicide is lower (Kristianto, 2017).

The toxicity of the extract against Aedes aegypti larvae was assessed based on the LC value$_{50}$ (Lethal Concentration 50%), namely the concentration that causes the death of 50% of the larvae in the test population.

Table 2. LC50 Value of Biolarvicide Papaya Leaf and Durian Peel Extracts

Concentration (ppm)	Total Larva	Dead Larvae	% Mortality	Concentration Log
0	11	0	0	0.00
25	10	3	30	1.40
50	11	6	55	1.70
100	10	9	90	2.00
200	10	10	100	2.30

The toxicity of papaya leaf and durian peel extracts against Aedes aegypti larvae was evaluated through Probit analysis, resulting in an LC50 value of 131.95 ppm, which indicates toxic potential. This LC50 value is included in the toxic category (LC50 ≤ 1000 mg/L), confirming that this extract can function as a natural biolarvicide. The test results showed that there was a positive relationship between extract concentration and larval mortality rate; at a concentration of 200 ppm, larval mortality reached 100% (Chakraborty et al., 2021). This is in line with previous findings showing that increasing the extract concentration can increase the death rate of mosquito larvae, each of which serves as an effective indicator of the larvicidal activity carried by the bioactive compounds in the extract (Alafia et al., 2022).

The linear probit regression analysis obtained, namely $y = 17.166x – 31.399$, indicated that there was a significant and strong relationship between the concentration of papaya leaf extract and durian peel on the death rate of Aedes aegypti larvae (Redo et al., 2019). The effectiveness of this extract is thought to be due to the content of bioactive compounds such as flavonoids, alkaloids and saponins, which have been shown to have larvicidal activity through various mechanisms, including inhibition of the nervous system and disruption of larval metabolism (Priya & Jones, 2021). This compound functions by disrupting the physiological processes and feeding behavior of larvae, which leads to death (Valverde et al., 2022).

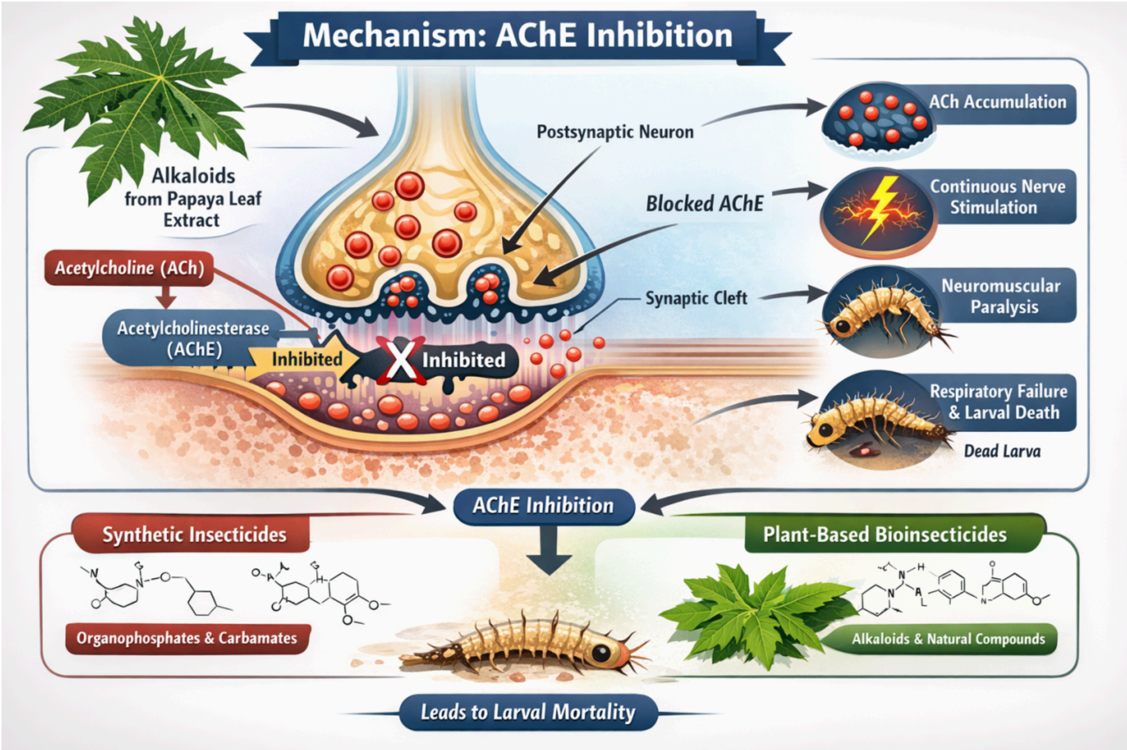

Figure 3. Illustration the AChE inhibition mechanism by papaya leaf extract and durian peel extract

The larvicidal activity observed in this study is closely associated with the presence of bioactive secondary metabolites, particularly alkaloids identified in Carica papaya leaf extract. Alkaloids are well-documented for their neurotoxic effects on insects, primarily through the inhibition of the acetylcholinesterase (AChE) enzyme (Isman, 2006; Rattan, 2010). AChE plays a crucial role in the nervous system by hydrolyzing the neurotransmitter acetylcholine at synaptic junctions, thereby terminating nerve impulse transmission (Casida & Durkin, 2013).

Inhibition of AChE leads to the accumulation of acetylcholine in the synaptic cleft, resulting in continuous stimulation of postsynaptic neurons. This persistent excitation disrupts normal neuromuscular function, causing paralysis, loss of coordination, respiratory failure, and ultimately death in larvae (Pimentel, 2005; Casida & Durkin, 2013). This mode of action is comparable to that of synthetic organophosphate and carbamate insecticides, which also target AChE. However, plant-derived compounds are generally considered more environmentally friendly and biodegradable, making them promising alternatives for sustainable vector control (Isman, 2006).

The higher larvicidal efficacy of C. papaya leaf extract compared to Durio zibethinus peel extract suggests a greater abundance or diversity of neuroactive compounds, particularly alkaloids, as well as other secondary metabolites such as terpenoids and phenolic compounds. These compounds have been reported to exhibit insecticidal activity through multiple mechanisms, including neurotoxicity, enzyme inhibition, and disruption of metabolic processes (Pavela, 2015; Rattan, 2010).

Furthermore, the combination treatments may produce additive or synergistic effects by targeting multiple physiological pathways simultaneously, such as interference with the nervous system and metabolic regulation. Such interactions can enhance overall larvicidal efficacy, although further statistical validation—such as the application of Abbott's correction and factorial analysis—is required to confirm the presence of true synergism (Pavela, 2015).

In the context of the development of biolarvicides from natural sources, it is important to consider that compounds such as flavonoids are not only limited to the currently studied plants, but are also present in many other plant species that show similar potential in controlling populations of dangerous mosquitoes such as Aedes aegypti (Derua et al., 2019). Further research on the combination and interactions between bioactive compounds in plant extracts can produce innovative and nature-based strategies for controlling mosquitoes. This paves the way for a more environmentally friendly and effective approach in the use of biolarvicides compared to conventional synthetic pesticides which have a negative impact on the ecosystem (Paulraj et al., 2020).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified several main compounds in papaya leaf and durian peel extracts that have the potential to act as insecticides.

Table 3. GC-MS Results of Papaya Leaf Extract and Durian Peel

No	Papaya Leaf Extract		Durian Skin Extract	
	Compound	Concentration % Per 1 ml	Compound	Concentration % Per 1 ml
1	Prilocaine	14.98	2-hydroxy-gamma-butyrolactone	10,01
2	Udecanoic acid, 10-methyl-, methyl ester	8,03	5-bromovaleric acid, 4-methoxyphenil ester	10,56
3	3-propoxyamphetamine	8,67	Lidocaine	10,27
4	Methyl stearate	7,12	Bis(2-ethylhexylo)phthalate	13,53
5	Hetriacontane	16,07		
5	Sulfurous acid. Dodecyl 2-propyl ester	8,04		
6	MDMA Methylene homolog	6,60		

The results of GC–MS analysis revealed the presence of a spectrum of bioactive compounds in papaya (Carica papaya) leaf extract which showed insecticidal activity through several mechanisms, including disturbances in the nervous system, metabolism, hormones and larval cell membranes. Research on Carica papaya phytochemistry confirms the presence of metabolites that act as bioactive agents, thus supporting the finding that this natural extract has potential as a biolarvicide (Kesuma et al., 2024; , Dhenge et al., 2021; , Simarmata et al., 2023). Although specific data regarding durian skin is still limited, recent studies indicate that tropical plants can produce compounds that correlate with insecticidal activity and have the potential to be an environmentally friendly alternative in controlling disease vectors (Kesuma et al., 2024; , Dhenge et al., 2021;).

The detection of compounds such as Prilocaine and Lidocaine in papaya leaf extract suggests a possible neurotoxicity mechanism, where these compounds can interfere with the transmission of nerve impulses in larvae. However, the presence of Bis (2-ethylhexyl) phthalate in durian skin extract and its relationship as an endocrine disruptor needs to be studied further, considering that it may require stronger supporting data regarding its impact on larval development through disruption of hormonal regulation Ammari et al., 2021).

This synergy between compounds with different mechanisms strengthens the proposition that the resulting insecticidal activity does not only depend on one mechanism, but is the result of a combination of complex interactions of various metabolites detected by GC–MS (Ammari et al., 2021). It is important to acknowledge that Bis(2-ethylhexyl)phthalate (DEHP) is a common plasticizer and environmental contaminant. Therefore, the possibility of its introduction as a contaminant during the extraction process from plasticware or other laboratory materials cannot be ruled out. Future investigations should include rigorous control measures and analytical procedures to differentiate between naturally occurring compounds in durian peel and potential contaminants. Further research is warranted to definitively confirm whether DEHP is a genuine natural product of Durio zibethinus or an artifact of the experimental process, and to elucidate its precise role, if any, in the observed larvicidal activity

With a combination of active compounds that operate through different mechanisms, natural extracts from papaya leaves offer the prospect of being an effective and more environmentally friendly alternative biolarvicide compared to synthetic larvicides such as temephos. This multifactorial approach not only reduces potential toxicity to humans and non-target animals, but also has the potential to reduce the emergence of resistance in vector larvae through exposure to compounds with diverse targets and mechanisms (Kesuma et al., 2024; , Dhenge et al., 2021; , Ammari et al., 2021)

4. CONCLUSION

The formulation of papaya leaf extract and durian peel extract shows potential as a natural biolarvicide against Aedes aegypti larvae with LC values$_{50}$ 131.95 ppm which is classified as toxic. The combination of papaya leaf extract and durian peel at a ratio of 50%:50% provides one of the most effective combinations, achieving 70% mortality. While these results are promising, future studies should incorporate statistical analysis to confirm the presence and extent of synergistic effects. GC-MS analysis identified the active compounds in the formula containing Prilocaine and Lidocaine which play a role in neurotoxic mechanisms and hormonal disruption to larvae.

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