GC–MS Profiling of Secondary Metabolites from Catharanthus roseus and Annona muricata Leaves and Their Larvicidal Potential Against Aedes aegypti

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HOW TO CITE:

Mei Ahyanti, Endah Setyaningrum, Gregorius Nugroho Susanto, Jhons Fatriyadi Suwandi (2026). GC–MS Profiling of Secondary Metabolites from Catharanthus roseus and Annona muricata Leaves and Their Larvicidal Potential Against Aedes aegypti. International Journal of Special Education, 41(17s), 1145-1158

ABSTRACT:

Mosquito-borne diseases remain a major public health challenge in tropical regions, while increasing resistance to synthetic insecticides has intensified the search for environmentally friendly alternatives. This study aimed to identify the secondary metabolites present in ethanol leaf extracts of Catharanthus roseus and Annona muricata using gas chromatography–mass spectrometry (GC–MS) and to evaluate their larvicidal activity against Aedes aegypti larvae. Mature leaves were shade-dried, powdered, and extracted with ethanol. The concentrated extracts were analyzed by GC–MS, and compounds were identified by comparison with the NIST mass spectral database. Larvicidal bioassays were conducted using extract concentrations ranging from 0.5% to 2.5%, with larval mortality observed for 48 hours. Median lethal concentration (LC_{50}) values were determined using probit analysis. GC–MS analysis of C. roseus revealed several bioactive compounds, including phenol, 4-ethenyl-2-methoxy; tristrimethylsilyl-P-glycolate; and 3',4',5,6,7,8-hexamethoxyflavone, the latter being the dominant constituent. In A. muricata, 1,1,3,3-tetramethyl-1,3-diethoxydisiloxane was detected as the major compound, although its presence may be associated with analytical artifacts. Both extracts exhibited concentration-dependent larvicidal activity. The LC_{50} values were 3.259% for C. roseus and 2.842% for A. muricata, indicating greater potency of the latter. These findings suggest that both plant extracts have potential as botanical larvicides against Aedes aegypti. Further studies are needed to confirm active compounds, optimize formulations, and evaluate environmental safety.

Keywords: Annona muricata; Catharanthus roseus; botanical larvicide; GC-MS; LC50; mosquito control.

Introduction

Vector-borne diseases remain a major global public health problem, especially in tropical and subtropical regions, where mosquitoes act as the main vectors of pathogens that cause malaria, dengue fever, and other infectious diseases. The increasing resistance of mosquito larvae to synthetic insecticides, along with their environmental toxicity and non-target effects, has prompted the search for safer, more environmentally friendly alternatives, particularly bioactive compounds of plant origin. Secondary metabolites of plants have received considerable attention due to their diverse chemical structure and biological activity, including insecticidal and larvicidal properties (Gao et al., 2018; WHO, 2017).

Medicinal plants are recognized as a rich source of secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds, which play important roles in plant defense and exhibit significant pharmacological and bioactive potential. These compounds have been extensively explored not only for therapeutic applications but also as natural pesticides due to their biodegradability and lower environmental impact compared to synthetic chemicals (Abdallah et al., 2024a; Rani et al., 2023a). People are looking for ways to deal with these problems. So they are trying to use plant-based products. For example, they are using biolarvicides. These are things that happen naturally. They break down easily. They are also better for the environment. They do not hurt other living things as much.

Some plants are particularly interesting to people conducting this research. The periwinkle and soursop plants are two of them. This is because they have many helpers within them that can do good things. The periwinkle plant contains alkaloids such as vinblastine and vincristine. These are good for fighting cells and making people feel better. The soursop plant has leaves rich in special helpers called acetogenins, such as annonacin. These are very good at killing insects. People think that periwinkle and soursop plants can be very useful in making things that can help with these problems (Dey et al., 2023; Goswami et al., 2024; Moghadamtousi et al., 2015).

Catharanthus roseus (*Apocynaceae*) is a well-known medicinal plant that produces a variety of bioactive secondary metabolites, most notably the indole terpenoid alkaloid (TIA), with more than 130 compounds identified (Chen et al., 2017; Joshi et al., 2025). These metabolites are associated with a variety of biological activities, including anticancer, antimicrobial, and antioxidant effects, and are considered important clues for drug development (Joshi et al., 2025). In addition, *C. roseus leaves* contain a diverse array of phytochemicals, including phenolics, flavonoids, fatty acids, and alkaloids, which can contribute to its bioactivity against insects and other biological targets (Ahyanti & Yushananta, 2023a; Rani et al., 2023a; Riddick, 2024).

Similarly, *Annona muricata* (*Annonaceae*), commonly known as soursop, is widely distributed in the tropics and has been used traditionally for a variety of medicinal purposes. The leaves of *A. muricata* are rich in secondary metabolites, including alkaloids, flavonoids, phenolics, essential oils, and, in particular, annonaceous acetogenins, which are known for their potent biological activity (Abdallah et al., 2024a; Ilango et al., 2022; Syazana & Porusia, 2022). Previous studies have identified compounds such as β-phytol and hexadecanoic acid in plants using GC-MS analysis. These compounds are said to have insecticide and biopesticide properties (Deewatthanawong, Kongchinda, Deewatthanawong, & Insung, 2019). Our understanding of plant metabolites is much better now thanks to tools like Gas Chromatography–Mass Spectrometry. This tool is really good at identifying and analyzing the

parts of plants that are hard to see. Gas Chromatography–Mass Spectrometry is widely used to study plant metabolites. This is because it is very good at identifying compounds and consistently gives reproducible results. It also provides a detailed picture of what is in plant extracts. We use Gas Chromatography–Mass Spectrometry to analyze plant metabolites because it's highly informative (Chen et al., 2017). Analyzing metabolite profiles can identify potential bioactive compounds that can enhance larvicide effectiveness and support vector management strategies for plants. Although they are widespread, only a few studies have examined the secondary metabolites of *C. roseus* and *A. muricata* for larvicide effectiveness. However, the use of GC-MS to identify compounds with enhanced metabolite profiles for larvicide activity remains poorly understood. This is one of the main steps in developing plant-derived vector control substances by identifying bioactive compounds that may improve larvicide effectiveness. We are interested in discovering whether these secondary metabolites, such as those found on the leaves of *Catharanthus roseus* and *Annona muricata*, can be used to natural larvicidal agents through analysis using GC-MS. The most significant aspect of this study is the integration of GC-MS with biological knowledge regarding larvicide potential, emphasizing an essential compound that could be utilized for mosquito larval control. This research is expected to advance environmentally friendly mosquito control strategies.

Methodology

Design

This study employed a quantitative laboratory-based experimental design consisting of two complementary stages. The first stage involved chemical profiling of ethanol leaf extracts of Catharanthus roseus and Annona muricata using Gas Chromatography–Mass Spectrometry (GC–MS). The second stage involved controlled larvicidal bioassays to evaluate the concentration-dependent toxicity of the extracts against third- to early fourth-instar Aedes aegypti larvae.

The GC–MS analysis was performed to identify the major secondary metabolites present in each plant extract and determine their relative abundance. The larvicidal experiment was subsequently conducted using five extract concentrations, appropriate negative and solvent controls, and repeated experimental measurements. Median lethal concentration values (LC_{50}) were estimated to compare the larvicidal potency of the two plant extracts.

Participants/Sample

The biological materials used in this study consisted of mature leaves of *C. roseus* and *A. muricata* and laboratory-reared *Ae. aegypti* larvae. Fresh leaves were collected from Kemiling District, Bandar Lampung City, Indonesia. Only mature, healthy, and physically undamaged leaves were selected. The plant species were identified based on their morphological characteristics before sample preparation. The collected leaves were washed thoroughly under running water to remove adhering dust and other surface contaminants.

The Ae. aegypti larvae used in this study were sourced from a laboratory colony at the Public Health Laboratory Center in Baturaja, South Sumatra, Indonesia. The colony was kept in a controlled environment with a temperature of 27 ± 2°C, relative humidity of 75 ± 10%, and a 12-hour light, 12-hour dark cycle. Only healthy, active third- to early fourth-instar larvae were selected for the larvicidal bioassays. For each extract concentration, 20 larvae were used per replicate. The test involved five concentrations—0.5%, 1.0%, 1.5%, 2.0%, and 2.5%. Each concentration was tested in four replicates, and the entire experiment was independently repeated three times under consistent laboratory conditions.

Instruments

Chemical profiling was performed using a GC-MS QP2020 gas chromatography–Mass Spectrometry system manufactured by Shimadzu Corporation, Kyoto, Japan. Chromatographic separation was achieved using an Rxi®-5Sil MS capillary column with dimensions of 30 m × 0.25

mm internal diameter and a film thickness of 0.25 µm.

Helium with a purity of 99.999% was used as the carrier gas. Mass spectra were obtained through electron ionization at 70 eV and recorded in full-scan mode across a mass range of m/z 40–600. The National Institute of Standards and Technology Mass Spectral Library, NIST 2020, was used as the reference database for compound identification.

Additional laboratory equipment included an analytical balance, electric blender, sealed maceration containers, Whatman No. 1 filter paper, rotary evaporator, dark glass storage bottles, micropipettes, larval exposure containers, and fine needles for assessing larval responsiveness. A temperature-controlled storage unit was used to maintain the concentrated extracts at 4°C before chemical analysis and bioassay testing.

Procedure

Plant Sample Preparation

The collected leaves of Catharanthus roseus and Annona muricata were thoroughly washed under running water to remove dust and other surface contaminants and were subsequently shade-dried at room temperature (approximately 25–30°C), for five to seven days. Shade drying was performed to reduce the moisture content while minimizing the degradation of potentially bioactive plant constituents. After drying, the leaves of each plant species were ground separately into a fine powder using an electric blender, transferred into airtight containers, and stored at room temperature until extraction.

Preparation of Ethanol Extracts

A total of 100 g of powdered leaves from each plant species was separately extracted through maceration using 500 mL of 96% ethanol in sealed containers at room temperature for 24 h. After maceration, each mixture was filtered using Whatman No. 1 filter paper, and the resulting filtrate was concentrated under reduced pressure using a rotary evaporator at 60°C until a viscous crude extract was obtained. The concentrated extracts were then transferred into dark glass bottles and stored at 4°C until they were used for GC–MS profiling and larvicidal bioassays.

GC–MS Profiling

A 1-µL aliquot of each plant extract was injected into the GC–MS system in split mode using a split ratio of 10:1, with the injector temperature maintained at 250°C and helium supplied as the carrier gas at a constant flow rate of 1.0 mL min^{-1}. The oven temperature was initially set at 60°C and held for 2 min, increased to 200°C at a rate of 10°C min^{-1}, and subsequently raised to 280°C at a rate of 5°C min^{-1}, with the final temperature maintained for 10 min, resulting in a total chromatographic run time of approximately 40 min. Mass spectrometric detection was performed using electron ionization at 70 eV, with the ion-source and interface temperatures maintained at 230°C and 280°C, respectively, while spectra were acquired in full-scan mode over an m/z range of 40–600. Compounds were identified by comparing the obtained mass spectra with reference spectra in the NIST 2020 database, and only compounds with a similarity index of at least 80% were accepted. Each extract was analyzed in triplicate under identical analytical conditions to ensure the reproducibility of the chromatographic results.

Larvicidal Bioassay

The larvicidal activity of the ethanol extracts was evaluated in accordance with the World Health Organization guidelines for mosquito larval susceptibility testing using extract concentrations of 0.5%, 1.0%, 1.5%, 2.0%, and 2.5%. Twenty healthy third- to early fourth-instar *Aedes aegypti* larvae were introduced into each exposure container; each concentration was tested in four replicates, and the entire experiment was independently repeated three times. A negative control containing dechlorinated water and a solvent control containing the highest solvent concentration used for extract dilution were included in each experimental series. The larvae were exposed for up to 48 h, and mortality was assessed based on the absence of movement or response following gentle stimulation with a fine

needle. Bioassays were discarded and repeated when control mortality exceeded 20%, whereas mortality was corrected using Abbott's formula when control mortality ranged from 5% to 20%:

$$\text{Corrected Mortality (\%)} = \frac{(\text{Observed Mortality} - \text{Control Mortality})}{(100 - \text{Control Mortality})} \times 100$$

Data Analysis

GC–MS chromatographic data were analyzed based on retention time, molecular ion characteristics, mass spectral fragmentation patterns, and spectral similarity with reference compounds available in the NIST 2020 database, with compound identification restricted to peaks exhibiting a similarity index of ≥80%. The relative abundance of each identified compound was determined using peak-area normalization, and because each extract was analyzed in triplicate, the mean relative peak area from the three analytical replicates was used to characterize the metabolite profile of each plant species. Larval mortality was calculated by dividing the number of dead larvae by the total number of exposed larvae and expressing the result as a percentage, while Abbott's correction was applied when control mortality ranged from 5% to 20%. The concentration–mortality relationship for each plant extract was evaluated using probit analysis, from which the median lethal concentration (LC_{50}) and its corresponding 95% confidence interval were estimated. A lower LC_{50} value was interpreted as indicating greater larvicidal potency against *Aedes aegypti* larvae.

$$\text{Relative peak area (\%)} \frac{\text{Individual compound peak area}}{\text{Total area of all identified peaks}} \times 100$$

Results

GC–MS Chromatographic Profiles

GC–MS analysis produced distinct total ion chromatogram profiles for the ethanol leaf extracts of *Catharanthus roseus* and *Annona muricata*. The chromatogram of the *C. roseus* extract contained multiple peaks distributed across the chromatographic run, with greater signal intensity observed between approximately 20 and 30 min. An early peak was recorded at approximately 1 min, followed by several peaks of varying intensity. The highest-intensity peaks were observed within the retention-time range of approximately 25–28 min.

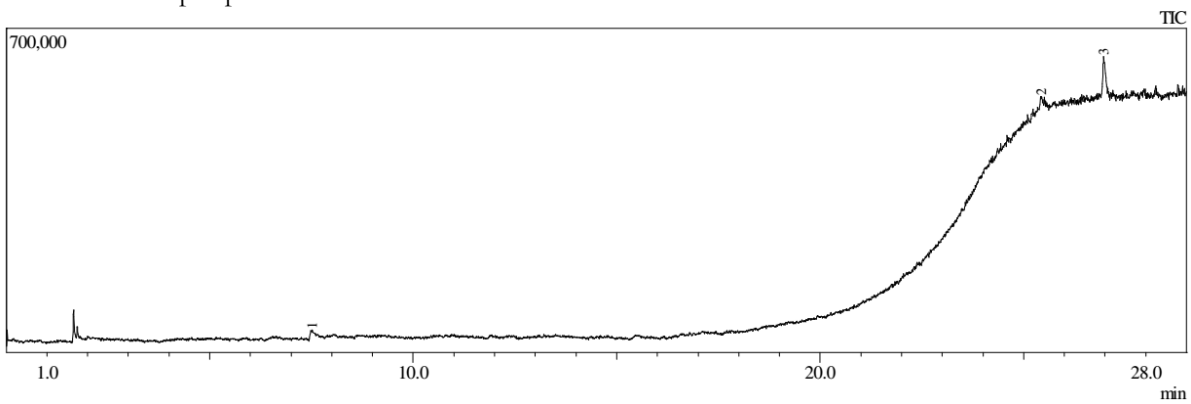

Figure 1. GC–MS chromatogram of the ethanol leaf extract of *Catharanthus roseus*.

The chromatogram of the *A. muricata* extract displayed a different peak-distribution pattern. Several low-intensity signals were recorded between approximately 1 and 10 min, whereas a progressive increase in signal intensity occurred after 20 min. The maximum chromatographic response was observed within the retention-time range of approximately 27–29 min.

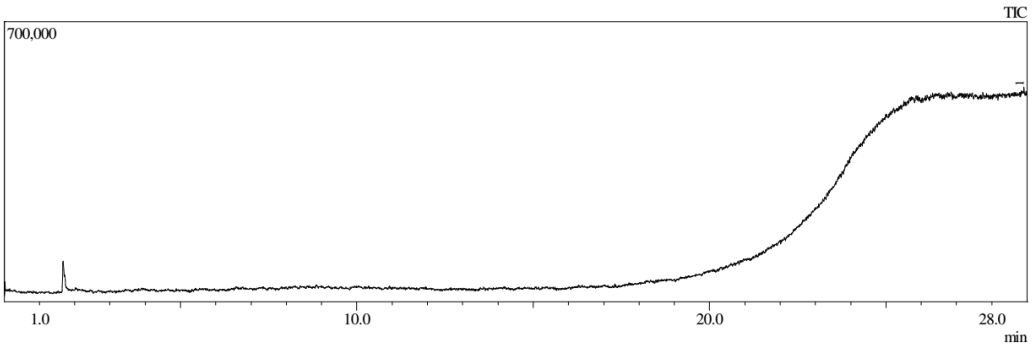

Figure 2. GC–MS chromatogram of the ethanol leaf extract of *Annona muricata*.

Compounds Identified by GC–MS

GC–MS analysis identified three compounds in the ethanol leaf extract of *C. roseus* and one major compound in the ethanol leaf extract of *A. muricata*. The identified compounds, retention times, molecular weights, molecular formulas, and relative peak areas are presented in Table 1.

Table 1. Compounds identified in the ethanol leaf extracts of *Catharanthus roseus* and *Annona muricata* by GC–MS analysis

Plant species	Retention time (min)	Molecular weight	Identified compound	Molecular formula	Relative peak area (%)
C. roseus	7.518	150	Phenol, 4-ethenyl-2-methoxy	$C_9H_{10}O_2$	17.13
C. roseus	25.433	372	Tristrimethylsilyl-P-glycolate	$C_{11}H_{29}O_6PSi_3$	14.15
C. roseus	26.967	402	3',4',5,6,7,8-Hexamethoxyflavone	$C_{21}H_{22}O_8$	68.72
A. muricata	28.909	222	1,1,3,3-Tetramethyl-1,3-diethoxydisiloxane	$C_{10}H_{32}O_4S_{15}$	100

Among the compounds detected in the C. roseus extract, 3',4',5,6,7,8-hexamethoxyflavone had the highest relative peak area at 68.72%. Phenol, 4-ethenyl-2-methoxy accounted for 17.13% of the total identified peak area, whereas tristrimethylsilyl-P-glycolate accounted for 14.15%. In the A. muricata extract, the chromatographic peak assigned to 1,1,3,3-tetramethyl-1,3-diethoxydisiloxane represented 100.00% of the identified peak area.

Larvicidal Activity

Both ethanol leaf extracts produced increasing mortality of third- to early fourth-instar *Aedes aegypti* larvae across the tested concentration range. The observed mortality after 48 h of exposure is presented in Table 2.

Table 2. Mortality of *Aedes aegypti* larvae after 48 h of exposure to *Catharanthus roseus* and *Annona muricata* leaf extracts

Plant extract	Concentration (%)	Larvae per replicate (n)	Mean larval deaths (n)	Mortality (%)
C. roseus	0.5	20	1	5
C. roseus	1	20	2	10

C. roseus	1.5	20	5	25
C. roseus	2	20	7	35
C. roseus	2.5	20	11	55
A. muricata	0.5	20	2.5	13
A. muricata	1	20	4.5	23
A. muricata	1.5	20	8	40
A. muricata	2	20	10.5	53
A. muricata	2.5	20	15.5	78

For the *C. roseus* extract, larval mortality increased from 5.0% at a concentration of 0.5% to 55.0% at 2.5%. Mortality levels of 10.0%, 25.0%, and 35.0% were recorded at concentrations of 1.0%, 1.5%, and 2.0%, respectively. For the *A. muricata* extract, mortality increased from 13.0% at 0.5% to 78.0% at 2.5%. Mortality levels of 23.0%, 40.0%, and 53.0% were recorded at concentrations of 1.0%, 1.5%, and 2.0%, respectively. At every tested concentration, the mortality recorded for the *A. muricata* extract was numerically higher than that recorded for the *C. roseus* extract. The greatest difference between the two extracts was observed at a concentration of 2.5%, at which mortality reached 78.0% for *A. muricata* and 55.0% for *C. roseus*.

4. Discussion

The present study set out to characterize the secondary metabolites contained in the ethanol leaf extracts of Catharanthus roseus and Annona muricata and to evaluate their larvicidal potential against Aedes aegypti. This study's findings indicate that the two plant species produced distinct gas chromatographic–mass spectrometry (GC–MS) chromatographic profiles and demonstrated concentration-dependent larvicidal activity. Despite the chemical profile of C. roseus being characterized by a greater number of identifiable chromatographic compounds, the A. muricata extract consistently produced a stronger larval mortality rate. This discrepancy between chromatographic detectability and biological activity is significant because it suggests that the number or relative abundance of GC–MS-detectable compounds cannot be interpreted directly as a measure of larvicidal potency. Conversely, it can be argued that biological activity may depend not only on the intrinsic toxicity of particular constituents, but also on interactions among multiple metabolites, in addition to the presence of non-volatile compounds that are not adequately detected by GC-MS.

The chromatographic profile of C. rosea was found to be predominantly characterized by 3',4',5,6,7,8-hexamethoxyflavone, accompanied by phenol, 4-ethenyl-2-methoxy, and tristrimethylsilyl-P-glycolate. The presence of a methoxylated flavonoid is consistent with the wider phytochemical variability previously documented in C. roseus, encompassing flavonoids, phenolic compounds, fatty acids, terpenoids and indole alkaloids (Deewatthanawong, Kongchinda, Deewatthanawong, Pumnuan, et al., 2019; Hari & Mathew, 2018; Louis et al., 2020; Shibula & Velavan, 2015). The utilization of ethanol for the extraction of these constituents is compatible with the use of ethanol, a method which can recover compounds across a relatively broad polarity range, including semi-polar and some non-polar metabolites (Agu & Okolie, 2017; Olasehinde et al., 2022; Ruiz-López et al., 2025; Shibula & Velavan, 2015).

The presence of 3',4',5,6,7,8-hexamethoxyflavone in the C. roseus extract may provide a molecular-level explanation for the observed larvicidal effect. It is generally accepted that methoxylated flavonoids are more lipophilic than their non-methylated counterparts. This property may facilitate their interaction with lipid-rich biological membranes and insect cuticular structures. Flavonoids have also been associated with insecticidal, antimicrobial, and antioxidant

activities, although these effects are contingent on molecular structure, concentration, and target organism (Pereira et al., 2024; Yadav et al., 2024). In the context of mosquito larvae, lipophilic plant metabolites have been observed to penetrate the cuticle, alter membrane function, or interfere with enzymes involved in neurological and metabolic regulation (Huang et al., 2016; Nguyen-Ngo et al., 2020; Thomann et al., 2024). However, the present findings do not establish a direct causal relationship between hexamethoxyflavone and larval mortality (Bugel et al., 2016). To identify this metabolite as the principal larvicidal agent, confirmatory isolation and compound-specific bioassays would be required.

The larvicidal activity observed in the C. roseus extract may be attributable to the phenolic compound identified in the extract. Phenolic constituents can interact with proteins and cell membranes, potentially disrupting membrane integrity, enzyme activity, and cellular homeostasis (Liu et al., 2024). Such effects may act in combination with flavonoids and other constituents that have not yet been detected. The biological response observed for the crude extract may therefore reflect additive or synergistic interactions, rather than the effect of one dominant compound (Caesar & Cech, 2019; Chaachouay, 2025). This interpretation aligns with evidence indicating that the application of complex plant extracts can result in stronger or more widespread biological activity through interactions between multiple secondary metabolites (El-Akhal et al., 2024; Hadidy et al., 2022; Haikal et al., 2025). Such chemical complexity may prove advantageous in the development of botanical larvicides, as it could potentially reduce reliance on a single biochemical target.

The interpretation of the A. muricata chromatographic profile requires greater caution. The principal compound assigned by the spectral library was 1,1,3,3-tetramethyl-1,3-diethoxydisiloxane. Siloxane-related compounds are frequently associated with analytical contamination, septum bleeding, column degradation, solvent impurities, or sample-handling materials rather than native plant metabolism. As indicated in the relevant literature, concerns regarding siloxane detection have been raised in phytochemical GC–MS studies, particularly when a siloxane derivative dominates the identifiable chromatogram (Abdallah et al., 2024b; Gavamukulya et al., 2014; Rani et al., 2023b; Syeda & Riazunnisa, 2020). Consequently, it can be concluded that the siloxane detected should not be regarded as the active larvicidal constituent of A. muricata without confirmation using authentic standards, procedural blanks, and complementary analytical techniques.

The stronger larvicidal response exhibited by A. muricata, despite its restricted GC–MS profile, is presumably attributable to metabolites that were either insufficiently volatile or lacked sufficient thermal stability for conventional GC–MS detection. Annona muricata leaves have been reported to contain a range of chemical compounds, including annonaceous acetogenins, alkaloids, flavonoids, terpenoids, and phenolic compounds (Abdallah et al., 2024b; Bharathithasan et al., 2021; Botelho et al., 2022; Ganesan et al., 2023; Zuharah et al., 2021). Acetogenins are of particular relevance in this context, given their ability to inhibit mitochondrial complex I, disrupt electron transport, reduce ATP production, and ultimately impair cellular survival. This mechanism provides a biologically plausible explanation for the larvicidal activity of A. muricata extracts (Zuharah et al., 2021). However, due to the absence of direct identification of acetogenins in the present GC–MS analysis, their contribution remains as a literature-supported hypothesis rather than a confirmed mechanism.

It has been previously reported that other constituents are present in A. muricata, including phytol, β-caryophyllene, hexadecanoic acid, linoleic acid, and related lipophilic metabolites (Olasehinde et al., 2022; Shibula & Velavan, 2015). These may also contribute to larvicidal activity. The aforementioned compounds have been associated with alterations in membrane

permeability, energy metabolism, cellular integrity, and insect detoxification systems (Bae et al., 2017; Milugo et al., 2021, 2024). The absence of these expected constituents from the present chromatographic identification underscores the necessity for complementary chemical analyses. The most appropriate methods to detect thermolabile, polar, and high-molecular-weight metabolites that may be responsible for the observed biological activity would be Liquid Chromatography–Mass Spectrometry, High-Resolution Mass Spectrometry, or targeted metabolomic profiling.

The concentration-dependent mortality produced by both extracts supports their potential as botanical larvicides. Concentration-response patterns analogous to those previously reported in studies evaluating plant-derived substances against mosquito larvae have been observed in this study. These patterns demonstrate that increasing exposure to crude extracts results in greater disruption of physiological and metabolic processes (Ahyanti & Yushananta, 2023b; Amir et al., 2025; Felipe Oliveros-Díaz et al., 2022; Mgbemena, 2010; Syifa Zulfiani Zakiyah Susilo & Aminah Asngad, 2024). It can be posited that the markedly augmented response evidenced for A. muricata may imply that its crude ethanol extract contains constituents with more pronounced larvicidal properties, or that it comprises a combination of active compounds that is more efficacious than that of the C. roseus extract. Nevertheless, discrepancies in extraction yield, compound solubility, penetration through the larval cuticle, and stability in the exposure medium may also affect the relative activity of the two extracts.

From a mechanistic perspective, the larvicidal effects of the extracts may be attributable to the engagement of multiple physiological targets. The association between flavonoids and terpenoids and neurological disturbance, as indicated by possible interference with acetylcholinesterase-mediated neurotransmission, has been documented (Altemimi et al., 2017; Oladimeji & Valan, 2020; Ribeiro et al., 2023; Sasidharan et al., 2011). In contrast, phenolic compounds and fatty acids have been shown to alter membrane permeability and intracellular stability (Sasidharan et al., 2011). The potential of acetogenins to impede mitochondrial respiration and cellular energy production is a salient consideration. These mechanisms should be interpreted with caution, as they were not directly measured in the present study. However, the potential for multi-target toxicity is a salient consideration in the context of mosquito-control research, given that botanical extracts comprising chemically diverse constituents can impose multiple physiological stresses concurrently. This could result in a slower rate of resistance development compared with insecticides that act through a single target. However, further research in the form of prolonged selection studies and resistance-monitoring experiments is required to validate this hypothesis (Lim et al., 2023; Martínez Rodríguez et al., 2024).

The results of the study indicate that the findings have practical implications for the development of environmentally oriented vector-control products. The geographical distribution of both plants extends to tropical regions, and their leaves have the potential to serve as raw materials for formulating botanical larvicides. The deployment of a plant-derived larvicide has the potential to enhance existing mosquito management programs, particularly in contexts where resistance to conventional insecticides has compromised the efficacy of control measures. However, the inherent safety of crude plant extracts, derived from natural sources, should not be assumed without rigorous evaluation. Prior to operational implementation, a comprehensive evaluation of standardized extraction procedures, formulation stability, residual activity, appropriate application doses, and effects on non-target aquatic organisms is imperative. The adoption of a policy would also require establishing quality-control specifications, a toxicological assessment, production standards, and evidence of field effectiveness under variable environmental conditions.

It is imperative to acknowledge the limitations that are inherent to this methodology. First,

compound identification was primarily based on spectral library matching rather than confirmation with authentic analytical standards. Consequently, the identified compounds must be regarded as tentative. Second, GC-MS has an inherent capacity that makes it especially suitable for detecting volatile and semi-volatile constituents; however, it may not necessarily detect important non-volatile metabolites, a category that includes a significant number of alkaloids and acetogenins. Thirdly, the predominant siloxane signal in the A. muricata extract may indicate an analytical artifact, underscoring the need for procedural blanks and enhanced contamination control measures. Fourthly, the study utilized crude ethanol extracts, thereby precluding the ability to attribute larvicidal activity to a specific compound or to ascertain whether the observed effect resulted from additive, synergistic, or antagonistic interactions. The fifth point to consider is that the bioassay was conducted under controlled laboratory conditions using a mosquito colony that was maintained in a laboratory setting. This may mean that the results of the study are not necessarily an accurate prediction of the efficacy of the product when used against field populations, which may have different physiological characteristics or insecticide-resistance profiles. Finally, the effects on non-target organisms, the stability of the extract, the residual activity of the extract, and its environmental persistence were not evaluated.

Future research should combine GC–MS with LC–MS-based metabolomic analysis, isolate the major candidate compounds, and evaluate purified fractions through compound-specific and combination bioassays. Mechanistic assays examining acetylcholinesterase activity, mitochondrial function, oxidative stress, and membrane integrity would clarify the pathways involved in larval mortality. Field-based evaluations and ecotoxicological testing are also necessary before either extract can be recommended for practical mosquito-control applications. Overall, the study provides preliminary evidence that *C. roseus* and *A. muricata* leaves contain biologically active metabolites with potential for botanical larvicide development, while also demonstrating that chromatographic identification must be interpreted alongside biological testing and methodological limitations.

Conclusion

This study demonstrated that ethanol leaf extracts of Catharanthus roseus and Annona muricata possess distinct secondary-metabolite profiles and measurable larvicidal activity against Aedes aegypti larvae. GC–MS profiling of C. roseus identified several compounds, with 3',4',5,6,7,8-hexamethoxyflavone as the predominant constituent, accompanied by phenolic and silylated compounds. In contrast, the chromatographic profile of A. muricata was dominated by a siloxane-related compound whose biological relevance remains uncertain because it may represent an analytical artifact. Despite the limited number of compounds detected in its GC–MS profile, A. muricata produced greater larval mortality than C. roseus across the tested concentrations. Both extracts exhibited concentration-dependent activity, indicating their potential as sources of plant-derived larvicidal agents.

The findings further show that GC–MS-detectable chemical diversity does not necessarily correspond directly to biological potency. The stronger larvicidal activity of A. muricata may be associated with non-volatile or thermolabile metabolites that were not adequately detected under the analytical conditions applied. Therefore, the identified compounds should be regarded as preliminary chemical markers rather than definitively confirmed active larvicidal constituents.

Future studies should employ complementary analytical techniques, particularly LC–MS or high-resolution mass spectrometry, to identify non-volatile metabolites and confirm compound assignments using authentic standards. Bioassay-guided fractionation is also recommended to isolate the active fractions and determine whether larvicidal activity results from individual compounds or synergistic interactions among multiple metabolites. Additional investigations should assess mechanisms of action, formulation

stability, residual effectiveness, toxicity to non-target aquatic organisms, and performance against field-derived mosquito populations. These steps are essential before either extract can be developed or recommended as a safe, standardized, and operational botanical larvicide for mosquito-control programs.

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Acknowledgments

The authors gratefully acknowledge the institutional and laboratory support provided by Poltekkes Kemenkes Tanjungkarang, Universitas Lampung, and the Public Health Laboratory Center in Baturaja, South Sumatra, Indonesia, particularly for facilitating plant-sample preparation, GC–MS analysis, and the provision and maintenance of Aedes aegypti larvae used in the larvicidal bioassays. The authors also appreciate the technical assistance of laboratory personnel who contributed to the successful completion of this study.

Author Declaration

The authors declare that the manuscript entitled "GC–MS Profiling of Secondary Metabolites from Catharanthus roseus and Annona muricata Leaves and Their Larvicidal Potential Against Aedes aegypti" represents original work that has not been published previously and is not currently under consideration

for publication elsewhere. All authors have read and approved the final version of the manuscript and have agreed to its submission for publication. The authors further declare that there are no financial, personal, professional, or institutional conflicts of interest that could have influenced the conduct, analysis, interpretation, or reporting of this study.