**JPPI (Jurnal Penelitian Pendidikan Indonesia)**
Volume 11, Number 3 2025


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Publication details, including author guidelines
URL: https://jurnal.iicet.org/index.php/jppi/about/submissions#authorGuidelines
Editor: Jeki Haryanto

Article History
Received: 13 Jul 2025
Revised: 11 Aug 2025
Accepted: 14 Sept 2025

How to cite this article (APA)
Zen, S., & Sulistiani, W. S. (2025). Learning the isolation of bioactive compounds and the utilization of the seven winds plant (polygala paniculata l) as an anti-mosquito innovation of aedes aegypti l. Jurnal Penelitian Pendidikan Indonesia.11(3), 189-197. https://doi.org/10.29210/020255424

The readers can link to article via https://doi.org/10.29210/020255424

**JPPI (Jurnal Penelitian Pendidikan Indonesia)**
ISSN: 2502-8103 (Print) ISSN: 2477-8524 (Electronic)

Vol. 11, No. 3, 2025, pp. 189-197
DOI: https://doi.org/10.29210/020255424

# Learning the isolation of bioactive compounds and the utilization of the seven winds plant (polygala paniculata l) as an anti-mosquito innovation of aedes aegypti l

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## Article Info

### History of the article:

Received Jan 21th, 2025
Revised Sept 3th, 2025
Accepted Oct 7th, 2025

### Keywords:

Polygala paniculata
Bioactive compound
Isolation
Anti-mosquito
Aedes aegypti

## ABSTRACT

This study aims to explore the isolation of bioactive compounds from Polygala paniculata L. (Seven Winds plant) and evaluate its potential as an environmentally friendly anti-mosquito innovation. The research employed a laboratory-based experimental approach, including precise steps for sample preparation, extraction, partitioning, and phytochemical testing. A total of 500 grams of Polygala paniculata plant material were used, sourced from rice field areas where the plant naturally grows. The plants were washed, air-dried in the shade, and ground into simplicia. The simplicia was extracted using the maceration method with methanol for 24 hours, repeated four times. The extract was then evaporated using a rotary evaporator at 40–45°C, yielding 28.72 grams of viscous extract. The extract was partitioned using n-hexane and ethyl acetate solvents to separate compounds based on their polarity. Phytochemical analysis identified several bioactive compounds, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolics. Among these, flavonoids, saponins, and terpenoids were identified as having significant potential biological activity, including natural insecticide properties. Although direct testing on Aedes aegypti was not conducted, the findings suggest that these compounds could play a role in future mosquito control applications. The study also contributes to educational practice by enhancing students' understanding of bioactive compound isolation techniques. This study provides an example of how local biodiversity can be explored to develop eco-friendly solutions, with the potential for natural mosquito repellent products in the future. Further studies are needed to test the toxicity of these compounds and their practical efficacy in field conditions.



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# Introduction

Research-based learning in the field of science offers students the opportunity to understand the application of scientific knowledge in addressing real-world issues (Isaac et al., 2022). One pressing problem relevant to this context is the control of disease vectors, particularly Aedes aegypti, the primary vector of dengue hemorrhagic fever (DHF) (Pérez-Guerra et al., 2024). Current mosquito population control methods still rely heavily on synthetic insecticides, which are non-selective and can harm non-target organisms, including humans (Şengül Demirak & Canpolat, 2022). Common synthetic insecticides such as DEET and icaridin, while effective, pose health risks when used over extended periods. This raises concerns about the long-term sustainability of chemical-based mosquito control methods.

Given these challenges, there is a pressing need for safer, more environmentally friendly alternatives (Kurul et al., 2025). One promising alternative is the development of bioinsecticides derived from local plants. Polygala paniculata L. (Seven Winds plant), known for its diverse bioactive compounds, offers significant potential as a natural mosquito repellent and an anti-mosquito agent. Beyond its potential in aromatherapy and as a preventative measure to minimize mosquito-human contact (Luker et al., 2023). Polygala paniculata is an economical alternative to more expensive solutions, such as lavender essential oil, due to its short growth cycle (4–5 months) and ease of cultivation (Tokumoto et al., 2020).

While previous studies have documented the bioactive compounds in Polygala paniculata, including those with cytotoxic, anticancer, antibacterial, antimycotic, antioxidant, and anti-inflammatory properties (de Paula Nogueira Cruz et al., 2021), there is a gap in understanding the plant's specific potential as an anti-mosquito agent. This study seeks to build upon these findings by focusing on the isolation and identification of bioactive compounds in Polygala paniculata that may be effective in controlling Aedes aegypti populations.

The research will test the hypothesis that specific bioactive compounds isolated from Polygala paniculata possess mosquito-control potential. By isolating these compounds and evaluating their biological activity, the study will contribute both to the advancement of phytochemistry and the practical development of environmentally friendly bioinsecticides (Nikolaou et al., 2021). The study aims to offer new insights into vector control strategies, specifically through the use of plant-based bioinsecticides.

The objectives of this study are to: 1) Isolate and identify bioactive compounds from Polygala paniculata; 2) Evaluate the phytochemical activity of these compounds against Aedes aegypti; 3) Assess their potential as effective bioinsecticides in controlling mosquito populations. This study will not only contribute to the academic field of phytochemistry and bioinsecticide development but also provide practical applications for public health, offering a sustainable and eco-friendly alternative to synthetic insecticides (Wuryantini et al., 2021).

# Method

This research utilizes an experimental design to explore the isolation and identification of bioactive compounds from Polygala paniculata L (Martins-Silva et al., 2025). (Seven Winds plant) for potential use as a bioinsecticide against Aedes aegypti (Wahyuni et al., 2022). The study is conducted in a controlled laboratory setting to ensure precise and reproducible results (Rosendo et al., 2020). The aim is to introduce students to the scientific process, including sample preparation, isolation techniques, and result analysis (Chengere et al., 2025). The study follows a structured approach with both treatment and control groups, and incorporates key steps in scientific research, from sample collection to data analysis (Thomas, 2023).

The first stage of the research involves the preparation of plant material (Abubakar & Haque, 2020). The Polygala paniculata plants are carefully selected based on inclusion criteria, including plant age (at least six months), health condition (free from visible disease), and harvest season (preferably during the peak growth period). The plants are then washed thoroughly with clean water to remove any dirt or contaminants, followed by natural drying in the shade to preserve the bioactive compounds. After drying, the plants are cut into small pieces and ground into a fine powder using a blender. A total of 500 grams of simplicia powder is used for each extraction trial, and the process is repeated with three separate plant samples to ensure replication and reduce variability (Royani et al., 2023).

The extraction process involves maceration, where the plant powder is soaked in methanol for 24 hours. This process is repeated four times, with the liquid being periodically shaken to promote the maximum dissolution of bioactive compounds (Paulo et al., 2022). The macerated extract is then filtered to separate the liquid from the residue. The resulting solution is evaporated using a rotary evaporator at 40–45°C to remove solvents, leaving behind a viscous extract. The extract is then re-dissolved in a methanol:water mixture (1:2 ratio) to facilitate the isolation of bioactive compounds.

To separate the compounds based on polarity, the solution is partitioned using n-hexane and ethyl acetate solvents. The fractions obtained are categorized as non-polar (n-hexane), semi-polar (ethyl acetate), and polar (methanol-water) (Nkwocha et al., 2024). Each fraction is subjected to phytochemical tests to identify bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and terpenoids. The presence of specific compounds is indicated by color changes or foam formation in response to the addition of appropriate reagents (Godlewska et al., 2022). For instance, saponins are confirmed by stable foam formation, while flavonoids and tannins are detected by specific color changes.

To ensure reliability and reduce bias, the research includes both negative and positive control groups (Landy et al., 2020). The negative control involves using a solvent without plant material, while the positive control includes a standard mosquito repellent compound (e.g., DEET) for comparison. All samples and controls undergo randomization in the testing phase, and phytochemical tests are conducted with blinding to prevent researcher bias (Omran et al., 2023). The methodology ensures that each fraction undergoes the same procedure to maintain consistency across all treatments.

The study also includes statistical analysis to validate the findings. Effect sizes, p-values, and confidence intervals are calculated to assess the significance of the results (Serdar et al., 2021). Statistical power analysis is conducted to determine the sample size needed for reliable conclusions. Data are analyzed using appropriate statistical methods to ensure the robustness of the results and minimize the risk of overinterpretation or bias.

This research method is designed not only to produce scientific data but also to enhance students′ understanding of the scientific process and the potential applications of local biological resources in sustainable solutions for mosquito control (Sundari et al., 2023). By integrating theoretical knowledge with practical experimentation, this study aims to foster critical thinking and an appreciation for biodiversity as an essential resource for environmental health innovations.

# Results and Discussion

This research used an experimental approach to demonstrate the isolation of bioactive compounds from Polygala paniculata L (Wahyuni et al., 2022). (Seven Winds plant) and assess its potential application as an environmentally friendly anti-mosquito innovation (Martins-Silva et al., 2025). The study provided learning opportunities for students in science-based methods for the isolation of natural compounds.

## Sample Preparation

The Polygala paniculata plants were obtained from areas surrounding rice fields, where they naturally grow under trees. The preparation process began with washing the plants to remove dirt and soil, followed by air-drying in the shade to preserve bioactive compounds that may be sensitive to sunlight (Sundari et al., 2021). After drying, the plants were cut into small pieces and ground into a fine powder, which was used for maceration.

## Extraction and Partitioning Process

Maceration was used as the primary extraction method. In this step, 500 grams of simplicia powder was soaked in methanol. Methanol was chosen because of its polar nature, which allows it to dissolve a wide range of bioactive compounds (Ferreira & Sarraguça, 2024). The maceration process lasted for 24 hours, repeated four times with periodic shaking to improve extraction efficiency. After maceration, the solution was filtered and evaporated using a rotary evaporator at 40–45°C, yielding 28.72 grams of a thick extract.

The thick methanol extract was then dissolved in a methanol:water mixture (1:2 ratio) to increase solubility based on polarity. Partitioning was carried out using n-hexane (for non-polar compounds) and ethyl acetate (for semi-polar compounds), separating the extract into three main fractions: the n-hexane fraction, the ethyl acetate fraction, and the methanol-water fraction.

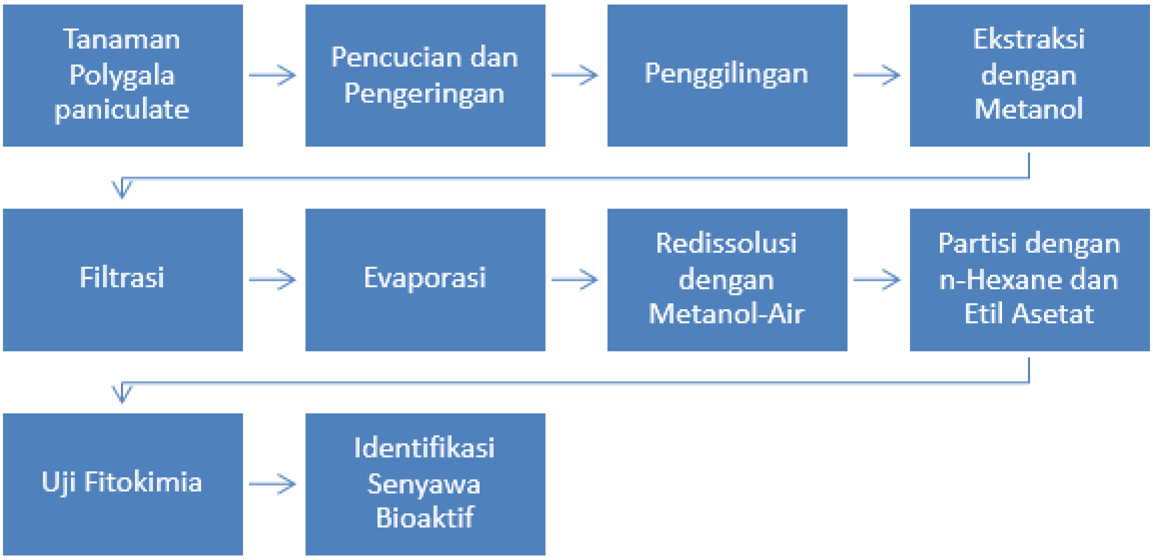


**Figure 1** The Thick Methanol Extract

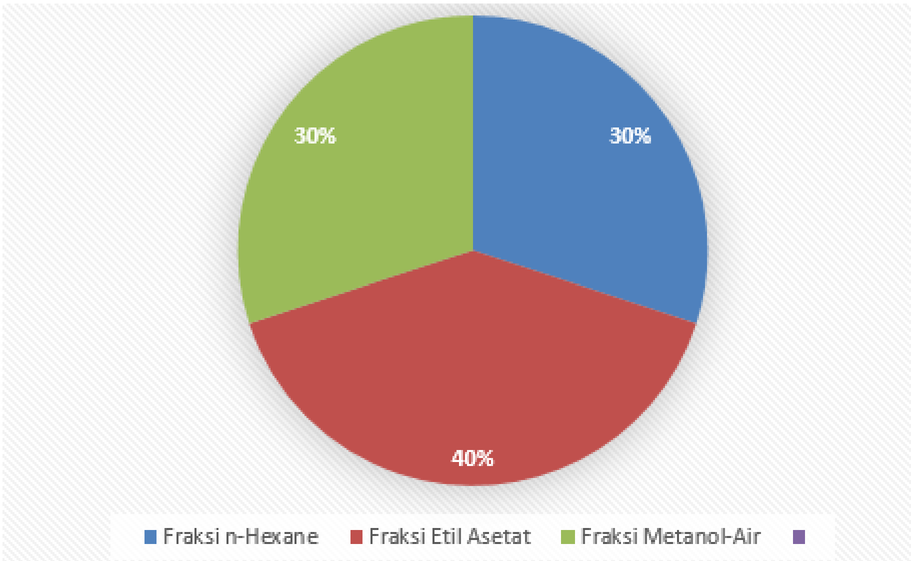


**Figure 2** Distribution of Fraction by Solvent

Fraksi n-Hexane: 30% Fraksi Etil Asetat: 40% Fraksi Metanol-Air: 30%

## Phytochemical Tests

Each fraction was evaluated using phytochemical tests to identify the presence of bioactive compounds, such as alkaloids, flavonoids, tannins, saponins, and terpenoids. The test results indicated specific color changes, which served as indicators for the presence of these compounds: (1) Saponins is stable foam formation, indicating natural surfactant activity; (2) Alkaloids, Color change to orange or brown when reacted with specific reagents; (3) Flavonoids: Bright yellow discoloration in testing (Giusti et al., 2023).

This table can show the bioactive compounds identified in each fraction obtained from the extraction and partitioning process. The table can include the compounds tested and indicate which fractions contain those compounds.

**Table 1.** Bioactive Compound Identification Table

| Bioactive Compound | n-Hexane Fraction | Ethyl Acetate Fraction | Methanol-Water Fraction |
|---|---|---|---|
| Saponin | (+) | (+) | (+) |
| Alkaloid | (+) | (-) | (+) |
| Flavonoid | (-) | (+) | (+) |
| Tannin | (-) | (+) | (+) |
| Terpenoid | (+) | (+) | (+) |
| Phenolic | (-) | (-) | (+) |

Notes: (+) indicates the compound was identified in the fraction and (-) indicates the compound was not identified in the fraction.

The results of these tests provided significant learning opportunities for students in understanding isolation techniques and the characterization of bioactive compounds: (1) Educational Context: This isolation process can be used as a practicum learning module that introduces students to solvent-based extraction technology as well as the concept of compound polarity; (2) Potential Applications: Bioactive compounds isolated from the seven winds plant, such as flavonoids and terpenoids, are known to have biological activity as mosquito repellents (Lowe et al., 2021). This confirms that the exploration of natural compounds from biodiversity can support environmentally friendly innovations in disease vector control; (3) Integration of Science and Environment: This study shows how chemistry can be applied to solve community problems, namely the control of Aedes aegypti mosquitoes as a vector of dengue hemorrhagic fever (DHF).

The process of isolating bioactive compounds from the seven winds plant not only provides results in the form of a fraction of active compounds that have the potential to be anti-mosquito, but also offers a learning experience that is integrated with laboratory practice. This method provides learners with insight into the importance of utilizing local resources for sustainable innovation.

This study aimed to provide insights into the process of isolating bioactive compounds from Polygala paniculata and evaluate its potential application as an environmentally friendly anti-mosquito innovation. The isolation process involved several stages, including extraction, partitioning, and phytochemical tests, providing a practice-based learning model for students to understand organic chemistry and pharmacology.

### Isolation of Bioactive Compounds by Chromatography

The initial stage of isolation involved thin-layer chromatography (TLC) to determine the optimal solvent ratio for separating compounds (Joshi et al., 2024). Various eluene ratios such as methanol:ethyl acetate (8:2, 7:3, 6:4, 5:5, 3:7, 2:8) and ethyl acetate:n-hexane (8:2, 7:3, 6:4, 5:5, 3:7, 2:8) were tested to assess the separation ability of compounds in viscous methanol extracts. The TLC results showed that the optimal solvent combinations for column chromatography were n-hexane:ethyl acetate (8:2, 7:3, 3:7) and ethyl acetate:methanol (7:3).

Column chromatography was performed using 45 grams of silica gel adsorbent with 28.72 grams of viscous extract. This separation resulted in 44 fractions, which were further analyzed using TLC. Based on this analysis, methyl salicylate was identified in the 6th fraction with an Rf value of 0.925, which was close to the standard methyl salicylate obtained via esterification of salicylic acid with methanol.

### Identification of Bioactive Compounds through Phytochemical Tests

Phytochemical tests revealed that the Polygala paniculata contained bioactive compounds such as saponins, alkaloids, flavonoids, tannins, terpenoids, and phenolics. Steroid compounds were not detected in the extract. This identification provides an understanding of the diversity of secondary metabolites in the plant: (1) Saponins, Indicated by stable foam formation. Saponins have natural pesticide activity, disrupting insect cell membranes, affecting the life cycle of Aedes aegypti mosquitoes (Matsuura & Fett-Neto, 2015); (2) Alkaloids, Indicated by brownish-white discoloration. Alkaloids serve as natural defenses against herbivores and pathogens and have insecticidal potential (Sangi et al., 2019); (3) Flavonoids, Indicated by yellow discoloration. Flavonoids have antioxidant and antimicrobial properties and may play a role in repelling mosquitoes (Weston & Mathesius, 2013); (4) Tannins, Indicated by bluish-black discoloration. Tannins inhibit insect digestive enzymes, potentially reducing mosquito survival (Ashok & Upadhyaya, 2012); (5) Terpenoids, Indicated by red or yellow discoloration. Terpenoids act as natural mosquito repellents, repelling mosquitoes through their aromatic properties (Vergara-Jimenez et al., 2017); (6) Phenolic, Indicated by blackish-green color when reacting with FeCl3. Phenolic compounds have antioxidant activity and provide additional protection against environmental damage and pathogens (Ersam, 2004).

**Table 2.** Phytochemical Test Results with Color Indicators Table

| Bioactive Compound | Color Indicator | Fraction |
|---|---|---|
| Saponin | Stable foam formation | All fractions |
| Alkaloid | Brown color change | n-Hexane, Methanol-Water |
| Flavonoid | Bright yellow color | Ethyl Acetate, Methanol-Water |
| Tannin | Bluish-black color | Ethyl Acetate, Methanol-Water |
| Terpenoid | Red or yellow color | Ethyl Acetate, Methanol-Water |
| Phenolic | Greenish-black color | Methanol-Water |

### Mosquito Control Learning and Innovation Applications

The process of isolating bioactive compounds provides valuable hands-on learning experiences for students, particularly in understanding natural compound extraction and analysis techniques (Matrose et al., 2021). The use of separation based on polarity, qualitative testing, and TLC identification methods provides a deeper understanding of organic chemistry and its applications in real-world scenarios.

In terms of application, the results of the study show that Polygala paniculata has great potential as an environmentally friendly anti-mosquito innovation. The presence of compounds like saponins, flavonoids, and terpenoids supports the use of this plant as a natural alternative to Aedes aegypti control. This aligns with efforts to reduce the negative impact of synthetic insecticides on human health and the environment.

### Challenges and Future Directions

While the study successfully identified bioactive compounds in Polygala paniculata, several challenges were encountered during the experiment. For example, variations in compound yields and the limited availability of plant material posed difficulties in optimizing the extraction process. Future studies could focus on enhancing extraction methods, increasing yields, and conducting field testing to confirm the real-world effectiveness of these compounds as mosquito repellents (Adjei et al., 2025).

This research identified bioactive compounds in Polygala paniculata and provided a structured learning model for students in natural product chemistry. It contributes to the development of eco-friendly mosquito control alternatives, addressing the need for sustainable solutions to combat diseases like dengue hemorrhagic fever.

## Conclusion

This study successfully demonstrated the process of isolating bioactive compounds from the seven winds plant (Polygala paniculata L) as a potential environmentally friendly anti-mosquito innovation. Through a series of stages that included extraction using the maceration method, partitioning based on polarity, and phytochemical tests, compounds such as saponins, flavonoids, alkaloids, tannins, terpenoids, and phenolics were successfully identified. These compounds have shown potential biological activity as mosquito repellents and natural insecticides, supporting the use of this plant as a biodiversity-based solution in the control of disease vectors like Aedes aegypti.

While the findings are promising, there are several key limitations to consider. The research design was constrained by the absence of control groups and the relatively small sample size, which may limit the generalizability of the results. The experimental methods, including the maceration process and phytochemical testing, were carried out under specific conditions, which may not fully represent real-world applications. Additionally, the instruments used for extraction and analysis were not subjected to rigorous validation procedures, which could affect the precision of the results.

Despite these limitations, this research offers a valuable practice-based educational approach, benefiting students by providing insights into organic chemistry, pharmacology, and solvent-based extraction techniques. The isolation and analysis of bioactive compounds serve as an engaging learning experience, bridging theory with practice.

In terms of application, the study highlights the potential of Polygala paniculata as a natural source of bioactive compounds for developing anti-mosquito products. This aligns with efforts to reduce the negative environmental and health impacts of synthetic insecticides. However, further studies are needed to optimize extraction and separation methods to improve efficiency and yield. Additionally, toxicity testing of the isolated bioactive compounds against humans and the environment is necessary to ensure their safety before any practical application.

Future research should also explore the formulation of mosquito repellent products derived from these natural compounds, such as sprays, lotions, or aromatherapy candles. Field testing of these products under various environmental conditions will be an essential step to validate their effectiveness and determine their potential as a sustainable mosquito control solution.

Overall, while the findings are promising, further exploration and refinement of the methods and testing procedures are necessary to move toward real-world applications and the development of practical, safe, and sustainable mosquito control products.

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