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Insecticidal Activity of Extracts and Fractions of the Anting-Anting Plant (*Acalypha indica*) with Variations in Drying Methods Against *Aedes aegypti* Mosquitoes

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ABSTRACT

The Anting-Anting plant (Acalypha indica) is a shrub commonly used by the indigenous people of West Kalimantan as a mosquito repellent. This study aims to reveal the insecticidal capabilities of extracts and fractions from the leaves and roots of the Anting-Anting plant (Acalypha indica) with and without drying. Previous research has shown that the leaves of the Anting-Anting plant exhibit insecticidal activity against Aedes aegypti larvae. The extracts used were only water and methanol extracts, obtained through various extraction processes, but no publication has yet addressed the insecticidal activity of the roots with different drying methods. This study used samples of the leaves and roots of the Anting-Anting plant, differentiated by oven drying at 40°C, air drying, and no drying. Extraction was performed through maceration using methanol as the solvent, followed by liquid-liquid partitioning using n-hexane, dichloromethane, and ethyl acetate solvents. The active fractions with the highest insecticidal activity were analyzed for phenolic and flavonoid content. The results showed that the ethyl acetate fraction from the air-dried roots of the Anting-Anting plant could kill 100% of the larvae at a concentration of 40 ppm. Phytochemical tests indicated that the ethyl acetate fraction from the air-dried roots contained alkaloid, phenolic, and flavonoid compounds. Based on the analysis of phenolic and flavonoid content in the ethyl acetate fraction of the air-dried roots, the values were found to be 79.75±0.23 mg GAE/g and 68.45±0.41 mg OE/g, respectively. This indicates that the air-dried roots of the Anting-Anting plant have the potential to be an insecticide against Aedes aegypti.

Keywords: Acalypha indica, Insecticide, Aedes aegypti, Drying

INTRODUCTION

The tropical regions of the world have the highest incidence of dengue fever. This is due to weather and climate, sanitation, and human behavior. Most tropical regions consist of developing countries with low levels of education, leading to inadequate health practices. Dengue fever, transmitted by the *Aedes aegypti* mosquito, frequently occurs in areas with poor sanitation (Wahyuni, 2020).

Various studies have been conducted to address dengue fever, ranging from prevention to control, across different scientific disciplines, including social and health studies. One preventive approach involves the search for extracts, fractions, and insecticidal compounds that can effectively kill the dengue fever vector, the *Aedes aegypti* mosquito.

One plant that shows potential as an insecticide against *Aedes aegypti* is the Anting-Anting plant (*Acalypha indica*). The Anting-Anting plant grows easily in both wet

and dry environments. The indigenous people of West Kalimantan Province commonly use the Anting-Anting plant as a raw material for mosquito control as a substitute for abate, but they do so using traditional methods.

Previous research has shown that a combination of 97% ethanol extract from the leaves and roots of the Anting-Anting plant in a 1:1 ratio has an LC50 of 766 ppm against *Aedes aegypti* larvae. The phytochemical content includes alkaloids, flavonoids, triterpenoids, and steroids (Wahyuni, 2020; Chekuri et al., 2020; Kalaivani et al., 2022). Research on the methanol extract of Anting-Anting (*Acalypha indica*) leaves combined with Achyranthes aspera has shown that a 1:1 ratio has an LC50 of 277 ppm (Kamalakannan et al., 2011). Meanwhile, a 5% concentration of water extract from Anting-Anting leaves was found to kill *Aedes aegypti* larvae within 24 hours (Muthaiyan et al., 2020).

Different extraction methods also influence the insecticidal activity of Anting-Anting leaf extracts.

Research has shown that water extracts from Anting-Anting leaves, extracted by sonication and Soxhlet extraction using acetone as a solvent, were able to kill 100% of *Aedes aegypti* larvae (Teklani and Perera, 2017). These results were better than those obtained by the maceration and steam distillation of Anting-Anting leaves (Teklani and Perera, 2017).

The insecticidal activity of Anting-Anting leaf extracts has also been studied using various solvents and tested on the malaria vector mosquito, *Anopheles stephensi*. Research shows that, in a 24-hour test, the methanol extract of Anting-Anting leaves had the best LC50 value at 15.03 ppm compared to benzene (19.25 ppm), chloroform (27.76 ppm), and ethyl acetate (23.26 ppm) (Govindarajan et al., 2008).

The secondary metabolites most involved in insecticidal activity include phenolics (Singh et al., 2021), flavonoids (Inaba et al., 2022; Pereira et al., 2024), and terpenoids (Achimon et al., 2022; Yadav & Upadhyay, 2022; Popescu et al., 2024).

Based on a literature review, it is evident that research on the Anting-Anting plant as an insecticide has largely focused on its leaves, with little attention given to its roots. Additionally, although extracts have been obtained using various extraction methods, no publications have been found that explore variations in drying methods.

This study investigates the insecticidal activity of extracts and partitioned fractions from the leaves and roots of the Anting-Anting plant, with variations in drying methods, against *Aedes aegypti* larvae.

METHODS

This experimental laboratory study was conducted at the Chemistry Laboratory and Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak. The Anting-Anting plant samples were obtained from Rasau Jaya Umum Village in Rasau Jaya District, Kubu Raya Regency, West Kalimantan Province. Fourth instar larvae samples were bred in the Faculty of Mathematics and Natural Sciences Biology Laboratory, Tanjungpura University.

Materials and Equipment

The materials used include samples of Anting-Anting leaves and roots, solvents such as n-hexane, dichloromethane, ethyl acetate, and methanol (E. Merck, redistilled), sulfuric acid (E. Merck), hydrochloric acid (E. Merck), magnesium (E. Merck), sodium hydroxide (E. Merck), acetic anhydride (E. Merck), chloroform (E. Merck), ferric chloride (E. Merck), quercetin (E. Merck), gallic acid (E. Merck), Dragendorff reagent, Mayer reagent, and Liebermann-Burchard reagent. The equipment includes laboratory glassware, a macerator, an evaporator, and a UV-Vis spectrophotometer (Shimadzu).

Research Stages

The research stages encompass sample preparation, maceration extraction, partition extraction, phytochemical testing, and insecticidal testing. Plant samples included leaves and roots, subjected to drying variations using an oven and air drying without direct sunlight.

Plant Samples

The plant samples consisted of Anting-Anting leaves and roots. Selected plants reached heights of 30-50 cm. The leaves and roots were separated and thoroughly washed with running water without damaging the samples.

Extraction

Each 5 kg sample of Anting-Anting leaves and roots was dried in an oven at 40°C and air-dried without direct sunlight until the moisture content was below 10%. The dried leaves and roots were then powdered. The powdered leaves and roots underwent maceration extraction using methanol as the solvent. The resulting maceration extracts were subsequently partitioned using solvents of varying polarities, namely n-hexane, dichloromethane, and ethyl acetate. The maceration extracts, and the partitioned fractions were subjected to phytochemical testing and insecticidal assays.

Phytochemical Testing

Phytochemical tests were conducted on the maceration extracts and partition fractions from the leaves and roots of the Anting-Anting plant to identify secondary metabolites. These tests targeted specific groups of compounds: phenolics (using ferric chloride reagent), flavonoids (using NaOH, H₂SO₄, and Mg-HCl reagents), alkaloids (using the Liebermann-Burchard reagent), and steroids (using the Liebermann-Burchard reagent).

Insecticidal Testing

Insecticidal tests were performed on all maceration extracts and partition fractions from the leaves and roots of the Anting-Anting plant. Each insecticidal test was repeated five times. The tests were conducted on the fourth instar larvae of *Aedes aegypti*.

Phenolic and Flavonoid Content Analysis

The most active fractions exhibited the highest insecticidal activity and were analyzed for their phenolic and flavonoid content. This analysis used a UV-Vis spectrophotometer with quercetin and gallic acid as standards. Calibration curves for quercetin and gallic acid were generated using a range of concentrations: 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mg/mL.

Data Analysis

Data analysis was conducted on the results of the insecticidal tests of the extracts and fractions from the leaves and roots of the Anting-Anting plant to determine the average values and standard deviations.

RESULTS AND DISCUSSION

Table 1 shows that the highest percentage yield of maceration extract was obtained from the air-dried leaf samples, which yielded 2.54%. This higher yield is likely because the air-drying process allows the leaves to retain their mass better than oven-drying for the same sample weight of leaves and roots. Additionally, during the maceration extraction process, the diffusion and osmosis mechanisms occur more rapidly in the leaf samples than

in the root samples. This difference is influenced by the varying degrees of cell lysis between leaves and roots.

Table 1

Weight of Anting-Anting Plant Samples and Yield of Maceration Results

Sample	Weight of Plant Sample	Weight of Maceration Yield		
	(kg)	gram	%	
Fresh leaves	5	99	1.98	
Oven-dried leaves	5	111	2.22	
Air-dried leaves	5	127	2.54	
Fresh roots	5	93	1.86	
Oven-dried roots	5	101	2.02	
Air-dried roots	5	117	2.34	

The maceration extracts and partition fractions were subjected to phytochemical tests to determine the distribution of secondary metabolites based on the solvents' polarity. This analysis helps predict which secondary metabolites are responsible for the observed insecticidal activity.

Table 2
Phytochemical Test of Methanol Extract from Maceration
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		Results		
Methanol	Secondary metabolites			
Extract	Phenol Flavonoid Alkaloid Terpenoid / Steroid			•
Fresh leaves	+	+	+	+++
Oven-dried leaves	+++	+++	+	+
Air-dried leaves	+++	+++	+	+
Fresh roots	+	+	+	+
Oven-dried roots	+++	+++	+	+
Air-dried roots	+++	+++	+	+

As shown in Table 2, the phytochemical test results indicate that fresh leaf samples have a higher content of terpenoids and steroids than those that underwent oven and air drying. This is likely due to the loss of volatile terpenoids and steroids during drying. Additionally, leaf samples have a higher content of terpenoids and steroids than root samples, likely because leaf cells are more prone to lysis, which facilitates the extraction of volatile secondary metabolites more quickly (Nkumah et al., 2016; Sahukari et al., 2021).

Table 3 shows that the ethyl acetate fraction, across all drying variations of the leaf samples (fresh, oven-dried, and air-dried), consistently exhibits high levels of phenolic and flavonoid content. This suggests that secondary metabolites, particularly phenolics, and flavonoids, are predominantly distributed in the ethyl acetate solvent. Ethyl acetate, being less polar than methanol but more polar than dichloromethane, is effective in dissolving both glycosidic and non-glycosidic phenolic and flavonoid secondary metabolites (Sahukari et al., 2017; Sahukari et al., 2021).

Table 3
Phytochemical Test of Partition Fractions of Leaf Sample

Phytochemical	Secondary metabolites			
Sample	Phenol			Terpenoid / Steroid
Methanol Fraction from Fresh Leaves	+	+	+	-
Ethyl Acetate Fraction from Fresh Leaves	+++	+++	+	-
DCM Fraction from Fresh Leaves	+	+	+	+++
n-Hexane Fraction from Fresh Leaves	-	-	-	+++
Methanol Fraction from Oven-dried Leaves	+	+	+	-
Ethyl Acetate Fraction from Oven-dried Leaves	+++	+++	+	-
DCM Fraction from Oven- dried Leaves	+	+	+	+
n-Hexane Fraction from Oven-dried Leaves	-	-	-	+
Methanol Fraction from Air-dried Leaves	+	+	+	-
Ethyl Acetate Fraction from Air-dried Leaves	+++	+++	+	+
DCM Fraction from Air-dried Leaves	+	+	+	+
n-Hexane Fraction from Air-dried Leaves	-	-	-	+++

As shown in Table 4, the ethyl acetate fraction from fresh and dried root samples (dried using oven or airdrying methods) consistently exhibits high levels of phenolic and flavonoid content. This indicates that ethyl acetate effectively dissolves glycosidic and non-glycosidic phenolic and flavonoid compounds.

Table 4Phytochemical Test of Partition Fractions from RootSamples					
	Secondary metabolites				
Sample	Phenol Flavonoid Alkaloid Terpenoid / Steroid				
Methanol Fraction from Fresh Roots	+	+	+	-	
Ethyl Acetate Fraction from Fresh Roots	+++	+++	+	-	
DCM Fraction from Fresh Roots	+	+	+	+	
n-Hexane Fraction from Fresh Roots	-	-	-	+	
Methanol Fraction from Oven-dried Roots	+	+	+	-	
Ethyl Acetate Fraction from Oven-dried Roots	+++	+++	+	-	
DCM Fraction from Oven-dried Roots	+	+	+	+	
n-Hexane Fraction from Oven-dried Roots	-	-	-	+	
Methanol Fraction from Air-dried Roots	+	+	+	-	
Ethyl Acetate Fraction from Air-dried Roots	+++	+++	+	+	
DCM Fraction from Air-dried Roots	+	+	+	+	
n-Hexane Fraction from Air-dried Roots	-	-	-	+	

The distribution of these metabolites in the ethyl acetate fraction suggests that it likely has a high insecticidal activity. This is because phenolic and flavonoid secondary metabolites are known to inhibit protein and DNA synthesis in larvae, which can contribute to their insecticidal properties (Adnyana et al., 2021; Khan et al., 2020; Hematpoor et al., 2017).

Table 5

Mortality Test of Methanol Extract from Maceration				
Results				
Methanol Extract	Larval Mortality after 24 Hours (at Various Concentrations			
	20 ppm	30 ppm	40 ppm	

45±0.2	56±0.2	65±0.2
56±0.1	65±0.1	77±0.1
65±0.5	70±0.5	80±0.5
62±0.2	70±0.1	86±0.2
65±0.1	72±0.1	80±0.1
70±0.2	80±0.2	90±0.2
	56±0.1 65±0.5 62±0.2 65±0.1	56±0.1 65±0.1 65±0.5 70±0.5 62±0.2 70±0.1 65±0.1 72±0.1

Table 5 shows that the methanol extract from macerated roots of Anting-Anting, particularly from airdried samples, exhibits the highest insecticidal activity. This is likely due to the roots' high levels of phenolic and flavonoid compounds. Additionally, air-drying minimizes damage to secondary metabolites because it avoids excessive heat exposure, preserving the insecticidal properties of these compounds (Boekoesoe et al., 2022; Cui et al., 2019).

Table 6

Mortality Test of Partition Fractions from Leaf Samples Larval Mortality after 24 Hours (%) at Various Concentrations Sample 20 ppm 30 ppm 40 ppm 60±0.1 70±0.2 Methanol Fraction 50±0.1 from Fresh Leaves Ethyl Acetate 60±0.4 75±0.2 85±0.2 Fraction from Fresh Leaves 75±0.2 DCM Fraction from 50±0.2 60±0.2 Fresh Leaves n-Hexane Fraction 40 ± 0.2 50±0.2 57±0.2 from Fresh Leaves 60 ± 0.1 Methanol Fraction 65±0.1 72±0.1 from Oven-dried Leaves Ethyl Acetate 72±0.2 75±0.2 80±0.2 Fraction from Ovendried Leaves DCM Fraction from 50±0.3 55±0.1 70±0.2 **Oven-dried Leaves** n-Hexane Fraction 30±0.2 40±0.2 50±0.1 from Oven-dried Leaves Methanol Fraction 60±0.2 65±0.2 72±0.2 from Air-dried Leaves 75±0.1 80 ± 0.1 Ethyl Acetate 85±0.2 Fraction from Airdried Leaves DCM Fraction from 65±0.2 70±0.2 75±0.2 Air-dried Leaves n-Hexane Fraction 40 ± 0.3 50 ± 0.2 55±0.2 from Air-dried Leaves

Table 6 indicates that the ethyl acetate fraction from air-dried leaf samples shows the highest insecticidal activity. This is likely due to the high levels of phenolic and flavonoid secondary metabolites in these samples, which

inhibit protein and DNA synthesis in the fourth instar larvae of *Aedes aegypti*. Additionally, phenolic and flavonoid compounds and saponins can accelerate the lysis of the larvae's cells (Adewoyin et al., 2021; Oberemok et al., 2015; Masaki, 2008).

Tahle 7

	Table 7				
Mortality Test of Partition Fractions from Root Samples					
	Larval Mortality after 24 Hours				
Sample	(%) at Various Concentrations				
	20 ppm	30 ppm	40 ppm		
Methanol Fraction	50±0.2	60±0.2	70±0.2		
from Fresh Roots					
Ethyl Acetate	55±0.1	62±0.1	75±0.2		
Fraction from Fresh					
Roots					
DCM Fraction from	50±0.4	55±0.4	60±0.2		
Fresh Roots					
N-Hexane Fraction	49±0.1	50 ± 0.1	55±0.2		
from Fresh Roots					
Methanol Fraction	50±0.2	60±0.2	72±0.2		
from Oven-dried					
Roots	60 1 0 1	6510.4			
Ethyl Acetate Fraction from	60±0.1	65±0.1	80±0.1		
Oven-dried Roots					
DCM Fraction from	60±0.2	65±0.2	70±0.2		
Oven-dried Roots	00±0.2	05±0.2	70±0.2		
N-Hexane Fraction	45±0.3	50±0.3	60±0.2		
from Oven-dried	-JT0.5	J0±0.J	00±0.2		
Roots					
Methanol Fraction	55±0.2	65±0.2	75±0.2		
from Air-dried	00-012	00-012	, <u>0</u> _0.2		
Roots					
Ethyl Acetate	65±0.2	80±0.2	100±0.0		
Fraction from Air-					
dried Roots					
DCM Fraction from	60±0.2	70±0.2	85±0.2		
Air-dried Roots					
N-Hexane Fraction	50±0.2	60±0.4	70±0.2		
from Air-dried					
Roots					

Table 7 shows that the ethyl acetate fraction from air-dried root samples exhibits the highest insecticidal activity, achieving 100% larval mortality at 40 ppm. This can be linked to phenolic and flavonoid secondary metabolites, known for their effective insecticidal properties (Pintong et al., 2020; Rattan, 2010). Additionally, other secondary metabolites like saponins, terpenoids, steroids, and alkaloids also contribute to the insecticidal effects, each with distinct mechanisms of action (Popescu et al., 2024; Sari et al., 2023; Valverde et al., 2023; Singh et al., 2022).

The content of secondary metabolites present in the samples influences insecticidal activity. These metabolites can have synergistic or antagonistic effects, depending on factors such as the concentration ratio of secondary metabolites and the test animal specimen (Zain et al., 2023; Sari et al., 2023).

Based on the analysis of phenolic and flavonoid content in the ethyl acetate fraction of air-dried Anting-Anting root, values of 79.75±0.23 mg GAE/g and 68.45±0.41 mg QE/g were obtained. These results indicate that the ethyl acetate fraction of air-dried Anting-Anting root contains high levels of phenolic and flavonoid secondary metabolites. This suggests that the Anting-Anting root has a high content of these secondary metabolites, and the air-drying method without direct sunlight effectively preserves the number of secondary metabolites. Previous research has shown that different plant parts can vary in secondary metabolite content depending on their biosynthetic pathways (Chekuri et al., 2020).

CONCLUSION

The research results show that the fraction obtained from the root part of the Anting-Anting plant using the airdrying method has better insecticidal activity compared to the extract obtained from the leaf part using oven-drying or no drying. The ethyl acetate fraction obtained from the air-dried root of the Anting-Anting plant has the best insecticidal activity, achieving 100% mortality at a concentration of 40 ppm over 24 hours against *Aedes aegypti* larvae.

RECOMMENDATION

The results of this study indicate that both the leaf and root parts of the Anting-Anting plant have potential as insecticides for the dengue fever vector, *Aedes aegypti*, and could be used as an alternative adjunct to abate.

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