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SUSCEPTIBILITY OF THE DENGUE VECTOR, *Aedes aegypti* ON THE LARVICIDAL AND REPELLENT ACTIVITY OF RHIZOME PLANT EXTRACTS

Sayono Sayono[1*], Risyandi Anwar[2] & Othman Wan-Norafikah[3,4,5*]
[1]Faculty of Public Health and Postgraduate Program of Public Health of
Universitas Muhammadiyah Semarang,
Semarang, Indonesia.
[2]Herbal Research Unit,
Faculty of Dentistry,
Universitas Muhammadiyah Semarang,
Semarang, Indonesia.
[3]Faculty of Medicine,
Universiti Teknologi MARA (UiTM),
Selangor Branch, Sungai Buloh Campus,
Jalan Hospital,
47000 Sungai Buloh, Selangor, Malaysia.
[4]HW ReNeU,
Universiti Teknologi MARA (UiTM),
40450 Shah Alam, Selangor, Malaysia.
[5]Institute of Medical Molecular Biotechnology (IMMB),
Faculty of Medicine,
Universiti Teknologi MARA (UiTM),
Selangor Branch, Sungai Buloh Campus,
Jalan Hospital,
47000 Sungai Buloh, Selangor, Malaysia.
*Corresponding authors: *say.epid@gmail.com, norafikah@uitm.edu.my*

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ABSTRACT

The insecticidal potential of several plant secondary metabolites provides new hope for dengue vector control in endemic areas. This study evaluated the susceptibility of *Aedes aegypti* mosquitoes to the larvicidal and repellent activity of rhizome plant extracts. Sixteen extract types of *Kaempferia galanga, Alpinia galanga, Zingiber zerumbet,* and *Curcuma aeruginosa* resulted from a maceration extraction based on methanol, ethyl acetate, n-hexane, and butanol solvents were occupied. The temephos-susceptible third-instar-larvae of *Ae. aegypti* were subjected to larvicidal bioassay. Concentration levels of 50, 100, 250, 500, and 1,000 ppm were used in the initial bioassay test five times replicated where each replicate consisting of 20 larvae. Larval mortality was calculated in 24h and 48h post-exposure. A total of 50 laboratory strain *Ae. aegypti* aged 3-5 days were placed in a chamber. Repellent cream was applied to the right arm while the left arm served as a control. The arms were inserted into the chamber and the number of mosquitoes perched within 30 minutes were counted. Post-exposure of 50% and 90% lethal exposure (LC_{50}-LC_{90}) and 50% and 90% effective concentration (EC_{50}-EC_{90}) were

determined. Within 24 hours, seven extract types indicated effective larvicidal concentrations, namely n-hexane extracts of *K. galanga, A. galanga, Z. zerumbet, C. aeruginosa* with the LC_{50} of 18.693, 41.926, 109.247, and 205.500 ppm; methanol of *C. aeruginosa* (179.291 ppm); and ethyl acetate of *A. galanga* (306.200 ppm). Six extract types showed the lowest concentrations of repellents, namely ethyl acetate and methanol of *A. galanga* (1.558% and 2.629%); methanol, ethyl-acetate, and n-hexane of *Z. zerumbet* (2.525, 3.946 and 4.481%); and n-hexane of *K. galanga* (4.338%). *Aedes aegypti* larvae were susceptible to the hexane extract of four rhizome plants while the adults were susceptible to *Z. zerumbet* and *K. galanga* extracts. Of these, the hexane extract of the *K. galanga* affect most or kills/repell more both adult and larvae of Aedes. The stability of extracts, the practical formulation of larvicides and repellents; and the isolation of chemical compounds are important to be investigated in the future.

Keywords: Larvicidal activity, repellent, rhizome plant, dengue vector, *Aedes aegypti*

ABSTRAK

Potensi insektisid bagi sebilangan metabolit sekunder tumbuhan memberikan harapan baru kepada kawalan vektor denggi di kawasan endemik. Kajian ini telah dijalankan untuk menilai kerentanan nyamuk *Aedes aegypti* terhadap aktiviti larvisidal dan penghalau ekstrak tumbuhan rizom. Sejumlah 16 jenis ekstrak daripada *Kaempferia galanga, Alpinia galanga, Zingiber zerumbet* dan *Curcuma aeruginosa* yang dihasilkan daripada pengekstrakan maserasi berasaskan larutan metanol, etil asetat, n-heksana dan butanol telah digunakan. Larva *Ae. aegypti* instar ketiga yang rentan kepada temephos telah digunakan dalam bioasai larvisidal. Tahap kepekatan pada 50, 250, 500 dan 1000 ppm telah digunapakai dalam ujian bioasai awalan untuk lima replikasi yang mana setiap replikasi mengandungi 20 larva. Mortaliti larva telah dikira pada 24 jam dan 48 jam selepas pendedahan. Sebanyak 50 ekor nyamuk *Ae. aegypti* strain makmal berusia 3-5 hari telah diletakkan di dalam sebuah kebuk. Krim repelen telah disapu pada lengan kanan manakala lengan kiri bertindak sebagai set kawalan. Kedua-dua lengan telah dimasukkan ke dalam kebuk dan bilangan nyamuk yang hinggap di atas lengan dalam masa 30 minit telah dikira. Nilai kematian 50% dan 90% (LC_{50}-LC_{90}) dan 50% dan 90% kepekatan efektif (EC_{50}-EC_{90}) selepas pendedahan telah ditentukan. Dalam tempoh 24 jam, tujuh jenis ekstrak menunjukkan keberkesanan dari segi kepekatan larvisidal, iaitu ekstrak n-heksana bagi *K. galanga, A. galanga, Z. zerumbet* dan *C. aeruginosa* dengan nilai LC_{50} 18.693, 41.926, 109.247 dan 205.500 ppm; ekstrak metanol bagi *C. aeruginosa* (179.291-ppm); dan ekstrak etil asetat bagi *A. galanga* (306.200-ppm). Enam jenis ekstrak menunjukkan kepekatan repelen paling rendah iaitu ekstrak etil asetat dan metanol bagi *A. galanga* (1,558 dan 2,629%); ekstrak metanol, etil asetat dan n-heksana bagi *Z. zerumbet* (2,525, 3,946 dan 4,481%); dan ekstrak n-heksana bagi *K. galanga* (4.338%). Larva *Ae. aegypti* adalah rentan terhadap ekstrak heksana bagi empat tumbuhan rizom manakala nyamuk dewasa pula adalah rentan terhadap ekstrak *Z. zerumbet* dan *K. galanga*. Kestabilan ekstrak, formulasi praktikal bagi larvisid dan repelen, serta isolasi sebatian kimia adalah penting untuk dikaji pada masa hadapan.

Kata kunci: Aktiviti larvisidal, Repelen, tumbuhan rizom, vektor denggi, *Aedes aegypti*

INTRODUCTION

The increase in the global occurrence of Dengue in two decades (Zeng et al. 2021), mainly in Oceania and Asia (Gwee et al. 2021, Tian et al. 2022) has triggered preventive measures (Buhler et al. 2019) through vector control and environmental management in synergy with the use of insecticides and cleaning microhabitats (Mone et al. 2019, Srisawat et al. 2022). The

long-term use of insecticides has led to the widespread emergence of resistant strains of dengue vectors in America, Africa, and Asia (Moyes et al. 2017). The temephos-resistant *Aedes aegypti* have been reported in Brazil (Valle et al. 2019), Ecuador (Morales et al. 2019), and Southeast Asia (Gan et al. 2021) including Indonesia (Rahayu et al. 2021), Columbia (Morgan et al. 2022), Nigeria (Mukhtar & Ibrahim 2022), Mexico (Ciau-Mendosa et al. 2022), and Peru (Palomino et al. 2022) that hampered the vector control efforts to prevent dengue virus transmission (Rasli et al. 2021). This phenomenon has prompted researchers to study the use of phytochemical compounds as active, safe, environmentally friendly, and biodegradable insecticide ingredients (Alyahya et al. 2021; Schorkopf et al. 2016; Piazzoni et al. 2022).

In the context of larvicides, several plant extracts such as *Derris elliptica* root (Sayono et al. 2020), *Solanum nigrum* (Dinh et al. 2020), *Ocimum basilicum* leaf (Chan et al. 2022), *Eugenia astringent* and *Myrrhinium atropurpureum* (Carneiro et al. 2021), roots and leaves of *Saussurea costus* (Ali & Venkatesalu 2020), *Tabernaemontana cymosa* and *Mammea americana* (Oliveros-Diaz et al. 2022) have been found to possess highly effective larvicidal effect. The repellent activity of 74-87% was achieved by methanol extracts of *Ervatamia coronaria* and *Caesalpinia pulcherrima* 5 mg/cm^2 (Govindarajan et al. 2011), while the repellency of petroleum ether extracts *Tribulus terrestris* of 1.5 mg/cm^2 was equivalent to the commercial repellent (El-Sheikh et al. 2016). The repellent activity of 96.9% was demonstrated by *Euphorbia balsamifera* petroleum ether extract 25% against *Anopheles gambiae* (Idris et al. 2014). *Allium cepa* leaf ethanol extract of 20% had the highest repellent effect against *Ae. aegypti* (Adnani et al. 2020) while the acetone extracts of *Ocimum basilicum* and *Abizia amara* showed repellent activity of 98-100% and 97-98% against *Culex quinquefasciatus* (Ninthya & Dhivya 2019).

Evaluation of the larvicidal and repellent activity of rhizome plants against mosquito vectors has been reported in the form of extracts, essential oils, and endophytic bacteria (Fitri et al. 2023) with various results. The secondary metabolites of *Kaempferia galanga* extract showed effective larvicidal activity with LC_{50} of 40 ppm against *Ae. aegypti* (Kim et al. 2008), while ether and chloroform extracts result in LC_{50} of 64.08 and 105.02 ppm against the dengue vector (Satoto et al. 2013). The larvicidal efficacy of the hexane and ethyl acetate extracts of *Alpinia galanga* against *Ae. aegypti* was shown by LC_{50} values of 53.39 and 123.29 ppm (Poonsri et al. 2019), while its rhizome essential oils showed an LC_{50} of 47.18 ppm (Nguyen et al. 2022a). The methanol and ethyl acetate extracts of *Zingiber zerumbet* showed larvicidal activity with LC_{50} of 153.57 and 185.80 ppm (Murini et al. 2017), while its essential oil reached LC_{50} of 154 ppm against *Ae. aegypti* larvae (Khandagle et al. 2011). Studies on larvicidal activity of *Curcuma aeruginosa* ethanol extracts against *Ae. aegypti* showed an LC_{50} of 291.78 ppm (Prawirasudarga et al. 2018). Another study on the larvicidal activity of turmeric (*C. domestica*) against *Ae. aegypti* larvae showed an LC_{50} of 2.084 ppm (Susilowati et al. 2021), while *C. xanthoriza* essential oil showed an LC_{50} of 25.94 ppm (Pereira et al. 2022). Essential oils of the rhizome plant of *C. longa* and *Z. cassumunar* have repellent activity for 4 to 8 hours against adult mosquito vectors (Tawatsin et al. 2001; Phasomkusolsil & Soonwera 2010), including the compound of (−)-terpinen-4-ol in *C. cassumunar* against *Aedes albopictus* (Li et al. 2021). *Z. zerumbet* essential oil 10% had a repellent activity of 72-99.1% against *Ae. aegypti* within two hours (Phukerd & Soonwera 2014). Seven species of local rhizome plants are abundantly produced in Indonesia, namely *K. galanga*; *Z. zerumbet*; *A. galanga*, *C. longa*, and *C. aeruginosa* (Statistics Indonesia 2022). However, studies on the bio-insecticidal potential of these rhizome plant extracts as larvicides and repellents are still limited. This study aimed to determine the larvicidal and repellent activity of these rhizome plant extracts that are effective against dengue vector; *Ae. aegypti* larvae and adult mosquitoes.

MATERIALS AND METHODS

Preparation of Rhizome Plant Samples

This study was conducted for nine months from March until November 2022. The rhizome plants were obtained from three districts in Central Java, Indonesia, namely Semarang and Batang (*Z. zerumbet*) as well as Karanganyar districts (*A. galanga, K. galanga,* and *C. aeruginosa*). As many as 30 kg of *Z. zerumbet* rhizomes were purchased from farmers, while 15 kg of *A. galanga*, *K. galanga,* and *C. aeruginosa* were purchased in the form of simplicia from the Research Center for Medicinal Plants and Traditional Medicine, Ministry of Health in Tawangmangu, Central Java, Indonesia. The extraction process was carried out at the Natural Materials Chemistry Central Laboratory, Padjadjaran University, Bandung, West Java, Indonesia using a gradual maceration method sequentially based on four solvents, namely methanol, ethyl acetate, n-hexane, and butanol.

Rearing of *Aedes aegypti*

Aedes aegypti Grobogan strain from Central Java, Indonesia which was known susceptible to temephos larvicide and colonized at the Laboratory of Epidemiology of Tropical Diseases, Faculty of Public Health, University of Muhammadiyah Semarang, Indonesia was used in this study. Eggs of *Ae. aegypti* Grobongan strain (F14) was immersed in the water and hatched into larvae that were nurtured in plastic trays and fed with dog food. Some of the third instar larvae were used in the larvicidal bioassay test while the rest were further reared until the adult stage which were kept in a mosquito cage and fed with a 10% sugar solution. Some of the female adult mosquitoes were employed in the repellent bioassay test. The room air temperature and relative humidity (R.H.) conditions of the laboratory were maintained at a range of 26-30°C and 60-80%, respectively.

Larvicidal Bioassay Test

The larvicidal bioassay test was performed following the procedures of the World Health Organization (WHO) (2009). Twenty (20) healthy *Ae. aegypti* third instar larvae were subjected to each treatment group. In the preliminary study, the larvae were exposed to the larvicide solution of rhizome plant extract in a plastic cup with an initial concentration range of 50, 100, 250, 500, and 1000 ppm with a solution volume of 100 mL. The treatment for each concentration was prepared in five replicates and was accompanied by two control groups which were the distilled water as a negative control and 0.02 ppm temephos as a positive control. Larval mortality was observed and calculated after 24 and 48 hours of exposure. The concentration range for the next tests was determined based on the results of this bioassay test. The final experiment was stopped when the concentration range resulted in a <50 and >90% mortality range. If the larval mortality in the negative control group is >20%, the calculation of the larval mortality was corrected using Abbott's formula (1925).

Repellent Bioassay Test

The repellent used for this study was a topical lotion with a basic cream composition and rhizome plant extracts listed in Table 1. Following the repellent bioassay test procedure by the WHO (2009), a total of fifty (50) healthy *Ae. aegypti* laboratory strain female adults were collected using an aspirator from a mosquito cage and put into the repellent test chamber. The chamber was a box of 60 cm in length x 50 cm in width x 50 cm in height. The top, sides, and back were covered with tulle cloth while the front was equipped with two round holes with a diameter of 15 cm and were 20 cm apart from one another in which both holes were equipped with a cone-shaped cloth cover with a perforated end. The left arm of the proband served as a negative and positive control, while the right arm served as a treatment. The negative control

used was 70% alcohol while the positive control was a commercial 13% DEET. The left arm that was smeared with alcohol was put into the chamber and left for 30 seconds. The number of mosquitoes that perched on the left arm was counted with a counter. The experiment was repeated three times in different chambers. If the number of mosquitoes that landed was more than 10, then the experiment was continued. The repellent bioassay test for the positive control group was carried out with the same procedures. Meanwhile, the right arm was smeared with rhizome plant extract repellent with an initial concentration range of 1%, 15%, and 30%. The number of mosquitoes that perched on the right arm was counted. The mortality percentage of mosquitoes during the preliminary study was used to design the concentration of the repellent in this experiment until the repellent activity was <50% and >90%.

Table 1. The composition of the repellent formula of rhizome plant extracts

Components	Materials Composition (%) In Each Formula					
	A	B	C	D	E	F
Cera alba	2	2	2	2	2	2
Asam stearate	5	5	5	5	5	5
NaOH	0.2	0.2	0.2	0.2	0.2	0.2
Carbomer	0.5	0.5	0.5	0.5	0.5	0.5
Tween 80	8.9	8.9	8.9	8.9	8.9	8.9
Span 80	1.1	1.1	1.1	1.1	1.1	1.1
Metil paraben	0.18	0.18	0.18	0.18	0.18	0.18
Propil paraben	0.02	0.02	0.02	0.02	0.02	0.02
Aquadest Ad	100	100	100	100	100	100
Extract	5	10	15	20	25	30

Extract concentration in each formula: A=5%, B=10%, C=15%, D=20%, E=25%, and F=30%

Data Analysis

Simple statistical analysis and description of extract quantity, larvicidal potency, and repellent activity data were performed and displayed in detail using Microsoft Excel version 10 software in the form of charts and tables. The effective concentrations of larvicides and repellents were determined by probit analysis using SPSS version 16.0.

RESULTS

Preparation of Rhizome Plant Samples

In this study, 16 extracts from four rhizome plants were obtained which represented three classifications of solvent polarity, namely polar (methanol and butanol), semi-polar (ethyl acetate), and nonpolar (n-hexane) (Table 2). Based on their quantities, *A. galanga* and *C. aeruginosa* produced 5-6 times more extract than *Z. zerumbet* and *K. galanga* (Figure 1). The quantity of the extracts varied according to the rhizome plant species and the solvent. The n-hexane extract type showed the highest quantity in the three species of rhizome plants while ethyl acetate was produced the most only in *A. galanga*. The least quantity of extract was produced from butanol solvent (Table 2).

Table 2. The extract weight of rhizome plants using different extraction solvent

No.	Rhizome Plants	Extraction Solvent	Extract Weight (Gram)
1	*Z. zerumbet*	Methanol	15.8
		Ethyl acetate	149.0
		n-Hexane	190.2
		Butanol	9.7
2	*A. galanga*	Methanol	195.6
		Ethyl acetate	901.9
		n-Hexane	173.0
		Butanol	26.4
3	*K. galanga*	Methanol	47.8
		Ethyl acetate	24.3
		n-Hexane	135.4
		Butanol	3.9
4	*C. aeruginosa*	Methanol	208.3
		Ethyl acetate	358.6
		n-Hexane	449.3
		Butanol	25.3

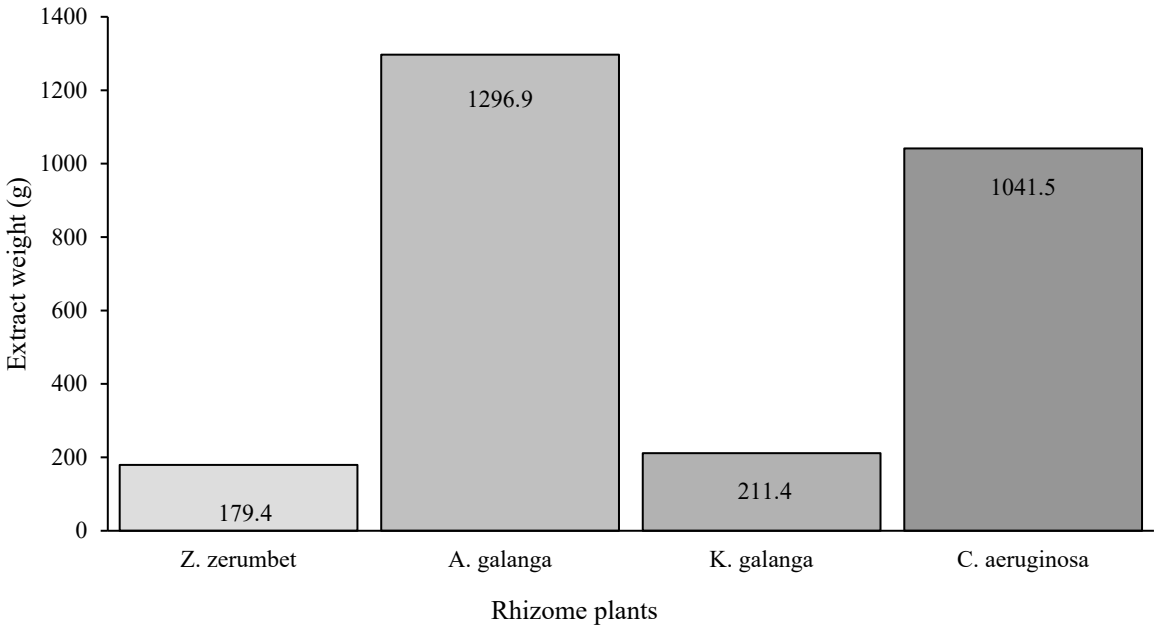

Figure 1. The total extract quantities of the rhizome plants

Larvicidal Bioassay Test

Results of the rhizome plant larvicidal bioassay test showed variations in the concentration and mortality of *Ae. aegypti* by species and extract type (Table 3). The n-hexane extract showed the highest larvicidal activity among the four rhizome plant species studied with various concentrations and post-exposure larval mortality ranges at 24h and 48h. The best concentration range of n-hexane extract was found in the rhizome of *K. galanga* and *A. galanga* with 37.5 – 67.5 ppm and 30 – 90 ppm, respectively, which caused post-exposure mortality of 24 hours of 83-100% and 23-100%, respectively. These were followed by *C. aeruginosa* and *Z. zerumbet* each with a concentration of 40-200 ppm and 175-275 ppm, respectively, with 24h

post-exposure mortality of 6-97% and 9-100%. Butanol extract showed low activity wherein the high concentration range resulted in the mortality of *Ae. aegypti* which was low for three species of rhizome plants. The limited amount of *C. aeruginosa* caused the butanol extract to be insufficient for the bioassay test. The low larvicidal activity was also demonstrated by the methanol extracts of *K. galanga* and ethyl acetate of *Z. zerumbet* and *C. aeruginosa*.

Table 3. Results of larvicidal bioassay test using rhizome plant extracts against *Aedes aegypti* larvae

Extract types	Concentration (ppm)	Dead Larvae After 24h of Exposure to Larvicide			Larval Mortality (%)	Dead Larvae After 48h of Exposure to Larvicide			Larval Mortality (%)
		Min	Max	Mean		Min	Max	Mean	
Z. zerumbet									
Methanol	50	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0
	250	0	0	0	0	0	0	0	0
	500	0	0	0	0	0	1	0.2	1.66
	1000	0	1	0.2	1.66	0	2	0.4	3.33
Ethyl acetate	100	0	1	0.01	0	2	7	4.2	21
	200	1	1	0.2	1	5	8	6.2	31
	500	1	2	0.3	1.5	3	9	6.2	31
	1000	2	2	0.4	2	7	11	8.0	40
	2500	5	14	9.6	48	11	17	13.6	68
n-Hexane	40	1	2	1.8	9	3	8	5.0	25
	70	1	6	2.8	14	5	20	13.0	65
	100	8	14	9.8	49	19	20	19.6	98
	130	5	18	10.8	54	19	20	19.8	99
	160	5	19	12.6	63	20	20	20.0	100
	200	20	20	20.0	100	20	20	20.0	100
Butanol	100	0	0	0.0	0	0	0	0.0	0
	600	0	0	0.0	0	0	0	0.0	0
	1200	0	0	0.0	0	0	0	0.0	0
	2000	1	1	0.6	3	1	3	2.0	10
	3000	2	7	5.0	25	3	9	6.0	30
A. galanga									
Methanol	50	0	1	0.2	1.66	0	1	0.2	1.66
	100	0	1	0.2	1.66	0	1	0.4	3.33
	250	0	1	0.2	3.33	0	1	0.4	3.33
	500	0	1	0.4	3.33	0	2	0.4	3.33
	1000	0	2	0.4	3.66	1	3	0.8	6.66
Ethyl acetate	50	0	0	0.0	0	0	3	1.4	7
	100	0	1	0.4	2	1	6	3.2	16
	200	4	11	7.4	37	7	13	9.8	49
	400	7	15	10.2	51	13	17	14.8	74
	600	16	20	18.2	91	20	20	20.0	100
n-Hexane	30	0	9	4.6	23	6	11	8.6	43
	45	6	14	10.4	52	15	18	16.6	83
	60	12	18	15.4	77	18	20	18.8	94
	75	19	20	19.6	98	20	20	20.0	100
	90	20	20	20.0	100	20	20	20.0	100
Butanol	100	0	0	0	0	0	0	0	0
	600	0	0	0	0	0	0	0	0
	1200	0	0	0	0	0	0	0	0
	2000	0	0	0	0	0	0	0	0
	3000	0	0	0	0	0	0	0	0
K. galanga									
Methanol	2600	0	3	0.8	4	0	20	9.6	48

Extract types	Concentration (ppm)	Dead Larvae After 24h of Exposure to Larvicide			Larval Mortality (%)	Dead Larvae After 48h of Exposure to Larvicide			Larval Mortality (%)
		Min	Max	Mean		Min	Max	Mean	
	3000	1	4	3.0	15	8	18	10.6	53
	3400	1	20	7.0	35	8	20	12.2	61
	3800	9	19	15.6	78	14	20	17.4	87
	4000	14	20	17.2	86	14	20	18.8	94
Ethyl acetate	200	3	12	7.0	35	15	19	17.6	88
	250	8	17	11.8	59	16	20	18.4	92
	300	11	`19	16.4	82	19	20	19.8	99
	350	16	20	19.0	95	20	20	20.0	100
	400	20	20	20.0	100	20	20	20.0	100
n-Hexane	37.5	10	20	16.6	83	13	20	17.6	88
	45.0	14	20	17.6	88	18	20	19.4	97
	52.5	15	19	17.8	89	18	20	19.6	98
	60.0	19	20	19.8	99	20	20	20	100
	67.5	20	20	20.0	100	20	20	20	100
Butanol	40	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0
	100	0	0	0	0	0	0	0	0
	500	0	10	3.33	16.65	3	11	6.33	31.65
	1000	10	13	11	55.0	15	17	14.66	73.3
C. aeruginosa									
Methanol	100	1	5	2.8	14	8	12	10.0	50
	150	6	9	7.6	38	12	17	15.0	75
	200	10	14	11.6	58	15	19	16.6	83
	250	10	15	13.0	65	16	18	17.4	87
	300	17	20	19.2	96	20	20	20.0	100
Ethyl acetate	500	1	5	3.0	15	10	17	13.6	68
	1000	3	8	5.0	25	14	17	15.8	79
	1500	7	15	10.8	54	15	18	16.8	84
	2000	13	20	15.2	76	20	20	20.0	100
	2500	17	20	18.2	91	20	20	20.0	100
n-Hexane	175	0	2	1.2	6	0	5	2.2	11
	200	4	10	8.0	40	14	18	16.0	80
	225	16	19	17.4	87	19	20	19.4	97
	250	16	20	18.8	94	19	20	19.8	99
	275	18	20	19.4	97	20	20	20.0	100
Butanol	-	-	-	-	-	-	-	-	-
Temephos*	0.02	100	100	100		-	-	-	
Aquadest#	0	0	0	0		0	0	0	

After 24h of exposure, seven out of sixteen rhizome plant extracts showed high larvicidal activity against *Ae. aegypti* as indicated by LC_{50} of less than 750 ppm, namely n-hexane extract of *K. galanga, A. galanga, Z. zerumbet*, and *C. aeruginosa*, as well as methanol extract of *C. aeruginosa*, and also ethyl acetate extracts of *K. galanga* and *A. galanga*. Two extracts showed LC_{50} <50 ppm (n-hexane extracts of *K. galanga* and *A. galanga*); four extracts showed LC_{50} <250 ppm (methanol extract of *C. aeruginosa*, ethyl acetate of *K. galanga*, n-hexane extracts of *Z. zerumbet* and *C. aeruginosa*); and ethyl acetate extract of *A. galanga* with LC_{50} <750 ppm. Extract types that resulted in LC_{50} >750 ppm were butanol extracts of all rhizome plants, ethyl acetate extracts of *Z. zerumbet* and *C. aeruginosa*, and methanol extract of *K. galanga* (Table 4).

Table 4. The lethal concentrations obtained for rhizome plant extracts larvicides against *Aedes aegypti* larvae

No	Rhizome plants	Extract types	Lethal Concentration (ppm)			
			LC_{50}		LC_{90}	
			24h	48h	24h	48h
1	*Z. zerumbet*	Methanol	5125.266	2398.890	20605.453	8376.527
		Ethyl acetate	2851.995	1196.740	7549.773	46317.150
		n-Hexane	109.247	54.435	227.068	88.991
		Butanol	5510.043	4622.484	13727.866	11325.241
2	*A. galanga*	Methanol	112830.399	9582.149	1.386E8	1592560.506
		Ethyl acetate	306.200	196.960	705.459	528.420
		n-Hexane	41.926	32.122	66.973	50.774
		Butanol	-	-	-	-
3	*K. galanga*	Methanol	3470.942	2811.690	4159.423	4136.760
		Ethyl acetate	228.305	57.341	321.198	191.614
		n-Hexane	18.693	13.655	46.522	32.757
		Butanol	922.574	677.491	2068.714	1485.321
4	*C. aeruginosa*	Methanol	179.291	101.221	328.374	230.991
		Ethyl acetate	1269.421	364.481	3028.015	1322.509
		n-Hexane	205.500	190.515	239.180	211.702
		Butanol	-	-	-	-

Repellent Bioassay Test

The repellent activity varied according to the rhizome plant species and the concentration of the extract whereas a dose-response effect occurred according to the concentration. The lowest and highest concentration ranges were found in the ethyl acetate extracts of *K. galanga* (2.5-20.0%) and the n-hexane extract of *C. aeruginosa* (15.0-30.0%). The average of perch mosquitoes in the treatment group ranged from 0.2 to 8.5, while in the negative control group, it ranged from 10.2 to 16.0. There were no mosquitoes perched on the skin in the positive control. Seven types of extracts namely two types of *Z. zerumbet* (ethyl acetate and n-hexane extracts), two types of *A. galanga* (methanol and ethyl acetate extracts), and three types of *K. galanga* (methanol, ethyl acetate, and n-hexane) extracts showed high repellent activities in which at a concentration of 20%, they were able to avoid 92.0-98.5% exposure to mosquitoes. The ethyl acetate extracts of *Z. zerumbet* and *K. galanga* indicated the highest repellent activity, which was 98.5% and 98.21%, respectively, at a concentration of 20% (Table 5). Variations in the repellent activity of rhizome plant extracts according to the plant species and extract type are shown in Table 6 in which four extracts showed EC_{50} <5% (ethyl acetate extract of *Z. zerumbet* and *A. galanga*, n-hexane extract of *Z. zerumbet* and *K. galanga*), three extracts with EC_{50} between 5-10% (methanol and ethyl acetate extract of *K. galanga*, and butanol extract of *C. aeruginosa*), and four extracts with EC_{50} >10% (n-hexane extract of *A. galanga* and *C. aeruginosa*, methanol and ethyl acetate extracts of *C. aeruginosa*). Butanol extract was the only effective repellent on *C. aeruginosa* due to its limited quantity.

Table 5. Repellent activity of rhizome plant extracts against *Aedes aegypti* adult mosquitoes

Extract types	Concentration (%)	Number of Landing Mosquitoes on The Arm Skin						Positive Control	ΔA*			Repellent Activity (%)
		Treatment Group			Negative Control							
		Min	Max	Mean	Min	Max	Mean		Min	Max	Mean	
Z. zerumbet												
Methanol	5	3	6	5.00	12	13	12.67	0	7	11	8.33	66.11
	10	2	4	3.00	11	13	12.33	0	9	10	9.67	76.49
	15	1	4	2.00	11	13	12.67	0	9	12	10.67	84.39
	20	1	3	1.33	11	13	12.33	0	10	12	11.00	86.96
Ethyl acetate	5	5	5	5.0	12	14	13.6	0	8	9	8.6	63.24
	10	2	3	2.6	13	14	13.6	0	10	12	10.6	77.95
	15	1	2	1.2	13	15	13.6	0	12	13	12.4	91.18
	20	0	1	0.2	11	14	13.6	0	13	14	13.4	98.53
n-Hexane	5	4	8	5.4	10	15	12.4	0	6	8	7.2	58.06
	10	3	5	3.4	11	14	12.8	0	8	10	9.2	73.02
	15	0	2	0.8	11	15	12.6	0	10	13	11.4	90.48
	20	0	1	0.4	10	14	12.8	0	10	14	12.2	96,83
Butanol	-	-	-	-	-	-	-	-	-	-	-	-
A. galanga												
Methanol	5	3	6	3.83	11	14	12.33	0	6	11	8.50	68.46
	10	2	4	2.67	11	14	12.33	0	8	12	9.67	78.01
	15	1	4	1.83	11	13	12.33	0	8	13	10.50	85.00
	20	0	1	0.67	11	14	12.33	0	10	14	11.67	94.42
Ethyl acetate	2.5	3	6	5.2	11	15	12.8	0	6	9	7.8	60.94
	7.5	2	5	4.4	10	15	12.8	0	7	11	9.2	71.43
	15	1	3	2.3	11	15	12.7	0	8	13	10.3	81.10
	20	0	2	1.0	11	15	12.7	0	10	14	11.7	92.13
n-Hexane	10	6	11	8.5	15	18	16.0	0	5	10	7.7	48.13
	15	3	8	5.8	15	18	15.8	0	7	13	10.2	64.56
	20	2	5	3.5	14	18	15.8	0	11	14	12.5	79.11
	25	0	4	1.5	15	18	16.0	0	11	16	14.5	90.63
Butanol	-	-	-	-	-	-	-	-	-	-	-	-
K. galanga												
Methanol	5	5	8	6.5	11	14	13.0	0	3	9	6.3	48.46
	10	3	4	3.1	11	15	12.8	0	8	11	9.8	76.56

	15	1	2	1.3	11	14	13.0	0	9	13	11.3	86.92
	20	0	1	0.5	11	14	12.8	0	10	14	12.5	97.66
Ethyl acetate	5	6	9	7.5	10	13	11.3	0	2	5	3.8	33.63
	10	3	5	4.5	10	13	11.2	0	6	9	7.2	64.29
	15	1	3	2.5	10	13	11.3	0	7	11	8.8	77.88
	20	0	1	0.3	10	13	11.2	0	9	13	11.0	98.21
n-Hexane	5	3	8	4.8	10	14	11.5	0	2	9	6.7	58.26
	10	1	5	5.3	10	15	11.3	0	6	12	8.3	73.45
	15	0	4	1.8	10	14	11.3	0	7	13	9.7	85.84
	20	0	2	0.5	10	14	11.5	0	10	13	11.0	95.65
Butanol	-	-	-	-	-	-	-	-	-	-	-	-
C. aeruginosa												
Methanol	10	6	10	7.3	12	18	15.5	0	5	12	7.8	50.32
	15	3	8	4.7	11	18	15.3	0	8	15	10.8	70.59
	20	2	5	2.5	12	18	15.5	0	7	15	12.0	77.42
	25	0	3	1.7	12	18	15.2	0	9	18	13.8	90.79
Ethyl acetate	10	5	8	6.7	10	14	13.1	0	4	9	6.5	49.39
	15	3	6	4.7	10	14	13.2	0	8	11	8.5	64.39
	20	2	5	3.3	11	14	13.2	0	9	12	10.2	77.27
	25	0	1	0.7	11	14	13.3	0	10	14	12.5	93.99
n-Hexane	15	4	6	4.5	9	11	10.2	0	5	7	6.0	58.82
	20	2	5	3.2	10	11	10.3	0	6	9	7.3	70.87
	25	0	3	2.0	10	11	10.5	0	7	11	8.5	80.95
	30	0	1	0.3	10	12	10.5	0	9	11	10.2	97.14
butanol	10	5	8	6.5	10	20	14.7	0	5	14	8.2	55.78
	15	3	5	4.0	10	20	14.3	0	7	16	10.7	74.83
	20	2	3	2.5	11	20	14.5	0	8	17	12.2	84.14
	25	0	1	0.5	10	19	14.3	0	10	19	14.2	99.30

Table 6. The effective concentration for rhizome plant extract repellents against *Aedes aegypti* adult mosquitoes

No	Rhizome plants	Extract types	Effective Concentration (%)	
			EC_{50}	EC_{90}
1	*Z. zerumbet*	Methanol	2.525	23.005
		Ethyl acetate	3.946	13.334
		n-Hexane	4.481	15.950
		Butanol	-	-
2	*A. galanga*	Methanol	2.629	17.812
		Ethyl acetate	1.558	27.236
		n-Hexane	10.781	26.780
		Butanol	-	-
3	*K. galanga*	Methanol	5.329	15.059
		Ethyl acetate	7.215	18.163
		n-Hexane	4.338	17.105
		Butanol	-	-
4	*C. aeruginosa*	Methanol	10.064	26.930
		Ethyl acetate	10.739	25.789
		n-Hexane	14.070	27.720
		Butanol	9.610	20.438

DISCUSSION

The quest for new active compounds that are effective, safe, and environmentally friendly is an important effort in the formulation of phyto-insecticides (Dohutia et al. 2015). The development of bio-larvicides is part of these efforts to overcome the problem of mosquito vector resistance to insecticides (Rasli et al. 2021). Findings from this study complement the previously reported information, specifically on the larvicidal and repellent activity of extracts from four species of rhizome plants. There were seven of sixteen and six of thirteen types of rhizome plant extracts that showed effective larvicidal and repellent activities in this study. Based on previously reported criteria for the larvicidal effectiveness of herbal ingredients (Komalamisra et al. 2005), the n-hexane extracts of *K. galanga* and *A. galanga* exhibited larvicidal activity with a high level of effectiveness. Both types of extracts were more effective than ether and chloroform extracts of *K. galanga* (Satoto et al. 2013) and secondary metabolites ethyl cinnamate and 3-carene (Kim et al. 2008) which have been reported previously. This study also demonstrated that the larvicidal activity of *A. galanga* extract was on par with previous reports, both in extract form (Poonsri et al. 2019) and essential oil (Nguyen et al. 2022a). Only one *Z. zerumbet* extract showed high larvicidal activity, namely n-hexane extract. These findings differed from previous reports which showed high larvicidal activity in methanol and ethyl acetate extracts (Murini et al. 2017) and essential oils (Khandagle et al. 2011). Variations and differences in larvicidal activity from the same plant (*Z. zerumbet*) are very likely to occur and have been reported by several researchers. This phenomenon could be caused by several factors, including habitat conditions (Karahan et al. 2016), abiotic environment (Kumar et al. 2017), climate (Sampaio & Da-Costa 2018), and the geographical origin of plants (Qi et al. 2018).

Two of the three types of *C. aeruginosa* extract obtained in this study also indicated higher larvicidal activity than the previously reported ethanol extract (Prawirasudarga et al. 2018). This condition could be due to several factors including differences in solvents and extraction methods. Even though methanol and ethanol are universal solvents that are polar, the yield and total flavonoid content of methanol solvent is slightly higher (Nguyen et al. 2022b). Different types and levels of solvent polarity bind to different types and quantities of secondary metabolites (Iloki-Assanga et al. 2015). Although studies of *C. aeruginosa* larvicidal activity are still limited, these findings can add new information to several similar reports on larvicidal activity from the genus *Curcuma*, namely *C. domestica* (Susilowati et al. 2021) and *C. xanthoriza* (Pereira et al. 2022) in which only *C. xanthoriza* showed high larvicidal activity so far.

Results from this study complement various reports on the larvicidal and repellent activity of rhizome plants. Findings from this study are more complete than previous studies which covered on thirteen types of extracts from four species of rhizome plants with EC_{90} ranging from 13.334% to 27.720%. The high repellent activity was found in seven of thirteen types of rhizome plant extracts in which at a concentration of 20%, they were able to repel at 92-98.5% from exposure to mosquitoes. The highest repellent activity was shown by the *Z. zerumbet* ethyl acetate extract with an EC_{90} of 13.334%. These findings are very promising for the development of repellent products as its repellent effect is equivalent to diethyltoluamide in commercial repellent products circulating in Indonesia, which is 13%. These results are also equivalent to the repellent activity of the petroleum ether extract of *Tribulus terrestris* (El-Sheikh et al. 2016). Studies on the repellent activity of rhizome plants including *Z. zerumbet* against *Ae. aegypti* mosquitoes are still limited. The repellent activity of several rhizome plant species that have been studied only covered on *C. longa* and *Z. cassumunar* in which the

essential oils of both species had a repellent activity of 4-8 hours. Another study reported the repellent activity of the compound (−)-terpinen-4-ol on *C. cassumunar* (Li et al. 2021), while *Z. zerumbet* essential oil 10% had a repellent activity of 72-99.1% against *Ae. aegypti* within 2 hours (Phukerd & Soonwera 2014). The repellent formulation in this study used a different material from previous studies which was the rhizome extract. According to the WHO bioassay repellent test procedure (World Health Organization 2009), the repellent activity is determined from the comparison between the average difference of mosquitoes perching in the control group and the experimental group to the control group based on the concentration of the extract in the total volume of lotion. This thinking refers to commercial repellent formulations with active DEET ingredients whereby the concentration of the active ingredient in each sachet pack is 13%. This concentration is very close to the repellent activity of 90% in ethyl acetate and n-hexane extract of *Z. zerumbet* and methanol extract of *K. galanga* achieved in this study.

The extracts of the rhizome plants in this study were obtained by the gradual maceration of extraction procedure; an inexpensive and optimum conventional method, especially for obtaining total flavonoids and phenolics (Alipieva et al. 2010), although it required a longer time, and more materials and solvents (Rasul 2018). Overall, the results of this study provided added value to the benefits of rhizome plants both inside and outside the health sector. The added value of these findings in the health sector is more focused on diversifying technological products that are useful for controlling mosquito-borne diseases. So far, various species of rhizome plants have been studied for their benefits in strengthening and maintaining the body's metabolic system so that they can prevent worm and bacterial infections, suppress inflammation, reduce pain, diarrheal diseases, sedative, cytotoxic, and insecticide (Kumar et al. 2017; Shetu et al. 2018). The added value also includes aspects of function diversification and utilization of local potential. Currently, the production of rhizome plants in Indonesia has reached 54,400 tons per year in 2021 with the five provinces that recorded the highest production being Central Java, West Java, Lampung, South Kalimantan, and East Java (Statistics Indonesia 2022). This study also supports the diversification of functions of rhizome plants in the health sector, especially in strengthening its function as an insecticide (Shetu et al. 2018) when the traditional use by the community is still limited to only as herbal drinks and cooking spices (Sumarni et al. 2019).

CONCLUSION

A total of seven extract types of rhizome plants showed effective larvicidal activity in which two extract types were highly effective (n-hexane extract of *K. galanga* and *A. galanga*), four types were moderately effective (n-hexane extracts of *Z. zerumbet* and *C. aeruginosa*, and methanol extracts of *C. aeruginosa* and *A. galanga*), while the one low effective type was ethyl acetate extract of *A. galanga*. Six extract types showed effective repellent concentrations with EC_{50} <5%, namely methanol and ethyl acetate extracts of *A. galanga*; methanol, ethyl acetate, and n-hexane extracts of *Z. zerumbet*; and n-hexane extract of *K. galanga*. Further studies on extracts' stabilities, larvicidal and repellent formulations for practical application in the field, and the isolation of chemical compounds from the most active extracts are necessary to be performed in the future.

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Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
This research protocol was reviewed and obtained ethical approval from the Health Research Ethics Committee, Faculty of Public Health, University of Muhammadiyah Semarang number: 698/KEPK-FKM/UNIMUS/2022.

Data Availability Statement
The manuscript has no associated data.

Authors' Contributions
Sayono Sayono (SS) designed the research, collected natural materials, reared mosquitoes, conducted experiments, analyzed data, and wrote and revised the manuscript. Risyandi Anwar (RA) extracted plant rhizomes and provided experimental support, data analysis, and manuscript writing. Othman Wan-Norafikah (OWN) finalizes manuscripts and supports submission to journals and manuscript revision.

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