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by Indonesian Journal of Dentistry Universitas Muhammadiyah Semarang

Submission date: 11-Dec-2023 04:22PM (UTC+0700)
Submission ID: 2236721686
File name: pbjs_P1.pdf (619.42K)
Word count: 4784
Character count: 26156

http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS

Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2022.1040.1046

Research Article

Evaluation of Larvicidal Activity of *Kaempferia galanga* Extracts Against *Aedes aegypti* Larvae

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Abstract

Background and Objective: The resistance of *Aedes aegypti* larvae against temephos become an obstacle in controlling the arboviral vector. This condition triggered researchers to investigate the larvicidal activity of phytochemical compounds that are effective, safe, biodegradable and eco-friendly from various medicinal plants. This study evaluated the larvicidal activity of *Kaempferia galanga* extracts against *Ae. aegypti* larvae. **Materials and Methods:** Four solvents with different polarities, namely ethanol, ethyl acetate, n-hexane and water were used in the sequential extraction. The final larvicidal bioassay test of the four extract types was designed in five replicates of five concentration ranges, namely 1.0, 8.75, 17.5, 35.0 and 70.0 ppm. A total of 20 3rd instar larvae of *Ae. aegypti* were contacted with each replicate in a plastic cup. Larval mortality and effective concentration of larvicide were calculated and determined after 24 and 48 hrs of exposure. **Results:** The average range of larval mortality according to the concentration of larvicide extracts of ethanol, ethyl acetate, n-hexane and water was 40-91, 2-36, 7-83 and 44-86% after 24 hrs and 88-100, 11-84, 12-99 and 77-100% after 48 hrs of exposure. The data yielded LC_{50} for 24 and 48 hrs of exposure at 1,563 and 0.061 ppm, 206,739 and 7,623 ppm, 47,579 and 38,063 ppm and 1.33 and 0.300 ppm, respectively. **Conclusion:** The polar extract of *K. galanga* showed high effectiveness so it is necessary to design the right formulation for field application, potency stability and active period of this larvicide residue.

Key words: Larvicidal activity, *Kaempferia galanga*, extract types, *Aedes aegypti*, arboviral vector

Citation: Anwar, R., J.N. Hida, D. Sumanto and S. Sayono, 2022. Evaluation of larvicidal activity of *Kaempferia galanga* extracts against *Aedes aegypti* larvae. Pak. J. Biol. Sci., 25: 1040-1046.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The role of the *Ae. aegypti* mosquito as the vector for some of the most important arboviral diseases such as Dengue, Chikungunya and Zika[1,2] and in addition to Rift Valley[3] and Yellow fever[4] has been well known by the community in endemic areas of the disease, so they carried out various efforts for vector control[5]. They also have very high confidence that temephos larvicide and fumigation are effective in suppressing the *Aedes* mosquito population[6], so the larvicide is one of the most widespread methods used in *Aedes* control measures in endemic areas, including Indonesia[7]. Long-term use of temephos has given rise to resistant strains in *Ae. aegypti* mosquito populations in various countries, such as recent reports in Brazil[8], Cuba[9], Indonesia[10], Malaysia[11] and Peru[12], in addition to genotoxic effects in humans[13].

The emergence of temephos-resistant strains in the *Ae. aegypti* populations have hampered the efforts to control these arboviral vectors and triggering researchers to study new herbal-based phytochemical compounds that are effective, safe, eco-friendly and biodegradable[14-16]. As a general criterion, the larvicidal activity effectiveness of a phytochemical compound is classified into three levels based on the lethal concentration of 50% (LC_{50}) which is high, medium and low if LC_{50} is less than 50, 250 and 750 ppm[17]. Several studies report various types of plant extracts that meet the criteria including Thai plants[17], local *Derris elliptica*[18-20], several medicinal plants in India[21] and Brazilian plants[22,23]. These plants contain a class of phytochemical compounds that have larvicidal potency, especially flavonoids, saponins, tannins, steroids, cardiac glycosides, alkaloids, anthraquinones and terpenoids. The compounds cause DNA damage and affect the detoxifying enzymes[24,25].

Kaempferia galanga is a member of the Zingiberaceae family[26] where the rhizome contains several secondary metabolite compounds such as steroids, saponins, flavonoids, terpenoids, phenols, alkaloids, tannins, cardiac glycosides and essential oils[27,28]. Traditionally, *K. galanga* rhizome is used as an antipyretic, anti-infection, digestive disorder and bioinsecticide[29,30]. During the COVID-19 pandemic, *K. galanga* became the medicinal plants that were mostly used by people in the world[31]. The potential for bioinsecticide of *K. galanga* rhizome extracts and essential oils has been reported by several researchers for vector control of arboviruses in various countries with varying effective concentrations. The lethal concentration (LC_{50}) of larvicidal activity of *K. galanga* essential oils was 39.22 ppm[32], ether and chloroform extracts of 64.08 and 105.02 ppm[33], methanol, hexane, chloroform, ethyl acetate and water extracts were 30.4, 27.9, 40.9, 133.7 and >200 ppm[34]. Production of medicinal plants in Indonesia is bountiful, including Zingiberaceae[35]. This fact strongly supports the efforts to diversify the use of *K. galanga* more broadly for health. This study aimed to evaluate the susceptibility of *Ae. aegypti* larvae to the larvicidal activity of ethanol, ethyl acetate, n-hexane and aqueous extracts of *K. galanga* rhizome.

MATERIALS AND METHODS

Plant material and extraction: *Kaempferia galanga* rhizome was purchased from a spice farmer in Gunung Kidul Regency, Yogyakarta Special Region Province, Indonesia. The rhizome is made simplicia, dried and made into powder. The technical grade ethanol, ethyl acetate, n-hexane and aqua dest were purchased from a local chemical distributor in Semarang City, Central Java, Indonesia. The extraction process was carried out at the Chemical Laboratory of Natural Materials, Garut University, West Java Province, Indonesia following the previous research procedure[19,34] with minor modifications. In short, the dry powder of *K. galanga* rhizome simplicia was macerated with ethanol for 3×24 hrs. The impurities are filtered and the solution is evaporated to produce ethanol extract. Part of the extract was dissolved in distilled water and liquid-liquid partitioned with ethyl acetate in a separating funnel to produce two layers: Water and ethyl acetate. The ethyl acetate layer is evaporated and yields the ethyl acetate extract. The water layer was partitioned with n-hexane solvent to produce water and n-hexane layers. Both are evaporated and produce aqueous and n-hexane extracts.

Determination of larvicidal activity: The initial bioassay test applied three levels of larvicide concentration of *K. galanga* extract in which five replicates were made, namely 85, 105 and 125 ppm and placed in a plastic cup. A total of 20 3rd instar *Ae. aegypti* larvae with healthy conditions were subject to each cup and contacted for 24 hrs. Larval mortality after 24 hrs exposure was 90, 98 and 100%. This data is used to design new concentration levels, namely 1, 8.75, 17.5, 35 and 70 ppm. The experiment was equipped with a positive control (0.02 ppm temephos solution) and a negative (aquadest) control. The effective concentrations (LC_{50} and LC_{90}) were determined by the Probit Test[36] and the results were compared with related references[17].

Review of ethics: The research protocol was reviewed and obtained ethical approval from the Health Research Ethics Commission, Faculty of Public Health, University of Muhammadiyah Semarang number: 516/KEPK-FKM/UNIMUS/2021.

Data analysis: The data were analyzed statistically according to the post-test-only control group experimental design using a two-way analysis of variance mean difference test. The difference test between groups used the least significant difference (LSD) at a probability level of 0.05. Determination of LC_{50} using the Probit Test. All data analysis using SPSS version 16.0 software. Visualization of data in graphs using Microsoft Excel.

RESULTS

In total, mortality data for *Ae. aegypti* larvae showed variations in larvicidal activity based on the types of *K. galanga* rhizome extract. The order from highest to lowest larval mortality was ethanol extract, water, n-hexane and ethyl acetate with the average mortality at the highest concentration reaching 91%. A significant increase in larvicidal activity occurred after 48 hrs of exposure where larval mortality reached 100%, even since the concentration of 35 ppm in ethanol and water extracts. This increasing phenomenon occurred in the n-hexane extract, although 99% mortality could be achieved at a twice higher concentration of 70 ppm. During the observation period, ethanol and water extracts showed equivalent larvicidal activity. This experimental condition was well controlled. It was shown that no dead larvae were found in the negative control group and 100% of dead larvae were found in the positive control group (Table 1). The temperature and pH of the water showed suitable conditions for the life of mosquito larvae (Table 2).

Figure 1 shows the description of larvicidal activity based on the concentration of *K. galanga* rhizome extracts. The ethanol and water extracts showed higher larvicidal potency and equivalence than ethyl acetate and n-hexane, even since the concentrations were low. All types of extracts showed an increase in larvicidal potency after 48 hrs of exposure, even ethanol and water extracts reached 100% mortality at a concentration of 35 ppm. Meanwhile, the ethyl acetate extract showed the lowest potency, while the n-hexane extract showed the highest potency after 48 hrs of exposure.

The larvicidal potency of *K. galanga* extract showed significant differences, both separately based on type and concentration, as well as the interaction between the two (Table 3). This difference can be seen in almost all types of extracts, except for ethanol and water extracts. These two extracts showed the lowest difference in the mean larval mortality (Table 4). The LC_{50} data after 48 hrs of exposure showed that all types of *K. galanga* extract were effective larvicides with high activity, although at 24 hrs of exposure the ethyl acetate extract showed moderate larvicidal activity (Table 5).

Table 1: Larval mortality of *Aedes aegypti* based on the types and concentrations of *K. galanga* extract

		Larval mortality percentage based on the exposure time					
		24 hrs			48 hrs		
Extract type	Concentration (ppm)	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Ethanol	1	35	55	40	65	100	88
	8.75	70	85	80	90	100	96
	17.5	80	90	85	95	100	99
	35	80	95	89	100	100	100
	70	85	95	91	100	100	100
Ethyl acetate	1	0	10	2	5	20	11
	8.75	0	10	5	10	30	15
	17.5	0	20	9	10	40	23
	35	0	30	17	30	45	37
	70	20	55	36	65	95	84
n-hexane	1	5	10	7	5	20	12
	8.75	5	10	8	10	40	28
	17.5	5	25	11	70	95	82
	35	15	50	32	75	100	94
	70	75	90	83	95	100	99
Water	1	25	75	44	55	90	77
	8.75	65	90	75	95	100	98
	17.5	65	90	81	95	100	99
	35	75	95	83	100	100	100
	70	80	95	86	100	100	100
Temephos*	0.02	100	100	100	-	-	-
Aquadest‡	0	0	0	0	0	0	0

*Positive control and ‡Negative control

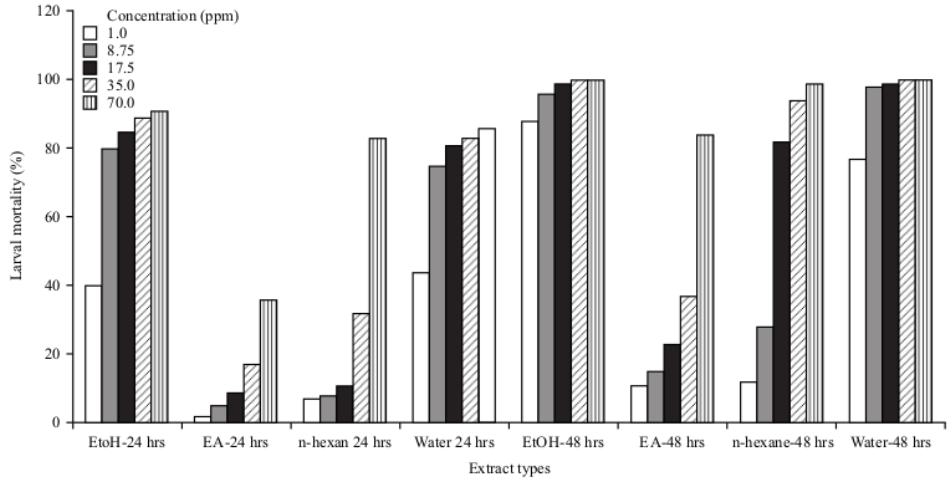

Fig. 1: Mortality rate of *Aedes aegypti* larvae after 24 and 48 hrs exposure to four extract types, namely Ethanol (EtOH), Ethyl acetate (EA) and n-hexane (Hex), EtOH extract type showed rapid progress in mortality of the research subject

Table 2: Physical conditions of the experiment

Variables	Pre	Post
Water temperature (°C)	26.0-27.0	26.2-27.0
Water pH	7.8-8.0	7.6-7.9

Table 3: Effect of extract type, concentration, and their interaction on larval mortality

Variables	F	p
Intercept	33.690	0.004
Extract types	26.071	0.000
Concentrations	7.032	0.004
Extract types and concentrations	10.867	0.000

Table 4: Multiple comparisons of extract types on larval mortality

Extract type comparisons	Mean difference	p	95% confidence interval
Ethanol-ethyl acetate	63.20	0.000	57.83-68.55
Ethanol-n-hexane	48.80	0.000	43.45-54.15
Ethanol-water	3.20	0.237	-2.15-8.55
Ethyl acetate-n-hexane	14.48	0.000	19.75-10.90
Ethyl acetate-water	-60.00	0.00	-50.95-54.65
n-hexane-water	-45.60	0.00	-50.95-40.25

Table 5: Lethal concentration (LC_{50} and LC_{90}) of the ethanol, ethyl acetate and n-hexane extract types on mortality of *Aedes aegypti* larvae

	Lethal concentration (ppm)			
Extract types	LC_{50} (95% CI)	LC_{90} (95% CI)	Chi-square	p
24 hrs exposure				
Ethanol	1.563 (0.853-2.411)	38.410 (25.689-66.490)	15.429	0.879
Ethyl acetate	206.739 (90.142-2656.154)	3026.409 (545.569-942044.641)	58.388	0.000
n-hexane	47.579 (29.006-116.513)	370.596 (140.397-4713.808)	109.982	0.000
Water	1.33 (0.375-2.683)	91.568 (44.037-350.320)	34.124	0.063
48 hrs exposure				
Ethanol	0.061 (0.003-0.217)	1.457 (0.575-2.648)	28.788	0.190
Ethyl acetate	7.623 (5.123-10.566)	36.932 (25.095-65.592)	72.2332	0.000
n-hexane	38.063 (23.598-79.283)	518.528 (187.215-4912.710)	72.096	0.000
Water	0.300 (0.107-0.532)	2.455 (1.672-3.780)	23.979	0.405

DISCUSSION

This finding proved that local *K. galanga* rhizome has good larvicidal activity whereas the extracts produced from polar solvents show higher larvicidal activity than semi-polar and non-polar. In general, larval mortality increased with the concentration of *K. galanga* rhizome extract, which indicates a dose-response effect phenomenon. On the other hand, the phenomenon of larval mortality in the experimental group, positive control, negative control and physical factors also showed the quality of controlled experiments. Temperature and pH data showed the optimum range for the life of *Aedes* larvae so that death was not caused by physical factors of water. *Aedes* larvae stopped moving and died at 8°C[37] and pH 3 or lower[38].

Ethanol and water are the polar extraction solvent. The literature stated that ethanol extracts contain higher phytochemical compounds than aqueous extracts, especially the presence of four classes of secondary metabolites of steroids, flavonoids, saponins and terpenoids. There are six secondary metabolites found in both extracts, namely alkaloids, tannins, phenols, coumarins, cardiac glycosides and phlobatinins[27]. The ethanol extract of *K. galanga* also contains seven minor compounds[39]. This larvicidal activity is related to secondary metabolites of alkaloids, phenols, coumarins, terpenoids and flavonoids that interfere with acetylcholinesterase (AChE) and octopaminergic receptors[40]. Cardiac glycosides interfere with the sensitivity of the performance of Na^+ and K^+-ATPase[41], while tannins interfere with the digestive system and the activity of detoxifying enzymes and AChE[42]. The larvicidal activity of the ethyl acetate extract of *K. galanga* rhizome is lower than the ethanol and water extracts because it is thought to contain only two important secondary metabolites, namely tannins and cardiac glycosides[27]. Ethanol is a broad-spectrum extraction solvent capable of capturing polar to non-polar compounds, while n-hexane only captures non-polar compounds. The data showed that the n-hexane extract of *K. galanga* rhizome had the weakest larvicidal activity. This indicated that the larvicidal activity of *K. galanga* rhizome extract is more determined by polar secondary metabolites. These findings indicate that *K. galanga* rhizome extracts have highly effective larvicidal activity indicated by an LC_{50} value of fewer than 50 ppm[17]. Overall, the literature stated that *K. galanga* rhizome contains 97 phytochemical compounds, namely 26 terpenoids, 15 phenolics, 16 cyclic dipeptides, 3 flavonoids, 8 diarylheptanoids, 9 polysaccharides and 9 other compounds including twelve volatile compounds and eighteen essential oils[28]. The presence of volatile compounds and essential oils raised the hypothesis of the repellent activity of the *K. galanga* extracts.

The results of this study add to the evidence of the non-medical benefits of *K. galanga* rhizome in the health sector, where there are promising prospects as a larvicidal raw material. Historically, the benefit value of *K. galanga* as a medicinal plant has long been known and felt by the wider community, especially in the South to Southeast Asia Region. In addition to its use for health and medicine, this plant also has culinary, cultural and economic value[43]. Traditionally, this medicinal plant has been used to improve the fitness of pregnant women and childbirth, relieve respiratory and digestive disorders and reduce swelling and joint pain[44]. Currently, the potential of this plant has been utilized for inflammatory, analgesic, diarrheal, bacterial and helminth infections, sedative, cytotoxic and insecticide[29,45]. *Kaempferia galanga* also has functional food value as a safe spice, highly nutritious, non-toxic antioxidant, high chemo-preventive potential and anti-nutritional properties[46]. In the Indonesian context, *K. galanga* has a high bio-cultural value, especially for the Batak and Javanese communities related to functional food and health[43]. The economic value of *K. galanga* is very promising because it is supported by the availability of bountiful raw materials. More than 5400 species of medicinal plants have been identified in Indonesia, including Zingiberaceae[35]. The use of *K. galanga* in traditional medicine is also a form of community creative economy, especially various herbal drinking in Central Java, Indonesia[47].

CONCLUSION

Kaempferia galanga rhizome extracts had effective larvicidal activity against *Ae. aegypti* larvae, whereas, the polar extracts were more active than semi-polar and non-polar. Further research is needed to determine the stability of the larvicidal potency and active period of the phytochemical residue and the appropriate formulation for field applications.

SIGNIFICANT STATEMENT

This study found high insecticidal potency in 2 types of *K. galanga* extract that can be beneficial for obtaining the specific phytochemical compounds as larvicide material for controlling the arboviral vectors, *Aedes* mosquitoes. This study will help the researchers to uncover critical areas of finding alternative methods for solving the resistance problems in mosquito vector control that many researchers are unable to explore. This finding reinforces that new theories on herbal chemical compounds can arrive in the near time.

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