

RESEARCH PAPER

The larvicidal activity of auraptene: oxygeranylated coumarin from *Limonia acidissima* L.

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DOI: <https://doi.org/10.29303/aca.v9i1.288>

Article info:

Received : 07/02/2026

Revised : 06/04/2026

Accepted : 08/05/2026

Available online : 31/05/2026

Abstract: *Limonia acidissima* L. (Rutaceae) is a plant rich in coumarin compounds and has been used in traditional medicine. One of the main coumarin compounds reported from the roots of this plant is auraptene (7-oxygeranylcoumarin), which has previously been reported to have antioxidant activity. With increasing cases of vector-borne diseases, especially dengue fever transmitted by *Aedes aegypti*, the development of effective and environmentally friendly natural larvicides is needed. This study aimed to evaluate the larvicide activity of auraptene, building on previous research. The larvicide test was carried out using the WHO larval bioassay method on third-instar *A. aegypti* larvae at concentrations of 25–500 ppm within an exposure time of 24 h. Mortality data were analyzed using probit analysis to determine the Lethal Concentration 50% (LC₅₀) value. The results showed that auraptene increased larval mortality with an LC₅₀ of 145.29 ± 0.02 ppm after 24 h of exposure. This value indicates that auraptene is classified as an active natural larvicide. In conclusion, auraptene has the potential to be developed as a natural larvicidal candidate against *A. aegypti* and may expand the spectrum of biological activity of coumarin compounds from *L. acidissima* L.

Keywords: *Aedes aegypti*, auraptene, coumarin, larvicide, *Limonia acidissima* L.

Citation: Ulfah, S. M., Tjahjandarie, T. S., Tanjung, M., Saputri, R. D., & Marlina, E. The larvicidal activity of auraptene: oxygeranylated coumarin from *Limonia acidissima* L. *Acta Chimica Asiana*, 9(1), 852–858. <https://doi.org/10.29303/aca.v9i1.288>

INTRODUCTION

Limonia acidissima L. (synonymous with *Feronia limonia*), locally known as kawista, was a plant in

the Rutaceae family widely distributed throughout Indonesia, including Java, Sumatra, Bali, Sulawesi, and Nusa Tenggara. This plant has long been used in traditional medicine to treat various health problems, such as diarrhea, dysentery, asthma, wounds, tumors, heart

problems, and hepatitis [1]. These therapeutic activities are related to its secondary metabolite content. Based on previous studies, *L. acidissima* L. contains secondary metabolite, such as alkaloids, flavonoids, terpenoids, and coumarins [2]. Coumarins are the main group in *L. acidissima* L., with structural variations such as isoprenylated, oxyphenylated, oxygeranylated coumarins, furanocoumarins, and pyranocoumarins. Coumarin compounds are known to possess diverse biological activities, including antioxidant, antimicrobial, antitumor, and hepatoprotective properties [3][4][5].

One of the main coumarin compounds reported in *L. acidissima* L. was auraptene (7-oxygeranyl coumarin). Previous research successfully isolated and identified auraptene from *L. acidissima* L. roots through fractionation and purification using various chromatographic techniques and confirmed its structure using spectroscopic analysis [1]. Auraptene reportedly exhibits significant antioxidant activity, thus strengthening its role as a bioactive compound in *L. acidissima* L. This finding was supported by a report stating that isoprenylated coumarins from the Rutaceae family have a broad spectrum of biological activities influenced by the presence of isoprenyl substituents on the coumarin skeleton [3]. The identification of auraptene as the dominant compound opened up opportunities to explore other biological activities of the same compound.

In contrast, the increasing number of vector-borne diseases in Indonesia, an endemic tropical country, particularly dengue fever, transmitted by the *Aedes aegypti* mosquito, has demanded the development of effective and environmentally friendly larvicidal agents. Current mosquito larval control efforts are dominated by synthetic insecticides, which, in the long term, can lead to resistance, environmental pollution, and risks to human health [6]. The World Health Organization (WHO) along with the Food and Agriculture Organization (FAO) through the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 2024 reported that several active ingredients in synthetic insecticides, such as dichlorvos, propoxuran, and diethyltoluamide, are toxic and carcinogenic and can cause disorders of the

nervous, respiratory, and cardiovascular systems. Therefore, exploring larvicides based on natural compounds is a safer and more sustainable alternative. Coumarin compounds have been reported to possess larvicidal activity by disrupting the metabolism and nervous system of insect larvae. Coumarin compounds from plants of the Rutaceae family have also been reported to have potential as natural insecticides, effective in suppressing mosquito populations during the larval stage [7].

Previous studies on the larvicidal activity of *L. acidissima* L. have generally focused on the use of extract; for example previous research reported that *L. acidissima* L. leaf extract at a concentration of 3% caused up to 90% mortality of *Culex quinquefasciatus* larvae [5]. Chloroform and methanol extracts at a concentration of 100 ppm have also been reported to cause up to 95% mortality of *A. aegypti* larvae. Meanwhile, n-hexane extract at a concentration of 500 ppm was also reported to exhibit larvicidal activity of 79.2% and 60% against *C. quinquefasciatus* and *A. aegypti* eggs, respectively [8].

Although auraptene compounds from the *L. acidissima* L. plant have been reported to possess antioxidant activity, there are no reports on the larvicidal potential of this compound. Therefore, this study aimed to evaluate the larvicidal activity of the auraptene compound from *Limonia acidissima* L. against third-instar *A. aegypti* larvae to a further develop previous research. The novelty of this study lies in the larvicidal activity of the auraptene compound isolated from Indonesian plants. It is hoped that this project will broaden the spectrum of auraptene biological activity and contribute to the development of safe and sustainable natural larvicidal candidates.

MATERIALS AND METHODS

Research Location

This study was conducted at the Entomology Laboratory of the Institute of Tropical Disease (ITD), Universitas Airlangga, Surabaya, East Java.

Equipments and Materials

The equipment used in this study comprised an analytical balance, Petri dish, stopwatch, filter paper, and standard laboratory glassware. The materials used in this study were auraptene, third-instar *A. aegypti* larvae, methanol, distilled water, and temephos.

Preparation of Test Compound

The compound used in the larvicidal test was a pure auraptene compound obtained from the roots of *Limonia acidissima* L. The extraction, fractionation, purification, and structural identification procedures for the auraptene compound have been described in detail in previous report [3].

Test Larvae

The *A. aegypti* larvae used in this study were obtained from a standardized laboratory colony maintained at the Entomology Laboratory, Institute of Tropical Disease (ITD), Universitas Airlangga, Surabaya. Species identification was based on larval morphology using the applicable mosquito taxonomic identification key. The larvae were maintained in containers filled with chlorine-free water under controlled environmental conditions at 25–27°C and a consistent photoperiod. During the rearing period, the larvae were fed a standard diet tailored to their developmental stage to support optimal growth and prevent contamination of the rearing medium. The larvae were monitored daily to ensure their health and uniform growth. The third-instar larvae were selected for their relatively uniform sensitivity to the test treatments. All procedures for rearing, monitoring, and selecting test larvae were performed in accordance with the 2005 World Health Organization (WHO) guidelines for laboratory testing of larvicides to ensure biological uniformity, reproducibility, and validity of the research results [9][10].

Larvicidal Activity Testing

The larvicidal activity of auraptene was tested using the WHO larval bioassay method, with adjustments for the test compound. A stock solution of auraptene was prepared by dissolving

1.0 mg of the isolated compound in 1.0 mL of methanol p.a., yielding a concentration of 1000 ppm. From this stock solution, test solutions were prepared at five concentrations: 25, 50, 100, 250, and 500 ppm. Subsequently, 20 µL of each test solution was added to a container containing the test medium, bringing the final volume to 100 µL. Next, 25 third-instar *A. aegypti* larvae were added to each container. Each treatment concentration was replicated five times. The larvae were exposed to room temperature for 24 h, and the number of dead larvae was counted. Larvae were considered dead if they did not respond to light mechanical stimulation. Mortality data for the larvae after 24 h of exposure were used to calculate the percentage of mortality at each concentration, which was then analyzed using probit regression against the logarithm of the test compound concentration to determine the LC₅₀ value of auraptene [9][11].

In addition to the test compound treatment, negative and positive controls were used to validate the test results. The negative control used a test medium of chlorine-free distilled water (aquadest) without any added compounds, whereas the positive control used temephos at a concentration of 0.02 ppm, in accordance with the WHO-recommended operational concentration for controlling *A. aegypti* larvae. The use of positive and negative controls aimed to ensure that larval mortality was caused by the test compound and to confirm the larvae's sensitivity to standard larvicides. The 50% lethal concentration (LC₅₀) of auraptene was determined based on the percentage of larvae mortality after 24 h of exposure. The corrected mortality data were then transformed into probit values and plotted against the logarithm of the test compound concentration. Probit regression analysis was used to calculate the LC₅₀ value. The obtained LC₅₀ value was used as the main parameter to evaluate the potential larvicidal activity of auraptene [12][13].

RESULTS AND DISCUSSION

Larvicidal Activity of Auraptene

The larvicidal activity of auraptene isolated from *L. acidissima* L. roots was tested against third-instar *A. aegypti* larvae using the WHO larval bioassay method for 24 h. The larvicidal test results showed that auraptene at five concentrations (25, 50, 100, 250, and 500 ppm) caused mortality of third-instar *A. aegypti* larvae in a concentration-response manner (**Table 1**).

Table 1. Probit analysis of larvicidal activity

Test Sample	Concentration (ppm)	Log ₁₀ Concentration (ppm)	Mortality (%)	Mortality Proportion	Mortality Probit (z)
Negative control	0	-	0	0.00	-
Positive control	0.02	-1.70	100	1.00	-
Auraptene	25	1.40	52	0.52	0.05
Auraptene	50	1.70	56	0.56	0.15
Auraptene	100	2.00	64	0.64	0.36
Auraptene	250	2.40	84	0.84	0.99
Auraptene	500	2.70	92	0.92	1.41

The negative control group showed no larval mortality, whereas the positive control using temephos at a concentration of 0.02 ppm resulted in 100% larval mortality after 24 h of exposure. This demonstrates the validity of the test method in accordance with the World Health Organization (WHO) guidelines (2022) and the principles of probit analysis by Finney (1971) [13]. Control data are presented as an indicator of bioassay quality but were not included in the probit analysis because they do not represent exposure to the test compound concentration and cannot be transformed logarithmically. Probit analysis was performed only on treatment groups with measured concentrations to model the dose-response relationship and estimate toxicity parameters in the form of LC₅₀ and LC₉₀ values [14].

The test results showed that the percentage of larval mortality increased with increasing auraptene concentration from 52% at 25 ppm to 92% at 500 ppm, indicating a clear dose-response relationship [14]. The good linearity between the log₁₀ concentration of auraptene and the probit value of mortality (**Figure 1**) indicated that the data met the requirements of

probit analysis for determining LC₅₀ and confirmed that larval mortality in the treatment group was caused by the larvicidal activity of the test compound [15].

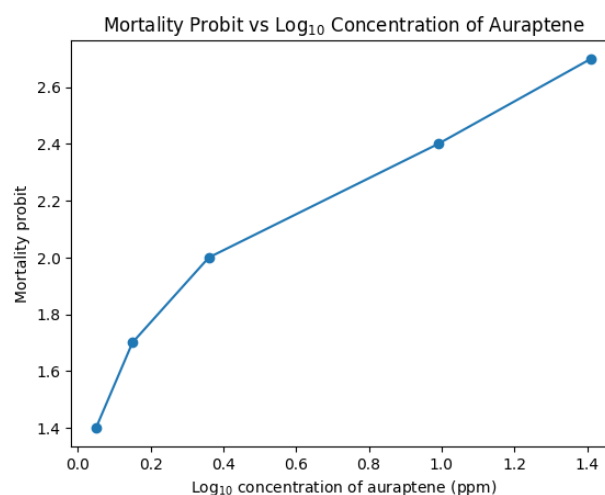


Figure 1. Relationship graph of log₁₀ concentration of auraptene and mortality probit

Based on the probit analysis, the LC₅₀ value of auraptene was 145.29 ± 0.02 ppm after 24 h of exposure. With regard to the criteria for natural insecticidal activity from natural compounds, compounds with LC₅₀ values <100 ppm are categorized as highly active, 100–500 ppm as active, and 500–1000 ppm as weakly active [9]. Thus, auraptene is classified as an active natural larvicide.

Relationship between The Structure of Auraptene and Larvicidal Activity

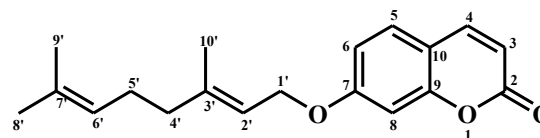


Figure 2. The structure of auraptene compound

The mechanism of action of auraptene as a larvicide is thought to be closely related to its chemical structure. The structure of the auraptene compound (**Figure 2**) shows the presence of an oxygenated substituent at the C-7 position of the coumarin skeleton, which is lipophilic [3]. The presence of this lipophilic group increases the molecule's lipophilicity, which is generally associated with its ability to interact with

the lipid phase and penetrate lipid-containing biological barriers. Lipophilic molecules tend to have higher partition coefficients and better penetration through the lipid-rich cuticle layer, thus facilitating auraptene diffusion through the larval integument and enabling accumulation in target tissues [16]. Coumarins are known to disrupt larval metabolism by disrupting mitochondrial function and inhibiting key enzymes, such as acetylcholinesterase (AChE), which plays a role in nerve impulse transmission. This disruption can cause paralysis and even death of the larvae, and this mechanism aligns with the mortality pattern of *A. aegypti* larvae observed in this study [17]. Further research is needed to examine the mechanism of action at the molecular level, assess toxicity to non-target organisms, and develop formulations to support the application of auraptene as a safe and sustainable vector control candidate.

Larvicidal Activity of Coumarin Compounds

Other coumarin compounds have been reported to exhibit larvicidal potential against *A. aegypti* and *C. quinquefasciatus* mosquito larvae. The complex coumarin compound C₂₉H₄₂O₈, in the form of (3,4-dihydroxy-5-[(3,6,8-trimethyl-2-oxo-2*H*-chromen-7-yl)oxy]methyl}oxolan-2-yl)methyl-3,7-dimethylnonanoate, isolated from *Eucalyptus deglupta* leaves, was reported to exhibit strong toxicity, with LC₅₀ values of 3.99 ppm and 4.99 ppm against *C. quinquefasciatus* and *A. aegypti*, respectively, after 24 h of exposure [18].

The strong larvicidal activity of this complex coumarin compound is thought to be closely related to its chemical structure. The aromatic and relatively lipophilic coumarin core (2*H*-chromen-2-one) allows effective interaction with the larval cell membrane, thereby facilitating the compound's penetration through the integument. The presence of an oxygenated substituent and a long aliphatic ester group (3,7-dimethylnonanoate) increases the lipophilicity of the molecule, which plays a crucial role in accelerating passive diffusion and accumulation of the compound in target larval tissues. Furthermore, the hydroxyl groups at positions 3 and 4 can form hydrogen bonds with vital enzymes or proteins, thereby disrupting

important physiological processes, such as cellular respiration and ion regulation. The combination of a reactive aromatic core, lipophilic substituents, and polar functional groups enables coumarin to efficiently penetrate larval biological systems and exert high toxicity [3][18].

The larvicidal activity of auraptene in this study aligns with the structure–activity relationship of this complex coumarin compound. Auraptene has a coumarin core (2*H*-chromen-2-one), which acts as the primary pharmacophore, and a lipophilic geranyl substituent at position C-7 that enhances penetration through the larval integument and accumulation in target tissues [3]. This mechanism aligns with the complex coumarins from *E. deglupta*, which have long aliphatic chains and exhibit high toxicity. The combination of a reactive aromatic skeleton and lipophilic isoprenoid substituents in auraptene is thought to enhance the interaction with membranes and physiological systems of larvae, thus explaining the significant larvicidal activity obtained in this study [18].

CONCLUSION

In this study, the auraptene compound from *Limonia acidissima* L. was shown to have larvicidal activity against third-instar *A. aegypti* larvae with LC₅₀ value of 145.29 ± 0.02 ppm. This activity indicates that the auraptene compound has the potential to be developed as a natural larvicide and may expand the biological activity spectrum of oxygenated coumarins from *L. acidissima* L.

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