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In vitro insecticidal activity of *Homonoia riparia* Lour leaf extract for use in controlling *Aedes aegypti* L. populations

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Abstract
Plant extracts and secondary metabolites extracted from plants can produce natural pesticides. Compared to synthetic insecticides, they degrade quickly and threaten the environment and public health less. It was feasible to discover the formation of the important secondary metabolites, phenols, flavonoids, and alkaloids by the *Homonoia riparia* leaf extract as the investigation progressed. Because phenolic chemicals are renowned for their antioxidant activity, they have a lot of interest. At low doses, the larvicidal activity of *Homonoia riparia* leaf extract implies that it could be utilized as a domestic larvicide to restrict the spread of the mosquito vector *Aedes aegypti*. Following the WHO guidelines, a dose-response bioassay was done on *Aedes aegypti* larvae collected from the municipality of Guntur rural areas. After 48 hours, the extracts of *Homonoia riparia* (692.6%±125.89) required less concentration to kill 95% of the larvae. Ethanolic extract (EE) obtained from *Homonoia riparia* leaves, and their effect on *Aedes aegypti* was evaluated at concentrations of 40, 60, 80, 85, and 95% for EE, in a completely randomized design with a 2x5 factorial arrangement. There were also substantial differences (p<0.01) across the concentrations tested, yielding a 95% death rate at 60%. At high concentrations, the secondary metabolites in the EE were effective, causing acceptable death values in *Aedes aegypti*, recommending its usage as a preventive control.

Keywords: *Homonoia riparia*, phytochemical analysis, *Aedes aegypti*, insecticidal activity, secondary metabolites

Introduction
Diseases transmitted by arthropods affect millions of people around the world annually. Among these diseases, dengue has the most significant epidemiological potential [1]. *Aedes* (Stegomyia) *aegypti* (Linnaeus, 1762) is an important vector of dengue, chikungunya and Zika [2]. In Asia, particularly in India, *A. aegypti* is the primary vector of Dengue and, recently, of chikungunya and Zika viruses [3]. Several studies are being conducted to develop an effective vaccine against these diseases [4], mainly dengue, which already has a vaccine on the market [5]. However, given the complexity in the preparation of these vaccines (e.g., several serotypes for each arbovirus, side effects in people never infected before and number of doses [6], currently the most effective means is to control vector population densities to reduce the number of cases of dengue, chikungunya and Zika. At a global level, including in India, vector control strategies are oriented toward community participation through education campaigns to reduce possible larval breeding grounds [7]. However, in epidemic situations, applying chemical insecticides on a large scale is suggested. However, the continuous use of insecticides in vector control programs has resulted in the selection of resistant *A. aegypti* populations in India and the world [8].
Insecticide options for replacement are scarce due to high development costs and inevitable insecticide resistance. Therefore, it is necessary to look for alternatives to control *A. aegypti* that reduce the problems caused by synthetic insecticides [9]. As alternative methods to control this mosquito vector, biological controllers have been evaluated in the laboratory, such as natural predators, entomopathogenic fungi. and natural plant extracts [10]. These methods have proven to be effective in controlling mosquito populations and preventing the development of resistance.

Several works [11] using plant extracts have been developed in India to search for new molecules to control *A. aegypti*. Among these methods, natural plant extracts constitute a source of new and varied bioactive structures (secondary metabolites), which can have intrinsic activity or serve as bests for the development of safer insecticides [12]. Secondary metabolites, among which alkaloids, terpenoids, coumarins and phenols stand out, are characterized by presenting biological activity to control insect vectors [13].

Human communities traditionally use insecticides of botanical origin in many places worldwide to manage mosquito vectors [14]. Given the high diversity of the Indian flora, including the Euphorbiaceae family, it is plausible to identify species with larvicidal potential to control natural populations of *A. aegypti* [15]. The search for new insecticides and the growing stimulus for research aimed at using plants as an alternative for controlling mosquitoes, vectors of diseases such as dengue, are motivated by the almost absence of toxicity of these natural products to animals and plants, and because they are biodegradable, which prevents contamination of the environment. In contrast, synthetic insecticides, to which insects are becoming increasingly resistant are toxic and polluting.

For a very long time, it was believed that plants in the Euphorbiaceae family might be used to heal a variety of ailments. *Homonoia riparia* Lour has become well known due to its application in medicine [16-18]. The powdered root of this plant has laxative, diuretic, and emetic effects. Even though *H. riparia* possesses medicinal properties, but very little is known about this plant's antibacterial activity and phytoconstituents [17]. *H. riparia* is best known for its Antioxidant and Nephroprotective [18]. The current research aimed to assess the larvicidal activity of *Homonoia riparia* Lour leaf extract (ethanolic) against III and IV instar larvae of *A. aegypti*.

2. Methodology

2.1 Chemicals and Reagents

All chemicals and reagents purchased from Sigma Aldrich, USA, with 98-99% purity, AR grade quality. Double distilled water (pH 7.02) was used to prepare solutions and reagents.

2.2 Plant material and Extraction Process

Homonoia riparia (Euphorbiaceae family) leaves (Figure 1) were collected in a rural area of Guntur district (Karempudi) reserve forest (16.431555° N, 79.704394° E), Andhra Pradesh, India, in February 18 to 22, 2018. Botanical identification was carried out by comparing the collected samples with a specimen of the species deposited in the Herbarium in the Department of Botany, Nagarjuna University, Andhra Pradesh, India. The material was cleaned and dried in an oven at 50 °C for 3 days. Once dry, it was ground to obtain fine particles subjected to ethanol extraction. For every 100 g of dry material, 700 ml of 96% ethanol was added. Two consecutive extractions were carried out on this material with fresh ethanol. The first extraction was performed by applying sonication for 30 minutes and the second by using sonication for 30 minutes and leaving it to rest for approximately 18 hours. The extract obtained from the two extractions was filtered under a vacuum in a Büchner funnel attached to a Kitasato flask. It was then concentrated in a rotary evaporator at 40 °C and under reduced pressure. Once the extract was focused, it was lyophilized and from each sample, a solution of 5000 mg/L was prepared in a mixture of 96% ethanol and water (1:1) to carry out the bioassays. This concentration was chosen according to the bibliographic review carried out on the plant species.

Fig 1: *Homonoia riparia*

2.3 Collection of *A. aegypti*

Natural inhabitants of *A. aegypti* were collected and used to verify the potential larvicidal effect of crude plant extract of the *H. riparia*, using natural populations of this vector. For this, *A. aegypti* larvae were collected from four locations in the city of Guntur rural mandal, Andhra Pradesh, India. The collections were carried out following the World Health Organization (WHO) methodology to determine the infestation rates of *A. aegypti*. These localities were used because they presented high rates of vector infestation [21]. From each locality, *A. aegypti* larvae were randomly collected from at least 10 water storage tanks in selected homes. Each storage tank water was located at least 100 m apart. The larvae from each location were taken to the laboratory and maintained under controlled conditions (27±1 °C, humidity 90±10% and photoperiod 12:12 h) until the emergence of adults. All *A. aegypti* adults were gathered to establish a base colony named AP-18. Adult mosquitoes were fed with a 10% sugar solution and blood (to induce oviposition in females) twice a week using mice (*Mus musculus Swiss*). The larvae of the F3 generation were used as material to determine, in dose-response bioassays, the larvicidal activity of the collected plant species.

2.4 Effect of ethanolic extract of *H. riparia* on *A. aegypti*

The dose-response bioassays for concentrated crude extract were carried out following the methodology proposed by the WHO [22]. In these experiments, *A. aegypti* larvae (initial third or fourth instar) were exposed to 5 concentrations (40, 60, 80, 85 and 95%) to determine larval mortality at 24 and 48 h of exposure. The larvae were considered dead when they did not react to physical contact in the cervical region and when they showed prolonged movements or inability to float. For each

concentration and for the control, four replicates of 20 larvae were evaluated. Additionally, for each bioassay, two control groups were included in the experiments, one using filtered water and the other 95% absolute ethanol (solvent used to obtain the ethanolic extracts). Each treatment was replicated 5 times and each repetition consisted of two experimental units. The insecticidal effect of ethanolic extracts on adults of *A. aegypti* larvae was considered to have a direct relationship with the percentage of dead insects.

The mortality data (expressed in number of deaths per dose) were used to calculate the lethal concentrations 50 and 95 (LC_{50} and LC_{95}) at 24 and 48 h of the exposed individuals and analyzed through Finney's log-probit method (1971) [23], using the Probit program of Raymond (1993). This analysis was performed for those plant extracts that showed mortality percentages >50% after 48 h.

Additionally, for the ethanolic extracts that showed mortality percentages >50% after 48 h, a completely randomized factorial analysis of variance (factorial ANOVA) was performed. Before the factorial ANOVA, data conflicts' normal distribution and homogeneity were checked. The factorial ANOVA evaluated the differences between the factors: species, time (24 and 48 h), and concentration (40, 60, 80, 85 and 95% with four repetitions. When the differences were significant, the Tukey HSD (Honestly-Significant-Difference) test was used to explain the differences between levels of each factor. All analyses were performed with STATISTICA 10 software.

3. Results

3.1 Effect of ethanolic extracts of *Homonoia riparia* leaves on the mortality of III and IV instar larvae of *A. aegypti*

The concentrations of the ethanolic extract of *Homonoia riparia* leaves (40, 60, 80, 85, and 95%) produced a significant effect on mortality in III and IV instar larvae of *A. aegypti*, showing differences between them ($P < 0.01$) (Table 1). Figure 2 shows that the mortality of the borer increased as the dose increased. However, the mortality percentage produced at 96% slightly exceeded 50% of the population studied. The effect of ethanolic extract at 80 and 85% was not significantly different ($p<0.01$) with mortality values of 45 and 44%, respectively. The lowest mortality percentages were 25.75 and 10.5% at 60 and 40% doses, respectively.

Table 1: ANOVA of the effect of the concentrations of the ethanolic extracts of *Homonoia riparia* on the percentage of mortality of III and IV instar larvae of *A. aegypti*

Parameter	Degrees of freedom	Sum of squares	Mean squares	F	p
Concentration	4	0.274	0.0685	17.35	0.0000***
Extract	1	0.0015	0.0015	0.37	0.5841^{ns}
Extract concentration*	5	0.0488	0.0122	3.09	0.0336*
Error	40	0.1659	0.0041		
Total	50	0.4902			

* Significant at $p < 0.05$; ** Significant at $P < 0.01$. ns: not significant. CV 24.45

Figure 2. Average mortality (%) of III and IV instar larvae of *A. aegypti* under the effect of the concentrations of ethanolic extract of *Homonoia riparia* (Averages with the same letter do not differ statistically, Tukey $p< 0.01$)

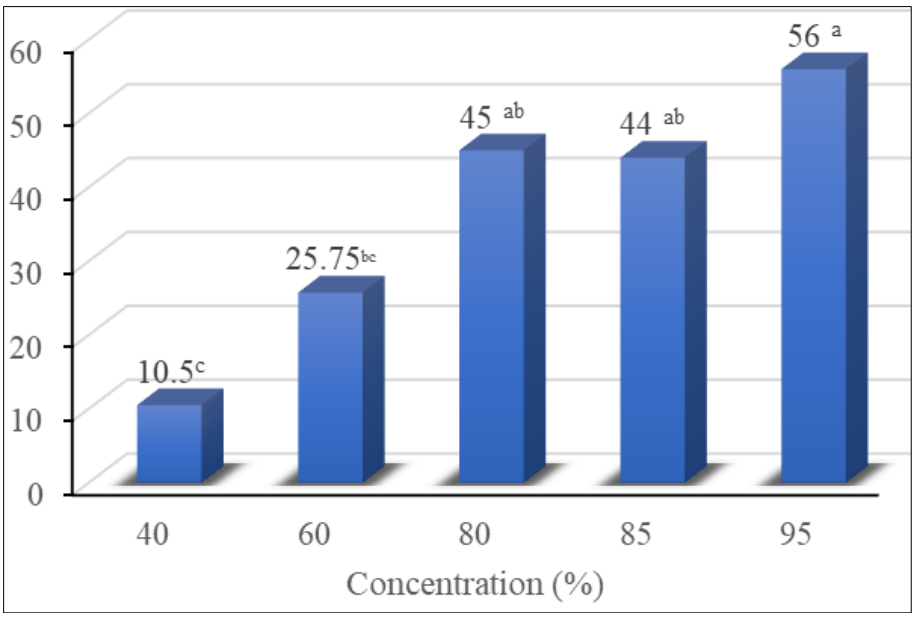

Fig 2: Average mortality (%) of III and IV instar larvae of *A. aegypti* under the effect of the concentrations of ethanolic extract of *Homonoia riparia* (Averages with the same letter do not differ statistically, Tukey $p< 0.01$)

The factorial ANOVA for the ethanolic extracts that showed mortality percentages >50% after 48 h showed significant differences between all the factors analyzed and their interactions (Table 1). In Figure 2, it can be seen that higher concentrations of ethanolic extract of *H. riparia* manage to eliminate a higher percentage of larvae. This trend was maintained in the two reading times (24 and 48 h). In contrast, lower concentrations did not cause larval mortality.

3.2 Morphological view of *A. aegypti* larvae

An optical microscope (Leica USA) with a magnification range of 40-400x was used to observe early third and fourth instar larvae of *Aedes aegypti* [25]. The morphological photogram of the midgut following food ingestion, as depicted in Figure 3a&b of this study, provides valuable insights into the impacts of larvicidal mechanisms. Following a 24-hour incubation period, the plant extract observed in Figure 3a & b within the midgut content of the larvae exhibited a resemblance to the findings of a prior investigation involving the utilization of *Azolla pinnata* plant extracts, as indicated by the presence of a green coloration. The lethal effects of the ethanol extract derived from *H. riparia* leaves on *Aedes* larvae are believed to be primarily attributed to ingestion. The findings obtained from the utilization of an ethanolic extract derived from *H. riparia* leaves indicate that liquid-based methodologies are most suitable for the commercialization of this extract.

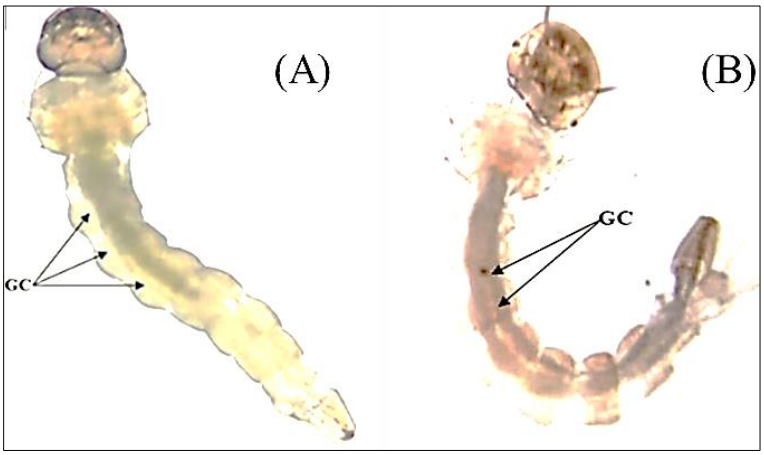

Fig 3: The morphological composition of the midgut was influenced by the administration of an ethanolic extract of *H. riparia* leaves.

Table 2: Analysis of variance for the percentage of mortality of 3-4 stage larvae of *A. aegypti* against different species and concentrations of ethanolic extract of *H. riparia* showed mortality percentages >50% after 48 h

Analysed factor	F	p
Species (S)	111.06	0.0001*
Time (T)	161.39	
Concentration (V)	1178.07	
S x T	8.59	
S x V	39.23	
T x V	58.54	
S x T x V	6.27	

* P -values with significant differences ($p< 0.05$).

The ANOVA (Table 2) also indicates significant differences (P<0.05) in the interaction between the factor's concentration by extract for the variable percentage of mortality of *A. aegypti*, which was directly affected by the increase of the attention of the ethanolic extract of *Homonoia riparia* (Figure 4). The highest percentage of mortality (65%) was produced by the ethanolic extract of *Homonoia riparia* at 95%.

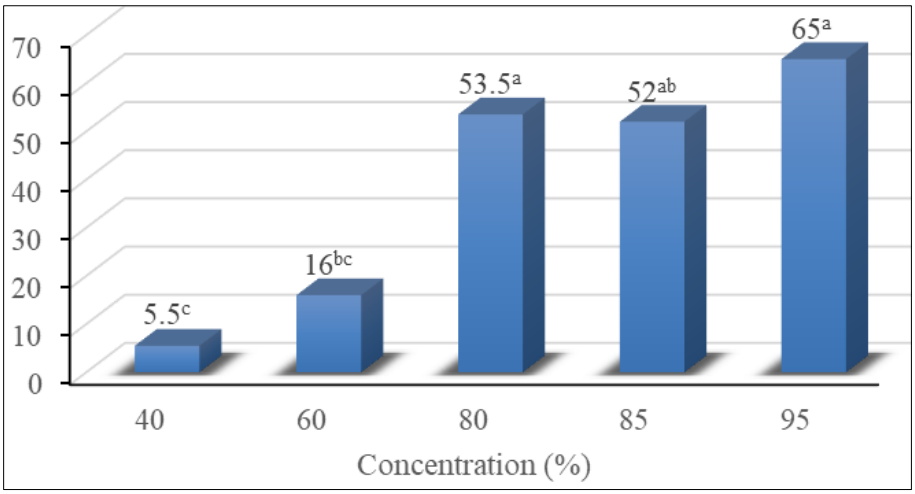

Fig 4: Average mortality (%) of III and IV instar larvae of *A. aegypti* under the effect of ethanolic extract, evaluated at five concentrations. (Averages with the same letter do not differ statistically; Tukey $p< 0.01$)

Table 3. Susceptibility profile of 3-4 stage larvae of *A. aegypti* against ethanolic extract of *H. riparia* showed mortality percentages >50% after 24 and 48 h. The standard deviation for each LC is presented in parentheses. In all experiments, there was no mortality in either of the two control groups.

Table 3: Susceptibility profile of 3-4 stage larvae of *A. aegypti* against ethanolic extract of *H. riparia*

Plant Name*	ET (hours)	LC (%)	
		50	95
H. riparia leaves	24	792.4 (132.9)	1802.91(369.67)

*Bioassay included larvae of the F_1 filial generation; ET= Exposure time.

At the 60% dose, mortality presented statistical differences, higher for ethanolic extract (16%). The concentrations of 60, 80 and 85% of *Homonoia riparia* did not register significant differences in the percentage of mortality. In comparison, the dose of 40% of the extract showed the lowest mortality of the doses evaluated.

4. Discussion

The present work evaluated the possible use of a selected plant (*H. riparia* leaves), present in the Indian flora, to control the vector of dengue, chikungunya and Zika viruses. The use of *H. riparia* leaves that grow predominantly in areas cultivated and controlled by man for the production of potential larvicides of plant origin has several advantages, including their easy achievement, cultivation, and little economic investment for their maintenance, a trend observed in South Asia [26]. Additionally, plants, in general, are a rich source of alternative (ecologically friendly) agents for the control of disease-vectoring mosquitoes since they have bioactive chemicals that act against a limited number of species, including target species [27].

A recent literature review [28] shows that compounds derived from plants such as tannins, quinones, flavonoids, sterols, coumarins and alkaloids are widely used as potential larvicides, secondary metabolites present in the species evaluated. Of these compounds, in the Asteraceae family, flavonoids and terpenes (and their derivatives, e.g., sterols, triterpenes) have been recorded as metabolites with larvicidal activity. Therefore, the results found here are promising for the control of *A. aegypti* larvae.

On the other hand, numerical calculations suggest, in terms of reducing the adult population size and time to do so, what is done every 15 days with the LC_{95}. However, the application of LC_{50} every 30 days also has a beneficial effect since it manages to reduce the population size in a short time (in four months it decimates the initial population of adults by 50%) and does not require extra expenditure of material for its potential implementation as LC_{95}.[19-20] Therefore, it is concluded that the extract of *H. riparia* leaves was the most efficient for control*ling A. aegypti* populations, so they deserve to be studied in depth given their potential larvicidal effect. Similar results were obtained by Alvarez-Valverde *et al*. (2023) [29] when they applied powdered leaves of *Ipomoea cairica* on having the lowest LC_{50} of 0.0341 mg/mL offered to *A. aegypti*, arguing that the differences were due to the protection that the wheat grain provided to the insect. The insecticidal effect produced by the EE of *H. riparia* on III and IV instar larvae of *A. aegypti* is associated with the toxicity caused by the phytochemicals of the extract, which could induce starvation and suffocation of the insect. Additionally, it is deduced that the more significant number of alkaloids, phenols [24], flavonoids and saponins in the EE of *Homonoia riparia* could contribute to this extract showing greater effectiveness in producing mortality. In this regard, Ileke and Bulus (2012) [30] mentioned that extracts can cause death due to contact toxicity or induce asphyxiation due to toxic Odors and obstruction of spiracles, as well as inhibit locomotion and consequently reduce the ability to search for food.

5. Conclusion

Based on the findings of this study, it can be inferred that the ethanolic extract derived from *H. riparia* leaves exhibits significant efficacy against primary dengue vectors in the early stages of 3rd and 4th instar larvae. The toxicity of the secondary metabolites in the extract, which could cause starvation and suffocation in the insect, is linked to the insecticidal effect of *Homonoia riparia* EE on *A. aegypti* larvae. Furthermore, it is inferred that *Homonoia riparia*'s EE contains more alkaloids, phenols, flavonoids, and saponins because these compounds are more effective at causing mortality.

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7. Conflict of Interest

The authors have no conflicts of interest regarding this investigation**.**

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