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Research Article

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Mosquito Larvicidal Activity of Leaf Extract of *Annona squamosa* against larvae of *Anopheles annularies* and *Aedes aegypti*

Priyanka Ramteke*

Department of Zoology, Shri Shivaji Science College, Amravati, India

***Correspondence:** Priyanka Ramteke, Department of Zoology, Shri Shivaji Science College, Amravati, India; E-mail: priyanka.ramteke753@gmail.com; DOI: https://doi.org/10.56147/jmcscr.2.2.26

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Abstract

Mosquito-borne diseases remain one of the most serious public health concerns worldwide, particularly in tropical and subtropical regions. Conventional vector control strategies largely rely on synthetic chemical insecticides; however, their continuous application has resulted in widespread insecticide resistance, environmental contamination and harmful effects on non-target organisms. In this context, plant-derived bioactive compounds offer promising alternatives due to their biodegradability, safety and eco-friendly nature. The present investigation was conducted to evaluate the larvicidal efficacy of leaf extracts of *Annona squamosa* against fourth instar larvae of the malaria vector *Anopheles annularis*.

Shade-dried and powdered leaves of *A. squamosa* were subjected to extraction using three solvents water, ethanol and methanol. A series of concentrations ranging from 20 to 100 ppm were prepared from the respective stock solutions. Larvicidal bioassays were performed under laboratory conditions following standardized WHO guidelines and larval mortality was recorded at 24 and 48 hours post-treatment. The percentage mortality was calculated and LC_{50} values were determined through regression-based dose-response analysis.

Qualitative phytochemical screening of the extracts revealed the presence of several important secondary metabolites including flavonoids, phenols, terpenoids, alkaloids, steroids and saponins. These compounds are known to possess insecticidal and growth-inhibitory properties and are likely responsible for larval toxicity. Spectroscopic characterization using UV-visible and Fourier Transform Infrared Spectroscopy (FTIR) further confirmed the chemical complexity of the extracts and identified functional groups such as O-H, C-H, C=O and aromatic C=C bonds associated with biologically active constituents.

The bioassay results demonstrated significant concentration-dependent and time-dependent larvicidal effects. Among the three extracts tested, methanolic leaf extract exhibited the highest larvicidal activity, achieving up to 90% mortality at 100 ppm within 24hrs and 95% mortality after 48hrs. Ethanolic extract showed the next best performance, while aqueous extract was comparatively less potent. The LC_{50} values obtained after 48hrs were 30.00 mg/L for methanol extract, 34.79 mg/L for ethanol extract and 53.95 mg/L for aqueous extract, clearly indicating superior efficacy of organic solvents in extracting active principles.

The findings of this study highlight Annona squamosa leaves as a potential source of natural larvicidal agents for mosquito vector management. The enhanced potency of methanolic and ethanolic extracts suggests their possible development into botanical larvicide formulations. Further studies involving isolation of specific bioactive molecules and field-level evaluations are recommended to establish practical applicability. Overall, the study supports the use of plant-based insecticides as sustainable tools in integrated mosquito control programs.

Keywords: *Annona squamosa*; Mosquito control; Larvicidal activity; Phytochemical

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Introduction

Mosquitoes belong to the family Culicidae in the class *Insecta*. They were responsible for transmitting the most relevant vector-borne diseases, including malaria, *Lymphatic filiariasis*, Japanese encephalitis, dengue and yellow fever [1]. These were common pests seen on every corner of the globe, having evolved to adapt to virtually any climatic conditions [2]. The majority of mosquito control programs target the larval stage in their breeding sites. Medicinal plants were a rich source of alternative agents for mosquito control whereas they contain bioactive chemicals that act against a limited number of species, involving specific target insects and were eco-friendly. Some plant extracts and isolated botanicals from certain plant families had been analyzed for their promising larvicidal activity, with no hazardous effects on the ecosystem [3]. Approximately 3500 species of mosquitoes had been described worldwide, with relatively few of them being significant vectors of human diseases. However, the worldwide problem of mosquito-transmitted diseases was quite severe [4]. The only effective approach to minimizing the incidence of these diseases was to eradicate and control mosquito vectors, mainly by applying insecticides to larval habitats and educating the public [5]. *Anopheles annularies* was responsible for transmitting *Lymphatic filiariasis* and malaria fever to humans.

The most effective approach to minimizing the incidence of these diseases was to eradicate and control mosquito vectors, primarily by applying insecticides to larval habitats and educating the public. However, the control of vector populations using synthetic an insecticide has led to insecticide resistance and has negative effects on humans and the environment. Therefore, the development of eco-friendly control tools was crucial for public health [6,7]. More attention has been focused on the search for eco-friendly compounds that could serve as alternatives to synthetic insecticides. In pursuit of this objective, numerous studies on the efficacy of phytochemicals against mosquito vectors had been conducted worldwide, demonstrating their larvicidal, pupicidal, adult emergence inhibition and repellent properties [8].

Thus, phytochemicals could be utilized as alternatives to synthetic insecticides or in conjunction with other insecticides within integrated vector control programs. Consequently, research into alternative methods utilizing plants for the development of environmentally safe, biodegradable insecticides for mosquito control and encouraged. Botanical insecticides may emerge as viable alternatives to synthetics in the future, given their relative safety, ease of degradation and widespread availability in many parts of the world [9].

Annona squamosa was a small deciduous or semi-evergreen tree in the plant family *Annonaceae*, renowned for its fruit known as custard apple, 'Sharifa' in Hindi and Sitaphalam in the Telugu language, is cultivated throughout India, mainly for its edible fruit. It is a tropical fruit tree originating from Asia. The literature from several research studies demonstrates that all parts of A. squamosa have therapeutic properties [10]. This study aims to assess and compare the larvicidal activity of aqueous, ethanol and hexane extracts of *A. squamosa* against the third instars larvae of the *Anopheles annularies*.

This plant species has historically been used to cure a variety of ailments, including tumours, cancer, fever, ulcers and constipation. Additionally, it is said to have hepatoprotective, antibacterial, antimicrobial and antioxidant effects. The delayed larvae growth-regulating properties of the larval-pupal intermediate phase and partial ecdysis adults found in the roots and leaves of *A. squamosa* are well known [11,12]. This plant also attracts insects, especially *Coleopterans* and *Lepidopterans.* and dizziness are treated with leaves [13]. The fruits of Annona are tonics for maturation, expectorants, cooling, sedatives, stimulants and *Haematinics*. It is helpful in alleviating searing pain and anaemia [14].

The seeds are abortifacient and insecticidal and are useful in destroying lice in the hair. Fruit is used in making ice creams and milk beverages [13,14]. An alkaloid known as *Annonaine* is found in the bark and leaves and has been discovered to have many of these qualities [15]. The extract of A. squamosa leaf was discovered to have hypoglycemic and anti-diabetic effects [16]. A. squamosa's bark contains a bioactive *Acetogenin* that has anticancer properties [17]. Also, from leaves, flavonoids aporphine alkaloids glycoside and *Squamoline* were isolated from this plant [18-20]. In the Ayurvedic system of medicine, herbal extracts but not purified compounds have been used for centuries because many constituents with more than one mechanism of action are considered to be beneficial. It was selected for the research since there are no significant reports on the phytoconstituents of A. squamosa leaf. The goal of this study is to utilize the GC-MS technique to determine the compounds present in A. squamosa leaves, which may provide insights into how it is used in traditional medicine.

Aim and Objective of the Study

The study's goal was to evaluate the larvicidal potential of leaf extract for the management of mosquito vectors. The primary objective was to explore the potential of A. squamosa as a natural, eco-friendly larvicidal agent for controlling the population of *Anopheles annularies*, a vector for serious diseases such as vector-borne diseases, including malaria, lymphatic filiariasis, Japanese encephalitis, dengue and yellow fever". This study was aiming to use UV and FTIR to identify the active phytochemicals present in the leaf extracts, which may contribute to their larvicidal efficacy.

Scope of the study

- **Phytochemicals profiling:** The study was sought to identify the secondary metabolites in the A. squamosa

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leaf extracts that may be responsible for the larvicidal activity. Techniques such as UV-visible spectrophotometry and Fourier Transform Infrared Spectroscopy (FTIR) was employing to detect the functional groups and compounds in different solvent extracts (aqueous, ethanolic and methanolic).

- **Larvicidal activity:** The focus was on testing the effectiveness of different concentrations of A. squamosa leaf extracts (20 ppm-100 ppm) against the 4th instar larvae of *Anopheles annularies*. The study was to quantify the mortality rates and determine the most effective concentrations for larvicidal activity.
- **Comparison of extracts:** Three different solvent extracts (aqueous, ethanolic and methanolic) was compared to determine which one would have the highest larvicidal potential.
- **Ecological and practical benefits:** The use of natural plant extracts as biopesticides was offered a potential alternative to synthetic chemical insecticides, with reduced environmental harm and toxicity to non-target organisms.

Limitations of the study

Geographic limitation: The larvae collection and experimental work was restricted to the Amravati region. Results may vary in other regions with different environmental conditions and mosquito species.

Materials and Methods

Study area

The study was conducted in Shri Shivaji Science College, Amravati, Maharashtra, India. Amravati was located in the Vidarbha region. The specific location of the study was the artificial water tank in the botanical garden in the college campus. The given species *Anopheles annularies* was found in the fresh water bodies like lake, ponds, *etc.*

Preparation of *A. squamosa* leaf extract

Fresh *A. squamosa* leaves were collected. The leaves were shade-dried, powdered in a blender, sifted and stored in an air-tight bottle **(Figure 1)**.

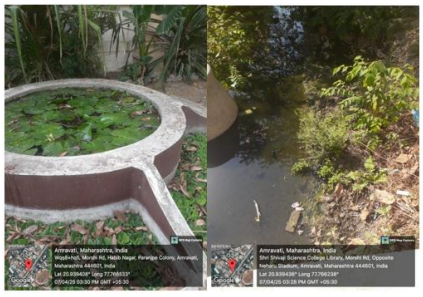


Figure 1: Fresh *A. squamosa* leaves.

Collection and maintenance of mosquitoes

Selection of mosquito species

The important vector species of mosquito borne which was *Anopheles annularies* was selected for the present study. They were responsible for transmitting the most important vector-borne diseases, including malaria, *Lymphatic filiariasis*, Japanese encephalitis, dengue and yellow fever.

Collection and culture of mosquito larvae

Anopheles annularies larvae were collected from the pond, riverbeds, pools and stream water near Amravati, the collected larvae were reared from egg to larval stage and then to adults in the laboratory itself, to avoid the species mixture next f1 generation larvae were used for the present study. This procedure facilitates to maintain the uniform age of larval instar. The 4th instars larvae were used to test the various concentrations of extracts **(Figure 2)**.

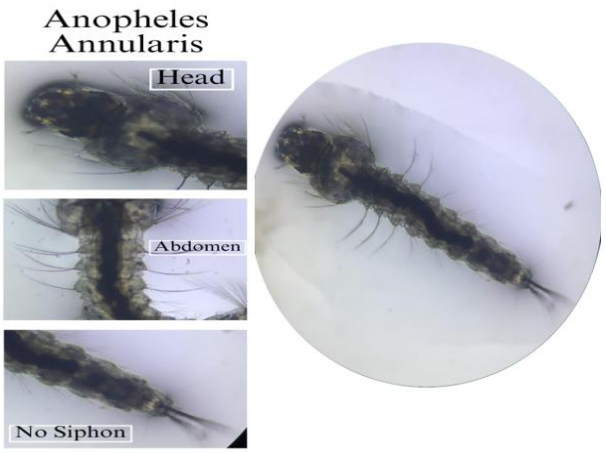


Figure 2: *Anopheles annularies* larvae.

Preparation of aqueous extract of the plant material

The dried leaves of *A. squamosa* were weighted and 100g powder was soaked in 500 ml distilled water for 24 hours and then filtered through filter paper and the aqueous extract was stored in bottles (to avoid degradation). These solutions were considered at stock (20%) from which various concentrations (20, 40, 60, 80, 100 ppm) were made with distilled water for evaluating its larvicidal potential.

Preparation of ethanolic extract of plant material

By percolation method powdered leaves of *A. squamosa* were weighted and 100 g of the leaf powder was soaked in 500ml ethanol for 24hrs. The extract was stored at room temperature. These solutions were considered at stock from which various concentrations (20, 40, 60, 80, 100

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ppm) were made with distilled water for evaluating its larvicidal activity.

Preparation of methane extract of plant material

Powdered leaves of *A. squamosa* were weighted and 100 g of the leaf powder was soaked in 500 ml Methane for 24hrs. The extract was stored at room temperature. These solutions were considered at stock from which various concentrations (20, 40, 60, 80, 100 ppm) were made with distilled water for evaluating its larvicidal activity.

Percentage of mortality=(Number of dead larvae/Number of larvae introduced) × 100.

Phytochemicals study

A phytochemical analysis was conducted to determine whether specific secondary metabolites, like tannins, flavonoids, terpenoids, cardiac glycosides and alkaloids, *etc.,* using standard protocol as defined by Trease and Evans Harborne and Sofowara.

UV spectral study

One gram of plant powder was added to 10 ml of solvent such as Methanol, ethanol and aqueous and then filtered. To identify the distinctive wavelength of the plant extract, an aliquot of the filtered material was scanned using a UV-visible spectrophotometer (Cecil Corporation, England) between 190 nm and 900 nm.

Fourier Transform Infrared Spectrophotometer (FTIR)

To identify the functional groups (Chemical bonds) present in the compounds Fourier Transform Infrared Spectrophotometer (FTIR) analysis was performed. Dried powder of the various solvent extracts was used for the analysis. FTIR studies were carried out in the Shri Shivaji Science College Amravati, India.

Bioassay

The larvicidal activity was assessed by the standard guidelines of World Health Organization, with some modifications and as per the method of Rahuman, Prabakar and Jebanesan. A laboratory colony of *Anopheles annularies* was used for the larvicidal activity studies. For bioassay test, twenty numbers of fourth instar larvae were taken in six batches for the treatment. Bowls of 100 ml capacity were kept in series and tested for each desired plant extract concentration of 20, 40, 60, 80 and 100 ppm. The control was set up with Aqueous, methanol, Ethanol and distilled water. The experimental media, in which 100% mortality rate of larvae occurred were selected for a dose-response bioassay. Based on the screening results, the numbers of dead larvae were counted after every 12hrs of treatment up to 48hrs. The percentage mortality and standard error of mean had been calculated for all the results obtained by this study **(Figure 3)**.



Figure 3: A laboratory colony of *Anopheles annularies*.

Data Analysis

The present study's data analysis evaluated the larvicidal potential of *Annona squamosa* leaf extracts (aqueous, ethanolic and methanolic) against the fourth-instar larvae of *Anopheles annularity*. The analytical approach focused on measuring mortality rates, determining Lethal Concentration (LC_{50}) values and interpreting the dose-response relationship through regression analysis.

- **Mortality rate:** Calculation larval mortality was recorded after 24hrs and 48hrs of exposure to each concentration of the extracts (20, 40, 60, 80 and 100 ppm). The percentage mortality for each treatment was calculated using the following formula:

  Percentage of mortality=(Number of dead larvae/Number of larvae introduced) × 100

- **Dose-response assessment:** A dose-response test was conducted for each extract type. The relationship between extract concentration and larval mortality was analyzed to assess the effectiveness of each extract in a concentration-dependent manner.

- **LC_{50} determination:** The LC_{50} (lethal concentration to kill 50% of the larvae) values for each extract were determined using regression analysis. Figures were plotted showing concentration (ppm) on the x-axis and percentage mortality on the y-axis. The LC_{50} was estimated from the regression equation derived from these plots.

- **Comparative evaluation:** The results of aqueous, ethanolic and methanolic extracts were compared based on their LC_{50} values and mortality percentages. This helped determine the most effective solvent extract for larvicidal activity.

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Observation and Result

Mosquitoes in the larval stage are desirable targets for pesticides because mosquitoes breed in water, which makes it simple to deal with them in this habitat. However, using traditional pesticides poses significant risks to the environment as well as human health. Vector control was facing a serious threat due to the growing resistance of mosquitoes to commonly used synthetic insecticides; better alternative means are sought. Tikar et al., had reported that the development of insecticide resistance in populations of *Aedes aegypti* indicates the need for the search for safe and effective alternative measures [21]. Natural pesticides, especially those derived from plants, were more promising in this aspect. The result presented in **Table 1** reveals the presence of various phytoconstituents of aqueous, ethanol and methanol crude leaf extract of Annona squamosa. The presence of flavonoids, terpenoids, tannins, phenols and steroids, was identified in ethanol and methanol leaf extract.

FTIR analysis of methanolic leaf extract of Annona squamosa was given in **(figure 7)**. Analysis of the figure reveals the presence of characteristics of absorbance bond of O-H stretch at 3416 cm^{-1} with the functional group representing phenols. Two bands were absorbed at 2924 and 2854cm^{-1} characteristics of C-H stretch representing alkanes. C=O saturated carbonyl groups at 1730cm^{-1}. C=C stretch at 1624cm^{-1} represents aromatic compounds. Two bands were absorbed at 1454 cm^{-1} and 1377 cm^{-1} with the functional group representing alkyl chains. Characteristics peak at 1036cm^{-1} represent amines or alcohols. Infrared rays' spectrum of ethanolic leaf extract **(figure 5)** reveals the presence of characteristics peak indicate presence of carbonyl group which strong C=O at 35587.19cm^{-1}. A medium bond C=C stretch represents aromatic was absorbed in 46082.95cm^{-1}. The presence of phenolic group was identified with the presence of O-H stretch at 40322.58cm^{-1}. The infrared spectrum of the aqueous extract of Annona squamosa shown in **(figure 6)** showed the characteristic absorption bond at 36764.71cm^{-1} with bonding of strong C=C stretch representing the aromatic possibly flavonoid or phenolic functional group.

The activity of plant extract was mostly attributed to the complex mixture of active compounds. In the primary screening, the potential larvicidal activity of the different solvent extracts of the *Annona squamosa* plant was noted. Nowadays, the control of mosquitoes at the larval stage is focused on plant extracts. The advantage of targeted mosquitoes at the larval stage was that they cannot escape from their breeding sites until the adult emergences and also reduce the overall pesticide use to control adults by aerial applications of adulticidal chemicals. Bioactive crude extract or isolated phytoconstituents from the plant could be used as an alternative to the currently used synthetic insecticides. The biological activity of plant extract might be due to the various compounds, including phenolic, terpenoids and alkaloids present in plants [22]. The present research that was done reveals the presence of phenols and terpenoids in ethanol and methanol extracts were the larvicidal activity was observed **(Figure 8)**. The **table 2** and **3** shows the finding that all the extract tested for observing the larvicidal activity showed significant effect on the larvae of *Anopheles annularies*. evaluated larvicidal activity of extract of medicinal plant *Plumbago zeylanica* and *Cestrum nocturnum* against *A. aegypti* the LC_{50} values of both the plants were less than 50 ppm [23]. The present research done results are in agreement with the previous finding as the LC_{50} values after 48hrs amount less than 50 ppm mg/L in ethanol and methanol extract and the maximum LC_{50} value was observed with aqueous extract was about 53.95 mg/L **(Figure 9)**.

The larvicidal potential of Annona squamosa leaf extracts was evaluated using methanol, ethanol and aqueous solvents at varying concentrations (20 ppm-100 ppm) and observations were recorded at 24hrs and 48hrs post-treatment. As shown in **Table 4**, at 24hrs, the methanolic extract exhibited the highest larvicidal activity, with mortality rates increasing from 25% at 20 ppm to 90% at 100 ppm. The ethanolic extract also showed significant larvicidal effects, with mortality rising from 20% to 85% across the same concentration range. The aqueous extract displayed comparatively lower efficacy, with mortality ranging from 5% at 20 ppm to 60% at 100 ppm **(Figure 10)**.

At 48hrs, a significant enhancement in larvicidal activity was observed for all extracts, as presented in **Table 5**. The methanolic extract continued to demonstrate the highest efficacy, achieving 95% mortality at 100 ppm. The ethanolic extract followed closely, with 90% mortality at the same concentration. The aqueous extract showed a noticeable increase in effectiveness over time, rising from 15% mortality at 20 ppm to 80% at 100 ppm. These results suggest a clear dose-dependent and time-dependent relationship. Among the three solvents, the methanolic extract proved to be the most effective, followed by the ethanolic and aqueous extracts, respectively.

The larvicidal efficacy of Annona squamosa leaf extracts was tested using methanol, ethanol and aqueous solvents across a concentration gradient of 20 ppm-100 ppm, with mortality rates recorded at both 24hrs and 48 hrs. As presented in **Table 4** and visualized in **Figure 11**, the methanolic extract demonstrated the highest larvicidal potential at 24hrs, with mortality increasing steadily from 25% at 20 ppm to 90% at 100 ppm. Ethanol extract also showed considerable activity, with mortality ranging from 20% at 20 ppm to 85% at 100 ppm. In comparison, the aqueous extract exhibited significantly lower mortality rates, beginning at 5% for the lowest concentration and rising to only 60% at 100 ppm. The results suggest that both methanol and ethanol serve as more efficient solvents for extracting active larvicidal compounds compared to water, with methanol leading in overall effectiveness.

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After 48hrs, as shown in **Table 5** and **figure 12**, the larvicidal activity of all extracts increased across all concentrations, confirming a time-dependent effect. The methanol extract continued to display the highest potency, reaching a peak mortality of 95% at 100 ppm and showing considerable impact even at lower concentrations (35% at 20 ppm). Ethanol extract followed a similar pattern, achieving 90% mortality at the highest concentration. The aqueous extract, though still the least effective, showed notable improvement compared to its 24-hour performance, with mortality rates rising from 15% at 20 ppm to 80% at 100 ppm. These patterns across both time intervals and concentration gradients underscore a clear dose-response relationship and highlight the increasing effectiveness of the extracts over time.

Comparative analysis of **figures 11** and **12** illustrates that the methanolic extract consistently outperformed the ethanol and aqueous extracts, both in rapid onset (24hrs) and sustained impact (48hrs). The ethanol extract, while slightly less effective than methanol, still demonstrated strong larvicidal activity. The aqueous extract, though comparatively weaker, exhibited a gradual increase in effectiveness over time. Overall, the findings strongly suggest that methanolic extracts of Annona squamosa possess the most significant larvicidal potential, making it a promising candidate for natural vector control methods. The data further reinforces the importance of solvent choice in phytochemical extraction, particularly when targeting vector-borne disease management strategies.



Figure 4: Mosquitoes in the larval stage.

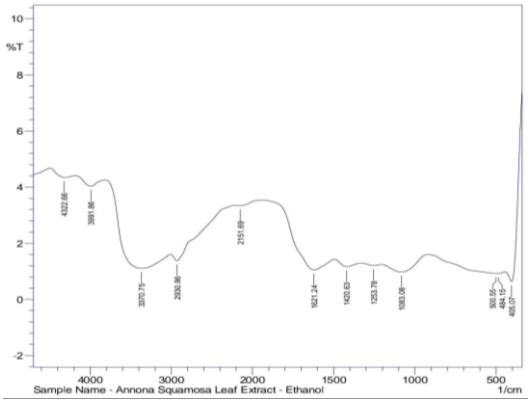


Figure 5: FTIR.

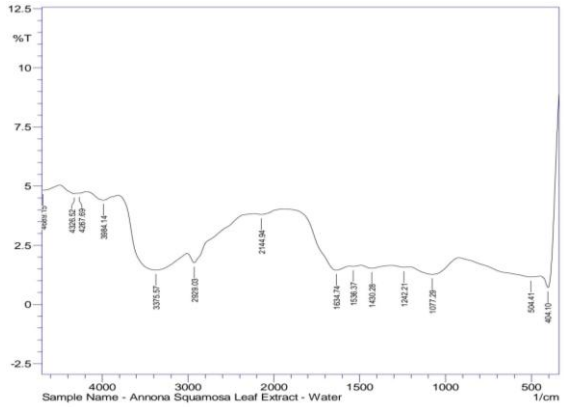


Figure 6: FTIR.

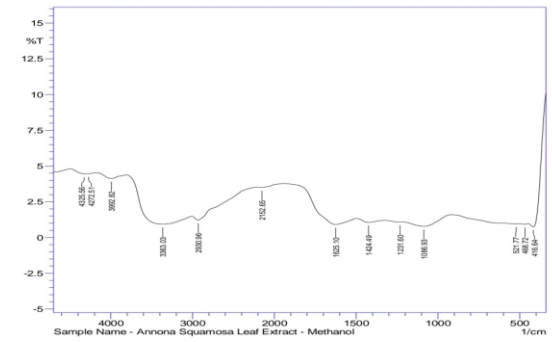


Figure 7: FTIR.

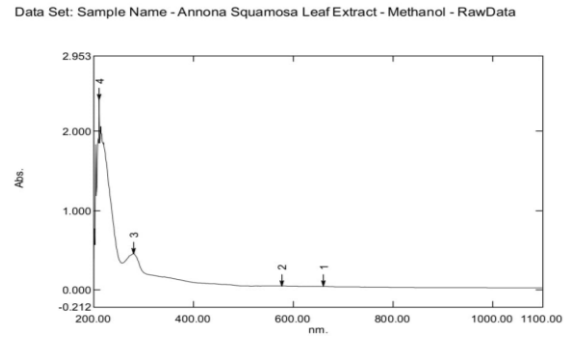


Figure 8: UV.

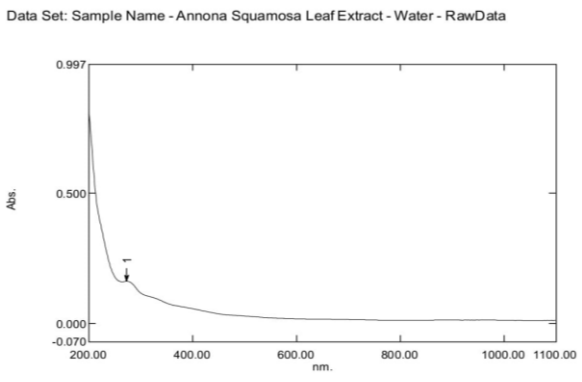


Figure 9: UV.

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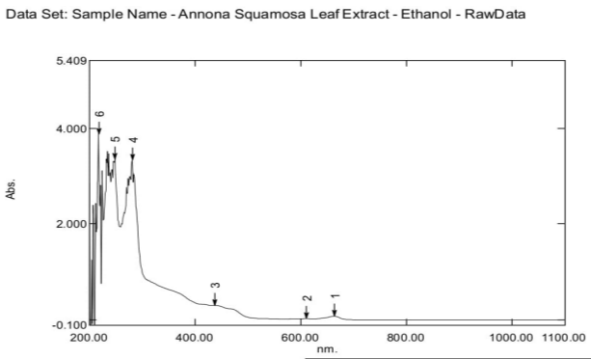


Figure 10: UV.

Table 1: Qualitative phytochemical analysis of *Annona squamosa*.

Phytoconstituents	Aqueous	Ethanol	Methanol
Flavonoids	-	+	+
Terpenoids	-	+	+
Cardiac glycosides	+	-	+
Phenol	-	+	+
Alkaloids	+	+	-
Quinones	-	+	-
Tannins	-	-	-
Steroid	-	+	-
Saponins	+	-	+

Table 2: Dose-Response larvicidal bioassay (24hrs) of different solvents of leaf extract of Annona squamosa against larvae of Anopheles annularis.

	LC50	Lower bound	Upper bound
Aqueous	80.75+2.39	78.36+1.22	83.14+3.23
Ethanol	45.75+1.88	43.87+0.96	47.63+1.97
Methanol	39.90+2.05	37.85+1.05	41.95+2.36

Table 3: Dose-response larvicidal bioassay (48hrs) of different solvents of leaf extract of Annona squamosa Against larvae of Anopheles annularis.

	LC50	Lower bound	Upper bound
Aqueous	53.95+2.12	37.262+1.78	83.14+2.23
Ethanol	34.79+1.29	43.87+0.96	47.63+1.97
Methanol	30.00+1.10	37.85+1.05	41.95+1.82

Table 4: Mortality rate at 24hrs of various extracts at 20-100 PPM.

	Methanol	Ethanol	Aqueous
20 ppm	25%	20%	5%
40 ppm	45%	40%	15%
60 ppm	60%	55%	40%
80 ppm	80%	75%	50%
100 ppm	90%	85%	60%

Table 5: Mortality rate at 48hrs of various extracts at 20-100 PPM.

	Methanol	Ethanol	Aqueous
20 ppm	35%	30%	15%
40 ppm	60%	50%	30%
60 ppm	75%	70%	50%
80 ppm	90%	85%	70%
100 ppm	95%	90%	80%

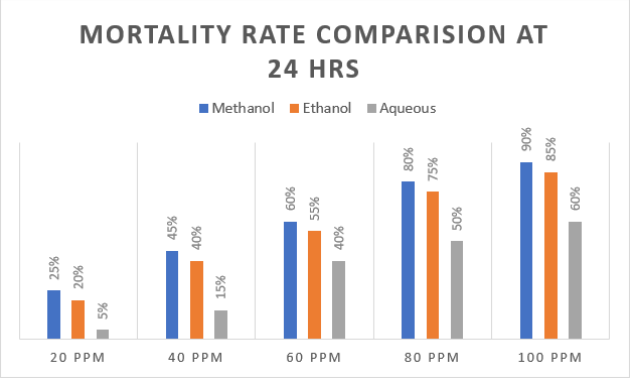


Figure 11: Mortality rate comparison of various extracts at 24hrs from 20 PPM-100 PPM.

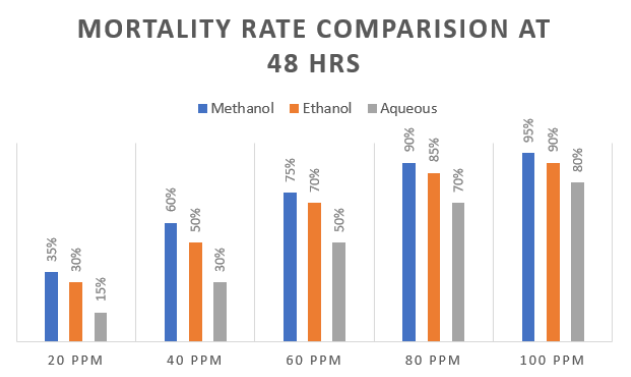


Figure 12: Mortality rate comparison of various extracts at 48hrs from 20 PPM-100 PPM.

Discussion

Annona squamosa leaf extracts' larvicidal activity in this study provides compelling evidence of the plant's potential as a natural, eco-friendly alternative to conventional synthetic insecticides. The effectiveness of the extracts, particularly the methanolic and ethanolic ones against fourth instar larvae of Anopheles suggests the presence of potent bioactive compounds capable of disrupting the larval development cycle.

Amitabh et al. researched and their findings indicate the average larval mortality rate percentage of 24hrs of exposure to the leaf extract of *A. squamosa* was recorded as 20%, 30%, 50%, 68% and 100% [12]. The results of the

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present study revealed that the ethanolic leaf extracts of the A. squamosa plant can be used effectively as a potential, eco-friendly, biodegradable and economic larvicide in an integrated mosquito control program. The present research done by us disagrees with these findings; the comparative analysis of mortality rates across different solvents and exposure durations revealed that methanol was the most effective extractant. This can be attributed to its high polarity, which enables the dissolution of a broader range of phytochemicals, including phenols, flavonoids and terpenoids. These compounds are well-documented in entomological research for their larvicidal, neurotoxic and growth-inhibitory properties. Flavonoids, for example, interfere with oxidative stress mechanisms, while terpenoids can inhibit acetylcholinesterase activity, leading to larval paralysis and death.

Bavani Govindarajulu et al. researched and used Annona reticulata to study its larvicidal efficiency [24]. The Fourier Transform Infrared Spectroscopy (FTIR) result reveals the presence of various functional groups in the extracts. The presence of phenol, aromatics and carboxylic acid was seen in the methanol extract by them. The presence of alkanes, aromatic compounds and aliphatic groups was seen in the ethanolic group extract. Then, the presence of alcoholic or phenolic compounds and alkyl or aromatic compounds was seen in the aqueous extract by them. The present study, done by us, agrees with these findings. Phytochemical screening confirmed the presence of these active secondary metabolites, particularly in methanolic and ethanolic extracts. FTIR analysis further validated the chemical nature of these constituents, highlighting functional groups such as hydroxyl (-OH), carbonyl (C=O) and aromatic C=C bonds, which are frequently associated with insecticidal activity.

Govindarajan et al. observed similar UV-Vis absorption profiles in methanolic extracts of Azadirachta indica, with maximum absorbance around 270 nm-300 nm, correlating with potent larvicidal effects [25]. The resemblance of spectral patterns suggests a shared mechanism of action through oxidative damage pathways and disruption of metabolic functions in larvae, the UV-visible spectroscopy findings, which indicated strong absorbance peaks in relevant spectral ranges, complement these observations by reinforcing the presence of light-absorbing phytochemicals that may participate in larval toxicity through photoactive mechanisms or oxidative pathways.

The results demonstrated a clear dose- and time-dependent larvicidal effect. Mortality rates increased progressively with higher concentrations and longer exposure, suggesting a cumulative toxic effect on the larval population. At 100 ppm concentration and 48-hour exposure, methanolic extracts achieved a 95% mortality rate, while the aqueous extract, though less effective, still caused up to 80% mortality. This gradient of efficacy among solvents underlines the critical role of the extraction method in maximizing bioactivity. Furthermore, the LC_{50} values obtained particularly 30.00 mg/L for methanol and 34.79 mg/L for ethanol fall well within the threshold considered effective for botanical larvicides, as per WHO guidelines.

These findings align with previous studies, such as those by Nayak and Govindarajan et al. which also reported significant larvicidal efficacy of Annona species extracts [25,26]. Interestingly, the phytochemical profiles and larvicidal effects observed in this study not only confirm earlier observations but also highlight A. squamosa as an underutilized botanical resource with strong potential for public health applications. The presence of saponins and alkaloids in the methanolic extract is particularly notable, as these compounds are known to cause cytolysis, interfere with neurotransmission and impair nutrient absorption in insect larvae.

Another crucial aspect of this study is its implication for sustainable vector management. With growing resistance to pyrethroids organophosphates and other synthetic insecticides, plant-based alternatives like A. squamosa offer a promising solution. They are biodegradable, relatively safe for non-target organisms and culturally acceptable in many communities. Moreover, the use of locally available plants could empower community-based vector control initiatives, especially in endemic regions like Amravati.

However, while the laboratory results are promising, the transition to practical application requires further research. The exact mode of action of the phytochemicals must be elucidated through bioactive compound isolation and characterization. Additionally, field studies are necessary to test efficacy under natural environmental conditions, including variables like water quality, sunlight exposure and larval density. The potential toxicity of these extracts to aquatic organisms, human handlers and beneficial insects must also be evaluated before large-scale implementation.

Govindarajan et al. has reported that the methanol extract of *Cassia fistula* exhibited LC50 value of 17.97 and 20.57mg/L against *Anopheles stephensi* and *Aedes aegypti*, respectively [25]. It had been observed from the research done presently that the ethanol and methanol extracts of *Annona squamosa* the LC50 value was about 17.97 mg/L and 6.91 mg/L with reference to ethanol and methanol respectively.

The variation in the larvicidal activity of the extract could be due to the presence of secondary metabolites constituents of the extract. No death was observed with control. Larval mortality was noted after the treatment of the extract of Annona squamosa, after 24hrs exposure, LC_{50} value was 45.75, 39.90 and 80.75 mg/L with reference to ethanol, methanol and aqueous extract respectively.

48hrs after the exposure to the extract the LC_{50} values of Annona squamosa leaf extract were impressive as the LC50 values of aqueous ethanol and methanol were 53.95, 34.79 and 30.

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Nayak reported the larvicidal activity of *A. reticulata* leaf crude extract at different concentration shows 100 % mortality rate of larvae was observed at 5, 10, 25, 50, 100 and 200 ppm concentrations of crude extract [26]. Present result is in agreement with these findings as nearly 100% mortality was observed after 48hrs of treatment. Identifying plant-based insecticides that are efficient as well as suitable and adaptive to local ecological conditions, biodegradable and have the widespread insecticidal property will obviously work as a new weapon in the arsenal of insecticide and in the future may act as a suitable alternative product to fight against mosquito-borne diseases [22]. The biological activity of plant extracts might be due to various compounds, including phenolics, terpenoids and alkaloids presenting plants. The research done presently shows the finding that the study aligns with previous report, showing that the presence of phytoconstituents such trapezoids, phenols, alkaloids and saponins were evident in all the extract of the plant potentially influencing the response of the tested organism. The larvicidal activity depends on the presence of several bioactive chemicals in different parts of the plant. The presence of cytotoxic compounds saponins was observed and the presence of saponin along with other phytoconstituents may be the reason for 100% mortality observed, with reference to the extract of the tested plant. current research reveals that the extract of leaves of Annona squamosa possesses exceptional larvicidal activity against anopheles annuaries.

In conclusion, the findings of this study reinforce the larvicidal potential of Annona squamosa, particularly when extracted with methanol and ethanol. The phytochemical-rich profile and significant mortality rates suggest that the plant could be developed into an effective biopesticide. Its integration into mosquito management programs could enhance the sustainability and ecological safety of vector control strategies, particularly in tropical and subtropical regions where mosquito-borne diseases remain a persistent threat.

Conclusion

The present investigation establishes the larvicidal efficacy of *Annona squamosa* leaf extracts against the fourth instar larvae of *Anopheles annularies*, a prominent vector of several vector-borne diseases including malaria, lymphatic filariasis, dengue and Japanese encephalitis. The study utilized aqueous, ethanolic and methanolic extracts to evaluate larvicidal activity through standardized bioassay protocols. Among the three solvent extracts, the methanolic extract demonstrated the highest larvicidal potency, followed by the ethanolic extract, while the aqueous extract exhibited comparatively lower activity.

Phytochemical analysis confirmed the presence of bioactive secondary metabolites such as flavonoids, phenols, terpenoids, alkaloids and saponins, which are likely contributors to the observed larvicidal effects. The findings were further supported by UV-visible spectroscopy and FTIR analysis, which revealed key functional groups associated with larvicidal properties.

The results of the bioassay indicated significant larval mortality, with LC_{50} values for methanolic and ethanolic extracts falling well below 50 mg/L after 48hrs, underscoring the strong larvicidal potential of *A. squamosa*. These findings corroborate previous research and highlight the plant's promise as a viable, eco-friendly alternative to synthetic insecticides. Given the increasing resistance of mosquito populations to conventional chemical insecticides and the environmental concerns associated with their usage, the use of plant-based larvicides such as *A. squamosa* offers a sustainable and environmentally compatible vector control strategy.

Nevertheless, further research is warranted to elucidate the precise mode of action of the active compounds, assess their long-term ecological impacts and explore their effectiveness under field conditions. The development of standardized formulations and integration into existing vector control programs could greatly enhance the applicability of such botanical insecticides in public health efforts.

References

1. WHO (2006) Pesticides and their application for the control of vectors and pests of public health importance. WHO Pesticides Evaluation Scheme. [Google Scholar]

2. Mahadev PV, Fulmali PV, Mishra AC (2004) A preliminary study of multilevel Geographic distribution of prevalence of *Aedes aegypti* (Diptera: Culicidae) in the state of Goa. Ind J Med Res 120: 173-182. [Google Scholar] [Indexed]

3. Indian Council of Medical Research (2003) Prospects using herbal products in the control of mosquito vectors. ICMR Bull 33: 1-12. [Google Scholar]

4. Goddard J (2007) Physician's guide to arthropods of medical importance (5th edtn). CRC Press: 480.

5. Corbel V, Duchon S, Zaim M, Hougard JM, Dinotefuran (2004) A potential neonicotinoid insecticide against resistant mosquitoes. J Med Entomol 41: 712-717. [Crossref] [Google Scholar] [Indexed]

6. Gratz NG (2004) Critical review of the vector status of *Aedes albopictus*. Medical and Vertinary Entomology 18: 215-227. [Crossref] [Google Scholar] [Indexed]

7. Nasir S, Nasir I, Iqra Y (2017) Medicinal plant extracts: Control strategies against dengue mosquitoes. Bangladesh J Bot 46: 27-33. [Google Scholar]

8. Ghosh, Nandita C, Goutam C (2012) Plant extracts as potential mosquito larvicides. Indian J Med Res 135: 581-598. [Google Scholar] [Indexed]

9. Sivagnaname S, Kalyanasundaram M (2004) Laboratory evaluation of methanolic extract of *Atlanta monophylla* (Family: Rutaceae) against immature stage of mosquitoes and

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non-target organisms. Mem Inst Oswaldo Cruz 99: 115-118. [Crossref] [Google Scholar] [Indexed]

10. Durai PV, Ayyanar M, Ignacimuthu S (2006) Antimicrobial activity of some ethno medicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complement Altern Med 6: 35. [Crossref] [Google Scholar] [Indexed]

11. Babu PBS, Rao JM (1996) Evaluation of some plant extracts as feeding deterrents against adult *Longitarsus nigripennis* (Coleoptera: Chrysomelidae). Entomon 21: 29-1294. [Google Scholar]

12. Shad A, Andrew J (2017) Larvicidal efficacy of ethanolic extracts of the plant *Annona Squamosa (Annonaceae)* over the filarial vector *Culex quinquefasciatus* say (Culicidae). Journal of Entomology and Zoology Studies 5: 373-377. [Google Scholar]

13. Bhattacharya A, Chakraverty R (2016) The pharmacological properties of *Annona squamosa* Linn: A review. Int J Pharm Eng 4: 692-699. [Google Scholar]

14. Vanitha VKJ, Uma Devi KV (2011) Determination of bioactive components of *Annona squamosa L* leaf by GC-MS analysis. Int J Pharm Sci Drug Res 3: 309-312. [Google Scholar]

15. Vohora S, Kumar I, Naqvi S (1975) Phytochemical, pharmacological, antibacterial and anti-ovulatory studies on *Annona squamosa*. Planta Med 28: 97-100. [Google Scholar] [Indexed]

16. Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, et al. (2005) Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa L.* in experimental animals. J Ethno Pharmacol 99: 75-81. [Crossref] [Google Scholar] [Indexed]

17. Hopp DC, Zeng L, Gu Z, Kozlowski JF, McLaughlin JL (1997) Novel mono-tetrahydrofuran ring *Acetogenins*, from the bark of *Annona squamosa*, showing cytotoxic selectivities for the human pancreatic carcinoma cell line, PACA-2. J Nat Prod 60: 581-586. [Crossref] [Google Scholar] [Indexed]

18. Seetharaman TR (1986) Flavonoids from the leaves of *Annona squamosa* and *Polyalthialongifolia*. Fitoterapia 57: 189-198. [Google Scholar]

19. Bhakuni DS, Tewari S, Dhar MM (1972) Aporphine alkaloids of *Annona squamosa*. Phytochemistry 11: 1819-1822. [Crossref] [Google Scholar]

20. Forgacs P, Desconclois JF, Provost J, Tiberghien R, Touché A (1980) A new nitro-glycoside extracted from *Annona squamosa*. Phyto-Chemistry 19: 1251-1252.

21. Tikar SN, Mendki MJ, Chandel K, Parashar BD, Prakash S (2008) Susceptibility of immature stages of *Aedes aegypti*: The vector of dengue and Chikungunya to insecticides from India. Parasitol Res 102: 907913. [Crossref] [Google Scholar]

22. Joseph JM, Innocent E, Otieno JN (2009) Mosquito larvicidal and cytotoxic activities of 3 Annona species and isolation of active principle. Journal of Medicinal Plants Research 3: 674-680. [Google Scholar]

23. Patil CD, Patil SV, Salunke BK, Salunkhe RB (2011) Bioefficacy of *Plumbago zeylanica (Plumbaginaceae)* and *Cestrum nocturnum (Solanaceae)* plant extracts against *Aedes aegypti (Diptera: Culicidae)* and *Poecilia reticulata*. Parasitol Res 108: 12531263. [Crossref] [Google Scholar] [Indexed]

24. Govindarajulu B, Srimathi A, Bhuvana R, Karthikeyan J (2015) Mosquito larvicidal efficacy of the leaf extracts of *Annona reticulata* against *Aedes aegypti*. [Google Scholar]

25. Govindarajan M, Jebanesan A, Pushpanathan T (2008) Larvicidal and ovicidal activity of *Cassia fistula Linn.* Leaf extract against filarial and malarial vector mosquitoes. Parasitol Res 102: 28992. [Crossref] [Google Scholar] [Indexed]

26. Nayak JB, Sunil R, Ramkishan G (2015) Larvicidal efficacy of *Annona squamosa* plant extracts against Culex *Quinquefasciatus* mosquito larvae.

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