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Larvicidal efficacy of *Stachys byzantina* extracts against *Aedes aegypti*: A potential alternative to synthetic insecticides

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Abstract
Mosquito-borne diseases are a significant global health challenge, with a substantial burden in regions like India, where environmental factors and urbanization exacerbate the risk of such diseases. Among the myriad mosquito species, *Aedes aegypti* is a prominent vector responsible for transmitting dengue fever, a disease with serious health implications. Conventional mosquito control strategies, including chemical insecticides, are fraught with issues such as resistance development, environmental impact, and high costs. As a response, there is a growing interest in exploring plant-based alternatives for mosquito management.
This study focuses on the larvicidal potential of *Stachys byzantina*, a plant from the *Lamiaceae* family known for its medicinal properties. The study assessed the effectiveness of methanol, ethanol, chloroform, acetone, and hexane extracts of S. byzantina at multiple concentrations. The results revealed that the methanol extract exhibited the highest larvicidal activity, achieving a mortality rate of 85.71% at the highest concentration (400 ppm) and demonstrating the lowest LC50 (124.63 ppm) and LC90 (408.52 ppm) values among the solvents tested. Ethanol also showed considerable efficacy, though slightly less than methanol. Chloroform and acetone extracts displayed moderate activity, while hexane was the least effective. These findings suggest that S. byzantina possesses potent bioactive compounds with significant larvicidal properties, highlighting its potential as an effective and sustainable alternative to synthetic insecticides.

Keywords: Mosquito-borne diseases, *Aedes aegypti*, *Stachys byzantina*, larvicidal activity, botanical insecticides, phytochemicals, mosquito control, vector management, methanol extract, dengue fever

Introduction

Almost 80% of the world's population is at risk of contracting mosquito-borne diseases. Mosquito-borne diseases, particularly those transmitted by mosquitoes, are the largest contributors to the burden of vector-borne diseases. These diseases can have severe impacts on public health and can lead to significant mortality and morbidity, especially in regions where they are endemic (Franklinos, *et al*., 2019) [6]. India faces significant challenges with mosquito-borne diseases due to its climate, rapid urbanization, infrastructure development, and mosquitoes' adaptability. There are currently 58 families in the genus *Anopheles*, 111 *Aedes* species, and 57 *Culex* species known to exist in India (Nagpal and Sharma, 1995) [11].

Mosquitoes, classified into 41 genera and around 3,500 species within the Culicidae family, play a crucial role in spreading these viruses (Xia, *et al*., 2018) [14]. Zika, dengue, yellow fever, chikungunya, and Japanese encephalitis, are primarily transmitted by *Aedes* species such as *Aedes albopictus* and *Aedes aegypti* (Li, *et al*., 2023) [8]. *Aedes* mosquitoes are vectors for various diseases, including dengue, yellow fever, Zika virus, and chikungunya virus. These mosquitoes can spread germs through bites, transmitting potentially deadly pathogens to humans and other animals. *Aedes aegypti*, in particular, is responsible for spreading dengue fever, which is a global health threat (Becker, *et al*., 2010) [3].

While various mosquito control strategies—physical, chemical, and biological—have been developed globally, their implementation in India is limited by high costs and potential environmental hazards. Among these strategies, physical control is noted for its effectiveness and minimal environmental impact. It can be applied at both public and individual levels by reducing breeding sites and using mosquito traps or electrocuters. Another effective method is using larvivorous fish, especially in water bodies like ponds and tanks. Though cost-effective, this approach may impact local aquatic fauna. Biological control using bacteria is useful but costly, and resistance can develop. Genetically modified mosquitoes have been tested but are expensive, target specific species, and may pose environmental risks. Chemical control should be a last resort due to its environmental and health hazards (Dey, *et al*., 2023) [4].

Plant-based phytochemicals with mosquito control potential can be employed instead of synthetic insecticides or in conjunction with other pesticides as part of an integrated vector control program (Dharmagadda *et al*., 2005) [5]. The hunt for plant-based phytochemicals with repellent properties against vector mosquitoes has been fuelled primarily by the fact that some of the most commonly used chemical repellents, such as DEET and DDT, have significant resistance and toxicity issues. Phytochemical investigations show that traditional medicinal herbs contain chemicals with therapeutic effects. Phytochemicals from diverse plant species are harmless, biodegradable, and may be tested for insecticidal and mosquito-repellent properties, as well as mammalian toxicity (Mittal 2003 [10]; Macêdo *et al*. 1997 [9]).

Woundwort (*Stachys*) plants from the *Lamiaceae* family (Jassbi, *et al*., 2014) [7]. They are traditionally used in Anatolian medicine for a range of ailments, including coughs, infections, tumors, ulcers, inflammatory conditions, and wounds. The main volatile compounds identified in the extracts of *S. byzantina* were sesquiterpenes, followed by monoterpenes and secondary compounds. Germacrene D was the most abundant compound. They have significant antioxidant properties and antimicrobial activity, particularly against Gram-positive bacteria (Stegăruș, *et al*., 2021) [12].

Materials & methods

Initially, fresh leaves of *Stachys byzantina* were gathered and thoroughly rinsed under running tap water to remove any surface dirt. This was followed by a final rinse with distilled water to ensure complete cleanliness. After washing, for 15 days, the leaves were left to air-dry in a shaded area to prevent degradation of their active components from direct sunlight. Once the leaves were completely dry, they were ground into a fine powder using an electric blender. The powdered leaves were then stored in a clean, dry container to maintain their quality until further use.

Stock solutions of 1000 ppm were prepared by dissolving 1 gram of the extract with 10 ml of respective solvent. Later by made it to 1000 ml by adding 990 ml of distilled water. Test solutions of 25 ppm, 50 ppm, 100 ppm, 200 ppm, and 400 ppm were prepared by serial dilution method. Control solutions for each test extract were prepared by mixing 10 ml of respective solvent in 990 ml of distilled water.

Eggs of *Aedes aegypti* were collected from Osmania University by placing egg traps. The collected eggs were reared in the laboratory. Early 4^{th} instar larvae (Figure No. 1) were used in the larvicidal bioassays. The World Health Organization's (2005) [13] guidelines were followed in conducting larvicidal bioassays. 20 healthy and same-sized larvae of early 4th instar were taken into each of the 250 ml plastic bowls filled with 100 ml of distilled water. Test solutions of different concentrations were added to test cups 1 ml each separately to each of the test bowls. For each concentration, five replicates were conducted simultaneously, with a total of 100 larvae. The control cup was also maintained without adding any test solutions. Test cups were observed for larval mortalities for two days at intervals of 12 hours. In addition to observing and recording symptoms, no food was offered to the larvae after treatment. A larva was considered dead if, after 48 h, it showed no swimming movements, even when gently touched with a glass rod. Each concentration's mortality rate was calculated by combining the dead larvae in five replicates. These bioassay tests were performed at 25 ± 5 C and 60 - 70% relative humidity. For synergistic effects studies, extracts collected by using different solvents were used in different combinations to prepare test solutions.

The obtained results of the larvicidal bioassays were analyzed using Microsoft Excel (Office 2007). The mortality rates were corrected using Abbott's (1925) [1] formula. Data were subjected to one-way ANOVA with Tukey's honest significant difference (HSD) $P < 0.05$. Probit analysis was carried out to determine the LC50 and LC90 values of the test compounds.

Results & discussion

Table No. 1 and Figure No. 1 present the dose-response relationship between the concentrations of different solvent extracts of *S. byzantina* leaves and the mortality of *Ae. aegypti* larvae. The dose-response relationship clearly shows an increase in larval mortality with higher concentrations across all extracts, but distinct differences in efficacy are observed.

Comparatively, the methanol extract exhibited the highest larvicidal activity, with a mortality rate of 29.59% at the lowest concentration (25 ppm) and reaching 85.71% at the highest concentration (400 ppm). The LC50 and LC90 values, calculated at 124.63 ppm and 408.52 ppm, respectively, indicate that methanol is the most potent solvent, requiring significantly lower concentrations to achieve 50% and 90% mortality. This suggests that methanol is highly effective at extracting potent bioactive compounds from S. byzantina leaves, leading to enhanced larvicidal activity.

Ethanol, though slightly less effective than methanol, also demonstrated strong larvicidal properties. The ethanol extract caused 21.43% mortality at 25 ppm and increased to 77.55% at 400 ppm, with LC50 and LC90 values of 185.69 ppm and 471.61 ppm, respectively. While not as potent as methanol, ethanol's lower LC50 and LC90 values compared to other solvents indicate its effectiveness in extracting bioactive compounds, making it a promising alternative.

The chloroform extract exhibited a comparable mortality trend to ethanol, starting at 18.37% mortality at 25 ppm and reaching 71.43% at 400 ppm. The LC50 and LC90 values of 222.31 ppm and 522.39 ppm reveal that chloroform is less effective than ethanol and methanol but still demonstrates significant larvicidal activity. This suggests that chloroform can extract moderate levels of larvicidal compounds, though at higher concentrations than methanol and ethanol.

Acetone extract showed a similar pattern to chloroform, with mortality rates starting at 18.56% at 25 ppm and peaking at 72.16% at 400 ppm. The LC50 and LC90 values were slightly lower at 218.60 ppm and 515.78 ppm, respectively. Although the acetone extract demonstrated moderate larvicidal activity, it was not as effective as methanol or ethanol, suggesting that acetone may extract fewer active compounds.

The hexane extract displayed the least efficacy among the tested solvents, with the lowest initial mortality of 17.35% at 25 ppm and reaching a maximum of 69.39% at 400 ppm. Its LC50 and LC90 values were the highest, at 235.91 ppm and 540.79 ppm, respectively, indicating that much higher concentrations were required to achieve the same mortality rates observed with the other solvents. This implies that hexane extracts fewer or less potent bioactive compounds from S. byzantina.

S. byzantina plant extracts are reported to possess a wide variety of bioactive secondary metabolites and exert antioxidant and anti-microbial effects (Stegăruș, *et al*., 2021) [12]. In a previous study, Asnaashari, *et al*. (2010) [2] reported on the insecticidal properties of fractions from the methanol extract of *S. byzantina*. The fraction eluted with 20% methanol in water exhibited the highest mortality rate, which increased with concentration. At 20 mg/mL, the mortality rates for this fraction were 26.6%, 30.0%, 30.0%, and 33.3% after 4, 8, 24, and 48 hours, respectively. In light of the previous studies and the results of the present study, it is clear that the extracts of *S. byzantina* have secondary metabolites that can be used in the control of the larvae of Ae. aegypti.

Conclusion

The present study demonstrates that *S. byzantina* leaf extracts possess significant larvicidal activity against *Ae. aegypti* larvae, with methanol extract showing the highest potency. Methanol and ethanol extracts were effective in causing larval mortality at lower concentrations, indicating their ability to extract bioactive compounds with strong larvicidal properties. The results align with previous studies on the insecticidal properties of *S. byzantina* and suggest that the plant could serve as a promising botanical resource for developing environmentally friendly mosquito control strategies. Further research is recommended to explore the mechanisms of action, optimize extraction methods, and assess the field applicability of these extracts in vector control programs.

Fig 1: Larvae and Pupae of *Aedes aegypti* mosquito

Table 1: Results of the larvicidal bioassays using different solvent extracts of *Stachys byzantina* leaves against the 4th instar larvae of *Aedes aegypti*

Conc.	*S. byzantina* extracts				
	Hexane	**Acetone**	**Chloroform**	**Ethanol**	**Methanol**
25 ppm	17.34 ± 2.04	18.56 ± 2.04	18.37 ± 1.84	21.43 ± 2.35	29.59 ± 2.42
50 ppm	25.51 ± 2.35	27.84 ± 2.05	27.55 ± 2.04	31.63 ± 2.35	39.80 ± 3.00
100 ppm	36.73 ± 1.79	38.14 ± 1.84	37.76 ± 1.58	42.86 ± 2.09	51.02 ± 2.39
200 ppm	47.96 ± 2.42	50.52 ± 2.01	50.00 ± 2.04	55.10 ± 2.46	65.31 ± 3.21
400 ppm	69.389 ± 3.04	72.16 ± 2.35	71.43 ± 2.01	77.55 ± 2.21	85.71 ± 2.93
LC 50	222.31	218.60	222.31	185.69	124.63
LC 90	522.39	515.78	522.39	471.61	408.52
Regression Equation	y = 0.1333x + 20.366	y = 0.1346x + 20.576	y = 0.1333x + 20.366	y = 0.1399x + 24.022	y = 0.1409x + 32.44
R^2 Value	0.9654	0.9654	0.9654	0.9597	0.9524

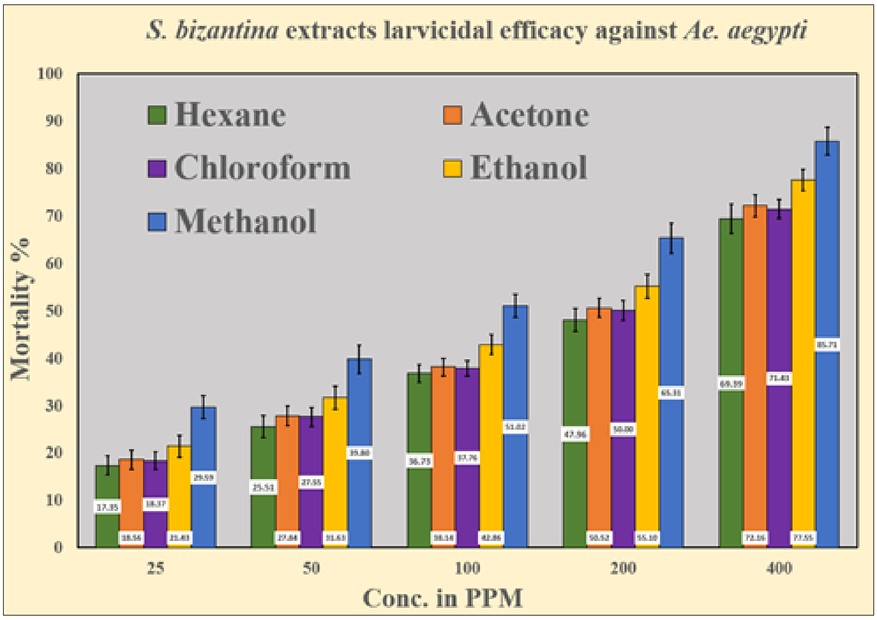

Fig 2: Graphical representation of the results of the larvicidal bioassays using different solvent extracts of *Stachys byzantina* leaves against the 4th instar larvae of *Aedes aegypti*

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