https://doi.org/ 10.33472/AFJBS.6.8.2024.52-61

African Journal of Biological Sciences

Larvicidal activity of *Byttneria herbacea* Roxb leaf extract against insect-borne mosquitoes *Aedes aegypti* and *Anopheles stephensi*

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Article History
Volume 6,Issue 8, 2024
Received:17 Feb 2024
Accepted : 28 Mar 2024
doi: 10.33472/AFJBS.6.8.2024.52-61

Abstract

Vector-borne infections remain to be a main contributor to disease and loss worldwide. The emergence of tolerances to synthetic pesticides and the resulting risks have long been viewed as an obstacle in the control of mosquito vectors. Botanicals have a wide variety of phyto-compounds that allow them to efficiently manage and prevent diseases transmitted by vectors by eradicating eggs of insects and larvae. This current study was aimed to evaluate the toxicity of methanolic and ethanolic extracts of *Byttneria herbacea* on the two types mosquito larvae (*Aedes aegypti* and *Anopheles stephensi*). The larvae's mortality was reported after being exposed for 24 hours. Our study findings demonstrate that the leaf extract of *B. herbacea* has significant larvicidal effect, with a rate of 93.1 ± 0.8 percent in *A. stephensi* and 83.061 ± 0.29 percent in *A. aegypti*. In addition, the ethanolic extract caused a decrease in protein levels after 24 hours of exposure to 4th instar larvae. The LC_{50} values for *A. stephensi* were 72.3 ppm and 81.2 ppm for *A. stephensi*, while the LC_{90} values were 144.7 ppm and 110.4 ppm for *A. stephensi* and *A. aegypti*, respectively. The data unequivocally demonstrate that the active extract from *Byttneria herbacea* can be employed as an effective method for controlling pests and vectors, hence aiding in the prevention of the transmission of infectious diseases.

Keywords: *Byttneria herbacea*, Larvicidal activity, Mosquito, *Aedes aegypti* and *Anopheles stephensi*

INTRODUCTION

Mosquitoes serve as the primary carriers of Carried by vectors illnesses and significantly pay to the overall prevalence of diseases in India. The propagation of diseases can be halted by implementing diverse ways to regulate the vectors. Nevertheless, the excessive and disproportionate utilization of chemical pesticides has resulted in issues such as the amplification of mosquito population resistance to synthetic insecticides, environmental contamination, and harmful impacts on the non-target plant and animal species residing in the same aquatic environment. The increasing challenges necessitate a thorough exploration for novel items that are ecologically friendly, specifically targeted, and capable of degradation. Mosquito-borne diseases continue to pose significant public health challenges in Asian countries due to their tropical or subtropical climates, inadequate drainage systems, particularly during rainy seasons, and the abundance of mosquito breeding grounds such as fish ponds, irrigation ditches, and rice fields. Millions of rupees are being used on acquiring personal protective measures such as mosquito coils, vaporizing mats, or liquids to avert insect bites. These control measures have a temporary effect on the adult population alone. Hence, a more effective approach to manage the mosquito population is to specifically focus on the larvae. Synthetic mosquito control programs have a negative impact on the surroundings by polluting soil, water, air, and by causing harmful effects on non-target creatures, including humans. Plants provide as an economical source of bioactive chemicals. Botanical larvicides are efficacious against target species, environmentally safe, readily biodegradable, and cost-effective. Hence, it is necessary to conduct a study in order to extract a larvicidal component derived from plants.

Every year, a substantial number of individuals are infected with insect-borne illnesses, resulting in noteworthy social and economic consequences. Mosquito-borne infections are a significant health issue in numerous countries. The use of chemical has resulted in several ecological issues, including the emergence of insect strains that are resistant to these chemicals, disruption of ecological balance, and harm to mammals. Therefore, it is crucial to continuously create plant materials with biological activity as larvicides. These materials are anticipated to decrease the risks to humans and other creatures by decreasing the buildup of residues in the environment. The majority of mosquito control efforts focus on eradicating the larval stage of mosquitoes in their breeding grounds with larvicides, as adulticides only provide temporary reduction of the adult population (Chilakam *et al.,* 2023). Mosquito-borne infections remain a major cause of human mortality worldwide, affecting around 700 million individuals each year (Onen *et al.,* 2023). Vector-borne illnesses transmitted by mosquitoes exert an economic impact by causing a decline in commercial and labor productivity, especially in nations characterized by tropical and subtropical climates. Nevertheless, vector-borne illnesses are present in every corner of the world (Chilakam *et al*., 2023). Reports indicate that *C. quinquefasciatus* annually infects approximately 100 million individuals globally (Lupenza *et al.,* 2021), with 44 million people suffering from typical chronic symptoms (Bernhard et al., 2003). Chemical vector control programs have been implemented for a significant period of time. However, mosquito-borne diseases persist due to households declining to

undergo house spraying with artificial insecticides, changes in the biting patterns of mosquitoes (Tabashnik, 1994). Researchers in countries with a strong herbal tradition have become increasingly interested in finding new control agents for insects. They have focused on natural products, particularly plant secondary metabolites. The main study is to identify a novel candidature for larvicidal activity against *A. aegypti* and *A. stephensi* from *B.herbacea*.

Materials and Methods

The leaves of *Byttneria herbacea* are collected and cleaned using tap water, followed by a surface sterilization procedure with a 10% solution of sodium hypochloride. This is done to effectively to hinder the presence of any microorganisms and prevent contamination. A crude extract of the plant material is made utilizing several solvent systems such as ethanol, methanol, chloroform, acetone, and distilled water, using the Soxhlet apparatus. The solvents are volatilized and the desiccated extracts will be preserved at 4°C until subsequent analysis.

Preparation of Extract

Methanol, acetone, chloroform, ethanol, and distilled water extracted the powder for 24 hours. The supernatant was put into a china dish after 24 hours. By holding the china dish over a boiling water bath at 40° C, the supernatant was eliminated. Following the complete removal of alcohol/solvent, a semi-solid extract was produced. The resulting residue was diluted in solvents to a predetermined volume (25ml) for phytochemical analysis.

Preliminary Phytochemical

The preliminary phyto-chemicals like tannins, saponins, flavonoids, steroids, terpenoids, total phenols, alkaloids, anthraquinones, cardiac glycosides and coumarins were performed on the various extracts using standard procedures, as reported by Sofowara (1993), Trease and Evans (1989), and Harborne (1989).

Larvicidal assay

The larvicidal activity was assessed using the World Health Organization's (WHO) standard technique outlined in their 2005 guidelines. The larvae will be nourished with Brewer's yeast and dog biscuit in a ratio of 1:3. The larvae in the early 4th instar stage will be utilized for the larvicidal assay. Aqueous extract of *Byttneria herbacea* leaves was dissolved in 1 ml of methanol and subsequently diluted with double distilled water to get the specified concentrations. For each concentration, 25 young larvae of the fourth instar were placed into 100 ml beakers. The experiment was conducted at a temperature of 26-28°C, with three replicates for the control group. The larvae perished after being exposed for 12 and 24 hours. There was a lack of food offered to the larvae throughout the two exposure periods, and the proportion of deaths was determined for each period (Veni *et al.*, 2017). The average larval mortality was calculated using the chi-square test using LC_{50} and LC_{90}.

DPPH Radical Scavenging Assay

The activity of DPPH in scavenging radicals has been evaluated utilizing the method developed by Baliyan *et al.* (2010). 0.1 mL of the solvent extracts was transferred to separate test tubes, and methanol was used to increase the volume in each test tube to 100 µL. Three milliliters of a solution containing 0.1 millimoles per liter of Methanol has been added to each test tube, and the mixture was forcefully agitated a left to stand for 20 minutes. The absorbance of the solutions was determined at a wavelength of 517nm using a Shimadzu UV-2550 spectrophotometer. A solution of ascorbic acid at a concentration of 0.1mg/mL was employed as a control for the test.

RESULT AND DISCUSSION

Preliminary Phytochemical Analysis

Preliminary phytochemical screening is crucial in determining the functional phyto chemical elements present in the crude plant extract. The data help decide on an extraction technique and perform specific assays or estimations. The finding of qualitative preliminary chemical tests on five different extracts of *Byttneria herbacea* was performed. The key notion gained from qualitative phytochemical analysis of *B. herbacea* is the kind of primary and secondary metabolites present in the leaves of *B. herbacea.*

The results were obtained from qualitative preliminary phyto-chemical tests done to assess several phyto-constituents in various extracts of *B. herbacea* (Table 1). Secondary metabolites in *B. herbacea* were evaluated qualitatively in extracts (ethanol, methanol, water, chloroform, acetone, and distilled water). The preliminary phytochemicals such as tannin, saponin, flavonoids, terpenoids, phenols, anthraquinone, and coumarin were conformed to their presence in all five extracts. Among the alkaloids was found in only acetone and distilled water extracts. Glycosides were to confirm their presence in ethanol, methanol, and aqueous extract, but in chloroform and acetone extracts were not done their presence flavonoids. The steroids were conformed in almost all the extract except the aqueous extract of *B. herbacea*. Secondary nitrogenous compounds, such as alkaloids, have been used for ages in folk medicine as an anti-oxidant (Tiong *et al.,* 2013). Antioxidative stress-related issues have been used as a preventative agent (Gonzalez-Burgos and Gomez-Serranollos, 2012). Tumor cell death is facilitated by saponin, an antioxidant found in nature that acts as a growth inhibitor (Podolak *et al.*, 2010). Quercetin monoglycosides, and flavonol glycosides are powerful anti-lipid peroxidants (Plumb *et al.*, 1999).

Table 1: Initial analysis of the chemical components of *B. herbacea*

S.No.	Phytochemical constituents	Name of the Extracts				
		Ethanol	**Methanol**	**Chloroform**	**Acetone**	**Distilled Water**
1.	Tannins	+	+	+	+	+
2.	Saponins	+	+	+	+	+

3.	Flavonoids	+	+	+	+	+
4.	Steroids	+	+	+	+	-
5.	Terpenoids	+	+	+	+	+
6.	Phenol	+	+	+	+	+
7.	Alkaloid	-	-	-	+	+
8.	Anthraquinones	+	+	+	+	-
9.	Coumarin	+	+	+	+	+
10.	Glycoside	+	+	-	-	+

The symbol (+) denotes presence, (-) means the absence of phytoconstituents.

Larvicidal Activity

Quantification of the lethal concentration (LC_{50}) and concentration at which 90% mortality occurs (LC_{90}) the mortalities, expressed as percentages, were recorded for each concentration after 30 minutes. These data were then used to create a log-probit graph using Polo-plus 2.0 software from LeOra Software Company, located in Petaluma, CA. Specifically, the total number of individuals and the total number that died in each bottle were analyzed by considering the aggregate of the four treated bottles per assay. The value for control mortality was inputted into Polo-plus as the sum of the five replicates for each concentration. The parameters used for analyzing the data files in Polo-plus were as follows: a probit model was employed, the natural response was considered, and the concentrations were transformed to logarithms. The LC_{50} and LC_{90} values were subsequently determined, along with a 95% confidence interval indicating the upper and lower bounds. A study was done to investigate the effectiveness of methanolic extract of *B.herbacea* leaves in killing larvae. The findings from Table-2 demonstrated that the extracts exhibited efficacy against 4^{th} instar larvae following 12 and 24 hours of exposure. The methanol extract exhibited a larvicidal activity of 84.3 on 0.5ml and an average LC_{90} of 161.1 against *A. stephensi* after 12 hours of exposure. Additionally, the activity was 198.2 on 0.5ml with an average LC_{90} of 246.2 after 12 hours of revelation. The LC_{50} values after 24 hours of exposure time were 72.3 ppm against *A. stephensi* and 81.2 ppm against *A. aegypti*. The LC_{90} values were 144.7 ppm against *A. stephensi* and 110.4 ppm against *A. aegypti*. The larvicidal activity of the plant *B.herbacea* was quantified as 93.1% at a volume of 0.5ml. The larvicidal activity of the leaf extract was substantially higher when tested against *A.aegypti*. Due to the ability of vectors to acquire resistance to commonly used pesticides, vector management has emerged as a significant concern. These molecules range from simple compounds like phenolic acids, phenyl propanoids, and flavonoids to more complex substances such as lignins, melanins, and tannins. Flavanoids are the predominant and extensively

spread subgroups. The correlation between increased antioxidant activity and a high concentration of total phenol compounds content has been thoroughly examined. The antioxidant capability of phenolics is primarily determined by their structures, specifically the presence of hydrogen-donating hydroxyl groups. Phenolics with a higher number of hydroxyl groups exhibit a larger antioxidant capacity (Al-Mamary *et al.,* 2012).

Because of the ability of vectors to develop resistance to commonly used insecticides, vector management is one of the most pressing problems (Veni *et al*., 2017). The larvicidal activity of leaf extracts was investigated, and the results revealed that the extracts possessed effective larvicidal properties. After 12 hours of exposure, A. *stephensi* and *A.aegypti* were combined to form LC_{50} and LC_{90} concentrations. The findings revealed that the extract of *B. herbacea was* more effective against the larvae of the 4th instar. The larvicidal activity of the plant *B. herbacea* extract was expressed as 93.1 percent at 0.5mL concentration. In their study, Raj *et al*. (2014) discovered that *Nigella sativa* leaf extract had larvicidal activity against *A. aegypti* and *A. stephensi* in their *A.aegypti*. The larvicidal activity of the leaves of *Eclipta prostrata* was found to be the most effective against the 4th instar larvae of *C. quinquefasciatus* and *A. subpictus* (Bhuvaneswari *et al*., 2016; Alois *et al*., 2022). This demonstrates that the methanol extract of *B. herbacea* can suppress the rested larvae. Using chemical agents in sources is harmful to the environment and humans. So plant-based pesticides are the potential candidates, especially for mosquito larvae management.

Table-2: Larvicidal activity of *B.herbacea* leaf methanol extract against *A. stephensi* and *A. aegypti* reared larvae.

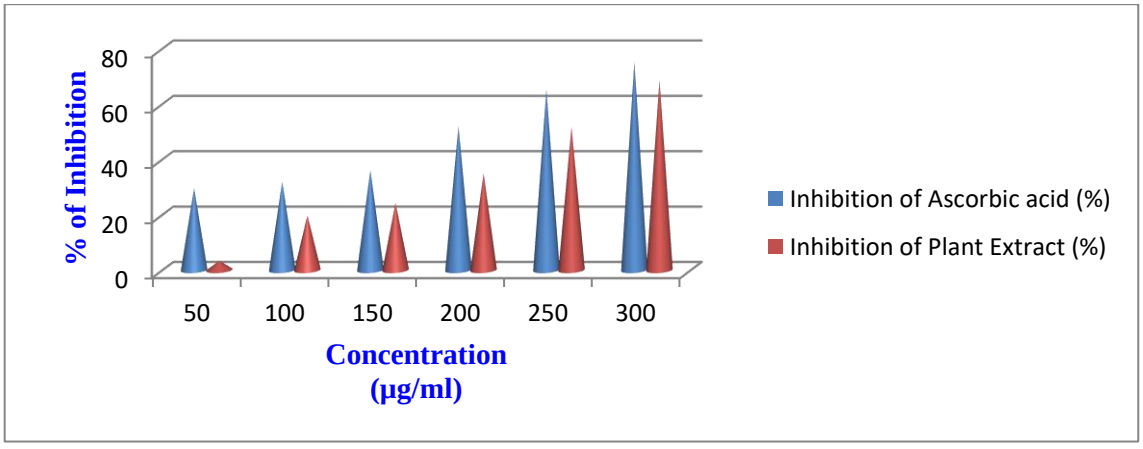
Mosquito	**Period of (h)**	**Concentration (**mL/5ml**)**	**Percentage of Mortality ± SE**	**LC_{50} (LCL-UCL)[a]**	**LC_{90} (LCL-UCL)[a]**	**$v^2(d=4)$[b]**
Anopheles stephensi	12	Control	0±0	85.1 (71.2—121.3)	161.1 (142.8-180.4)	7.6
		0.1	16.1±0.12^{a}			
		0.2	33.4±0.32^{ac}			
		0.3	45.4±0.32^{bc}			
		0.4	59.2±0.31^{a}			
		0.5	84.3±0.46^{ec}			
	24	Control	0±0	72.3 (59.1—86.2)	144.7 (114.0—140.2)	1.22
		0.1	22.0±0.78^{ac}			
		0.2	40.2±0.33^{b}			
		0.3	55.9±0.15^{e}			
		0.4	82.1±0.64^{d}			
		0.5	93.1±0.81^{c}			
Aedes aegypti	12	Control	0±0	198.2 (68.5—	246.2 (96.1—	4.17
		0.1	12.4±0.19^{bc}			

		0.2	23.1±0.75[ab]	93.7)	136.4)	
		0.3	37.1±0.65 [cd]			
		0.4	51.1±0.33 [b]			
		0.5	71.4 ±0.51 [a]			
	24	Control	0±0	81.2 (61.5—86.4)	110.4 (79.1—136.7)	3.9
		0.1	16.0±0.11 [a]			
		0.2	28.2±0.15 [b]			
		0.3	43.0±0.18 [c]			
		0.4	59.2±0.14 [e]			
		0.5	83.0±0.61[ab]			

DPPH Radical Scavenging Assay:

The DPPH radicals scavenging capacity was assessed by measuring the reduction in absorption of the DPPH free radicals at a wavelength of 517 nm. The study conducted an analysis of the hydrogen peroxide radical scavenging capabilities of the *B. herbacea* extract. The DPPH radical scavenging capabilities of the extracts rose gradually in a dose concentration-dependent way (50-300µg/ml), which is noteworthy. A variation in antioxidant activities ranged from 3.36 to 68.61%, the inhibition rate of *B. herbacea* extract was 68.61%, and ascorbic acid was 75.31% at the concentration of 300µg/ml, respectively. A lower IC_{50} value represents a higher free radical scavenging activity. The plant extract showed 50% inhibition (IC_{50}) at 177.13µg/ml and ascorbic acid, which was used as a standard antioxidant compound, exhibited an IC_{50} value at 154.224 µg/ml. The DPPH inhibition in *B. herbacea* was 3.36, 19.82, 24.34, 35.04, 51.80 and 68.61%of different concentration in 50, 100, 150, 200, 250, 300µg/ml respectively. The free radical scavenging assay, usually referred to as the DPPH assay, is a straightforward, rapid, and highly thoughtful technique for evaluating the antioxidant capacity of a particular substance or plant-based extract (Koleva *et al.*, 2002). These results are in obeyed with previous findings in other plants reported by a number of workers Wong-Paz *et al.*, 2015; Zahin *et al.*, 2009; and Ashafa *et al.*, 2010.)

Graph 1: DPPH Radicals Scavenging Activity of *B. herbacea* Plant Extract



Conclusion

Plants are an essential component of modern medicine, and as compared to current pharmaceuticals, plant medicines have a reduced likelihood of causing negative side effects. The analysis revealed that the extracts of *B. herbacea* included many preliminary phytochemicals, including tannins, saponins, flavonoids, steroids, terpenoids, phenols, alkaloids, anthraquinones, coumarins and glycosides. The study on the larvicidal efficiency of *B. herbacea in vitro* demonstrates that the use of a biological pathway for producing larvicidal products on a wide scale is an environmentally benign, though time-consuming and cost-effective, alternative to chemical processes. In conclusion, the strong larvicidal activity and antioxidant capabilities of the methanolic extract of *Byttneria herbacea* can be attributed to the presence of substantial levels of secondary phyto-compounds. Additional purification and characterisation of the active compound must be conducted.

Acknowledgement:

The Author is grateful to the Management of the The New College (Autonomous), Chennai, India, for providing financial support (as Seed Grand under CMRI) to carry out this research work.

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