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Original Article

Evaluation of bioactivity of aqueous extracts of *Bougainvillea spectabilis*, *Saraca asoca*, and *Chenopodium album* against immature forms of *Aedes aegypti*

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ABSTRACT

Background: Increasing rate of vector transmission of dengue has led to the exponential rise in the mortality and morbidity scales in the past five years. There are various natural and synthetic chemical agents available commercially as potent larvicides, but they are either highly toxic, difficult to obtain, have a high manufacturing cost, or show cross-resistance, hence do not suffice as ideal larvicides. The objective was to screen aqueous extracts of *Bougainvillea spectabilis* (*B. spectabilis*), *Saraca asoca* (*S.asoca*), and *Chenopodium album* (*C. album*) for larvicidal activity against *Aedes aegypti* (*A. aegypti*).

Methods: The larvae were exposed to increasing concentrations of aqueous extracts of *B. spectabilis*, *S. asoca*, and *C. album* under controlled laboratory environment. The mortality was checked after 24 hours, results were statistically analyzed, and LC50 and LC90 were determined.

Results: *B. spectabilis* and *S. asoca* were found effective as larvicides against *A. aegypti* with LC50 values of 0.22% and 0.26%, respectively.

Conclusion: The aqueous extracts of *B. spectabilis* and *S. asoca* are efficient larvicides against *A. aegypti* and can be used as effective, accessible, and eco-friendly control options for management of *A. aegypti*, the vector of dengue/chikungunya.

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Introduction

The past decade has seen an exponential rise in the incidence of mosquito-borne diseases, especially in the tropical and subtropical regions of the world, despite prime advancements

in the health-care sector.¹ With rise in urbanization and subsequent growth in population density, poor sanitation, easy availability of breeding sites, and lack of awareness among general population, *Aedes aegypti* (*A. aegypti*) poses itself as the most dangerous disease-carrying vector because of

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its breeding pattern, day-biting behavior, and global presence. The dengue vector, *A. aegypti*, is the primary vector for several tropical and subtropical diseases such as yellow fever, dengue fever, dengue hemorrhagic fever, Zika fever, and Chikungunya. Approximately 3.9 billion people, forming two-third of the world's population in tropical and subtropical countries, are at risk of these diseases. Infection rate during epidemics often reaches up to 80–90% with evidence of cocirculation of multiple serotypes. In 2016, a total of 129,166 dengue cases were reported in India, leading to 245 deaths, which respectively increased in number to 15722 and 250 in 2017. The varied clinical presentation in the form of fever associated with arthralgia is often misinterpreted by the patients and ignored, causing a rise in the subsequent morbidity and mortality. The absence of a vaccine and definitive cure against the diseases transmitted by *A. aegypti* has enforced vector management to be the only viable strategy to alleviate morbidity and mortality due to *Aedes*-borne illnesses. *A. aegypti* is a highly domesticated, strongly anthropophilic, nervous feeder and a discordant species. The extensive use of larvicide (Temefos; 1 ppm) as an effective means of vector abatement is likely to render it ineffective due to development of resistance. It is thus necessary to find alternatives to insecticides.^{2,3} Although plant extracts are promising tools for vector management, accessibility of plants, local availability, and correct botanical identification before extraction pose major issues and hence limit their evaluation as potent larvicides. Three commonly occurring plants in India, viz. *Bougainvillea spectabilis* (flowering plant), *Saraca asoca* (Sita Ashok), and *Chenopodium album* (Bathua), easily identified by their characteristic inflorescence and leaf pattern were screened for their larvicidal potential.

Material and methods

Rearing of *A. aegypti* in laboratory

The laboratory evaluation was undertaken in the entomology laboratory of a government medical college over a period of six months (April 2014–September 2014). Bioassay of the plant extracts was tested against third to early fourth larval instar of laboratory-reared *A. aegypti*. The adults were fed on glucose solution, along with which intermittent blood feed was provided to the female adults. The eggs were collected on Whatman filter paper strips lining the water-filled enamel bowl and were transferred to the enamel trays for hatching. The hatched larvae were provided yeast tablets as food. The pupae were collected and kept in the muslin cloth cages for the emergence of adults. Bioassay was performed as per the standard method of WHO under laboratory conditions at a temperature of 27–32 °C and relative humidity of 60–80%.

Investigation of larvicidal potential

The fresh plant leaves after cleaning with distilled water were oven-dried at 150 °C for 30 min and were ground to fine powder in an electric grinder. A 10% stock solution was prepared and was left for 24 h. Thereafter, it was filtered through Whatman filter paper no. 1, and the filtrate was stored. Twenty, 3rd to

early 4th instar larvae of laboratory-reared *A. aegypti* were placed in test containers (plastic cups) with holding capacity of 200 mL of dechlorinated tap water. A total of 60 third to early fourth instar *A. aegypti* larvae (3 replicates) were exposed to the respective plant extracts initially at a screening concentration of 1%. Concurrent positive control with Temefos at the recommended concentration of 1 ppm and negative controls with normal tap water were set up. A total of 40 larvae (2 replicates) of negative and positive control were set up for each treatment. After screening, fresh lots of mosquito larvae were exposed to six increasing concentrations of the plant extracts, i.e., 0.05%, 0.1%, 0.2%, 0.4%, 0.6%, and 0.75% with similar laboratory setup and procedure. All the experiments were repeated three times. Larval mortality was monitored after 24 h by checking for active and passive movements of the larvae. During each bioassay, control and experimental, monitoring for larval motility was performed carefully for behavioral modifications. The observations were focused on the wriggling speed of larvae, aggregation behavior at corners, horizontal and vertical movements, and larval knockdown.

Statistical analysis

The tests recording 20% mortality in control assays, and over 20% pupal formation were rejected and assays were re-conducted. The control mortality that ranged between 5% and 20% was corrected by Abbott's formula.¹⁴ The mortality data were used for computation of lethal concentration (LC) of the plant extracts (LC₅₀ and LC₉₀ values) and confidence intervals for the same. One-way analysis of variance was carried out to determine the significant difference in the insecticidal property of the screened plant extracts and the positive control Temefos. A probit analysis was undertaken for computation of LC₅₀ and LC₉₀ values.

Results

The potential larvicidal properties of the three plants were investigated under laboratory conditions against larval forms of *A. aegypti*. The preliminary screening of plant extracts at 1% was undertaken to determine larvicidal properties of the three candidate plants considered in the study, i.e., *B. spectabilis*, *S. asoca*, and *C. album*. The result revealed only 31% mortality at the screening concentration of 1% in case of *C. album* and hence was not subjected to further evaluation. The larval mortality with *B. spectabilis* and *S. asoca* at 1% was found to be 100%; based on these results, further evaluations were undertaken. The results of the further assays with the aqueous extract at different concentrations are presented in Fig. 1. Cent percent mortality was recorded in both *B. spectabilis* and *S. asoca* at 0.75% aqueous extract, with a minimum of 5% and 20% larval mortality achieved at 0.05% extract concentration, respectively. The larval mortality with *B. spectabilis* and *S. asoca* at the test concentrations reflected a proportional rise of mortality with the concentration of the aqueous extract. The LC values of the two test plants show similar potency to *B. spectabilis* and *S. asoca* with LC₅₀ values of 0.26% and 0.22%, respectively (Table 1). No alteration of behavior was noticed during immediate exposure of the larvae to the extract. The

Fig. 1 – Percentage mortality of *Aedes aegypti* larvae against aqueous extracts of *Bougainvillea spectabilis* and *Saraca asoca*. The sequential mortality rates of larval forms of *Aedes aegypti* on exposure to increasing concentration of aqueous extracts of *Bougainvillea spectabilis* and *Saraca asoca*.

observation showed that there was no instant or rapid mortality. The results showed the noteworthy ability of the aqueous extracts of *B. spectabilis* and *S. asoca* as the prospective control and management agent of *A. aegypti*.

Discussion and conclusion

There is a constant burden on the health-care setups to manage cases of dengue that show an exponential rise during

Table 1 – LC values of test plants.

Values	<i>Bougainvillea spectabilis</i>	<i>Saraca asoca</i>
LC ₅₀ (95% CI)	0.26% (0.16–0.36)	0.22% (0.11–0.32)
LC ₉₀ (95% CI)	0.59% (0.47–0.77)	0.54% (0.43–0.73)

CI, confidence interval; LC, lethal concentration.

outbreaks in the postmonsoon season. Owing to nonavailability of a vaccine and a definitive treatment, primary prevention in the form of vector management remains to be the gold standard approach. The conventional use of chemical insecticides has its own side effects on the environment and human health. The use of aqueous extracts of plants is relatively an easy and quick method of screening a large number of plants for their larvicidal potential with nil adverse effects. The plants chosen in the study are commonly grown for ornamental purposes in the country and are well documented for their bioactivity. The candidate plants, viz. *B. spectabilis* and *S. asoca*, are perennial plant varieties and almost available year round at low costs. Phytochemical properties of the two plants show the presence of certain phytochemicals such as saponins, glycosides, steroids, anthraquinones, and volatile oil in *S. asoca*,^{4,5} whereas alkaloids, flavonoids, phlobatannins, and terpenoids were found as major constituents in case of *B. spectabilis*.⁶

Similar studies have been conducted in the west as well as southeast Asian region with wild as well as native plants with different extraction solvents, viz. ethanol, petroleum, and chloroform.^{7,8} The fact that the plants screened so far (Table 2) are not readily available tilts the balance in favor of *B. spectabilis* and *S. asoca* as plants with promising larvicidal potential.^{9–13} The efficacy of plant extracts as an efficient control tool for the management of *Aedes*-borne diseases affecting millions with minimum cost and effort and most importantly without any deleterious effect on the environment is a well-established fact; however, the availability of local plant flora and their identification coupled with techniques for evaluation adversely affect the exploitation of this effective option for vector control. A public health approach using these plants in the form of aqueous extracts along with personal protective measures is the key to develop an efficient, affordable, and reliable measure for limiting vector transmission. The need of the hour is to continue screening of plants as a safer alternative to toxic synthetic insecticides, which in turn can help address the issue of insecticidal resistance and also alleviate human suffering due to the diseases transmitted by *A. aegypti*.

Table 2 – Lethality of plant extracts against larvae of *Aedes aegypti*.

Mosquito	Plants	LC ₅₀ values	References
<i>A. aegypti</i>	<i>Ocimum sanctum</i>	0.0062%	Kelm & Nair (1998) ¹²
<i>A. aegypti</i>	<i>Eucalyptus camaldulensis</i>	0.0014%	Jantan et al. (2005) ¹²
<i>A. aegypti</i>	<i>Magnolia salicifolia</i>	0.001%	Kekm et al. (1997) ¹²
<i>A. aegypti</i>	<i>Triphyophyllum peltatum</i>	0.00005%	Francois et al. (1996) ¹²
<i>A. aegypti</i>	<i>Microcos paniculata</i>	0.00021%	Bandara et al. (2000) ¹²
<i>A. aegypti</i>	<i>Vitex trifolia</i>	0.00047%	Kannathasan et al. (2011) ¹²
<i>A. aegypti</i>	<i>Abutilon indicum</i>	0.001149%	Ghosh et al. (2012) ¹⁰
<i>A. aegypti</i>	<i>Piper longum</i>	0.000025%	Lee (2000) ¹²
<i>A. aegypti</i>	<i>Solanum villosum</i>	0.074%	Chowdhury (2008) ⁷
<i>A. aegypti</i>	<i>Solanum nigrum</i>	0.036%	Raghavendra K (2009) ⁸
<i>A. aegypti</i>	<i>Tradescantia zebrina</i>	7.59%	Iannacone J (2004) ⁹
<i>A. aegypti</i>	<i>Paullinia Clavigera</i>	1.19%	Iannacone J (2004) ⁹

LC, lethal concentration.

Conflicts of interest

The authors have none to declare.

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