Original Research

Evaluation of larvicidal activity of *Bakuchi* (*Psoralea corylifolia* Linn.) *beeja* extracts against *Aedes aegypti* larvae

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ABSTRACT

Mosquitoes transmit numerous diseases such as malaria, chikungunya, dengue fever, and yellow fever. Indiscriminate use of synthetic insecticides to eradicate mosquitoes nowadays steered to diminution in physiological resistance, which has led to investigate a safe and environment friendly alternative. In the current investigation, *bakuchi* (*Psoralea corylifolia* Linn.), an ayurvedic drug having *krimighna* (act against worms) action, was evaluated for larvicidal bioefficacy against *Aedes aegypti* larvae. Hence, the study was attempted to evaluate the larvicidal bioefficacy of petroleum ether, ethanol, and aqueous extracts of *bakuchi beeja* against *Aedes aegypti* larvae. Petroleum ether, ethanol, and aqueous extracts of *bakuchi* were collected by soxhlet extraction and cold maceration, respectively. The extracts were evaluated for the larvicidal activity. The larvicidal bioassay was performed by following WHO (2005) guidelines with slight modification and the mortality was recorded after 24 h. The obtained mortality data were subjected to log-probit analysis to determine the median lethal concentrations—LC_{50} and LC_{90}. Aqueous extract exhibited LC_{50} at 699.915 ppm, LC_{90} at 971.635 ppm; ethanolic extract exhibited LC_{50} at 66.71 ppm, LC_{90} at 127.80 ppm; and petroleum ether extract exhibited LC_{50} at 25.8 ppm and LC_{90} at 61.0 ppm against late third and early fourth instar *Aedes aegypti* larvae. From the results obtained, it was revealed that among the three extracts of *bakuchi*, petroleum ether extract exhibited higher mortality at a lower dose when compared to ethanol and aqueous extract. Hence, the study reasonably concludes that *bakuchi* carry potential as a mosquito larvicide and could be explored for the development of safer larvicide.

Keywords: *Aedes aegypti, bakuchi* (*Psoralea corylifolia Linn*.), larvicidal activity

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Introduction

Mosquitoes, an important group of insects in terms of public health importance, belong to the family Culicidae. They are the most redoubtable vectors, which transmit parasites and pathogens that continued to have devastating impact on humans (1, 2). They play a predominant role in the transmission of vector-borne diseases such as, malaria, dengue fever, chikungunya, yellow fever, filariasis, and several other diseases affecting millions of people in tropical and subtropical areas (3, 4). These diseases not only cause high levels of mortality and morbidity, but also impose great social disruption and economic loss in developing countries (5).

Aedes aegypti transmits the overwhelming diseases such as dengue, yellow fever, chikungunya and causes burden to the country and worldwide too (6). Development of vaccine against these diseases is still at an early stage and therefore the only method available for reducing incidence of the disease is the control of its mosquito vector (7). Controlling of these vectors has been attempted for long time using synthetic chemicals. Nevertheless, the chemicals in turn are causing environmental pollution and developing

resistance against the existing chemicals. Hence, these glitches have emphasized the prerequisite for the development of novel, effective, affordable, biodegradable, and selective mosquito control agents (8).

In Ayurvedic classics, we have ample references for the drugs, which have *krimighna* (act against worms) action (9, 10). *Bakuchi* (*Psoralea corylifolia* Linn.) has *krimighna* property (11). *Bakuchi*, commonly known as *Bavchi*, is widely distributed in the tropical and subtropical areas. The useful part of this annual erect herb is seeds, which has properties such as *Kushthaghnas* (anti-dermatosis), *Keshya* (improves quality of hair), *Krimighna*, *Pramehaghna* (anti diabetic) (12). Very few studies have conducted to assess the larvicidal activity of *Bakuchi*. One of the studies stated that the volatile oils of *Bakuchi* showed appreciable results in the larvicidal activity against *Culex Quinquefasciatus* (13). Therefore, the current study was performed using three extracts of *Bakuchi*—aqueous, ethanol and petroleum ether to evaluate larvicidal activity against the later instars of *Aedes aegypti* larvae.

Material and methods

Plant collection

The seeds required for the preparation of *Bakuchi* extract were procured from Agriculture College, Arabhavi and underwent authentication. The procured seeds were washed, shade dried, and later stored in airtight containers.

Preparation of extract

The extraction was prepared using two methods:

1. Continuous hot soxheletion using ethanol and petroleum ether as solvents individually.
2. Cold maceration using water as a solvent with chloroform in small quantities (2–5 mL) to each container as a preservative.

Continuous hot soxheletion

Coarsely powdered seeds (20 g) of *Bakuchi* were weighed and packed in a thick filter paper and placed inside the thimble. This was loaded into a Soxhlet extractor and extracted with solvents—petroleum ether and ethanol. The extraction solvent was taken in a round-bottom flask refixed with a condenser. The process was carried out until the solvent was colorless. For petroleum ether extracts, the temperature was maintained at 25°C; initial color was brown and after 22 cycles, it became colorless in the siphon. For ethanol extraction, the temperature was maintained at 30°C, initial color was brown and after 20 cycles, it became colorless. The extracts were separated from the residues by filtering through Whatman No. 1 filter paper. The insoluble portion of the extracted solid remained in the thimble, which was not further used. The excess solvent from the extracts was removed by drying on water bath and the dried crude extract was collected and weighed. The extracts were stored in desiccators and further used for larvicidal assay.

Cold maceration

The powdered drug (50 g) was added to 1000 mL of water and placed on an automatic shaker for 6 h. The solution was then kept standing still for 18 h and filtered with Whatman No. 1 filter paper. The obtained filtrate was heated on a water bath to get the extract. The extract was stored and further used for larvicidal assay.

Phytochemical screening

The extracts of *Bakuchi* seeds underwent qualitative phytochemical screening for the identification of several active chemical constituents. In addition, quantitative analysis was also performed to detect the inorganic elements.

Rearing and maintenance of Aedes aegypti colony

Mosquitoes were reared in the insectary of National Institute of Malaria Research, Bangalore. Adult *Aedes*

aegypti was cultured and fed with 10 % sucrose syrup solution soaked in cotton balls in a portable mosquito net cage (0.5 m × 0.5 m × 0.5 m). The female mosquitoes were periodically fed on blood meal. After feeding, ovitrap. a 400mL capacity bowl half-filled with water and lined with a 3" wide strip of filter paper, was placed in the cage to lay their eggs. Those eggs were placed in an enamel tray containing water and allowed to hatch to obtain the larvae. Newly hatched larvae were taken to white enamel trays with breeding source water (16 × 9 × 2 inches) maintained at 26 ± 2°C temperature and 12-h light/dark regime. Dead larvae and associated debris were removed timely and the larvae were transferred to clean trays containing water. Later, minimal larval food comprising powdered yeast was supplemented. The pupae were routinely collected and transferred to the net cage, so that no adults emerge in the larval rearing containers. They took 12–24 h to develop into adults.

Larvicidal bioassay

Larvicidal efficacy of ethanol, petroleum ether, and aqueous extracts of *Bakuchi* seed extracts was tested against late third or early fourth instar stages of *Aedes aegypti* larva, employing standard WHO (2005) guidelines. Experiments were conducted at 27±2°C, 12 h light/dark regime. Batches of 25 larvae were exposed to a known concentration of test solution (1 mL of dimethyl sulphoxide (DMSO) dissolved test extract in 249-mL tap water in bowls of 500 mL capacity). Five replicates sets were tested with a final tally of 125 larvae for each concentration, the fifth replicate served as the negative control (1 mL of DMSO and 249 mL of tap water).

Preparation of dose

For preparing a dose of 100 ppm of ethanol extracts, 100 mg was weighed accurately in the beaker and 4 mL of DMSO was added and mixed with a pipette to get a uniform solution. This solution without any precipitate was used for dosing. Similarly, other doses of ethanol and petroleum ether extracts were prepared. For Aqueous extract, tap water was used to make it soluble, no DMSO was used.

Dose

Solutions containing 1 mL of DMSO in 249 mL of tap water without plant extract in 500-mL bowls served as the control. The other four replicates were treated with solutions containing 4 mL of DMSO dissolved test solution in 996 ml of tap water, which served as the treated groups. No larval feed was provided to the larvae during the test period. Mortality and survival were recorded after 24 h.

Observations after 24 h

Dead: No movement, not responsive to a gentle prodding with a fine needle

Moribund: Not having the normal larval movement

Pupae: Comma shaped

Alive: Having a normal larval movement

The moribund and dead larvae in replicates were combined and expressed as percentage mortality. Like this, for various ppm, the dosing was done to find the least and highest mortality of each extracts, and the observations were noted carefully.

Statistical analysis

The data obtained was subjected to regression analysis of probit mortality on log dosage using SPSS Inc, Version 17.0 Statistical Software for log-probit analysis, which provided the LC50 and LD90.

Results

Phytochemical screening

The phytochemical constituents present in the aqueous, ethanol and petroleum ether extracts were shown in Table

1. The inorganic elements present in *Bakuchi* is given in Table 2.

Larvicidal bioassay

The results of larvicidal bioefficacy of aqueous, ethanol, petroleum ether extracts of *Bakuchi* against third and fourth instar larvae of *Aedes aegypti* are summarized in Tables 3–5. The larvicidal bioefficacy of aqueous, ethanol and petroleum ether extracts of *Bakuchi* showed 100 % of mortality with 1100 %, 125 % and 75 % concentrations, respectively. Among the three extracts, petroleum ether extract exhibited higher mortality at a lower dose.

The results obtained were subjected to log-probit analysis to attain the LC_{50} and, LC_{90} of the extracts. LC_{50} and, LC_{90} of petroleum ether was 25.8 and 61.0 ppm, respectively. LC_{50} and, LC_{90} of ethanol extract was 66.71 and 127.80 ppm, respectively. LC_{50} and, LC_{90} of aqueous extract was 699.91 and 971.63 ppm, respectively. The attained results shown the LC_{50} of petroleum ether was less than the LC_{50} of ethanol. (Table 6).

Discussion

Insecticides of botanic origin have been stated beneficial for selective control of mosquitoes. In the current study, a significant concentration-dependent larvicidal activity of *Bakuchi* seed extracts was demonstrated on third and early fourth instar larvae of *Aedes aegypti*. Among the three extracts (petroleum ether, ethanol, and aqueous extract) used, petroleum ether exhibited best results against the mosquito larva. Furthermore, the extractive value is more in ethanol (27.6%), followed by petroleum ether (24%) and aqueous extract (20.16%). It shows that ethanol and pet ether soluble contents are more in the seeds of *Bakuchi* than water-soluble contents. This could also be the reason for the ethanolic and petroleum ether extracts to act better than the aqueous extracts. Likewise, a review study by Sukumar et al. (14) reported a great correlation between solvent polarities and nature of compounds extracted. Among different solvents, petroleum ether extract exhibited maximum larvicidal activity than aqueous extract against the mosquito larva.

Larvicidal bioefficacy of *Bakuchi* extracts may be due to phytochemicals stored in the plant, which exhibit biological action either independently or jointly against larvae of *Aedes aegypti*. Azmathullah et al.(15) reported that alkaloids, saponins, and tannins are known to possess medicinal and pesticidal properties and protect the plants from insects, pests, and diseases. Bagavan et al.(16) reported that saponins isolated from *Achyranthes aspera* through bioassay-guided fractionation possessed a larvicidal efficacy against *Aedes aegypti* and *Culex Quinquefasciatus*. Furthermore, Kotkar et al.(17) reported that flavonoids isolated from water extracts of *Annona squamosa* were effective as insecticides against mosquitoes affecting 80% of *C. Chinensis*. In addition to these metabolites, *Bakuchi* possesses one of the major bioactive secondary metabolites belonging to the class of phenolic compounds called 'psoralene' (phototoxic furanocoumarins), which possess high insecticidal property (18).

In petroleum ether extracts, few of the larvae performed aggressive self-biting to their anal papillae with their mouth parts and form a ring shape (head to siphon), whereas the controls showed normal activity. The same observation was also seen in the study conducted by Kabir et al.(19), the probable reason may be due to the discharge of electrolytes from the anal region resulted from the photo-enhanced cytotoxic activity of the drug. It could have been the prime reason for the toxicity. Interestingly, similar observation was also reported by a study conducted by Choochote et al.(20), Becker et al.(21) and Rocha et al.(22). The study conducted by Kabir et al.(19) reported LC_{50} value was 238.15 ppm, whereas in a study conducted by Choochote et al.(20) the LC_{50} and LC_{95} values were 81.0 and 176.8 ppm, respectively. However, in the present study the LC_{50} and LC_{90} values were 25.8 and 61.0 ppm, respectively. The variation in the results may be due to high content of psoralen in *Bakuchi*. This observation does

not only have practical importance but also paves way for the mode of action of insecticides.

Conclusions

In the present investigation, all the three extracts showed larvicidal activity. Among the three, petroleum ether extracts exhibited higher mortality at a lower dose when compared to ethanol and aqueous extracts. Ethanol extract showed higher mortality at lower doses when compared to aqueous extract. Hence, the current study reasonably concludes that *Bakuchi* carries potential as a mosquito larvicide and could be explored for the development of safer larvicide.

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Table 1: Phytochemical analysis of *Bakuchi* extracts

Test for	Alcoholic extract	Aqueous extract	Petroleum ether
Carbohydrates	+	+	+
Reducing sugars	+	+	+
Monosaccharides	+	+	+
Non-reducing sugar	+	+	+
Proteins	+	+	+
Amino acids	+	+	+
Steroid	+	+	+
Cardiac glycosides	+	+	+
Saponin	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	+
Tannic & phenolic	+	+	+
Fats & oils	+	+	+

+ = Present

Table 2: Quantitative analysis of Inorganic elements

Test for	*Bakuchi*
Iron	+
Sulphate	+
Chloride	+
Sodium	+
Phosphate	+

+ = Present

Table 3: Larvicidal bioefficacy of aqueous extracts of *Bakuchi*

Dose	Total Ex (n)	Replicate 1 (n)		Replicate 2 (n)		Replicate 3 (n)		Replicate 4 (n)		Control (n)		% Mo
		D	P	D	P	D	P	D	P	D	P	
500	125	4	0	3	0	4	0	3	0	0	0	14
600	125	7	0	6	0	6	0	7	0	0	0	26
700	125	12	0	11	0	11	0	13	0	0	0	47
800	125	17	0	15	0	15	0	16	0	0	0	63
900	125	21	0	22	0	21	0	20	0	0	0	84
1000	125	22	0	25	0	23	0	22	0	0	0	92

Total Ex, Total number of larvae exposed; % Mo, % Mortality; D, Dead; P, Pupae

Table 4. Larvicidal bioefficacy of ethanol extracts of *Bakuchi*

Dose	Total Ex (n)	Replicate 1 (n)		Replicate 2 (n)		Replicate 3 (n)		Replicate 4 (n)		Control (n)		% Mo
		D	P	D	P	D	P	D	P	D	P	
25	125	2	0	1	0	2	0	1	0	0	0	06
50	125	5	0	9	0	7	0	6	0	0	0	27
75	125	13	0	12	0	11	0	13	0	0	0	49
100	125	19	0	18	0	17	0	19	0	0	0	73
125	125	25	0	25	0	25	0	25	0	0	0	100

Total ex, total number of larvae exposed; % mo, % mortality; d, dead; p, pupae

Table 5: Larvicidal bioefficacy of petroleum ether extracts of *Bakuchi*

Dose	Total Ex (n)	Replicate 1 (n)		Replicate 2 (n)		Replicate 3 (n)		Replicate 4 (n)		Control (n)		% Mo
		D	P	D	P	D	P	D	P	D	P	
10	125	3	0	3	0	2	0	3	0	0	0	11
25	125	10	0	12	0	11	0	10	0	0	0	43
50	125	20	0	17	0	20	0	21	0	0	0	78
75	125	25	0	25	0	25	0	25	0	0	0	100

Total ex, total number of larvae exposed; % mo, % mortality; d, dead; p, pupae

Table 6: Larvicidal bioefficacy of different *Bakuchi* extracts against *Ae. Aegypti*

Extract	LC_{50} (ppm)	95% Confidence Limits		LC_{90} (ppm)	95% Confidence Limits		Chi-square
		LCL	UCL		LCL	UCL	Value
Petroleum ether	25.8	8.12	51.8	61.0	35.3	1505.4	10.775
Ethanol	66.71	41.09	96.02	127.80	90.65	522.04	22.775
Aqueous	699.91	661.07	736.64	971.63	906.82	1072.47	9.201

LC = Lethal Concentration