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Phytochemical screening and larvicidal efficacy of methanolic extracts of folklore medicinal plants against *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT

In this study, we investigated the larvicidal activity of the methanolic extracts from traditionally used medicinal plants namely *Putranjiva roxburghii* Wall., *Coscinium fenestratum* Colebr, and *Nardostachys jatamansi* DC against larvae of *Aedes aegypti*. The larvae were taken in beakers were exposed to methanolic extracts of the plants at concentrations of 1, 2.5 and 5mg/ml for 24hours followed by counting of dead larvae and percentage mortality was calculated. The LC50 was also determined for each extract. All extracts showed larval mortality. Larval mortality was 100% with the use of 2.5 and 5% concentration of extract of each plant. The LC50 for *N. jatamansi, P. roxburghii* and *C.fenestratum* was found to be 0.83mg/ml, 0.90mg/ml and 1.25mg/ml respectively suggesting *N. jatamansi* is more potent followed by *P. roxburghii* and *C. fenestratum*. Qualitative analysis of the extracts revealed the presence of tannin, alkaloid, steroid and flavonoid in all the extracts. Saponin was not detected in *N. jatamansi*, while terpenoid was not detected in all the extracts. The larvicidal activity could be mainly due to the presence of phytoconstituents in the methanol extract. It is suggested that all the three plants possess larvicidal properties and could be used as natural insecticides for mosquito control.

KEYWORDS

Larvicidal activity;
Putranjiva roxburghii Wall;
Coscinium fenestratum Colebr;
Nardostachys jatamansi DC;
Aedes aegypti;
Mosquito larvae.

INTRODUCTION

Mosquitoes are the most important single group of insects well-known for their public health importance, since they act as vector for many tropical and subtropical diseases such as dengue fever, yellow fever, malaria, filariasis, Japanese encephalitis and others[1]. The approach to combat these diseases largely relied on interruption of the disease transmission cycle by either targeting the mosquito larvae through spraying of stagnant water breeding sites or by killing the adult mosquitoes using insecticides[2]. Killing larvae of mosquitoes is a suc-

cessful way of minimizing mosquito densities in breeding grounds before they reach adult stage. It largely depends on the use of synthetic chemical insecticides. But their repeated use has caused environmental problems and widespread development of resistance. Plants offer an alternative source of insect-control agents because they contain a range of bioactive chemicals, many of which are selective and have little or no harmful effect on non-target organisms and the environment[3,4]. *N. jatamansi*, a critically endangered rhizome-bearing medicinal plant, is restricted to specialized habitats in high altitudes of the Himalaya. The root is used for treatment of heart disease, high blood pressure and insomnia. The root and rhizome contain active compounds with carminative, sedative, antispasmodic and tranquilizing properties[5]. *Coscinium fenestratum* Colebr belongs to the family Menispermaceae and is a critically endagered dioecious medicinal liana found in Western ghats of India. The stem of the plant is used in curing several diseases and disorders like diabetes, wounds and ulcers, fever, jaundice, snake bite, piles etc in ethnomedicine. The chief constituent of *Coscinium* is the yellow crystalline alkaloid, berberine[6]. *Putranjiva roxburghii* Wall., commonly called Putranjiva, is a deciduous, evergreen tree of about 18m tall having grey bark. It is used in cold, fever and rheumatism[7,8] and seeds in inflammation[9]. The mosquito *Aedes aegypti* (Diptera: Culicidae) acts as a vector for an arbovirus responsible for yellow fever, dengue hemorrhagic fever[10]. The only successful way of reducing mosquito densities to a level where dengue or yellow fever epidemics do not occur is by attacking the larval breeding places[11]. The aim of this work was to investigate the larvicidal activity of the methanolic extracts essential from traditionally used medicinal plants namely *Putranjiva roxburghii* Wall., *Coscinium fenestratum* Colebr, and *Nardostachys jatamansi* DC against larvae of *A. aegypti* in the search for an alternative natural product, that can be used in the control of mosquito borne diseases such as dengue, chickungunya and yellow fever.

MATERIALS AND METHODS

Collection of plant materials

The plant materials of *P. roxburghii* (seed), *N. jatamansi* (rhizome) and *C. fenestratum* (stem) were obtained from local shops of Udupi city and authenticated to identity by Dept. of Botany, S.R.N.M.N College of Applied Sciences, Shivamogga. Voucher specimen was deposited in the department for future reference.

Extraction and phytochemical analysis of plant materials

The dried plant materials were powdered mechanically. About 150g of powdered material was subjected to soxhlet extraction and exhaustively extracted with methanol for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator, dried in the dessicator. The yield of the extracts was noted and the extract was kept in refrigerator until use[12]. The methanol extract was subjected to preliminary phytochemical analysis[13]

Larvicidal activity of methanolic extracts

Larvae of *Aedes aegypti* mosquito were collected from water stagnated area, and identified in the Dept. of Entomology, UAS, Shivamogga, Karnataka, India. The larvae were maintained under suitable temperature and humidity. Different concentrations of methanolic extracts (1, 2.5 and 5mg/ml) were prepared in 10% DMSO and added to sterile labeled beakers containing about 100ml of water. Twenty larvae were placed in each of the beakers containing extracts. A control was kept containing DMSO. After adding the larvae, the beakers were kept in the growth room maintained at room temperature. The larvicidal effect of extracts was determined by counting the number of dead larvae after 24 hours. Dead larvae were identified when they failed to move after probing with a needle in siphon or cervical region. Each test was repeated thrice, the percentage of larval mortality and Lethal concentration (LC50) for each concentration of extracts was calculated[14].

RESULTS AND DISCUSSION

The extract yield of 14%, 13.5% and 11% was obtained in case of *N. jatamansi*, *P. roxburghii* and *C. fenestratum* respectively (TABLE 1). The presence of various phytoconstituents in methanolic extracts of selected plants is shown in TABLE 2.

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Phytoconstituents namely tannin, alkaloids, saponins, steroids and flavonoids were detected in the seed extract of *P. roxburghii*. The *N. jatamansi* rhizome extract showed the presence of all phytoconstituents except saponins and terpenoids. In stem extract of *C. fenestratum* showed the presence of tannin, alkaloid, saponins, steroids and flavonoids. Terpenoid is not detected in extracts of all plants tested.

TABLE 1 : Yield of methanolic extracts of selected plants

Extract	Yield in %
N. jatamansi	14
P. roxburghii	13.5
C. fenestratum	11

TABLE 2 : Phytoconstituents present in methanolic extracts of selected plants

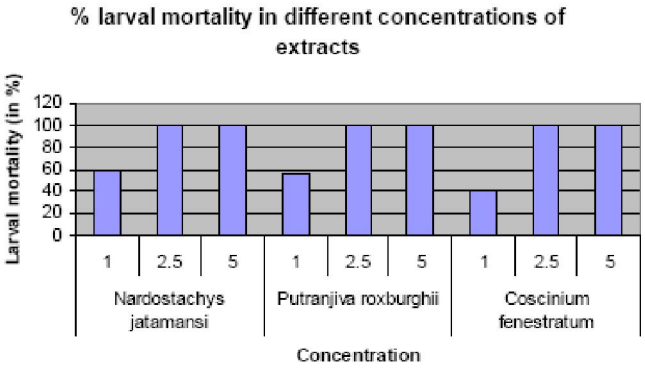
Phytoconstituent	*P. roxburghii*	*N. jatamansi*	*C. fenestratum*
Tannin	+	+	+
Alkaloid	+	+	+
Saponins	+	ND	+
Steroids	+	+	+
Terpenoids	ND	ND	ND
Flavonoids	+	+	+

'+' Detected; 'ND' Not detected

The methanolic extracts of selected plants have demonstrated promising activities against the larvae of *Aedes aegypti*. The results depicted in TABLE 3 and Graph 1 shows the dose depended activity of extracts. In case of extract concentration 2.5 and 5mg/ml, 100% mortality of larvae was observed in all plant extracts. At 1mg/ml concentration, 60%, 55% and 40% mortality was observed in case of *N. jatamansi, P. roxburghii* and *C. fenestratum* respectively. The LC50 values were found to be 0.83mg/ml, 0.90mg/ml and 1.25mg/ml for *N. jatamansi, P. roxburghii* and *C. fenestratum* respectively. From the result, it is clear that *N. jatamansi* is more potent in killing larvae followed by *P. roxburghii* and *C. fenestratum*. In control, no mortality of larvae was observed. Earlier studies observed that phytochemicals have major role in mosquito control programme[15,16]. It is observed the presence of carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins in the plant extract having mosquito larvicidal activity[14]. It is reported the use of commercial saponin from *Quillaja saponaria* bark as a natural larvicidal against *Aedes aegypti* and *Culex pipens*[17]. Cardiac glycoside was found to have an acaricidal effect against larva and adult stages of the camel tick[18]. It is suggested that the saponin molecules interact with the cuticle membrane of the larvae, ultimately disarranging the membrane could be the most probable reason for the larval death. The deficiency of dissolved oxygen and active presence of the antioxidant saponin molecule might be the reason for larval death. However, much study is required to find out the mechanism by which saponin kills the larvae[19]. Saponin extracted from the fruit of *Balanites aegyptiaca* showed 100% larvicidal activity against *A. aegypti* mosquito larvae[20]. Prenylated xanthones, tetracyclic phenols and saponins are reported to be effective in controlling mosquito *A. aegypti*, the vector of yellow fever[21]. Aluminium chloride, known for its phenolic complexing activity, obtained from alder leaf also reported to have the larvicidal activity against *A. aegypti*[22]. Monoterpene hydrocarbons showed a marked mosquito larvicidal activity against *C. pipiens* which is obtained from the fresh leaves of *Anthemis*

TABLE 3 : Larvicidal effect of different concentrations of methanol extract of selected plants

Treatment	Concentration (in mg/ml)	Number of larvae dead	% larval mortality	LC50 concentration (mg/ml)
Nardostachys jatamansi	1	12/20	60.00	0.83
	2.5	20/20	100.00	
	5	20/20	100.00	
Putranjiva roxburghii	1	11/20	55.00	0.90
	2.5	20/20	100.00	
	5	20/20	100.00	
Coscinium fenestratum	1	8/20	40.00	1.25
	2.5	20/20	100.0	
	5	20/20	100.0	



Graph 1 : Larval mortality (in %) in different concentrations of extracts

melampodina and *Pluchea dioscoridis*[23]. A piperidine alkaloid from *Piper longum* fruit was found to be active against mosquito larvae of *C. pipiens*[24]. An alkaloid derived from the tropical vine *Triphyophyllum peltatum* was found to have larvicidal activity against the malaria vector *Anopheles stephensi*[25]. From this study, we can conclude that the larvicidal property could be mainly due to phytoconstituents such as alkaloids, tannins, saponins present in the extracts.

CONCLUSION

Mosquitoes constitute a major public health menace. Several mosquito species belonging to genera Anopheles, Culex and Aedes are vectors for the pathogens of various diseases. Plant extracts have been used in the control of mosquito borne diseases as the chemical agents have caused some ill effects and also the mosquitoes developed resistance against them. The results of the present study are in justification of this and the extracts of plants selected in this study could be used in control of arboviral infections transmitted by *Aedes aegypti*.

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