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Short Report

Pharmaceutical and Biomedical Research

Larvicidal potential of *Cyathea* species against *Culex quinquefasciatus*

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Received: Jul 1, 2016, Revised: Sep 24, 2016, Accepted: Mar 4, 2017

Abstract

Resistance to insecticides has persuaded researchers to find new methods to control *Culex quinquefasciatus* proliferation. Plants may be a source of alternative agents for mosquito control due to ever-growing insecticide resistance in mosquito vectors and environmental imbalance caused by synthetic insecticides. The present study was intended to study the larvicidal activity of selected *Cyathea* species against the filarial vector *Culex quinquefasciatus.* Larvicidal potential of different extracts were evaluated and larval mortality were recorded. The larvae were more sensitive to ethanolic extracts of studied three *Cyathea* species when compared to other extracts. Acetone, chloroform and petroleum ether extracts were considered to be less effective. The LC_{50} values of different extracts ranged from 320.72 to 657.03 µg/ml. The results exhibited that the tested three *Cyathea* species showed concentration dependent potential larvicidal effects and also provide an indication of possible bioactive properties.

Keywords: Tree ferns, *culex quinquefasciatus,* larvicidal

Pharm Biomed Res 2017; 3(1): 48-51

Introduction

Vector-borne diseases are the major cause of morbidity in most of the tropical and subtropical countries. Mosquitoes are one of the important vectors responsible for the spread of several diseases viz., malaria, filariasis, dengue fever and Japanese encephalitis. *Culex* sp. is the most abundant mosquito species in urban areas. It is crucial to manage *Culex* population so that people can be protected from various mosquito borne diseases. These diseases can be controlled by targeting the causative parasites and pathogens. The chemical control was one of the most extensively used conventional methods for controlling mosquito borne diseases since chemical pesticides are relatively inexpensive and usually produces immediate control. Generally, the chemical control is carried out by the indoor residual spraying of insecticides such as temephos, insect growth regulators such as diflubenzuron and methoprene, which are still the most effective. Insecticide applications against the target species are facing a serious threat due to the development of resistance to chemical insecticides (1). Numerous studies have been carried out to identify safer mosquito control agents and to reduce environmental and human health concern (2, 3). An approach to obtain efficient, safe and selective insecticides is the study of natural products obtained from plants (4).In the world flora, next to angiosperms, pteridophytes occupy an important position in which many extant species were recorded (5). Pteridophytes are conspicuous and gorgeous elements of biodiversity which occurs in various kinds of habitats ranging from sea level to mountain top and tropical to subpolar regions (6). With reference to *Cyathea* species, Janakiraman and Johnson (7) studied the presence of phenolics, flavonoids, tannins, cardiac glycosides, terpenoids, steroids, saponins and alkaloids in *C. nilgirensis, C. gigantea* and *C. crinita*. Janakiraman and Johnson (8) determined the functional constituents of *C. nilgirensis, C. gigantea* and *C. crinita*. using FT-IR. Janakiraman and Johnson (9) studied the antioxidant potentials of *C. nilgirensis, C. gigantea* and *C. crinita*. Janakiraman and Johnson (10) revealed the phenolics profile of tree ferns using HPTLC analysis. Janakiraman and Johnson (11) studied the chemical constituents of tree ferns using GC-MS analysis. Janakiraman and Johnson (12) confirmed the cytotoxic activity of ethanolic extracts of selected *Cyathea* species against MCF 7 cell line cultures. Hence, the present study was undertaken to study the larvicidal potential of various extracts of *Cyathea nilgirensis* Holttum, *Cyathea gigantea* (Wall. ex. Hook.) Holttum and *Cyathea crinita* (Hook.) Copel.

Materials and methods

Collection of plant materials

Samples for the present study were collected from different parts of Tamil Nadu, South India.

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C. nilgirensis were harvested in and around Kakkachi stream (1,725 m), Tirunelveli hills (8° 44' N and 77° 44' E), *C. gigantea* from the road sides near Nadugani (2,637 m), Nilgiris hills (11° 24' N and 76° 44' E) and *C. crinita* from the Anglade Institute of Natural History, Shenbaganur, Kodaikanal (2,195 m), Palni hills (10° 13' N and 77° 32' E), Western Ghats, South India. The specimens were identified based on the "Pteridophyte Flora of the Western Ghats, South India" by Manickam and Irudayaraj (13). Herbarium specimens were deposited in the St. Xavier's College Herbarium (XCH), Palayamkottai for further reference (*C. nilgirensis* - XCH 25423; *C. gigantea* - XCH 25422 and *C. crinita* - XCH 25424).

Preparation of extracts

The collected species of *Cyathea* were thoroughly washed with tap water followed by distilled water. They were blotted on the blotting paper and spread out at room temperature in shade to remove the excess water contents. The shade dried plant samples were ground to fine powder using mixer grinder. The powdered materials were stored in refrigerator for further use. Thirty gram of powdered sample was extracted (1:6 ratio) successively with 180 mL of petroleum ether, chloroform, acetone and ethanol using the Soxhlet extractor for 8-12 h at a temperature not exceeding the boiling point of the solvent. The extracts were concentrated in a vacuum at 40 ºC using rotary evaporator.

Larvicidal activity

Culex quinquefasciatus (4th instar larvae) was collected from sewages of Tirunelveli district with the help of 'O' type brush. These larvae were brought to the laboratory and transferred to 18×13×4 cm size enamel trays containing 500 ml of water. It was maintained at 27 ± 2 °C, 75-85% RH and 14 h light and 10 h dark photoperiod cycles.

Larvicidal activity of different extracts (petroleum ether, chloroform, acetone and ethanol) with varied concentrations of *C. nilgirensis*, *C. gigantea* and *C. crinita* was evaluated as per the standard method described by WHO (14). Batches of twenty 4th instar larvae of *C. quinquefasciatus* were collected separately and transferred to small disposable cups each containing 200 mL of water. The appropriate volume of dilution was added in the cups to obtain the desired target dosage (concentrations ranging from 100-500 µg/ml) starting with the lowest concentration. Five replicates were set up for each concentration and simultaneously a control was maintained. The larval mortality in both treated and control were recorded after 24 h. The standard larvicide Temephos (Abate) was used as positive control. The control mortality was corrected by Abbott's formula (15).

Statistical analysis

The LC_{50} value was calculated by Probit analysis (16). The IC_{50} values of the crude extracts were found using MS-Excel 2007. Correlation analysis between LC_{50} and IC_{50} values were calculated using SPSS (Chicago, USA).

Results

The results of the larvicidal bioassay of crude petroleum ether, chloroform, acetone and ethanolic extracts of *C. nilgirensis*, *C. gigantea* and *C. crinita* against the fourth instar mosquito larvae *C. quinquefasciatus* were presented in Table 1. The larvae were more sensitive to ethanolic extracts of studied three *Cyathea* species when compared to other extracts. Acetone, chloroform and petroleum ether extracts were considered to be less effective. The LC_{50} values of different extracts ranged from 320.72 to 657.03 µg/ml. Among the three different species tested, the highest larval mortality was observed in ethanolic extracts of *C. crinita* with the LC_{50} value of 320.72 µg/ml followed by *C. gigantea* (361.07 µg/ml) and *C. nilgirensis* (373.99 µg/ml). The positive control Temephos showed 100% mortality rate at 0.025 mg/ml. The 95% confidence limits LC_{50} (LCL-UCL), LC_{90} and chi-square values were also calculated (Table 1). The Chi-square values for the studied extracts ranged from 0.35 to 7.37 for *C. quinquefasciatus*. Petroleum ether (0.35), chloroform (0.40) and ethanolic extracts (0.36) of *C. crinita*, chloroform (0.54) and acetone (0.38) extracts of *C. gigantea* and ethanolic extracts (0.69) of *C. nilgirensis* showed a strong positive correlation between the extracts and larval mortality (Table 1). The other tested extracts failed to show direct correlation between insect toxicity.

Discussion

The control of mosquito larvae by chemical substances is not safe at present because of environmental imbalance and insecticide resistance by vectors which leads to deleterious effects. The major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. Hence, an alternative mosquito control method is needed (17). The extracts which are obtained from plant parts have been used as conventional larvicide (18, 19). The observed results were also comparable with earlier reports. The fruit extract of *Croton caudatus*, flower extract of *Tiliacora acuminata* (20), leaf extract of *Typhonium trilobatum* (21) and flower extract of *Tagetes erecta* (22) were found to cause larval mortality against *C. quinquefasciatus*. In the present study, the different extracts of *C. nilgirensis*, *C. gigantea* and *C. crinita* exhibited a dose dependent activity. The results observed were similar to previous studies which have also reported dose

Table 1 Larvicidal activity of different *Cyathea* species against C*. quinquefasciatus*

Species	Extracts	LC_{50} (µg/ml)	95% Confidence Limits		LC_{90} (µg/ml)	χ^2	IC_{50} (µg/ml)
			Lower	Upper			
C.nilgirensis	Pet. ether	607.76	512.14	894.19	893.93	3.86	66.4
	Chloroform	592.26	482.18	917.90	1078.70	2.73	52.3
	Acetone	448.88	394.96	536.88	966.31	1.04	57.8
	Ethanol	373.99	338.65	417.91	758.69	0.69	51.9
C. gigantea	Pet. ether	639.92	561.76	784.12	1013.26	1.05	55.1
	Chloroform	624.63	532.47	813.44	1151.44	0.54	51.4
	Acetone	468.00	394.65	620.38	1175.06	0.38	141.6
	Ethanol	361.07	317.84	416.12	848.87	2.29	64.8
C. crinita	Pet. ether	657.03	556.55	868.54	1178.48	0.35	50.9
	Chloroform	568.04	489.18	722.23	1103.12	0.40	51.9
	Acetone	400.36	292.74	776.28	870.41	7.37	61.7
	Ethanol	320.72	291.11	351.94	645.79	0.36	50.5

dependency of plant extracts against mosquito larvae (2, 3). The results The phytochemical studies on the *Cyathea* species confirmed the various metabolites (7, 8, 10, 11).

Based on the existing phytoconstituents, the mortality rate of the present study showed varied percentage of mortality rate which may due to the occurrence of phytochemicals in the studied tree ferns. showed variation. The results suggest that the presence of several bioactive secondary metabolites may be responsible for the larval toxicity. Screening the tree fern crude extracts against mosquito larvae may pave a pathway to identify potential bioactive compounds to control mosquito that can be further employed as larvicides. Botanical derivatives have drawn attention as potential insect control agents targeting only larval stages in the mosquito control programme in the last three decades (20-22). The results also supported the previous observations on the larvicidal potential of plant extracts. The statistical analysis results of *C. nilgirensis* showed strong positive correlation between LC_{50} and IC_{50} values. The other two studied tree ferns failed to show the positive correlation. But all the three species demonstrated a significant relationship between larval mortality and crude extracts. The larvae were particularly more sensitive to ethanolic extracts of *Cyathea* species when compared to other extracts. It was also due to the presence of more number of phytochemicals in ethanolic extracts which was confirmed by previous researchers (7, 8, 10, 11). The mechanism of action exhibited by the studied *Cyathea* species may therefore possibly be due to its toxic effects on the larvae.

Conclusion

The tested three *Cyathea* species showed concentration dependent potential larvicidal effects. The results also provide an indication of possible bioactive properties of the tested extracts. Further investigation is needed to find out the active compounds responsible for the larvicidal activity.

Acknowledgement

The authors are thankful to University Grants Commission; New Delhi for the financial support through the Major Research Project Grant No. 41-416/2012 (SR) dated 16th July, 2012.

Conflict of interest

The authors declare that they have no conflict of interest.

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