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Larvicidal activity of *Cyclea peltata* against *Culex spp.*

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Abstract:
Methanolic extract obtained from mature leaves of *Cyclea pelata* was used for mosquito larvicidal effect against *Culex quinquefasciatus* at different concentrations and the mortality rate was calculated after 24 and 48hrs. Phytochemical analysis was also carried out for active methnolic extract of leaves. The results obtained from above experiment showed 100% moratality of leaf methanolic extract against *Culex quinquefasciatus*. After 48 hrs the LC_{50} value obtained for methanolic extract of leaves of *Cyclea peltata* was 279.42 µg/ml. Phytochemical analysis revealed the presence of alkaloids, flavonoids, sterols and phenolic compounds in methanolic extract of leaves of *Cyclea peltata*. Our study showed the potential use of *Cyclea peltata* for mosquito control as well as insecticide.
Keywords Alkaloids, *Cyclea peltata, Culex,* Flavonoids, Methanolic

Introduction:

Cyclea peltata is slender, climbing and twining shrub with sparingly stem and branches and belongs to the family menispermiaceae. The plant is found in south and east India & commonly known as Paatha in Hindi. A group of Asian indigenous people (Nagas) use this plant for fighting against evil spirits (Changkija *et al*; 2000). *Cyclea peltata* is reported to be used for skin diseases such as allergies, burns, cuts, wounds, inflammation, leprosy, leucoderma, scabies, smallpox and certain sexually transmitted diseases (STD) (Begum *et al*; 2000). The roots of *Cyclea peltata* have been examined for inhibitory properties on nephrolithiasis in rats, (Christina *et al*; 2002). It is also has been used in fever, jaundice, asthma (Hullatti *et al.*, 2010). Some members of the family Menisperamacae has been shown the larvicidal activity agaist *Aaedes egypti* and *Culex* (Ciccia *et al.*, 2000; Elango *et al.*, 2010). Here we established the larvicidal effect of *Cyclea peltata* against *Culex quinquefasciatus*.

Material and Methods:

Plant Material and preparation of extract:

Plants of *Cyclea peltata* (Lam) Hook F and Thoms were obtained from Kop's nursery, Dapoli, India. These plants are then established in the green house of North Maharashtra University campus. The plant was authenticated from a taxonomist of botany department, M. J. College, Jalgaon, India. The matured leaves of the plants were used as plant materials. All the plant materials were washed with distilled water, soaked on paper towel and then shed dried. The dried material was then powdered with the help of grinder. Each plant material (10 gm) was mixed with sterile distilled (500ml) methanol and kept at room temperature on an orbital shaker at 120 rpm for 3 days. Each crude extract was then filtered through whatman filter paper no. 1 and the filtrate was dried. Required concentrations of aqueous extracts were prepared by mixing each of the crude extract with a suitable amount of sterilized distilled water.

Rearing of mosquito larvae:

The immature stages of *Cx. quinquefasciatus* were collected from the stagnant water with rich organic pollution in and around NMU campus and also nearby places around University and identified by the macro and microscopic examinations (Harrison 2005; O'Meara and Cutwa, 2008). Mosquito larval collection was done from the breeding site by using large kitchen strainer and transferred to large plastic container and transported to the laboratory. In the laboratory the larvae was kept in enamel tray with tap water and a mixture of powdered dog biscuit with yeast powder in 3:1 ratio was provided as a feed. Once the acclimatization of the mosquito larvae was done in the laboratory conditions, subsequent experiments were carried out.

Mature Leaves from these plants were taken and shade dried. An infusion was prepared from fine powder of shade dried leaves. The larvicidal bioassay was done according to World Health Organization (WHO) standard protocols (WHO 1996). Twenty five *Culex* larvae from the late third instars were used in each 250 ml flask with 249 ml of dechlorinated water and 1ml of desired concentration of plant extracts to make the final concentration ranging 100, 200, 300, 400, 500 µg/ml. All the experiments were conducted in triplicate and control were performed at parallel condition in each series of experiment. Larval

mortality was recorded at 24 and 48 h of exposure. The percentage of mortality was calculated by with **Abbott's formula**,

$$\% \text{ mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100\%$$

Statistical analysis:

Statistical analysis of the experimental data was performed using the computer softwares MS Excel 2007 and Minitab version 16 to find out the LC50, mean larval mortality, standard error etc.

Photochemical Analysis:

Phytochemical analysis was carried out using different preliminary test (Khadelwal 2006; Rathore *et al.*, 2012).

Alkaloids

Iodine Test – Mix 3ml test solution and add few drops of dilute iodine solution. Blue colour appears; it disappears on boiling and reappears on cooling.

Flavonoids

NaOH Test – To 2-3ml of crude extract, few drops of NaOH solution were added in a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoid.

Glycosides

To small amount of crude extract, add 1ml water and shake well. Then aqueous solution of NaOH was added. Yellow colour appeared that indicate presence of glycoside. .

Phenols

Ellagic acid test – The test solution was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The solution turn muddy or niger brown precipitate occurred in the crude extract indicate the presence of phenol.

Saponins

Foam Test - The crude extract was diluted with 20ml of D/W and it was shaken in graduated cylinder for 15 minutes. A 1cm layer of foam indicates the presence of saponin.

Sterols

Salkowaski's Test – To 2ml crude extract, add 2ml chloroform and 2ml concentrated H_2SO_4 and was shaken well. The chloroform layer appeared red and acid layer show greenish – yellow fluorescence indicate the presence of sterols.

Tannins

Lead acetate test – To 5ml of crude extract, few drops of 10% lead acetate solution were added. Formation of yellow or red precipitate indicates the presence on tannins.

Results and discussion:

The mortality rate of *Cx. quinquefasciatus* third-instar larvae treated with 500 µg/ml methnaolic extract of *Cyclea pelata* was significantly higher than the mortality rates at 100, 200, 300 and 400 µg/ml concentrations of each extract at 24 and 48 h of exposure (Table 1). The LC_{50} and LC_{90} values calculated using probit analysis (at 95% confidence level) were found to be 279.42 µg/ml and 610 µg/ml after 48 h of exposure (Table 2). The larval stage is the most vulnerable stage to attack mosquitoes as they are concentrated in smaller areas (Rambabu *et al.*, 2014). There is no any successful vaccine technology for mosquito-borne diseases which makes them one of the leading cause for deaths specially in rural areas including tropical and subtropical regions (Ramathilaga *et al.*, 2012). *Culex spp.* are important vectors for transmission of deadly disease called Elephantiasis. Our results exploited the potential of *Cyclea peltata* leaves for larvicidal effect against these vectors. Also long term effect of chemical insecticides results in environmental contamination and threat to the aquatic organisms (Singha and Chandra, 2011). Larvicidal activity of *Cocculus hirsutus* (L.) Diels one of the member of Menispermaceae family against *Culex tritaeniorhynhus* was demonstrated by Elango *et al.*, (2010). Also Preliminary phytochemical analysis showed presence of alkaloids, sterols, tannins, phenols and flavonoids in the methanolic extract of leaves (Table 3).

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Table: 1. The effect of different concentration of *Cyclea peltata* leaves on mortality of *Cx. Quinquefasciatus* larvae

Sample	Concentration (µg/ml)	% Mortality	
		24h	48h
Cyclea peltata	100	5.33 ±	8.7 ± 0.8

methnolic extract of leaves		0.57	
	200	13 ± 1.2	17.96 ± 0.45
	300	15.3 ± 1.5	20.1 ± 0.83
	400	18.66 ± 0.89	24.2 ± 0.32
	500	23.34 ± 0.44	25 ± 0.1

Values are mean ± SD of three independent experiments.

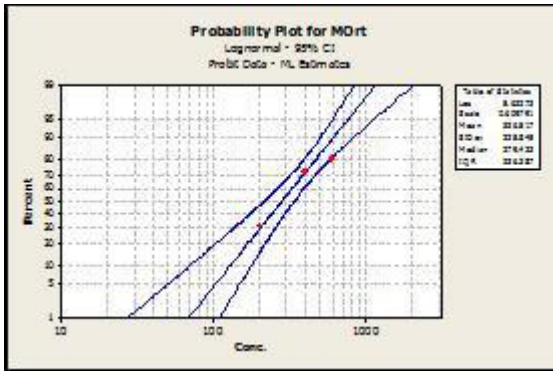
Table: 2. LC_{50} and LC_{90} values of methanolic extract agaist larvae of *Cx. Quinquefasciatus* calculated using Probit analysis after 48 hrs.

Percent	Concentration (µg/ml)	95% Confidence limit	
		Lower	Upper
1	67.63	27.0554	108.096
2	79.86	34.6570	122.741
3	88.75	40.5413	133.083
4	96.07	45.6096	141.458
5 (LC_5)	102.4	50.1895	148.678
6	108.2	54.4427	155.128
7	113.6	58.4623	161.027
8	118.6	62.3077	166.513
9	123.3	66.0196	171.675
10	127.9	69.6272	176.580
20	167.2	103.131	218.241
30	202.9	136.328	255.325
40	239.4	172.197	293.394
50(LC_{50})	279.4	212.777	336.340
60	326.1	260.247	389.535
70	384.7	317.576	463.321
80	466.8	390.809	582.264
90(LC_{90})	610.4	502.032	829.767
91	632.8	518.079	872.231
92	658.2	535.866	921.219
93	687.2	555.878	978.710
94	721.1	578.826	1047.69
95	761.8	605.825	1132.93
96	812.6	638.751	1242.77
97	879.7	681.161	1393.60
98	977.5	741.138	1624.53
99	1154.3	845.023	2072.43

Table: 3. Preliminary phytochemical analysis of Leaf extract of *Cyclea pelata*

Phytocostituents	Methanolic extract
Alkaloids	++
Flavonoids	+++
Sterols	+
Tannins	++
Phenols	+++
Saponins	++
Glycosides	++

+++ High; ++ moderate; + Low



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