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Evaluation of insecticidal activity of *Cleistanthus Collinus* plant extracts and their fractions against lepidopterian pests

KEYWORDS: Cleistanthus collinus, insecticidal activity, methanolic extract, cold percolation method.

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ABSTRACT *In the present study leaf and bark of Cleistanthus collinus were extracted with various solvents using cold percolation method. Methanol was found to be the best extractant amongst all the solvents. The crude methanol extract and solvent extract were then subjected to prescreen assays to check any insecticidal activity. Both leaf and bark extract showed some insecticidal activity against insects Plutella xylostella, Helicoverpa armigera, Spodoptera litura. Leaf extract showed more insecticidal activity (20-30%) compare to bark extract. When leaf and bark extract were partially purified using solvent extraction procedure, chloroform fraction of both leaf and bark extracts was found to have insecticidal activity against Plutella xylostella (40%). The present study has exploited the probability of having any insecticidal molecules present in the medicinally important plant Cleistanthus collinus.*

INTRODUCTION

In India the importance of trees have been recognized from the times immemorial. Derivatives of plants have traditionally been used by farmers to ward off insect pest of household, agricultural and medicinal importance. The plant biochemicals exhibit various properties such as insecticidal, repellent and growth disrupting activities against various pests and target a broad range of insects (Laxmikanta et al, 2009).

Cleistanthus collinus popularly known as Garadi(in Marathi) and Oduvan(in tamil) is a small deciduous tree of the family Euphorbiaceae. *Cleistanthus collinus* is abundantly found in many parts of India ,Malaysia and Africa. *C. collinus* is one of the most generally used and important trees in the state. The leaves, roots and specially the fruits act as violent gastro intestinal irritants. It is also used as cattle and fish poison and also for procuring criminal abortion. (Sarathachandra et al., 1997). The leaves are abortificinet and occasionally used for suicidal purposes (Modi and Coius,1940). Glycoside isolated from the leaves and bark have been reported to exhibit a wide range of biological action including cytotoxicity. The plant products such as leaf extracts and bark extract of *C. collinus* was reported to show antifeedant activity and insecticidal activity (Arivudainabi and Baskaran, 2004). Cleistanthin is also used as anticancer (Pradheepkumar et. al,2000).

In this paper, the insecticidal property leaf and bark extracts of *C. collinus* has been studied as part of the exploration for new and novel bio-active compounds. Therefore, this research regarding the insecticidal activity of this plant is expected to enhance the use of *C. collinus* against damages caused by the test insects.

MATERIALS AND METHODS

Plant materials

The authenticated plant material used for this study was collected from Forest department office at Purkabodi village situated in Bhandara taluka of Bhandara District (Maharashtra states) India.

Insect

The insects (*Lepidoptera*) were collected from Departmental fields of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra).

i) Plutella xylostella (DBM)

The larvae and pupae of *P. xylostella* were collected from cabbage and cauliflower field from outskirts of Akola. They were reared in the laboratory on the mustard seedlings upto F4 generations for establishing homologous laboratory population. The rearing procedure described by Lu and Sun (1984) was followed to maintain the test culture of *P. xylostella*..

ii) Helicoverpa armigera

Collection of *H. armigera* from fields. Third instar larvae of *S. litura* were collected from horticultural fields from Dr. P.D.K.V. campus, Akola.

iii) *Spodoptera litura*

Collection of *S. litura* from fields. Third instar larvae of *S. litura* were collected from horticultural fields from Dr. P.D.K.V. campus, Akola.

Preparation of plant extract

Fresh leaves and bark of *C. collinus* were collected from a 15 year old shrub growing in Purkabodi village situated in Bhandara taluka of Bhandara District (Maharashtra states), India and used for experimental work. Leaves and bark were dried at room temperature and then ground into fine powder using a grinder. About 20 g of dried powder of leaves and bark were taken in separate 250 ml conical flasks with screw cap, and then 200 ml of methanol was added to the flask. The flasks were kept at room temperature with shaking for 7 days. At the end of the extraction, extract was filtered using Whatman filter paper. The filtrate was concentrated in vacuum at 30°C and stored at 4°C until further use. The final crude extract was diluted with methanol to a standard volume and tested separately against the test organisms (Harborne, 1973).

Solvent extraction

Few amount (5 gm) of extract was taken and triturated with MeOH:H_2O (4:1) ratio, filtered and then the residue was separated and it was considered as fraction 1. The filtrate then acidified with 2M H_2SO_4 and was extracted again with $CHCl_3$ for three times. Thus chloroform and aqueous acid layer get separated. Chloroform was evaporated it was considered as fraction 2 while aqueous acidic layer was considered as fraction 3 (Harborne, 1998).

INSECT BIOASSAY

Preparation of sample extracts for bioassay

Accurately weighed 10 mg of extract and 1 ml of methanol was added to it and vortexed so that all the extract was dissolved to form a clear solution. This was 1 % solution. Similarly 1, 2.5, 5, 7.5 and 10% concentration of the extracts were prepared for the final bioassay. For the bioassay against *P. xylostella, S. litura* and *H. armigera* 1, 2.5, 5, 7.5 and 10%

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concentrations of the extracts were used. For crude extracts 1-10% concentration were used while for solvent extracted fraction 10% concentration was used.

a) Bioassay against *P. xylostella* (DBM)
The bioassay was carried out by cabbage leaf disc dip method as described by Tabashnik et al. (1987). Cabbage leaves were first washed with distilled water containing 0.1 % Triton X 100 and dried for about 1 hr. Cabbage leaf discs (approximately 5 cm diameter) were cut with a metal punch and then dipped in the test solution of various extracts prepared in methanol to facilitate uniform treatment of active ingredient for about 10 sec. The leaf discs were placed slanting for about 2 min over a blotting paper in a tray to drain excess solution for about 2 hrs. at room temperature. Ten 3rd instar larvae (6 hrs. starvation) were released on each disc in individual petri plate. Blotting paper was placed at the bottom of the petri plate. The plates were observed for 72 hrs for any insecticidal activity. The bioassay were conducted at temperature 27 ± 1 ^{0}C, relative humidity 75 ± 1%, dark and light regime of 13:11 hrs. Total 30 insect larvae were used for screening.

b) Bioassay against *Helicoverpa armigera*
Cotton leaf dip method of bioassay (Tabashnik et al., 1987) was adopted to assess any insecticidal activity. Cotton leaves were first washed with distilled water containing 0.1 % Triton X 100 and dried for about 1 hr., cotton leaves then dipped in the test solution of various extracts prepared in methanol to facilitate uniform treatment of active ingredient for about 10 sec. The leaves were placed slanting for about 2 min over a blotting paper in a tray to drain excess solution for about 2 hrs. at room temperature. Ten 3rd instar larvae (8 hr starvation) were released on each disc in individual petri plate. Blotting paper was placed at the bottom of the petri plate. The bioassay were conducted at temperature 27^{0}C ± 1 ^{0}C, relative humidity 75 ± 1%, dark and light regime of 13:11 hrs.

c) Bioassay against *Spodoptera litura*
Field collected *S. litura* larvae were used for leaf-dip bioassay. Castor leaves were first washed with distilled water containing 0.1% Triton-X-100, and dried for about 1 hrs. Castor leaves then dipped in the test solution of various extracts prepared in methanol to facilitate uniform treatment of active ingredients for about 10 second. Each leaf was kept in separate petriplate and then larvae were released in each petriplates. For, each extract, preliminary screening was done at a 1, 2.5, 5, 7.5 and 10% concentration to obtain mortality response of the test insect. Leaves were treated with methanol served as control. The petriplate containing treated leaves and released insects were then transferred to environmentally controlled growth chamber at a temperature 27^{0}C ± 1^{0}C, 65 ± 5 percent relative humidity for the assessment of insecticidal activity. Mortality counts were recorded at 24, 48, 72 hrs, after treatment and moribund insects were counted as dead (Birah et al, 2008).

RESULTS

Screening of crude methanolic extracts for insecticidal activity

a) Bioassay against *Plutella xylostella*
The leaf and bark extract were effective and showed mortality 23.33 and 20% at 10% concentration respectively. However mortality was not observed at low concentrations.

b) Bioassay against *Spodoptera litura*
The leaf extract was effective and showed 20 and 30% mortality at 7.5 and 10% concentration respectively and bark extract showed 20 and 26.66% mortality at 7.5 and 10% concentration respectively. However mortality was not observed at low concentrations of extracts.

c) Bioassay against *Helicoverpa armigera*
The leaf extract was effective and showed mortality 16.66 and 20% at 7.5 and 10% concentration respectively and the bark extract showed mortality 6.66 and 10% at 7.5 and 10% concentration respectively. However mortality was not observed with other extracts at low concentrations.

Table 1: Screening for Insecticidal activity of leaf extract

Test insects	7.5% extract		10% extract		Control (Methanol)	
	Mortality %	Pupation %	Mortality %	Pupation %	Mortality %	Pupation %
Plutella xylostella	0	40	23.33	20	0	0
Spodoptera litura	20	40	30	40	0	0
Helicoverpa armigera	16.66	0	20	0	0	0

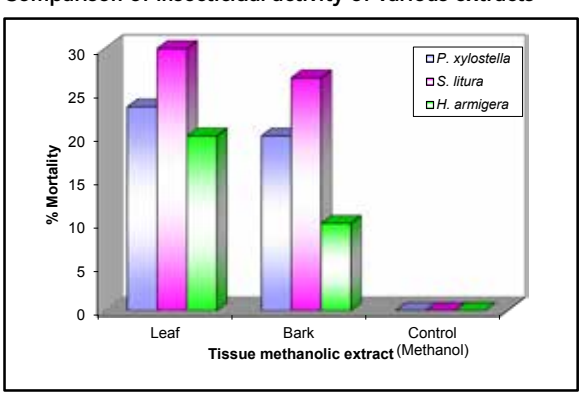
Each value represents the mean of three replicates, each set up with 10 adults (n = 30).

Table 2: Screening for Insecticidal activity of bark extract

Test insects	7.5% extract		10% extract		Control (Methanol)	
	Mortality %	Pupation %	Mortality %	Pupation %	Mortality %	Pupation %
Plutella xylostella	0	40	20	20	0	0
Spodoptera litura	20	40	26.66	20	0	0
Helicoverpa armigera	6.66	0	10	0	0	0

Each value represents the mean of three replicates, each set up with 10 adults (n = 30).

Graph
Comparison of insecticidal activity of various extracts



Insect bioassay of solvent extracted fractions
The chloroform (Fraction 2) and aqueous basic fraction (Fraction 3) of leaf and bark methanol extract were tested for insecticidal activity against *Plutella xylostella* using leaf disc bioassay method. Methanol was used as control. The chloroform (Fraction 2) of leaf and bark extract was found to have better insecticidal activity against *Plutella xylostella* (40%). Similarly the aqueous acid (Fraction 3) of leaf and bark extract was also found to have some mortality indicating that the bioactive molecules are distributed between the two fractions.

Table 3
Insect bioassay of fractions at 10% against *Plutella xylostella*

S.N.	Extract	% Mortality	% Pupation
1	$CHCl_3$-Leaf	40	40
2	$CHCl_3$-Bark	40	20
3	AC-Leaf	20	40

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4	AC-Bark	10	60
5	Control (Methanol) (DMSO)	0	0

$CHCl_3$: Chloroform fraction

AC: Aqueous acid fraction

DISCUSSION

The insecticidal activity of leaf and bark has been reported in literature (Arivudainambi and Baskaran 2004). 20-30 % mortality was observed with leaf and bark extract. The observed mortality may be due to starvation as a result of repellency which was observed at the higher concentration of leaf extracts tested. The difference in the observed activity may also be due to variation in the plant and place of collection. Although the work on efficacy of *C. collinus* bark extract against *P. xylostella* was not available, 20% mortality was observed with bark extract. In contrast to bioactivity against *P. xylostella* where larval mortality was not much more intense in case of leaf and bark extract, the results of bioassay against *S. litura* were not showing the same trend. On the contrarily, the larval mortality was higher in case of leaf extract (30%). The strong non feeding tendency on leaf extract treated cotton leaf was marked. The size and weight was found to be reduced especially with leaf extract.

All the extracts were found to have some insecticidal activity against all the insects tested. Therefore it was decided to fractionate these extracts with solvent extraction and proceed for further insect bioassay. The chloroform (Fraction 2) and aqueous basic fraction (Fraction 3) of leaf and bark methanol extract were tested for insecticidal activity against *Plutella xylostella* using leaf disc bioassay method. The chloroform (Fraction 2) of leaf and bark extract was found to have better insecticidal activity against *Plutella xylostella* (40%). Similarly the aqueous acid (Fraction 3) of leaf and bark extract was also found to have some mortality indicating that the bioactive molecules are distributed between the two fractions.

CONCLUSION

The *C. collinus* plant extract was not used earlier by the other workers although whole leaves application have been used in paddy recently. The information on effective solvent to extract the active ingredient at its maximum, so as to use it against insect pest is lacking. Both leaf and bark extract showed some insecticidal activity against insects *Plutella xylostella*, *Helicoverpa armigera, Spodoptera litura.* Leaf extract showed more insecticidal activity (20-30%) compared to bark extract. When leaves and bark extract were partially purified using solvent extraction procedure, chloroform fraction of both leaf and bark extracts was found to have insecticidal activity against *Plutella xylostella* (40%).

The present study has exploited the probability of having any insecticidal molecules present in the *Cleistanthus collinus.* It was observed that Garadi can be a good source to control agricultural pests and diseases. Such studies are important for exploration of new biomolecules to be used by pharmaceutical and agrochemical industry directly or can be used as a lead molecule to synthesize more potent molecules.

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