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Full Length Research Paper

Phytochemical profile and evaluation of insecticidal efficacy of *Calotropis procera* against defoliators

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Calotropis procera **belonging to family Asclepiadaceae, is one of the important medicinal plants that is being used since years to treat many ailments. It is used either alone or with other medicines to treat common diseases such as fever, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhea. According to ayurveda, dried whole plant is a good tonic, expectorant, depurative and anthelmintic. In the present study, different extractives of leaves of *C. procera* have been screened for their larvicidal activities against important defoliators (*Clostera cupreata*) of Poplar and (*Plecoptera reflexa*) of Shisham. The 3rd instar larvae of C*. cupreata* and *P. reflexa* were exposed to a wide range of concentrations (0.625 to 2.00%) of LC_{50} value of each extract along with their control. The phytochemical analysis of these extracts suggested the presence of alkaloids, glycosides, saponins, proteins, terpenoids, sterols and flavonoids. The data indicate that leaf extracts of *C. procera* may be utilized as the probable candidates for the development of bioinsecticides to control the population of Poplar and Shisham defoliators as safer and economic alternatives to the synthetic insecticides.**

Key words: *Calotropis procera*, *Clostera cupreata, Plecoptera reflexa,* Asclepiadaceae, Poplar, Shisham, larvicidal, defoliators, phytochemicals.

INTRODUCTION

Forests ecosystem comprise of varied biodiversity and varieties of fauna and flora. These resources are depleting very fast due to the attack of insects, in green standing trees/forest. The insects are the most dominating groups occupying highest position and constitute biggest number in the whole animal kingdom due to their versatility which plays a decisive role in determining the productivity of forest, forest resources, forest products and also affect the growth increment of the tree, at times even lead to the death of the tree. With a greater awareness of hazards associated with the use of synthetic organic insecticides, there has been an urgent need to explore suitable alternative products for pest control. Screening of plant extracts for deleterious effect on insects is one of the approaches in the search of novel biological insecticides (Ismam, 1995).

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Insecticidal activity of many plants against several insects has been demonstrated (Singh and Jain, 1987; Carlini and Grossi, 2002). The deleterious effect of plant extracts or pure natural/synthetic compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant, growth inhibitor, suppression of reproductive behavior and reduction of fecundity and fertility. *Calotropis procera* (family: Asclepiadaceae) commonly known as Madar or Aak, is a shrub widely distributed as a weed throughout India (in more or less warm dry places, predominantly in Sub-Himalayan tracts, Bihar, Orissa, West Bengal, Assam, Punjab, Sind, Rajasthan, Deccan to Kanya-Kumari), West Africa, Asia and other parts of the tropics. The plant is erect, branched and perennial with milky latex. A large quantity of latex can be easily collected from its green parts (Irvine, 1961). Local people use it successfully to combat some cutaneous fungal infections. The abundance of latex (containing alkaloids) in the green parts of the plant reinforces the idea that it produced and accumulated latex as a defense strategy against organisms such as virus, fungi and insects

Table 1. Yield (%) of leaves of *Calotropis procera.*

S/N	Solvents	Yield (%) of extracts
1.	Petroleum ether	6.40
2.	Acetone	3.84
3.	Methanol	2.24
4.	Distilled water	17.93

Table 2. Qualitative analysis of leaves of *C. procera.*

S/N	Plant constituents	Petroleum ether extract	Methanol extract	Aqueous extract
1.	Alkaloids	-	+	-
2.	Carbohydrates	-	-	-
3.	Glycosides	+	+	-
4.	Anthraquinones	-	-	-
5.	Saponins	-	+	+
6.	Phenolic compounds and tannins, flavonoids	-	+	-
7.	Sterols	-	+	-
8.	Terpenoids	-	-	-
9.	Phlobatannins	-	-	-

+ Indicates the presence of the constituents and – indicates the absence of the constituents.

(Larhsini et al., 1997). The development of integrated pest management (IPM) has been the most recent method of pest control. IPM is a combination of various methods being utilized in management of insect pests without disruption of the environment. Now-a-days entomologists are trying different new control compatible with IPM concepts without disturbing the ecosystem. These tools include the use of plant extracts, biopesticides, microbial control, pheromones and by genetic manipulation (Steinhous, 1936).

Keeping these facts in view, the present study was undertaken to investigate the larvicidal activities of different extracts of *C. procera* leaves against defoliators (*Clostera cupreata*) of Poplar and (*Plecoptera reflexa*) of Shisham under laboratory conditions as well to assess the chemical nature of the active components present in the extracts.

MATERIALS AND METHODS

Collection of defoliators

The larvae of Poplar defoliator - *C. cupreata* (Notodontidae: Lepidoptera) were collected from Haryana poplar plantations, Thano and Lacchiwala, Dehradun and Shisham defoliator – *P. reflexa* (Noctuidae: Lepidoptera) were collected from the Chichrauli (Haryana). The culture of both defoliators was maintained in the laboratory to lay down different experiments.

Collection of plant material

The leaves (500 g) of *C. procera* were collected from Thano, Dehradun, Uttarakhand. The plant material identified and authenticated from Head, Botany Division, Forest Research Institute (accession no. 162303), Dehradun. Leaves were cleaned and shade dried for 5 to 7 days at 32 to 35°C and re lative humidity at 50 to 60%. The dried leaves were powdered mechanically. The samples were stored in air tight container at room temperature in the dark for further analysis.

Extraction of leaves of *C. procera*

The dried and powdered leaves of *C. procera* were extracted with the solvents of elutropic series, namely petroleum ether, acetone, methanol and distilled water by using Soxhlet apparatus up to 6 h. The extracts were concentrated under reduced pressure and vacuum on rotatory evaporator. The yield percentages were determined on moisture free basis and are being given in Table 1.

Chemicals

All chemicals and solvents used for extraction and sample preparation were of analytical grade.

Phytochemical analysis of extracts

Qualitative phytochemical analysis of leaf extracts of *C. procera* was carried out by standard methods. In brief, the phytochemicals such as tannins, alkaloids, saponins, flavanoids, terpenoids and phenols/polyphenols were qualitatively determined as follows (Table 2):

Phlobatanins

An aqueous extract of leaves was boiled with 1% aqueous

hydrochloric acid to observe the deposition of red precipitate (Raman, 2006).

Flavonoids

20 mg of extract was heated with 10 ml of ethyl acetate over steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and observed a yellow coloration.

Alkaloids

50 mg of extract was stirred with few ml of diluted HCl and filtered. The filtrate was tested carefully with Wagner's reagent and reddish-brown precipitate indicates the presence of alkaloids.

Saponins

50 mg of the extract was shaken with distilled water in a test tube and it was warmed in a water bath and persistent froth indicates the presence of Saponins (Smolenski et al., 1974; Kapoor et al., 1969).

Tannins

50 mg of the extract was dissolved in 5 ml of distilled water and treated with ferric chloride; a blue-black precipitate was taken as evidence for the presence of tannins.

Terpenoids (Salkowski test)

5 ml of the extract was mixed with 2 ml of chloroform and 3 ml of concentrated sulphuric acid was added carefully to form a layer. Reddish- brown coloration at the interface was formed to show positive results for the presence of terpenoids (Harborne, 1973).

Steroids (Trease test)

50 mg of extract was dissolved in 4 ml of chloroform and then added equal volumes of concentrated sulphuric acid to the test tube along the sides of the tube. Appearance of pink color confirmed sterols.

Glycosides (Keller-killiani test)

Extract was stirred with 1 ml glacial acetic acid, after cooling added few drops of ferric chloride solution, contents were transferred to the test tube containing 1 ml of concentrated sulphuric acid. After standing reddish- brown layer acquires bluish green color indicating the presence of glycosides (Khirstova and Tissot, 1995).

Fixed oils and fats (Spot test)

A small quantity of extract was pressed between two filter papers. Oil stain on the paper indicated the presence of fixed oils (Raman, 2006).

Proteins and amino-acids

100 mg of the extract was dissolved in 10 ml of distilled water and filtered through Whatmann no. 1 filter paper and add few drops of Million's reagent in 1 ml of filtrate. A white precipitate indicates the presence of proteins (Raman, 2006).

Preparation of experimental concentration

Stock solution was [prepared by dissolving 1 g extract with different solvents in 100ml of their respective solvents. Further dilution was carried out to make 0.5, 0.25, 1.25, 0.065, 1.5 and 2.0%. The different extract concentrations were used for further study.

Method of treatment

The third instar larvae were tested in each treatment. Ten larvae of each Poplar and Shisham defoliator (3rd instar) were treated at 1% concentration of 2 ml solution with the help of hand sprayer/ automizer. After exposure to the test solution, larvae were released on fresh leaves of Poplar and Shisham in glass chimney cages. Observations were recorded on the mortality of larvae after 24, 48 and 72 h. Control was also maintained simultaneously. Finally, the bioassay of effective extracts, which were found effective at 1% concentration, was carried out. Each experiment was conducted in triplicates along with the control groups.

RESULTS

It is observed from Table 3 that out of four extracts CPPE, CPA, CPM and CPW, two extracts namely CPA and CPM gave 46.67 and 53.33% larval mortality of Shisham defoliator *(P. reflexa)*, respectively at 1% concentration (Table 3). CPPE and CPW were not found effective and provided 20.00 and 10.00% mortality, respectively. In the case of Poplar defoliator (*C. cupreata)* (Table 4), it is observed that out of four extracts CPPE, CPA, CPM and CPW, two extracts CPA and CPM were found effective at 1% concentration and gave 40.00 and 50.00% larval mortality. CPPE and CPW were not found effective and provided 16.67 and 6.67% larval mortality, respectively. Finally, bioassay of effective extracts (CPA and CPM) showing larval mortality at 40% or above were carried out against Poplar and Shisham defoliators with 7 concentrations (0.065, 0.125, 0.25, 0.5, 1.0, 1.5 and 2.0%). Each experiment was conducted in three replications (3rd instars larvae in each) with a control. Stastical analysis was carried out and the results of both the species and two effective extracts are being presented in Table 5. Average and standard deviation was calculated. It is evident from Table 5 that *C. procera* methanol extract has got very high variability and found to be most effective at 1% concentration in case of both the defoliators.

The phytochemical analysis of the extract (Table 2) showed the presence of alkaloids in maximum amount in methanol extract of *C. procera* leaves. The phenolic contents were also found in both the extracts (methanol and aqueous). In addition, the flavonoids and saponins were also found to be present in methanol and aqueous extract of *C. procera.* Carbohydrates, anthraquinones,

Table 3. Larval mortality of Shisham defoliator *(Plecoptera reflexa)* at 1% concentration.

S/N	Chemical extract	Replication	No. of larvae	Mortality after 24 h	Mortality after 48 h	Mortality after 72 h	% Mortality after 72 h	Average (%)	Effect
1.	CPPE	R1	10	2/10	2/10	2/10	20	20.00	Not effective
		R2	10	2/10	2/10	2/10	20		
		R3	10	2/10	2/10	2/10	20		
		Control	10	Nil	Nil	Nil	Nil		
2.	CPA	R1	10	4/10	4/10	4/10	40	46.67	Effective
		R2	10	4/10	4/10	4/10	40		
		R3	10	6/10	6/10	6/10	60		
		Control	10	Nil	Nil	Nil	Nil		
3.	CPM	R1	10	6/10	6/10	6/10	60	53.33	Effective
		R2	10	4/10	4/10	4/10	40		
		R3	10	6/10	6/10	6/10	60		
		Control	10	Nil	Nil	Nil	Nil		
4.	CPW	R1	10	Nil	Nil	Nil	10	10.00	Not effective
		R2	10	Nil	Nil	Nil	Nil		
		R3	10	1/10	1/10	1/10	20		
		Control	10	Nil	Nil	Nil	Nil		

terpenoids and phlotobatannins could not be detected in all the extracts.

DISCUSSION

In the present study, the methanol extract of the leaves of *C. procera* was found quite effective against Shisham defoliator (*P. reflexa)* and poplar defoliator *(C. cupreata)*. The methanol extract drastically affected the pupation and emergence of adults from pupae. Reports on nematicidal (Iqbal et al., 2005), antimicrobial and anti-helminthic (Kew, 1985) activities of *C. procera* extract and its use in the treatment of toothache, cough and subcutaneous diseases (Singh et al., 2005) exist. There is no report at all regarding the LC_{50} for the different extractives of *C. procera* leaf against defoliators in Uttarakhand State. The laboratory study on larvicidal properties of leaf extract of *C. procera* against mosquito larvae is known (Sripongpun, 2008). The results from the present study indicate the larvicidal property of the *C. procera* extract which may be due to the different compounds present in the extract possessing different bioactivities. The alterations in the rate of pupation and adult emergence due to treatment of the plant extracts were dose dependent.

This may be due to the effect of some active ingredients present in the extracts which exhibit potential to cause interference into the normal metabolism of the insects. The phytochemical analysis (Table 2) showed the presence of glycosides only in petroleum ether extract, while the presence of alkaloids, glycosides, saponins, phenolic compounds and tannins, flavonoids and sterols was detected in methanol extract of leaves of *C. procera.* Similarly, the alkaloids reported to

Table 4. Larval mortality of *Clostera cupreata* at 1% concentration.

S/N	Chemical extract	Replication	No. of larvae	Mortality after 24 h	Mortality after 48 h	Mortality after 72 h	Percentage mortality after 72 h	Average (%)	Effect
1	CPPE	R1	10	1/10	1/10	1/10	10	16.67	Not effective
		R2	10	2/10	2/10	2/10	20		
		R3	10	2/10	2/10	2/10	20		
		Control	10	Nil	Nil	Nil	Nil		
2	CPA	R1	10	4/10	4/10	4/10	40	40.00	Effective
		R2	10	3/10	3/10	3/10	30		
		R3	10	5/10	5/10	5/10	50		
		Control	10	Nil	Nil	Nil	Nil		
3	CPM	R1	10	5/10	5/10	5/10	50	50.00	Effective
		R2	10	5/10	5/10	5/10	50		
		R3	10	5/10	5/10	5/10	50		
		Control	10	Nil	Nil	Nil	Nil		
4	CPW	R1	10	1/10	1/10	1/10	10	6.67	Not effective
		R2	10	0/10	0/10	0/10	-		
		R3	10	1/10	1/10	1/10	10		
		Control	10	Nil	Nil	Nil	Nil		

Table 5. Bioassay of effective extracts (CPM and CPA) against the larvae of *Clostera cupreata and Plecoptera reflexa*

Species	Extract	Mortality						
		0.06	**0.125**	**0.25**	**0.50**	**1.00**	**1.50**	**2.00**
PR	CPM	0.00	1.00	1.67	2.33	5.67	6.33	6.67
PR	CPA	0.00	0.67	1.00	2.00	5.67	6.00	6.33
CC	CPM	0.00	0.67	1.00	2.00	5.00	5.67	6.00
CC	CPA	0.00	0.67	1.00	1.67	4.33	5.00	5.67
Average		0.00	0.76	1.18	2.02	5.22	5.80	6.20
Maximum		0.00	1.00	2.00	3.00	7.00	7.00	7.00
Minimum		0.00	0.00	0.00	1.00	4.00	5.00	5.00
S.D.		0.00	0.41	0.53	0.39	**0.85**	0.57	0.53

CPPE - *Calotropis procera* petroleum ether; CPA - *Calotropis procera* acetone; CPM - *Calotropis procera* metanol; CPM - *Calotropis procera* wáter.

be present in the latex of *C. procera* have been shown to contain insecticidal properties (Larhsini et al., 1997). The data obtained from the present study the present study clearly indicate that *C. procera* leaf extracts were quite effective as larvicides for providing a better and excellent alternative for the control of Poplar and Shisham defoliators.

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