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ORIGINAL ARTICLE

Antifeedant and larvicidal activities of Rhein isolated from the flowers of *Cassia fistula* L.

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Abstract Antifeedant and larvicidal activities of rhein (1,8-dihydroxyanthraquinone-3-carboxylic acid) isolated from the ethyl acetate extract of *Cassia fistula* flower were studied against lepidopteron pests *Spodoptera litura* and *Helicoverpa armigera*. Significant antifeedant activity was observed against *H. armigera* (76.13%) at 1000 ppm concentration. Rhein exhibited larvicidal activity against *H. armigera* (67.5), *S. litura* (36.25%) and the LC_{50} values was 606.50 ppm for *H. armigera* and 1192.55 ppm for *S. litura*. The survived larvae produced malformed adults.

1. Introduction

The growing awareness of the hazards of excessive use of pesticides globally has led researchers to search for safer and more environment friendly alternative methods for insect pest control. Therefore, extensive studies are carried out to screen plants as insect growth control agents. Over the last two to three decades, greater attention has been focused on the bioactivity of phytochemicals for their potential as pesticides against phytophagous insects (Padmaja and Rao, 2000). Research on natural products, that could be alternatives to synthetic pesticides and fungicides, for example, plant extracts and essential oils, has greatly increased during recent years (Wilson et al., 1997; Pradhanang et al., 2003; Cohen et al., 2006).

Cassia fistula L., (Leguminosae), a semi-wild Indian Labernum (also known as the Golden Shower), is distributed in various countries including Asia, South Africa, Mexico, China, West Indies, East Africa and Brazil. It is an ornamental tree with beautiful bunches of yellow flowers. The whole plant is used to treat diarrhea; seeds, flowers and fruits are used to treat skin diseases, fever, abdominal pain and leprosy by traditional people (Perry, 1980).

Kaempferol and proanthocyanidin have been isolated from the acetone extract of *C. fistula* flower (Narayanan and Seshadri, 1972). A bianthraquinone glycoside, fistulin together with kaempferol and rhein has been isolated from ethanol extracts of *C. fistula* flowers (Kumar et al., 1966). Besides phenolics and their derivatives, a certain amount of alkaloids have also been reported in *C. fistula* flowers

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(Asseleih et al., 1990); traces of triterpenes have been observed in both flowers and fruits (Guri-Fakim et al., 1994). A diterpene, 3B-hydroxy-17-norpimar-8(9)-en-15-one was isolated from the pods of *C. fistula* (Misra et al., 1996).

Besides its pharmacological uses, its extract is also recommended for pest and disease control (Jaipal et al., 1983; Sharma and Basandrai, 1999; Raja et al., 2000). The search for plants with novel insecticidal constituents has been intensive. Many leads from numerous plant species have been identified, with the most promising belonging to the families of Meliaceae, Rutaceae, Annonaceae, Asteraceae, Labiatae and Piperaceae (Isman, 1995; Jacobson, 1989; Schmutterer, 1992). Higher plants are a rich source of novel natural substances that can be used to develop environmentally safe compounds for insect control (Arnason et al., 1989).Our preliminary evaluation of ethylacetate extract from *C. fistula* flowers showed good antifeedant activity. In the present work we report the separation and identification of rhein from *C. fistula* flowers and its antifeedant and larvicidal effects on insects.

2. Materials and methods

2.1. Plant material

C. fistula flowers were collected from Loyola College Campus, Chennai, India in May 2006. It was authenticated by a plant taxonomist from the Department of Botany, Loyola College, Chennai. A voucher specimen (ERIC-D-73) is deposited at the herbarium of Entomology Research Institute, Loyola College, Chennai.

2.2. Preparation of plant extract

Flowers were collected and shade dried at room temperature and ground in a manual mill. The powder (1 kg) was extracted with 3 L (1:3 w/v) of ethyl acetate for 48 h. The extract was filtered through a Buchner funnel with Whatman number 1 filter paper. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator at 40 °C. The crude extracts were stored at 4 °C until further use.

2.3. Isolation of active compound

The crude ethyl acetate extract (20 g) was subjected to column chromatography over silica gel (200 g-acme's 100–200 mesh) and eluted with hexane followed by the combination of hexane: ethyl acetate ranging from 95:5 to 100. 117 fractions were collected in 200 ml conical flasks. After checking TLC, the fractions were combined into 24 fractions. Fraction 10 showed a crystal that was subjected to crystallographic analysis identified and also reported (Duraipandiyan and Ignacimuthu, 2007). Fraction 18 showed single spot on TLC and yielded 210 mg; this fraction was eluted using hexane: ethyl acetate (55:45) solvent system. The compound was subjected to spectroscopic analysis.

2.4. Spectroscopic analysis

UV spectra were measured with Hitachi-2010 Spectrophotometer in ethanol. IR spectra were taken using Shimadzu by KBr pellet method. NMR studies were performed in AL-300 MHz, JEOL spectrometer. ^{1}H NMR was run at either 300 or 400 MHz and ^{13}C NMR at 75 MHz using the solvent signal as reference. Mass spectrometric studies have been performed in Shimadzu with the temperature of EI method.

2.5. Antifeedant activity

The crude ethyl acetate extract and Rhein were tested for antifeedant activity using leaf disc no choice method (Isman et al., 1997). Different concentrations of crude extracts and compound were prepared by dissolving in acetone and tested against *H. armigera* and *Spodoptera litura*. Fresh cotton leaf discs (*Gossibium hirsutum*) for *H. armigera* and fresh castor leaf discs (*Ricinus communis*) for *S. litura* were used. Leaf discs of 4 cm diameter were punched using cork borer and dipped in 0.625%, 1.25%, 2.50% and 5.0% concentrations of crude extracts and 125, 250, 500 and 1000 ppm of isolated compound. Azadirachtin was used as positive control. Leaf discs treated with acetone and without solvent (Water) were considered as negative control. After air-drying, each leaf disc was placed in petri dish (1.5 × 9 cm) containing wet filter paper to avoid early drying of the leaf disc and single 2 h pre-starved fourth instar larva of *S. litura* and *H. armigera* was introduced into petri dishes containing the respective leaf discs. For each concentration 10 replicates were maintained. Progressive consumption of leaf area by the larva after 24 h feeding was recorded in control and treated discs using Leaf area meter (Delta-T Devices, Serial No. 15736 F 96, UK). Leaf area consumed in plant extract treatment was corrected from the control. The percent antifeedant index was calculated using the formula of Ben Jannet et al. (2000).

$$\text{Antifeedant Index} = \frac{\mathrm{C} - \mathrm{T}}{\mathrm{C} + \mathrm{T}} \times 100$$

where, C and T represent the amount of leaf eaten by the larva on control and treated discs, respectively.

2.6. Larvicidal activity

The larvae that were fed with treated cotton (*H. armigera*) and castor (*S. litura*) leaf disc (different concentrations of compound) for 24 h were continuously maintained on untreated fresh leaves. Diet was changed every 24 h. Larval mortality was recorded after 96 h of treatment. Four replicates were maintained for each treatment with 5 larvae per replicate (total, $n = 20$). Per cent mortality was calculated using the formula of Abbott (1925). At the laboratory conditions were the same as in antifeedant activity study.

$$\text{Abbott's corrected mortality} = \frac{\%\ \text{mortality in treatment} - \%\ \text{mortality in control}}{100 - \%\ \text{mortality in control}} \times 100$$

2.7. Statistical analysis

The data collected were represented as mean ± SD. One-way analysis of variance (ANOVA) and Significant differences between treatments were determined by Tukey's multiple range tests ($P \leqslant 0.05$). LC_{50} value was calculated using Probit Analysis (Finney, 1971).

3. Results

The present study deals with the antifeedant and larvicidal activities of ethyl acetate extract of *C. fistula* flower and a compound rhein isolated from it. The active compound was identified as Rhein (1,8-dihydroxyanthraquinone-2-carboxylic acid) (Fig. 1). The structural identification of compound was carried out by IR, MS, ^{1}H NMR and ^{13}C NMR spectra. ^{1}H NMR and ^{13}C NMR spectral data corresponded to the molecular formula $C_{15}H_8O_6$, which is of rhein (Wei et al., 2003; Liu et al., 2004).

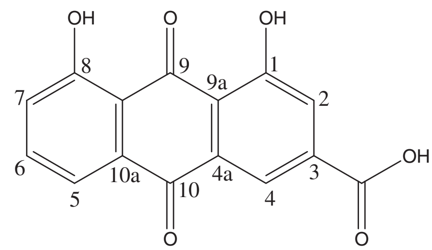

Fig. 1 Rhein (1,8-dihydroxyanthraquinone-3-carboxylic acid) isolated from *C. fistula* flower.

Antifeedant activity of crude extract and isolated compound was tested against two different insect pests and the results are presented in Table 1. The compound rhein showed moderate antifeedant activity (56.79%) against *S. litura* at 1000 ppm, whereas it showed significant activity against *H. armigera* (76.13%) at 1000 ppm. Rhein exhibited larvicidal activity of 67.5 % against *H. armigera* with LC_{50} value of 606.5 ppm and 36.25% against *S. litura* with the LC_{50} value of 1192.55 ppm. The larvae after treatment with compound rhein showed malformation and mortality in larval, pupal and adult stages (Table 2).

4. Discussion

Rhein compound was previously reported from some other plants. Sun et al. (2000) chromatographed and purified emodin, chrysophanol and rhein from *Rheum officinale* extract. Wang et al. (2001) separated and determined active anthraquinone components physcion, chrysophanol, aloe-emodin, emodin, and rhein from the Chinese herb *Polygonum multiflorum*.

Table 1 Percent antifeedant activity of ethyl acetate extract of *Cassia fistula* and compound rhein against *Helicoverpa armigera* and *Spodoptera litura* (mean ± S.D.).

Tested compounds	Concentration (%)	Test insects	
		H. armigera	*S. litura*
Cassia fistula (crude ethylacetate extract)	0.625	33.29 ± 12.92^{e}	26.97 ± 13.20^{c}
	1.25	40.25 ± 4.39^{cde}	29.41 ± 5.62^{c}
	2.5	49.81 ± 5.72^{cd}	32.68 ± 11.17^{c}
	5.0	68.27 ± 13.27^{a}	53.19 ± 4.98^{b}
Rhein (isolated compound)	125 ppm	47.82 ± 7.37^{fgh}	27.28 ± 12.27^{i}
	250 ppm	55.06 ± 5.79^{def}	33.05 ± 13.59^{ghi}
	500 ppm	59.98 ± 5.38^{cde}	40.85 ± 11.20^{f}
	1000 ppm	76.13 ± 13.43^{b}	56.79 ± 7.84^{de}
Azadirachtin (40%) (standard)	125 ppm	53.50 ± 6.34^{efg}	60.12 ± 7.12^{d}
	250 ppm	64.13 ± 3.11^{c}	70.08 ± 5.37^{c}
	500 ppm	75.88 ± 3.99^{b}	78.66 ± 5.44^{b}
	1000 ppm	86.47 ± 1.83^{a}	88.02 ± 3.83^{a}

Values carrying different alphabets in a column are statistically significant by LSD at 5% level.

Table 2 Percent larvicidal activity and LC_{50} values of tested compound rhein against *Helicoverpa armigera* and *Spodoptera litura* (mean ± SD).

Tested compounds	Concentration (%)	Test insects			
		H. armigera		*S. litura*	
		Larvicidal activity	LC_{50}	Larvicidal activity	LC_{50}
Rhein	125	22.5 ± 2.88^{a}	606.50	00.00 ± 00^{a}	1192.55
	250	32.5 ± 8.66^{ab}		15.00 ± 10.0^{b}	
	500	50.0 ± 8.16^{bc}		21.25 ± 2.5^{b}	
	1000	67.5 ± 8.66^{c}		36.25 ± 7.5^{c}	
Azadirachtin (40%)	125	38.75 ± 10.3^{ab}	186.16	52.5 ± 9.5^{d}	127.50
	250	61.25 ± 10.31^{c}		73.75 ± 9.46^{e}	
	500	95.00 ± 10.00^{d}		100.0 ± 0.00^{f}	
	1000	100.0 ± 0.00^{d}		100.0 ± 0.00^{f}	

Within the column, mean followed by the same letter do not differ significantly using Tukey's test, $P \leqslant 0.05$.

Similarly Wei et al. (2003) reported the isolation and purification of rhein from *Rheum officinale*. Kanokmedhakul et al. (2005) have isolated seven anthraquinone and triterpenoids from *Prismatomeris fragrans* with antifungal activity.

Isolated rhein showed significant antifeedant activity against *H. armigera*. Quinones and compounds with aldehyde groups have been reported to be insect antifeedants (Blaney et al., 1987; Morimoto et al., 1999).

Georges et al. (2007) have evaluated anthraquinone (emodin, citreorosein, and emodic acid) from *Cassia nigricans* against *Helicoverpa zea, Heliothis virescens, Bemisia tabacci* (white fly) and *Anopheles gambiaea* (mosquito larvae) and 80% mortality was recorded on *A. gambiaea*. Ethyl acetate extract of *C. fistula* and compound rhein did not show significant activity against *S. litura* but showed good activity against *H. armigera*. Raja et al. (2005) and Pavunraj et al. (2006) found that *Hyptis suaveolens* and *Excoecaria agallocha* possessed antifeedant activity against lepidopteron pests. Antifeedant assays for anthraquinones against carpet beetles significantly inhibited feeding (Morimoto et al., 2002). Rhein showed higher antifeedant activity (76.13%) against *H. armigera* than *S. litura* (56.79%).

The larvicidal activity of rhein showed 67.5% against *H. armigera* and 36.25% against *S. litura* at 1000 ppm concentration. Similar findings were recorded in hexane fractions of *A. monophylla* against *H. armigera* (Baskar et al., 2009).

The present findings show that the isolated compound rhein exhibited a significant antifeedant activity against *H. armigera*. It also has the potential to act as feeding deterrent against *H. armigera*.

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