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Antifeedant activity of a novel 6-(4,7-hydroxy-heptyl) quinone® from the leaves of the milkweed *Pergularia daemia* on the cotton bollworm *Helicoverpa armigera* (Hub.) and the tobacco armyworm *Spodoptera litura* (Fab.)

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Abstract The effects of crude extracts and an isolated compound from the leaves of milkweed, *Pergularia daemia* (Forssk) Choiv., on the antifeedant activity against two important lepidopteran pests, *Helicoverpa armigera* (Hub.) and *Spodoptera litura* (F.), were studied. Maximum antifeedant activity was recorded in ethyl acetate crude extract against *H. armigera* (70.3%) and *S. litura* (71.82%) at 1% concentration. Ethyl acetate crude extract was further subjected to column chromatography, which was performed using hexane as initial solvent and then by increasing the polar strength using ethyl acetate. Fractions collected at hexane and ethyl acetate (80:20) yielded 6-(4,7-hydroxy-heptyl) quinone, a novel compound which showed significant antifeedant activity against *H. armigera* (80.22% at 2000 ppm) and *S. litura* (68.31% at 2000 ppm).

Keywords Botanical pesticides · Medicinal plants · Plant-derived compounds

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Introduction

Chemical pesticides play a significant role in increasing agricultural production by controlling the insect pests. However, the chronic effects of chemical pesticides on living organisms and the environment prompt us to restrict the use of many pesticides (Isman et al. 1990; Katyal and Satake 1996). The threats posed by chemical pesticides demand an urgent search for an environmentally safer alternative method of crop protection. The injudicious use of synthetic pesticides can lead to secondary outbreaks of pests that are normally under natural control, resulting in their rapid proliferation. There have also been cases of pests becoming tolerant to insecticides, resulting in the use of double and triple application rates (Stoll 1988). In addition, problems such as health hazards, undesirable side effects and environmental pollution are caused by the continuous use of synthetic chemical pesticides. There is renewed interest in the application of botanical pesticides for crop protection (Nas 2004). Scientists are now experimenting and working to protect insect infestation by indigenous plant materials. The use of locally available plants in the control of pests is an ancient technology in many parts of the world (Roy et al. 2005). Most of these botanical pesticides are non-selective poisons that target a broad range of pests. Especially, the secondary metabolites of several plants

are found to be effective alternatives to synthetic pesticides (Leatemia and Isman 2004). The mode of action of these secondary metabolites has been studied in detail by many investigators. The action of the plant-derived compounds on pest insects is exerted through many ways such as antifeedant (Raja et al. 2005), larvicidal (Kabaru and Gichia 2001), ovicidal and oviposition deterrent activities (Pavunraj et al. 2006), repellent (Schmutterer 1995) and others. Some botanicals have an effect on juvenile hormone and ecdysone actions, but not on the hormones themselves (Williams et al. 1986). They also have substances that disrupt insect growth by antagonizing juvenile hormone action (Bowers et al. 1976).

The cotton bollworm *H. armigera* (Hübner) (Lepidoptera: Noctuidae) is a polyphagous pest of worldwide occurrence inflicting crop damage in India to the sum of one billion dollars annually (Subramaniyan and Mohankumar 2006). In India, this insect occurs as a major pest in many economically important crops including cotton, pigeonpea, chickpea, tomato, okra, and blackgram. The tobacco armyworm *S. litura* (Fabricius) (Lepidoptera: Noctuidae) was once recognized as a major pest of tobacco alone and now it has become a serious pest also of tomato (Alam 1969), cotton (Moussa et al. 1960), castor (Macleod et al. 1990) and bitterguard (Yasui et al. 1998). Recently, an outbreak of this pest was noticed in Kancheepuram District of Tamil Nadu, India, on brinjal, which is not a host plant of this pest.

The milkweed *Pergularia daemia* (Forssk.) Choiv. (Gentianales: Asclepiadaceae) is an important medicinal plant. Leaf juice of *P. daemia* is applied to treat rheumatic swelling and also used in the preparation of purgative medicinal oil given for rheumatism, amenorrhoea and dysmenorrhoea; the root bark is also used as a purgative in rheumatic cases (Wealth of India 1966). The root is used to treat mental disorders, anemia, leprosy, piles, and uterine and menstrual disorders (Yoganarasimhan 2000). The entire plant is used for pulmonary afflictions, asthma, biliousness, cough, piles, leprosy and syphilis, and leaves are used in infantile diarrhea, and as an expectorant, uterine tonic and emetic (Mohammed et al. 2004). The leaf aqueous extract of *P. daemia* showed some promising larvicidal activity against *H. armigera* (Ramya and Jayakumararaj 2009). Sakthivadivel and Danial (2008) reported that the petroleum crude extracts of *P. daemia* leaf exhibited a toxic effect on 4th instar larvae of some vector mosquitoes like *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti*. The present study was carried out with the objective of screening the extracts and a compound from *P. daemia* for their antifeedant activity against *H. armigera* and *S. litura.*

Materials and methods

Plant material The leaves of *P. daemia* were collected during October 2007 from Salem district of Tamil Nadu, India. A voucher specimen (LC/ERI/Herb.205) was deposited in the herbarium of the Entomology Research Institute, Loyola College, Chennai, India.

Extraction of crude extracts The leaves were dried in the shade at room temperature and ground in a hand mill. Two kilograms of plant material was powdered using an electric blender and 500 g leaf powder was soaked in 1.5*l* of n-hexane in an aspirator bottle for 72 h with occasional shaking. Then the content of the aspirator bottle was filtered using Whatman No.1 filter paper and the filtrate was concentrated using a rotary vacuum evaporator at 40°C until further use. The remaining residue was sequentially extracted with chloroform and ethyl acetate.

Isolation of active compounds Ten grams of crude ethyl acetate extract of *P. daemia* leaves were subjected to silica gel (60–120 and 230–400 mesh, Acme Synthetic Chemicals, Maharashtra, India) chromatography and eluted with hexane containing increasing amounts of ethyl acetate. Fractions were monitored by TLC using precoated silica gel plates (Merck silica gel 60 F_{254}, 0.25 mm thick) and the spots were visualized under UV (254 and 366 nm) followed by spraying with Panchal-D (Ceric ammonium sulphate (3.6 g), ammonium hepta molybdate (4.2g), Con. H_2SO_4 (6.2 ml) in 100 ml of water) is a spraying reagent for TLC and vanillin–sulfuric acid spray reagent. The following ratios of mobile phase were used for fractionation: hexane (100%); hexane and ethyl acetate (95:5); hexane and ethyl acetate (90:10); hexane and ethyl acetate (85:15); hexane and ethyl acetate (80:20); hexane and ethyl acetate (75:25). Six fractions were collected and concentrated; fraction five showed a single spot on TLC which was found to be a pure compound.

The structure of the compound was elucidated by spectroscopic methods such as ^{1}H NMR, ^{13}C-NMR and IR-spectrophotometer.

Establishment of insect culture *Helicoverpa armigera* and *S. litura* larvae were collected from vegetable fields in and around Chennai, India. *H. armigera* larvae were fed with artificial diet (distilled water [720 ml], chickpea [200 g], sorbic acid [1 g], L-ascorbic acid [3 g], methyl parabane sodium salt [2 g], streptomycin [0.01 g], formaldehyde [40% in 1 ml], agar [12 g], yeast [30 g] and multivitamin drops) and *S. litura* larvae were fed with castor leaves in the laboratory at 28±1°C, 11±1 h photoperiod and 65–70% r.h. Adults were released into the oviposition chambers for egg laying and provided with 10% honey solution. Cotton and castor leaves were kept inside the cages to facilitate egg laying. Eggs were collected, kept separately and newly hatched larvae were maintained on artificial diet and castor leaves for *H. armigera* and *S. litura*, respectively. Freshly moulted 4th instar larvae were used for the experiments.

Antifeedant activity Antifeedant activity of the crude extracts, fractions and pure compound was studied using the leaf disc no-choice method (Isman et al. 1990). Fresh leaf discs (4-cm diam) of cotton and castor were used for *H. armigera* and *S. litura,* respectively. For antifeedant bioassay, the leaf discs dipped in crude extracts at 1%, in fractions at 1000 ppm and in the isolated compound at 100, 250, 500, 1000 and 2000 ppm concentrations, were screened. Leaf discs treated with acetone were used as a negative control. Leaf discs treated with 1% aqueous neem seed kernel extract (NSKE) and azadirachtin 1000 ppm (40.86% purity, obtained from EID-Parry, India Ltd., Chennai) were used as positive controls for crude extracts and the isolated compound, respectively. Wet filter paper was placed in each petri dish (1.5 cm×9 cm) to avoid early drying of the leaf disc. A single 4th instar larva of *H. armigera* and *S. litura* was introduced individually. Five replicates were maintained for each treatment with five larvae per replicate. The experiment was conducted under laboratory conditions (27±2°C) with 14L:10D photoperiod and 75±5% r.h. Progressive consumption of leaf area by the larva was recorded after 24 h in control and treated discs using a leaf area meter (Delta–T Devices, Serial No. 15736 F 96, UK). The percentage of antifeedant activity was calculated according to the formula of Isman et al. (1990).

$$\text{Antifeedant activity} : = \frac{\text{Leaf area consumed in control} - \text{treated leaf}}{\text{Leaf area consumed in control} + \text{treated leaf}} \times 100$$

Statistical analysis The antifeedant activity data were subjected to one-way analysis of variance. Means with significant variance were separated using Duncan's Multiple Range *F*-test. Statistics were performed using SPSS package 11.5 version.

Results

The antifeedant activity of different crude extracts, different fractions and compounds of *P. daemia* was studied using the leaf disc no-choice method. The crude ethyl acetate extract of *P. daemia* leaves showed antifeedant activity against *H. armigera* and *S. litura*. All crude extracts showed antifeedant activity against 4th instar larvae of *H. armigera* (F-value: 139.95; $P<0.05$) and *S. litura* (F-value: 50.36; $P<0.05$) at 1% concentration with significant differences among solvents used (Table 1). In *H. armigera* and *S. litura*, the antifeedant activities of ethyl acetate extract were recorded as 70.3% and 71.82%, respectively; these were statistically significant ($P<0.05$) compared with

Table 1 Antifeedant activity[z] (mean ± SE) of different solvent crude extracts of *Pergularia daemia* leaves against the larvae of *Helicoverpa armigera* and *Spodoptera litura*

Extracts (1%)	*H. armigera*	*S. litura*
Hexane	27.84±3.7^{b}	19.2±3.3^{b}
Chloroform	54.04±2.6^{c}	48.2±1.4^{c}
Ethyl acetate	70.3±3.8^{d}	71.82±2.3^{d}
Reference (NSKE) for comparison	77.10±2.3^{d}	73.37±3.5^{d}
Reference (acetone) control	4.02±0.03^{a}	3.7±0.3^{a}
F-value	139.95	50.36
Significance (*P*)	<0.05	<0.05

Within columns, means ± SE followed by the same letter do not differ significantly (F-test; $P<0.05$; $n=5$)

[z] Percentage activity calculated as:[(Leaf area consumed in control − treated leaf)/(Leaf area consumed in control + treated leaf)]× 100, after consuming the antifeedant

other solvent extracts. Chloroform extract recorded 54.0% antifeedant activity against *H. armigera* and 48.2% against *S. litura*; hexane extract recorded 27.8% antifeedant activity against *H. armigera* and 19.2% against *S. litura*, suggesting that there was no difference between insect susceptibility among hexane and chloroform extracts. The NSKE aqueous extract treatment showed significant antifeedant activity against *H. armigera* (77.1%) and against *S. litura* (73.3%). There was not much variation in antifeedant activity of ethyl acetate extract and NSKE.

The effective ethyl acetate crude extract was subjected to column chromatography. Among the six fractions obtained, fraction 5 had the highest antifeedant activity against both insects tested (*H. armigera* [75.84%] and *S. litura* [66.33%]) (Table 2). There was no difference between insect susceptibility except for fractions 5 and 6. Fraction 1 showed poor antifeedant activity against both the insects tested. The F-values were 42.90 for *H. armigera* and 25.87 for *S. litura.*

Fraction 5 collected at the mobile phase ratio of 80:20 (hexane: ethyl acetate) was a single compound. The purity was further checked by thin layer chromatography (Merck silica gel 60 F_{254}, 0.25 mm thick) using hexane:ethyl acetate (4:1) mobile phase. When this pure compound was subjected to IR, ^{1}H and ^{13}C NMR spectral analyses, the following results were obtained.

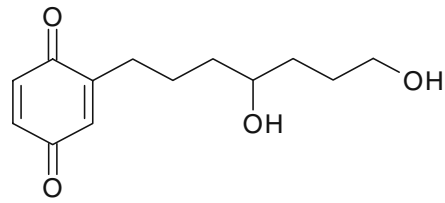

Fig. 1 The new quinone, 6-(4,7-hydroxy-heptyl) cyclohex-2,5-diene-ene-1,4-dione), isolated from *Pergularia daemia* leaves

^{1}H NMR (200 MHZ, $CDCl_3$): δ1.30 (m, 2H): δ1.36-1.70 (m-2H), δ1.44-1.48 (m, 2H); δ1.68-170 (m, 2H); δ2.16 (t, 2H); δ1.3-7. 41 (m, 1H); δ1.60-1.67 (m, 2H); δ 3.14 (broad singlet, 1-0H); δ3.40 (broad singlet 1-OH); δ6.42 (s, 1H); 6.50-6.72 (dd, 2H). ^{13}C NMR other carbon appear at 190 (C-12). The IR spectrum showed values of 546.0; 850.0; 952.10; 1108; 1296.6; 1351.7; 1408.4; 1455.0; 1724.30; 1792.40; 2875.0; 3440.5 (broad signal due to –OH group). The above spectral data confirmed the compound to be 6-(4, 7 hydroxy-heptyl) cyclohex-2-ene-1,4-dione) (Fig. 1).

Investigation of the antifeedant activity with the isolated compound showed a maximum antifeedant activity at 2000 ppm concentration against both *H.*

Table 2 Antifeedant activity[z] (mean ± SE) of different fractions isolated from the ethyl acetate extract of *Pergularia daemia* leaves against *Helicoverpa armigera* and *Spodoptera litura* at 1000 ppm

Fractions[x] (1000 ppm)	*H. armigera*	*S. litura*
1	34.04±2.00^{a}	31.64±0.77^{a}
2	53.02±0.94^{c}	45.82±2.07^{bc}
3	42.14±2.51^{ab}	37.73±0.80^{ab}
4	47.24±1.67^{bc}	51.24±1.34^{c}
5	75.84±1.75^{d}	66.33±2.15^{d}
6	68.33±1.66^{d}	48.73±2.37^{c}
F-value	42.90	25.87
Significance (*P*)	<0.05	<0.05

Within columns, means ± SE followed by a common letter do not differ significantly (F-test; $P<0.05$; $n=5$)

[z] Percentage activity calculated as: [(Leaf area consumed in control − treated leaf)/(Leaf area consumed in control + treated leaf)] × 100, after consuming the antifeedant

[x] 1, hexane (100%); 2, hexane and ethyl acetate (95:5); 3, hexane and ethyl acetate (90:10); 4, hexane and ethyl acetate (85:15); 5, hexane and ethyl acetate (80:20); 6, hexane and ethyl acetate (75:25)

Table 3 Antifeedant activity[z] (mean ± SE) of 6-(4,7-hydroxy-hyptyl) quinone isolated from *Pergularia daemia* against the larvae of *Helicoverpa armigera* and *Spodoptera litura*

Concentrations (ppm)	*H. armigera*	*S. litura*
100	64.04±3.2^{b}	52.18±1.3^{b}
200	67.02±3.0^{bc}	56.42±2.3^{bc}
500	76.13±1.8^{bc}	66.49±3.3^{cd}
1000	78.64±2.2^{bc}	63.71±2.1^{cd}
2000	80.22±2.2^{c}	68.31±2.4^{d}
Reference for comparison (azadirachtin 1000 ppm)	86.33±0.9^{c}	79.88±2.8^{e}
Negative (acetone) control	3.11±0.5^{a}	2.7±0.2^{a}
F-value	47.26	89.60
Significance (*P*)	<0.05	<0.05

Within columns, means ± SE followed by a common letter do not differ significantly (DMRT F-test; $P<0.05$; $n=5$)

[z] Percentage activity calculated as: [(Leaf area consumed in control − treated leaf)/(Leaf area consumed in control + treated leaf)] × 100, after consuming the antifeedant

armigera (80.22%) (F-value 47.26) and *S. litura* (68.31%) (F-value 89.60) (Table 3). Concentrations of 500 and 1000 ppm recorded antifeedant activities of 76.13% and 78.64%, respectively, against *H. armigera* and 66.4% and 63.7%, respectively, against *S. litura* larvae. A comparison with azadirachtin showed antifeedant activity of 86.33% against *H. armigera* and 79.88% against *S. litura* at 1000 ppm concentration.

Discussion

In the present investigation, ethyl acetate extract of *P. daemia* showed antifeedant activity of 70.3% and 71.82% against *H. armigera* and *S. litura*, respectively. Earlier, Jeyasankar et al. (2010) reported that ethyl acetate extract of *Syzygium lineare* showed antifeedant activity of 79.4% against *S. litura* at 5% concentration. Ethyl acetate extract of *Couroupita guianensis* showed promising antifeedant activity of 66.68% and 69.70% against *H. armigera* at 2.5% and 5% concentrations, respectively (Baskar et al. 2010). Bagavan et al. (2009) studied the larvicidal activity of hexane, chloroform and ethyl acetate extracts of *P. daemia* against tick, fluke and mosquitoes. They observed that ethyl acetate extract showed 100% mortality against *Anopheles subpictus* and *Culex tritaeniorhynchus* at 1000 ppm concentration and 76% and 71% mortality against *Haemaphysalis bispinosa* and *Paramphistomum cervi*, respectively, at 2500 ppm concentrations. Earlier, Muthu et al. (2010) reported that ethyl extract of *Atalantia monophylla* exhibited 78.67% antifeedant activity against *Earias vittella*.

In the present investigation, fractions eluted with the hexane:ethyl acetate combination showed antifeedant activity. Fraction 5 showed maximum antifeedant activity of 75.84% and 66.33% against *H. armigera* and *S. litura,* respectively, at 1000 ppm concentration. The results of the study agree with the earlier findings of Jeyasankar et al. (2010) who reported that the fractions eluted with hexane: ethyl acetate exhibited maximum antifeedant activity of 91.58% in fraction 6 at 1000 ppm concentration. Many researchers have also reported a similar type of findings (Caasi-Lit and Morallo-Rejesus 1990; Ignacimuthu et al. 2006; Morimoto et al. 2002; Ulrichs et al. 2008)

In our study, 6-(4,7-hydroxy-heptyl) quinone showed statistically significant antifeedant activity against *H. armigera* and *S. litura* at almost all the concentrations tested. Most of the potent insect antifeedants are quinoline, indole alkaloids, sesquiterpene lactones, diterpenoids, and triterpenoids (Schoonhoven 1982). Morimoto et al. (1999) reported that quinones such as furoquinones, cyperaquinone and scabequinone, isolated from the basal stem of *Cyperus nipponicus* and *C. distans*, exhibited insecticidal properties. Anthraquinone aldehyde isolated from *Galium aparine* showed promising antifeedant activity against polyphagous pests (Morimoto et al. 2002). A new amino-substituted p-benzoquinone isolated from the methanol extract of roots of *Cynanchum wilfordii* (Asclepiadaceae) was also insecticidal in nature (Yeo and Kim 1998). Seven quinone compounds (Physcion, Chrysophanol, Emodin, Ventiloquinone, Ventiloquinone B, Ventiloquinone E and Ventilone B) isolated from *Ventilago madaraspatana* were reported to be insecticidal against *S. litura* and *H. vigintioctopunctata* (Coccinellidae). The antifeedant activity of 6-(4,7-hydroxy-heptyl) quinone could be attributed to the presence of long chain aliphatic residue and of primary and secondary alcoholic functional groups. The long aliphatic chain is responsible for a hydrophobic nature; quinone ring improves the polarity character of the molecule. The presence of quinone moiety with secondary alcohol group and aliphatic side chain in the compound made the food unpalatable, with the result that the feeding rate of the larvae was greatly reduced. The isolated compound, 6-(4,7-hydroxy-heptyl) quinone also caused malformation and mortality in larval, pupal and adult stages. This is the first report on the bioactivity of the newly isolated compound from *P. daemia* and it could possibly be used as a component in biopesticide formulation.

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