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Evaluation of fractions and 5,7-dihydroxy- 4′,6-dimethoxy-flavone from *Clerodendrum phlomidis* Linn. F. against *Helicoverpa armigera* Hub.

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ABSTRACT

Twelve fractions from chloroform extract of Clerodendrum phlomidis *and 5,7-dihydroxy- 4′,6-dimethoxy-flavone (pectolinaringenin) were evaluated against* Helicoverpa armigera. *Maximum antifeedant (89.41%), larvicidal (83.77%) and ovicidal (69.25%) activities were observed in fraction 5. The least LC_{50} value for antifeedant (178.09 ppm) and larvicidal (198.23 ppm) were observed in fraction 5. No adult emergence was recorded in fractions 4-6 at 1000 ppm. The oviposition deterrent activity was 100% in fraction 5 at all the concentrations. Pectolinaringenin recorded maximum antifeedant (74.68%) and larvicidal (81.11%) activities at 100 ppm; it completely prevented the adult emergence of H. armigera at 100 ppm. Maximum ovicidal activity at 100 ppm concentration was 67.95%. The oviposition deterrent activity was 100% in 100 and 50 ppm concentrations.* C. phlomidis *could be effectively used to develop a new formulation to control the economically important pests.*

Key words: Adult emergence, antifeedant, larvicidal, ovicidal, oviposition deterrent, pectolinaringenin

INTRODUCTION

Continuous use of chemical insecticides cause negative impact on the environment, health and development of resistance by the insects and affect the natural enemies. The negative impact of toxic chemical insecticides leads to increase the strict measures on environmental regulation of insecticides resulted in searching an alternative strategy to develop and use the plant-based pesticides for the management of pests by agrochemical companies (Isman 2000). Plant-based pesticides are not accumulated in the food chain as by the synthetic chemicals which are the major concern for the environmental pollution (Malau and James 2008). Further, plant based secondary chemicals are non-toxic, targeted for a wide range of pests and could be potent alternatives to synthetic pesticides (Leatemia and Isman 2004). *Azima tetracantha* Lam. (syn. *Monetia barlerioides* L'Her.) derived friedelin did not shows any toxicity against *Cyprinus carpio* (Baskar et al. 2014). There are different kinds of insect pests which are causing extensive damage to crops and thereby reducing the productivity. David (2008) reported that some plants in nature possess secondary metabolites which act as antifeedants, oviposition deterrents, larvicides and insect growth regulators.

Many plants extracts, their fractions and isolated

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compounds present in them possess various pest control properties against many pests such as antifeedants (Raja et al. 2005), larvicides (Muthu et al. 2012a,b), ovicidal and oviposition deterrent activities (Muthu et al. 2013) and insect growth regulators (Baskar and Ignacimuthu 2012a; Munoz et al. 2013). The different flavonoids in the plants have diverse functions that include providing much of the colour to flowers and fruits, pollinator attraction mechanism, symbiotic relationship with N_2-fixing rhizobia, protection from UV, pathogens and insects, allelopathy and inhibition of auxin transport (Morimoto and Komai 2000; Buer et al. 2010). The presence of various groups of flavonoids such as flavones, flavonols, flavanones, anthocyanins and chalcones in the plants play a significant role in insect–plant interactions (Harborne and Grayer 1994; Simmonds 2003; Green et al. 2003). Gahukar (2010) reviewed the allelochemicals from some plants for their pesticide activities. Flavonoids from *Vitex negundo* (Lamiaceae), *Melia azedarach* (Meliaceae) and *Anacardium occidentale* had antifeedant, oviposition deterrent and pesticidal properties. 5,7-dihydroxyflavanone (pinocembrine) from *Teloxys graveolens* (Amaranthaceae) exhibited fasciolicide, ovicide and larvicidal activities against *Ascaridi galli* and *Stomoxys calcitrans* (Camacho et al. 1991).

Helicoverpa armigera Hub. is a polyphagous pest causing extensive damage to numerous crops worldwide. It has the potential to migrate long distance, facultative diapause, and high egg laying capacity, developing resistance to many conventional pesticides (Kranthi et al. 2002; Nimbalkar et al. 2009). It is a cosmopolitan and agronomically important pest affecting more than 300 plants worldwide (Pawar et al. 1986; Rajapakse and Walter 2007). The damage caused by *H. armigera* on cotton and pulses alone is estimated to US$ 300-500 million in India (King 1994). Estimation have revealed that annual loss caused by *H. armigera* alone was estimated at approximately US$ 5 billion on different crops worldwide and India and China spent nearly 50% of the total agricultural pesticides to control *H. armigera* (Lammers and MacLeod 2007).

Clerodendrum phlomidis (Lamiaceae) is a medicinal plant used traditionally for various ailments around the world. Juice of leaves is alterative and given in neglected syphilitic complaints (Shafi et al. 2001). Bitter tonic from the root is used as antidote, analgesic, anti-asthmatic and to treat inflammatory and rheumatic diseases (Katewa et al. 2004). The aim of this work was to evaluate the chloroform extract derived fractions from the leaves of *C. phlomidis* and a compound isolated from it against *H. armigera* for their antifeedant, larvicidal, adult emergence, ovicidal and oviposition deterrent activities since the chloroform crude extract had shown maximum antifeedant and larvicidal activities against *H. armigera* (Vendan et al. 2008) and *Earias vittella* (Muthu et al. 2012b). To the best of our knowledge, this is the first report on the effect of fractions and an isolated compound, pectolinaringenin isolated from *C. phlomidis* were studied against *H. armigera* for various biological activities.

MATERIAL AND METHODS

Extraction, fractionation and isolation of pectolinaringenin

Extraction, fractionation and isolation of pectolinaringenin from *C. phlomidis* were similar as reported by Muthu et al. (2012a).

Identification of Compound

Melting points of the compound were determined by an open capillary method on a heating block apparatus. The compound was subjected to UV-Visible Spectrophotometer (Model - Hitachi 2010), IR spectrum (Perkin-Elmer FT- IR instrument on KBr disc), ^{1}HNMR spectrum (500MHz) and ^{13}C NMR (75MHz) spectrum on a JEOL AL-300 instrument in $CDCl_3$. EI-MS was taken on SHIMADZU instrument at 70ev by the direct inlet method. All the chemicals and solvents used were of analytical grade (Muthu et al. 2012a).

Collection and rearing of *H. armigera*

Larvae of *H. armigera* Hub. were collected from the field in Melakondayar village in Tiruvallur district of Tamil Nadu and the collected larvae were reared individually in a plastic container (vials) and fed regularly with *Abelmoschus esculentus* L. (Malvaceae) till the larvae became pupae under the laboratory conditions (27±2°C and 75±5% relative humidity). Sterilized soil was used for pupation. After pupation, the pupae were collected from the soil and placed inside the cage for the emergence of adults. Cotton soaked with 10% honey solution mixed with a few drops of

multivitamins was provided for adult feeding to increase the fecundity. Potted cotton plant was kept inside the adult emergence cage for egg laying**.** After hatching, the larvae were collected from the cage and fed with standard artificial diet. Laboratory reared insect cultures was used for bioassay.

Antifeedant activity

Antifeedant activity of the chloroform extract fractions of the *C. phlomidis* and pectolinaringenin from fraction 5 was studied using leaf disc no choice method. Fresh cotton leaves were used. Leaf discs of 4.0 cm diameter were punched using cork borer and were dipped individually in 1000, 500, 250 and 125 ppm for fractions and 100, 50, 25, 12.5 ppm for pectolinaringenin. The leaf discs dipped in acetone + Tween 80 were used as negative control since they were used to dissolve the fractions and pectolinaringenin. In each plastic Petri dish (1.5cm x 9.0 cm) wet filter paper was placed to avoid early drying of the tested leaves. Single larva of *H. armigera* was introduced in each treated and control Petri dish. The consumption of leaf disc in the treated and control by *H. armigera* larvae after 24 h of the experiment was measured using Leaf Area Meter (Delta-T Devices, Serial No. 15736 F 96, U.K). Leaf discs consumed by the larvae in the treatment were corrected from the negative control. Five replicates were maintained for each treatment with 10 larvae per replicate (total, n = 50). The experiment was conducted at laboratory condition (27±2°C) with 14:10 h light and dark photoperiod and 75 ± 5% relative humidity (Baskar and Ignacimuthu 2012a)**.** Antifeedant activity was calculated according to the formula of Bentley et al. (1984).

Larvicidal bioassay

Larvicidal activity was studied using leaf disc no choice method**.** Cotton leaf discs (*Gossypium* sp.) were used; they were dipped in different concentrations of fractions and compound as used for the antifeedant bioassay. After 24 h treatment, the larvae were continuously maintained on untreated fresh cotton leaves. Diet was changed every 24 h. Larval mortality was recorded up to 96 h of treatment. The number of larvae, replicates used and laboratory conditions were same as antifeedant experiment. Per cent mortality was calculated using Abbott's formula.

Growth inhibitory activity

The treated larvae that attained pupae at different concentrations were kept in the adult emergence cage. Growth inhibitory activities like larval-pupal intermediates, malformation in adults and pupicidal were observed. The adults emerged with normal condition was recorded and was calculated using the following formula:

Adult emergence = No. of Emerged adults/Number of pupae X 100.

Oviposition deterrent activity

Oviposition deterrent activity was studied according to Raja et al. (2004). The oviposition deterrent activity was studied at the concentrations of 1000, 500, 250, 125, 100, 50, 25 and 12.5 ppm for fractions and compound, respectively. The treated materials were sprayed on fresh cotton plants (cultured in the pots) along with selected controls and placed inside the cage (60 cm X45 cm X 45cm) and covered with mosquito net. Ten pairs of adult *H. armigera* moths were introduced into a cage and were fed with 10% (w/v) sucrose solution with multivitamin drops. After 48 h, the number of eggs laid on the treated and control leaves were recorded and the percentage of oviposition deterrent was calculated. Five replicates were maintained for all the experiments. The oviposition deterrent activity was calculated using the formula of Williams et al. (1986):

$$\text{Oviposition deterrent activity} = \frac{\text{No. of eggs laid in control - No. of eggs laid in treated}}{\text{No. of eggs laid in control}} \times 100$$

Ovicidal activity

Twenty individual eggs of *H. armigera* were separated and dipped in 125, 250, 500 and 1000 ppm concentrations of fractions and 100, 50, 25 and 12.5 ppm of pectolinaringenin. Five replicates were maintained (n=100). Number of eggs hatched in the control and treatments were recorded. The laboratory conditions were the same as in the antifeedant experiment. Per cent ovicidal activity was calculated according to Abbott (1925).

$$\text{Ovicidal Activity} = \frac{\text{\% of eggs hatched in control - \% of eggs hatched in treated}}{\text{100-\% of eggs hatched in control}} \times 100$$

Statistical Analysis

All the biological data were subjected to analysis of variance (ANOVA). Significant differences

between treatments were determined by Tukey's multiple range tests ($p \leq 0.05$). LC_{50} value was calculated using Probit Analysis (Finney 1971). All the data were analysed using SPSS package version 11.5.

RESULTS

Fractions

Antifeedant activity

Antifeedant activity of 12 fractions isolated from the chloroform extract of *C. phlomidis* against *H. armigera* is presented in Table 1. Fraction 5 recorded maximum antifeedant activity of 89.41% followed by fractions 6 and 4 which recorded 86.15 and 85.22%, respectively, at 1000 ppm concentration. Fractions 5, 6 and 4 exhibited statistically similar antifeedant activity in 500 and 1000 ppm concentrations. More than 60% antifeedant activity was observed in fractions 4, 5, 6 and 7 at 1000ppm concentration. Less than 20% antifeedant activity was observed in fraction 2 and 12 at 1000 ppm concentration. Fraction 5 showed the least LC_{50} and LC_{90} values of 178.09 and 946.13 ppm followed by fractions 6 and 4 which showed LC_{50} and LC_{90} values of 236.39 and 273.48 ppm and 1049.46 and 1083.07 ppm, respectively. The highest LC_{50} value was recorded in fraction 12. All the fractions recorded statistically significant Chi-square values except fractions 1, 11 and 12.

Larvicidal activity

Results of the larvicidal activity of different fractions against *H. armigera* and are presented in Table 2. Statistically significant larvicidal activity of 83.77% was recorded in fraction 5 followed by fractions 4 and 6 which recorded 75.55 and 73.55%, respectively at 1000 ppm concentration. Fraction 5 exhibited more than 55% larvicidal activity in all the concentrations except 125 ppm which recorded 38.66%. All the other fractions recorded less than 50% larvicidal activity. Fractions 1, 2, 9, 10 and 11 showed very low larvicidal activity.

Effective concentrations (LC_{50} and LC_{90}) of fractions were evaluated against *H. armigera* showed that fraction 5 had least LC_{50} and LC_{90} values of 198.23 and 1094.23 ppm, respectively followed by fractions 6 and 4, which recorded the LC_{50} values of 417.83 and 480.49 ppm, respectively and the LC_{90} values of 1445 and 1343 ppm, respectively. Chi-square values were statistically significant for all the fractions except fraction 7.

Growth inhibitory activity

Fractions 4, 5 and 6 completely prevented the adult emergence of *H. armigera* at 1000 ppm concentration. Also 500 ppm concentration of fraction 5 showed no adult emergence. Fraction 6 at a concentration of 500 ppm recorded 26.66% adult emergence while concentrations 250 and 125 ppm of fraction 5 showed 31.0 and 36.66% adult emergence, respectively. Less than 50% adult emergence was observed in fractions 7, 8, 9 and 10 at 1000 ppm concentrations and fractions 7, 9 and 10 at 500 ppm concentrations. Irrespective of the concentrations tested, fractions 1, 2, 11 and 12 showed maximum adult emergence (Table 3).

Table 1 - Antifeedant activity (%) with LC_{50} and LC_{90} of different fractions isolated from chloroform extract of *Clerodendrum phlomidis* against *Helicoverpa armigera.*

Fractions	Concentrations (ppm)				Effective concentration (ppm)		X^2
	125	250	500	1000	LC_{50}	LC_{50}	
1	12.26±3.12^{bcd}	20.31±2.92^{bc}	21.38±2.94^{b}	27.40±3.69^{bc}	2136.57	4677.23	18.44
2	7.41±1.34^{ab}	15.18±2.89^{b}	18.66±2.05^{b}	19.32±5.35^{b}	2755.51	5562.21	29.07*
3	15.63±3.28^{cd}	25.62±3.87^{cd}	43.62±5.66^{d}	59.18±1.60^{e}	761.73	1731.19	31.14*
4	34.24±3.45^{et}	52.93±5.00^{t}	68.68±3.46^{t}	85.22±2.77^{t}	273.48	1083.07	30.53*
5	42.10±4.40^{g}	56.12±4.36^{t}	75.85±2.70^{t}	89.41±5.13^{t}	178.09	946.13	35.17*
6	37.38±2.65^{tg}	53.60±5.36^{t}	71.45±3.26^{t}	86.15±3.15^{t}	236.39	1049.46	29.59*
7	27.78±4.45^{e}	37.54±4.41^{e}	59.78±2.04^{e}	63.29±4.58^{e}	541.92	1799.57	50.43*
8	18.78±4.65^{d}	31.23±3.57^{de}	36.40±3.74^{cd}	43.80±4.37^{d}	1148.72	3029.64	29.32*
9	17.38±2.05^{d}	25.67±3.88^{cd}	33.52±4.87^{c}	36.18±5.92^{cd}	1507.45	3773.80	29.10*
10	13.95±2.83^{bcd}	22.51±5.74^{bcd}	33.02±1.24^{c}	39.28±5.45^{d}	1243.81	2834.54	33.32*
11	15.23±1.07^{cd}	18.43±3.04^{bc}	23.99±4.11^{b}	27.20±4.08^{bc}	2280.88	5155.99	13.39
12	9.89±1.93^{bc}	14.00±3.84^{b}	16.64±3.36^{b}	18.43±3.68^{b}	3372.13	6898.15	17.94
Control	2.72±0.64^{a}						

Values represent Mean ± SD of five replicates; Similar alphabets in a column do not differ significantly using Tukey's test ($P \leq 0.05$); * Chi-square values are significant at $P \leq 0.05$.

Table 2 - Larvicidal activity (%) with LC_{50} and LC_{90} of different fractions isolated from chloroform extract of *Clerodendrum phlomidis* against *Helicoverpa armigera.*

Fractions	Concentrations (ppm)				Lethal concentration (ppm)		χ^2
	125	250	500	1000	LC_{50}	LC_{90}	
1	6.22±5.69^{ab}	10.22±0.49^{b}	14.22±5.29^{bc}	18.44±4.81^{bc}	2387.93	4460.89	39.20*
2	00±00^{a}	00±00^{a}	6.22±5.69^{ab}	10.22±0.49^{b}	1817.01	2700.91	79.58*
3	14.22±5.29^{cd}	20.44±0.99^{cd}	40.88±1.98^{d}	49.11±5.63^{e}	930.73	2060.39	44.11*
4	22.44±4.33^{e}	42.88±4.41^{e}	55.11±5.01^{e}	75.55±5.15^{fg}	480.49	1343.93	43.66*
5	38.66±2.98^{g}	55.11±5.15^{f}	75.77±8.51^{f}	83.77±5.18^{g}	198.23	1094.23	64.17*
6	30.66±1.49^{f}	42.88±4.41^{e}	61.33±2.98^{e}	73.55±4.93^{f}	417.83	1445.05	29.55*
7	18.22±3.97^{de}	26.44±4.93^{d}	38.66±2.98^{d}	46.88±4.54^{e}	1012.38	2495.13	27.41
8	8.22±4.62^{bc}	14.22±5.29^{bc}	20.44±0.99^{c}	28.44±3.47^{d}	1648.17	3229.83	29.14*
9	00±00^{a}	10.22±0.49^{b}	14.22±5.29^{bc}	22.44±4.33^{cd}	1609.25	2755.03	61.03*
10	00±00^{a}	00±00^{a}	6.22±5.69^{ab}	14.22±5.29^{bc}	1583.93	2327.70	77.49*
11	00±00^{a}	8.22±4.62^{b}	14.22±5.29^{bc}	20.44±0.99^{cd}	1656.66	2799.79	70.32*
12	00±00^{a}	00±00^{a}	00±00^{a}	00±00^{a}	-	-	-

Table 3 - Percentage of adult emergence of *Helicoverpa armigera* treated with different fractions isolated from chloroform extract of *Clerodendrum phlomidis.*

Fractions	Concentrations (ppm)			
	125	250	500	1000
1	91.55±4.75^{de}	88.61±0.62^{f}	83.33±6.44^{f}	75.47±6.76^{c}
2	100±00^{e}	100±00^{g}	89.05±1.03^{f}	84.16±5.85^{c}
3	83.61±5.32^{d}	74.28±1.59^{e}	69.33±5.96^{e}	44.00±5.47^{b}
4	58.21±9.07^{bc}	50.66±10.90^{bcd}	46.00±5.47^{cd}	00±00^{a}
5	36.66±7.45^{a}	31.00±8.21^{a}	00±00^{a}	00±00^{a}
6	52.85±6.38^{b}	46.00±5.47^{b}	26.66±3.72^{b}	00±00^{a}
7	52.50±5.59^{b}	47.14±6.38^{bc}	40.00±9.12^{c}	38.66±2.98^{b}
8	64.50±3.09^{c}	59.72±3.80^{d}	51.42±3.19^{d}	45.71±6.38^{b}
9	59.11±1.98^{bc}	56.94±3.10^{cd}	47.77±3.04^{cd}	42.5±8.50^{b}
10	57.11±4.41^{bc}	51.11±2.48^{bcd}	43.77±4.12^{cd}	38.33±6.84^{b}
11	89.77±0.49^{de}	88.83±0.88^{f}	85.83±4.54^{f}	82.14±6.56^{c}
12	100±00^{e}	97.77±4.96^{fg}	89.77±0.49^{f}	83.77±5.18^{c}
Control	100±00^{e}	100±00^{g}	100±00^{g}	100±00^{d}

Values represent Mean ± SD of five replicates; Similar alphabets in a column do not differ significantly using Tukey's test (P≤0.05).

Ovicidal activity

Maximum ovicidal activity was noticed in fraction 5 at 1000 and 500 ppm concentrations which recorded 69.25 and 61.76%, respectively followed by fractions 4 and 6 against *H. armigera*. All the fractions had notable amount of ovicidal activity in a dose dependent manner. Among the fractions, fraction 9 showed poor ovicidal activity (Table 4).

Table 4 - Ovicidal activity (%) of different fractions isolated from chloroform extract of *Clerodendrum phlomidis* against *Helicoverpa armigera.*

Fractions	Concentrations (ppm)			
	125	250	500	1000
1	15.87±3.17^{bcd}	24.41±2.07^{def}	36.12±3.59^{efg}	43.56±3.21^{cde}
2	12.64±4.41^{abc}	21.19±3.00^{bcde}	30.68±5.86^{bcde}	36.01±5.67^{bc}
3	19.02±5.62^{cde}	28.80±3.53^{ef}	32.95±5.50^{def}	42.56±3.42^{cd}
4	25.41±4.96^{ef}	31.90±3.43^{f}	42.45±5.12^{g}	52.05±5.80^{e}
5	31.90±3.43^{f}	41.45±3.55^{g}	61.76±3.33^{h}	69.25±3.04^{f}
6	22.25±3.76^{de}	28.63±3.90^{ef}	40.40±4.03^{fg}	49.05±4.03^{de}
7	19.14±2.79^{cde}	23.47±6.51^{cde}	34.01±4.40^{defg}	45.61±5.64^{de}
8	8.43±4.43^{ab}	13.81±2.80^{ab}	21.19±4.78^{ab}	30.85±2.08^{ab}
9	7.38±2.64^{a}	11.76±4.63^{a}	18.14±3.26^{a}	24.47±2.80^{a}
10	13.87±4.82^{abc}	18.09±2.88^{abcd}	23.42±2.92^{abc}	35.07±1.75^{bc}
11	15.98±0.70^{bcd}	20.19±2.12^{bcd}	31.90±3.43^{cdef}	43.56±3.21^{cde}
12	9.54±2.26^{ab}	15.87±4.89^{abc}	25.41±4.96^{abcd}	36.12±3.59^{bc}

Values represent Mean ± SD of five replicates; Similar alphabets in a column do not differ significantly using Tukey's test (P≤0.05).

Oviposition deterrent activity

Oviposition deterrent activity of different fractions against *H. armigera* is presented in Table 5. Fraction 5 at all the tested concentrations exhibited 100% oviposition deterrent activity followed by fraction 4 at 1000 and 500 ppm concentrations and fraction 6 at 1000 ppm. Fractions 1 to 8 at all the concentrations revealed notable amount of oviposition deterrent activity in a dose dependent manner. Fractions 9-12 showed poor oviposition deterrent activity compared to other fractions. In control, oviposition deterrent activity was observed as 11.23%.

Table 5 - Oviposition deterrent activity of chloroform fractions of *Clerodendrum phlomoides* against *Helicoverpa armigera.*

Fractions	Concentrations (ppm)			
	125	**250**	**500**	**1000**
1	55.26 ± 4.13^{fg}	67.25 ± 3.60^{d}	74.11 ± 4.38^{cd}	88.24 ± 1.40^{ef}
2	51.43 ± 4.61^{ef}	69.57 ± 5.95^{de}	85.21 ± 3.29^{e}	89.33 ± 1.57^{ef}
3	64.09 ± 3.35^{hi}	79.67 ± 3.56^{f}	93.40 ± 1.20^{f}	96.18 ± 1.64^{gh}
4	85.22 ± 1.78^{j}	92.58 ± 1.13^{g}	100.0 ± 00^{f}	100.0 ± 00^{h}
5	100.0 ± 00^{k}	100.0 ± 00^{g}	100.0 ± 00^{f}	100.0 ± 00^{h}
6	71.40 ± 3.38^{i}	78.04 ± 2.39^{ef}	96.44 ± 1.26^{f}	100.0 ± 00^{h}
7	67.42 ± 3.51^{hi}	71.65 ± 3.68^{def}	78.57 ± 4.84^{de}	91.80 ± 2.03^{fg}
8	62.62 ± 5.11^{gh}	72.77 ± 3.42^{def}	79.48 ± 3.74^{de}	85.21 ± 0.50^{e}
9	38.81 ± 1.59^{cd}	43.16 ± 6.38^{b}	54.15 ± 4.03^{b}	63.04 ± 6.33^{bc}
10	33.37 ± 4.74^{bc}	38.34 ± 4.32^{b}	47.23 ± 5.38^{b}	56.88 ± 4.75^{b}
11	44.24 ± 5.30^{de}	51.78 ± 4.48^{c}	69.15 ± 4.09^{c}	74.48 ± 1.77^{d}
12	29.21 ± 3.84^{b}	37.48 ± 2.44^{b}	51.28 ± 2.20^{b}	65.88 ± 3.60^{c}
Control			11.23 ± 3.95^{a}	

Values represent Mean ± SD of five replicates; Similar alphabets in a column do not differ significantly using Tukey's test (P≤0.05).

Isolation and characterization of the compound

The fraction eluted with the solvent hexane: ethyl acetate (1:1) gave a compound as pale yellow crystal crystallized from methanol (melting point 210°C, Lit. mp 210 – 212°C). The yield was 850 mg. It gave positive ferric reaction by giving green colour with alcoholic ferric chloride for phenol. It also answered Shinoda test for flavonoid by giving reddish pink colour with mg/HCl. TLC over silica gel G with Chloroform: Ethyl acetate (9:1) as the developing system gave a single spot, pale yellow turning dark yellow, on exposure to ammonia (Rf = 0.35). The purity of the compound was (98.2%) checked using HPLC. Spectral analyses were performed using UV, IR, ^{1}HNMR, ^{13}CNMR and EI-MS.

The ^{1}HNMR spectrum showed the absence of substituent at C-3, H-3 appearing as singlet at δ = 6.56. The lone aromatic proton H-8 appeared as singlet at δ = 6.54. The A_2B_2 system in ring B was shown by two sets of ortho coupled protons at δ 7.85 and 7.03 (J=9.0 Hz) corresponding to H-2′ , H-6′ and H-3′ and H-5′. 4′-OMe appeared as three proton singlet at δ - 3.94 and 6-OMe appeared at 3.90. 7-OH appeared as a broad at singlet δ 9.39 and the chelated 5-OH appeared downfield at δ 12.96.

The ^{13}CNMR also confirmed the structure of the compound as pectolinaringenin in the present investigation. The flavonoid carbonyl appeared slightly downfield at δ = 182.33. This suggested the unsubstituted C-3 with 5-OH. The single peak at δ 93.09 was assigned to C-8 and the absence of slightly downfield peak around δ 97.0 found for C-6 in 5,7-oxygenated flavones showed that C-6 was substituted. C-6 OMe appeared at δ 131.08. C-5 attached to chelated OH appeared at δ 152.72. The δ_c values in the ring B corresponded to pectolinaringenin.

The EI-MS gave M^+, the molecular ion at m/z 314 corresponded to the molecular formula $C_{17}H_{14}O_6$. The peak at m/z 296 corresponded to $[M\text{-}H_2O]^+$. The above data confirmed the structure as pectolinaringenin (Fig 1). The molecular formula is $C_{17}H_{14}O_6$.

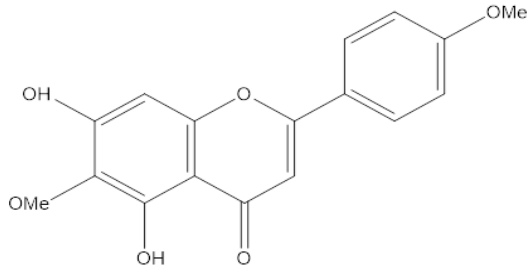

Figure 1 - Pectolinaringenin (5,7-dihydroxy- 4′,6-dimethoxy-flavone).

Bioeffficacy of pectolinaringenin

Antifeedant activity

Pectolinaringenin exhibited 74.68% antifeedant activity against *H. armigera* at 100 ppm concentration followed by 50 ppm, which was 66.89%. The LC_{50} and LC_{90} values were 33.95 and 140.44 ppm, respectively. Significant chi-square value was shown in Table 6.

Larvicidal activity

Maximum larvicidal activity of 81.11% was observed in pectolinaringenin against *H. armigera* at 100 ppm concentration followed by 50 ppm concentration, which was 68.22%. The LC_{50} and LC_{90} values were 27.31 and 122.72 ppm, respectively. The Chi-square value was significant (Table 6).

Growth inhibitory activity

Pectolinaringenin at 100 ppm concentration completely prevented the adult emergence of *H. armigera;* 50 ppm resulted 35% adult emergence. In the case of control, the adult emergence was observed as 95.55% (Table 6).

Table 6 - Percent antifeedant, larvicidal, adult emergence, ovicidal and oviposition deterrent activities of pectolinaringenin against *H. armigera.*

Concentrations (ppm)	Antifeedant activity (%)	Larvicidal (%)	Adult emergence (%)	Ovicidal (%)	Oviposition deterrent (%)
12.5	32.50±3.92^{b}	38.22±4.81^{a}	52.00 ±4.47^{c}	36.37±2.13^{b}	84.8±3.41^{b}
25	48.12±2.97^{c}	49.11±6.86^{a}	46.00±5.47^{bc}	44.09±4.94^{c}	86.84±4.00^{b}
50	66.89±3.71^{d}	68.22±6.45^{b}	35.00±9.12^{b}	56.67±4.23^{d}	100±00^{c}
100	74.68±5.02^{e}	81.11±8.01^{c}	00.00±0.00^{a}	67.95±4.82^{e}	100±00^{c}
Control	4.04±2.89^{a}	-	95.55±6.08^{d}	0.0^{a}	7.63±1.88^{a}
LC_{50}	33.95	27.31	-	-	-
LC_{90}	140.44	122.72	-	-	-
χ^2	46.81*	50.72*	-	-	-

Values represent Mean ± SD of five replicates; Similar alphabets in a column do not differ significantly using Tukey's test (P≤0.05); * Chi-square values are significant at (P <0.05)

Ovicidal activity

Pectolinaringenin at 100 ppm exhibited 67.95% ovicidal activity against *H. armigera* followed by 50 ppm concentration, which was 56.67% (Table 6).

Oviposition deterrent activity

The oviposition deterrent activity was 100% at 100 and 50 ppm concentrations against *H. armigera*. More than 84.8% oviposition deterrent activity was observed at 25 and 12.5% concentrations (Table 6).

DISCUSSION

Antifeedant activity

Fractions eluted with hexane: ethyl acetate (1:1) of chloroform extract of *C. phlomidis* recorded maximum feeding protection against *H. armigera* at 1000 ppm concentration. The result was in agreement with Baskar et al. (2010) who reported that hexane extract and effective fraction 8 from *Couroupita guianensis* exhibited more than 80% antifeedant activity against *H. armigera*.

Pectolinaringenin at 100 ppm concentration showed maximum antifeedant activity of 74.68% followed by 50 ppm with the LC_{50} and LC_{90} values of 33.95 and 140.44 ppm, respectively. This findings agreed with the report of Thoison et al. (2004) who isolated several triterpenes and flavonoids from *Nothofagus dombeyi* and *N. pumilio* using bioassay-guided fractionation and observed that pectolinarigenin and 12-hydroxyoleanolic lactone from *N. dombeyi* and dihydrooroxylin A from *N. pumilio* exhibited feeding deterrent to *Ctenopsteustis obliquana* larvae. Kumari et al. (2003) studied the antifeedant activity of six *neo*-clerodane diterpenoides isolated from *Clerodendrum* species against *E. vittella* and *S. litura*; they observed that all the compounds were exhibited more than 65% antifeedant activity at 10 µg/cm^2. Akhtar and Isman (2004) evaluated antifeedant effects of digitoxin, cymarin, xanthotoxin, toosendanin, thymol and trans-anethole against *Trichoplusia ni, Pseudaletia unipuncta, Plutella xylostella* and *Epilachna varivestis* and they found that xanthotoxin was most effective deterrent for *T. ni*, whereas thymol acted as good antifeedant against *P. unipuncta, P.*

xylostella and *E. varivestis*.

Pectilonaringenin isolated from *C. phlomidis* exhibited strong antifeedant activity against *H. armigera* at 100 ppm concentration with least LC_{50} and LC_{90} values. The present findings agreed with the reports of Singh et al. (2011), who evaluated eight essential oils and some compounds against *Chilo partellus* for their antifeedancy. They observed that thymol and 1,8-Cineole exhibited least LC_{50} values of 141.8 and 148.2 ppm, respectively. Similarly, Morimoto et al. (2000; 2003) reported the antifeedant activity of rotenone, flavone, and 5-hydroxy-3,6,7,8,40-pentamethoxyflavone and recorded the LC_{50} values of 59.1 and 24.6 l and 42.8 mg/cm^2, respectively against *S. litura.*

Proportionately little quantity of pectolinaringenin detected by HPLC was isolated from chloroform extract of *C. phlomidis* showed high antifeedant activity against *H. armigera* in the present study. This finding corroborated the earlier findings of Morimoto and Komai (2006) who isolated four flavonoids namely, 5-hydroxy-3,6,7,8,4‘-pentamethoxyflavone, 5-hydroxy-3,6,7,8-tetramethoxyflavone, 5,6-dihydroxy-3,7-dimethoxy flavone, and 4,4‘,6‘-trihydroxy-2‘-methoxychalcone from cudweed *Gnaphalium affine* and were screened against *S. litura* for feeding deterrent activity. Except 4,4‘,6‘-trihydroxy-2‘-methoxychalcone, all the other flavonoids showed strong antifeedant activity and were detected in small quantity by HPLC.

Positions of different groups in the structure decide the activity of the compound. In this study the presence of hydroxyl group at 5th and 7th position in A-ring and 4-pyran ring (a carbonyl in C-ring) contributed towards strong antifeedant activity. Methyoxyl group at C-4′ in the B-ring contributed moderate antifeedant activity (Fig. 1). Feeding deterrency of pectolinaringenin in the present study agreed with the findings of Ohmura et al. (2000) who reported that the presence of hydroxyl group at 7th position in the A-ring and carbonyl group at C-4 in the pyran ring in naringenin contributed strong antifeedant activity of *Coptotermes formosanus* than the C-4′ in the B-ring. Similarly, pinocembrin isolated from *Flourensia oolepis* having hydroxyl groups at 7th position in A-ring and carbonyl at 4th position in pyran ring C were responsible for the highest antifeedant activity against *Epilachna paenulata* (Georgina et al. 2009). Pectolinaringenin isolated from *Clerodendron siphonenthus* by Pal et al. (1989) showed feeding inhibition against the rice weevil, *Sitophilus oryzae* and from *C. phlomidis* by Muthu et al. (2013) showed ovicidal and oviposition deterrent activities against *E. vittella*, which also confirmed the present findings.

Larvicidal activity

Fraction 5 isolated from hexane: ethyl acetate system from chloroform extract of *C. phlomidis* exhibited the least LC_{50} value of 198.23 ppm against *H. armigera* larvae in the present study. This result coincided with the findings of Adeloma and Eloff (2011) who reported that acetone: chloroform fractions of *Cassia alata* exhibited the LC_{50} values of 0.505 mg/mL against the larvae of *Haemonchus controtus.* Recently, Anshul et al (2013) reported that methanol extract containing isomeric flavonoids had potent larval mortality against *H. armigera*.

Chloroform extract fractions 5, 6 and 4 from *C. phlomidis* showed potent larvicidal activity against *H. armigera* in the present investigation. This result correlated with the earlier findings of Calle et al. (1990), who studied the insecticidal activity of four fractions of *Ageratum conyzoides* petroleum extract, eluted with petroleum and increasing amount of chloroform. They reported that fraction 4 showed potent insecticidal activity of 80% against 3rd and 4th instar Lepidopteran larvae, *Cynthia carye.* They hypothesised that the activity might be due to the presence of flavonoidal compounds. Also, some flavonoids and flavonoid-containing extracts possessed anthelmintic and nematicidal activities which induced embryonic and larval lethality (Ayers et al. 2008; Kim et al. 2009).

Fraction 5 at 1000 ppm and pectolinaringenin at 100 ppm exhibited potent larvicidal activity with least LC_{50} values of 198.23 and 27.31ppm, respectively against *H. armigera* in this investigation. Similarly, Muthu et al. (2012a) reported that fraction 5 exhibited LC_{50} 5.02 and 61.63 ppm and pectolinaringenin LC_{50} 0.62 and 2.87 ppm against *C. quinquefasciatus* and *A. aegypti*, respectively. At lower concentration (12.5 ppm), the compound exhibited more than 38% larval mortality against *H. armigera*. It was in accordance with the earlier findings of Kumari et al. (2003) who reported that six *neo*-clerodane diterpenoides isolated from *Clerodendrum* species at 10 µg/cm^2 exhibited 20 and 44% larval mortality against *E. vittella* and *S. litura*,

respectively. There are many reports on various flavonoids isolated from plants which also supported the present findings: quercetin and kaempferol isolated from *Ricinus communis* showed insecticidal activity against *C. chinensis* (Upasani et al. 2003), bioassay with quercetin and rutin against *H. armigera* drastically reduced the larval survival were reported by Jadhav et al. (2012) which evidenced the larvicidal activity of flavonoids**.**

Growth inhibitory activities

Fractions and the compound either completely reduced the adult mergence or larval-pupal intermediate or pupicidal activities were observed in this study. This agreed with the earlier observation of Muthu et al. (2010) who reported very low adult emergence was observed in hexane and ethyl acetate extracts of *Atalantia monophylla* against *E. vittella;* Baskar et al. (2009) observed complete prevention of adult emergence of *H. armigera* at higher concentration. Further, the fractions from the chloroform extract of *Caesalpinia bonduc* reduced the emergence of *S. litura* (Baskar et al. 2012a).

In the present finding, pectolinaringenin completely prevented the adult emergence at 100 ppm concentration and low amount of adult emergence was observed in the lower concentrations against *H. armigera*. This was in agreement with the earlier findings of Muthu et al. (2012b) who found that chloroform extract of *C. phlomidis* completely prevented the adult emergence of *E. vittella*. Further, Isomeric flavonoid from methanol extract of *A. annua* exhibited promising growth inhibitory activities against *H. armigera* (Anshul et al. 2013).

Ovicidal activity

Maximum ovicidal activity of 69.25% was recorded in fraction 5 from chloroform extract of *C. phlomidis* at 1000 ppm concentration against *H. armigera*. This agreed with the findings of Raja et al. (2005) who studied the ethyl acetate derived fractions and their compounds from *Hyptis suaveolens* showed more than 60% ovicidal activity against *S. litura* and *H. armigera*. Ovicidal activity of different fractions from the hexane extract of *Atalantia monophylla* against *S. litura* and *H. armigera* was studied by Baskar et al. (2012b) and Baskar and Ignacimuthu (2012b); they reported that fraction 9 at 1000 ppm exhibited 75.61 and 72.21%, respectively. Jeyasankar et al. (2013) reported that *Syzygium lineare* derived fraction 3 and compound 3-(3-Hydroxy-hexyl)-detrahydro-pyron-4-one 3 showed ovicidal activity of 66.74 and 69.50% against *S. litura*.

Higher concentration of fraction 5 and 100 ppm of the compound reduced the egg hatchability in this study and the ovicidal activity was in dose dependent. Results of this investigation narrowly correlated with the results of Muthu et al. (2013) who observed maximum ovicidal activity of 69.25% at 1000 ppm of fraction 5 and 67.95% activity in pectolinaringenin at 100 ppm against *E. vittella*. Earlier, Malarvannan et al. (2009) observed higher concentration of plant derived extracts either completely or maximum percentage prevention of egg hatchability of *H. armigera*. Ovicidal activity may due to interference of the plant derived substances with the normal embryonic development of the eggs by suppressing hormonal and biochemical process, incomplete blastokinensis and abnormal breakage of extra embryonic membrane in the embryo (Enslee and Riddifold 1977; Fagoonee and Lauge 1981). Garlic volatile was studied against the eggs of *S. litura*, *Dysdercus koenigii*, *E. vittella* and *H. armigera* by Gurusubramanian and Krishna (1996) who observed that the chemicals present in the volatiles of garlic could diffuse into the eggs, thereby affecting the important physiological and biochemical processes associated with embryonic development.

Oviposition deterrent activity

Oviposition deterrency may be caused due to the adverse effects of secondary plant chemicals on ovarian development, fecundity and fertility of adults. In the present investigation, the fractions 4-6 and pectolinaringenin exhibited 100% oviposition deterrent activity against *H. armigera*. The observations of Muthu et al. (2013) confirmed the present findings. They reported that fractions 3-6 from chloroform extract of *C. phlomidis* completely prevented the oviposition of *E. vittella* at 1000 and 500 ppm concentrations and pectolinaringenin also showed 100% oviposition deterrent activity at 100 ppm concentration. Similarly, Singh et al. (2011) observed maximum oviposition deterrent activity in thymol against *Chilo partellus* both in choice and no-choice methods.

The presence of flavonoids in active fraction 5 and pectolinaringenin exhibited maximum oviposition deterrent activity against *H. armigera*. The present

finding agreed with the findings of Hashem et al. (2013) who studied the frass extract of *S. littoralis* fed with castor leaf against female adults of *S. littoralis* and *A. ipsilon* for oviposition deterrent activity; they observed that the presence of low amount of phenolics/ flavonoids effectively prevented the egg laying.

CONCLUSION

To the best of our knowledge, this is the first report on the fractions and pectolinaringenin against *H. armigera* for antifeedant, larvicidal, growth regulation, ovicidal and oviposition deterrent activities. The semi-purified extracts and an isolated compound from *C. phlomidis* exhibited various biological activities and could be effectively used to develop a new pesticidal formulation to control economically important agricultural pests and disease causing vector mosquitoes.

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