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Assessment of the Phytotoxicity and Antifeedant Properties of *Aristolochia bracteolata* Lamk. Leaf Extracts and their Derivatives against the Spotted Bollworm, [*Eariasvittella* (Fab.)], (Lepidoptera: Noctuidae)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The phytopesticidal effects of various solvent crude extracts and isolated fractions from the leaves of *Aristolochia bracteolata,* which were tested for their antifeedant and larvicidal activities on the fourth instar larvae of the shoot and fruit of *Earias vittella.*The antifeedant and larvicidal activities were performed by fruit disc no-choice methods at 0.625, 1.25, 2.5, and 5% and 125, 250, 500, and 1,000 ppm concentrations for crude and fractions, respectively. The dichloromethane (DCM) extract of *A. bracteolata* exhibited the maximum antifeedant (61.10%) and larvicidal (65.33%) activities at 5% concentration against *E. vittella,* followed by hexane (44.93%), acetone (40.40%) and aqueous (22.25%) extracts. It was subjected to fractionation using silica gel column chromatography with different combinations of hexane and ethyl acetate used as the mobile phase. Among the six fractions obtained, fraction 6 showed the maximum antifeedant (78.32%) and larvicidal (81.77%) activities against *E. vittella* at a 1000 ppm concentration. The preliminary phytochemical analysis of the plant also showed alkaloids, anthroquinones, diterpenoids, flavonoids, glycosides, polyphenols, saponins, steroids, and tannins in the extract. *A. bracteolata* leaves could be complemented with an eco-friendly pesticide/insecticide for an integrated pest management strategy.

Keywords: Antifeedant; larvicidal activity; Aristolochia bracteolata; Earias vittella; plant extracts; phytochemical screening; eco-friendly pesticide.

1. INTRODUCTION

Over the past four decades, India has significantly increased its agricultural output in an attempt to impose a system of food self-sufficiency. It has been suggested that synthetic pesticides and chemical fertilizers are probably part of the reason for this productivity increase [1]. Consequently, those synthetic chemical substances have been additionally influencing the improvement of resistance, especially in insect pests, which has caused extensive losses to agricultural manufacturing capacity [2]. Additionally, increasing evidence suggests that losses are rising even as the usage of chemical pesticides rises [3]. At the same time, there may be a growing public concern about the destructive effects of chemical insecticides on human health, the environment, and biodiversity [4]. However, these terrible externalities cannot be eliminated altogether; their intensity can be minimized via improvement, dissemination, and promotion of alternative technologies consisting of bio-insecticides as the right agronomic practices instead of depending entirely on chemical insecticides. India has a vast plant life and fauna that can grow into industrial technology [5].

Plants and their components are potential sources of new pesticidal materials, and the search for plant-based new insecticidal molecules or herbal products has heightened in the past decades [6-11]. Several herbivores target plants or plant parts that contain toxic compounds, so these compounds have a proven track record of effectiveness and survival value in preventing herbivore attacks on flora [12]. The control of insect pests will utilize a variety of secondary chemicals found in flora that can interfere with particular physiological mechanisms related to nutrition, reproduction, metamorphosis, and insect behavior. This will provide an environmentally friendly substitute for the use of conventional pesticides [13].

Bhendi (*Abelmoschu sesculentus* L. Moench) belonging to the family Malvaceae, is commonly known as Lady’s finger, an economically important vegetable crop that can boost small producers' farm earnings. The nutritious and financially important vegetable is commonly produced in tropical and subtropical climates [14-18]. Bhendi contains numerous minerals and vitamins in addition to carbohydrates, fiber, sugar, and fat. These include calcium, magnesium, phosphorus, potassium, iron, sodium, zinc, vitamins A, B (B1, B2, B3, B6, and B9), C, and K [19,20].

The shoot and fruit borer of okra, *Earias vittella*, is a reputable pest that causes more than 40-50 percent losses in cotton and okra crops, with 69% occurring solely in okra. *E. vittella*is a pest that attacks tender terminal shoots in early to mid-season, boring into the stem, and eating flowers and green shoots [21].*Earias vittella*, the shoot and fruit borer is accountable for inflicting damage that varies from 52.33 to 70.75 percent [22].

Aristolochia bracteolata is a shrub distributed throughout India and belongs to the Aristolochiaceae family. *A. bracteolata* is commonly known as Worm Killer in English and Aadutheendaapaalai in Tamil. In the indigenous system of medicine, the plant was used to cure skin diseases, inflammation, and purgatives [23] and antihelminthic activity and trypanocidal effect [24]. A root extract of *A. bracteolata* was documented earlier and stated to have an antimicrobial interest [25] and toxicity against *Aedesa egypti, Anopheles stephensi* and *Culexquinque fasciatus* [26]. This species has been proven to be nephrotoxic, mutagenic, and carcinogenic due to the cytotoxicity of the aristolochic acid constituents. Local tribes and villagers utilize the leaves of the plant. It is used intraditional drug treatments as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery, and snake bites [27]. Methanolic extracts of plant parts of *A. bracteolata* have been the source of physiologically active compounds. The use of the plant as an anti-malarial is not endorsed in its crude form [28]. The whole plant was used as a purgative, antipyretic, and anti-inflammatory. It additionally possesses potent anti-allergic activity[29] and has pronounced antibacterial and antifungal activities [30,31,32]. Hence, given the aforesaid, this present study aimed to authenticate the antifeedant and larvicidal activity of various crude extracts and fractions isolated from dichloromethane leaf extracts of*A. bracteolata* against the fourth-instar larvae of *E. vittella*.

2.MATERIALS AND METHODS

2.1 Plant Collection, Extraction, and Isolation of Fractions

Aristolochia bracteolata leaves were collected from Tirunelveli District, Tamil Nadu, India, based on information on their traditional insecticidal properties. The extraction and isolation of fractions were outlined in a prior study conducted by Pavunrajet al. [13].

2.2 Preliminary Phytochemical Analysis Test

A preliminary investigation of the phytochemical composition of *A. bracteolata* leaf extracts and fractions was conducted following the protocols outlined in Harbone [33].

2.3 Culture of *Earias vittella*

The insects were maintained as per the method of Pavunraj et al. [13]

2.4 Antifeedant Activity Test

The evaluation of antifeedant activity was assessed by following the procedure outlined by Bentley et al. [34]. Various concentrations ranging from 0.625% to 5% were utilized for the crude samples, while fractions were tested at concentrations of 125 ppm to 1,000 ppm. The actual consumption was determined using the methodology described by Pavunraj et al. [35].

2.5 Larvicidal Activity Test

A bioassay was conducted to assess the larvicidal effects of *E. vitella* using the fruit disc no-choice method. Bhendi fruit discs were subjected to various concentrations of crude (0.625%, 1.25%, 2.5%, and 5.0%) and fraction (125 ppm, 250 ppm, 500 ppm, and 1000 ppm) treatments. Following a 24-hour treatment period, untreated fruit discs were provided and replaced regularly until pupae formation. The larvicidal activity was evaluated for up to 96 hours, and the percentage mortality was calculated using the method described by Abbott [36].

2.6 Statistical Analysis

The data were subjected to a one-way analysis of variance (ANOVA) to find out the significance among treatments, and the effective treatments were separated by the least significant difference (LSD) ($P<0.05$).

3. RESULTS

3.1 Preliminary Phytochemical Analysis of *A. bracteolata*leaf Extracts and Fractions

The crude extracts and different fractions isolated from the DCM crude extracts of *A. bracteolata* were subjected to preliminary phytochemical analysis to confirm the major group of compounds present. The results are presented in Tables 1 and 2. The crude extracts and fractions obtained from the DCM crude extracts of *A. bracteolata* revealed the presence of various bioactive compounds, namely alkaloids, anthroquinones, diterpenoids, flavonoids, glycosides, polyphenols, saponins, steroids, and tannins.

3.2 Antifeedant Properties of Crude Extracts and Fractions

The hexane, DCM, acetone and aqueous extracts of *A. bracteolata* leaves exhibited antifeedant activity in a concentration-based manner. The results of the present investigation related to antifeedant activity towards *E. vittella* by using crude extracts of *A. bracteolata* are provided in Table 3. DCM extracts of *A. bracteolata* at 5% concentrations showed significant feeding deterrent activity (61.10%) towards *E. vittella*, followed by hexane (44.93%) and acetone (40.40%) extracts. While the aqueous extract confirmed poor antifeedant activity towards the tested larvae. The effective DCM crude extract was subjected to column chromatography. Among the six fractions obtained, fraction 6 had the highest antifeedant activity (78.32%) towards *E. Vittella* (Table 4). Increasing the concentration of fractions accelerated the antifeedant activity against *E. vittella*. Fractions 1, 2, and 3 confirmed poor antifeedant activity against the insect examined.

3.3 Larvicidal Activity of Crude Extracts and Fractions

The percent larval mortality of *E. vittella* larvae in different concentrations of crude extracts is provided in Table 5.Larval mortality was proportionately accelerated with increasing concentrations. The DCM extract showed maximum larvicidal activity against *E. vittella* (65.33%), and this was followed by acetone and hexane extracts at 5% concentration. The result of the larvicidal activity of the sixth fraction obtained from the DCM extract of *A. bracteolata* against the selected lepidopteran pest is provided in Table 6. Statistically significant larvicidal activity of 81.77% was recorded in fraction 6, followed by fractions 4 and 5, which recorded 79.55 and 59.11%, respectively, at 1000 ppm concentration. Fractions 1 and 2 confirmed very low larvicidal activity.

Table 1. Preliminary phytochemical analyses of different crude extracts of *A. bracteolata* leaves

Extracts	Yield of extracts by (gm)	Phytochemical constituents detected
Hexane	5.2	A,D,F,PP,S
DCM	8.5	A, AN, D, F, S, ST
Acetone	6.0	A,PP,ST,
Aqueous	5.8	A, ST,S,T

A=Alkaloids; AN=Anthroquinones; D=Diterpenoids; F=Flavonoids; G=Glycosides; PP=Polyphenol; S=Saponins; ST=Steroids; T=Tannins.

Table 2. Preliminary phytochemical analyses of different fractions isolated from the DCM extract of *A. bracteolata* leaves

Fractions	Yield of extracts by (gm)	Phytochemical constituents detected
Fraction 1	1.8	A,D,F
Fraction 2	1.3	D,F,G,PP
Fraction 3	1.6	F,GPP,
Fraction 4	2.0	A,F,S,
Fraction 5	2.3	A,AN,D,F,S,T
Fraction 6	2.7	A,AN,D,F,PP,S

A=Alkaloids; AN=Anthroquinones; D=Diterpenoids; F=Flavonoids; G=Glycosides; PP=Polyphenol; S=Saponins; ST=Steroids; T=Tannins.

Table 3. Percentage antifeedant activity (percentage reduction in leaf area consumption) of *A. bracteolata* leaf extracts against *E. Vittella*

Treatments	Concentration (%)			
	0.625	1.25	2.5	5
Hexane	12.03±3.80^{b}	18.24±2.55^{c}	24.74±2.64^{c}	44.93±4.72^{b}
DCM	25.01±3.58^{c}	36.66±3.30^{d}	52.83±4.27^{d}	61.10±4.49^{d}
Acetone	13.34±2.44^{b}	21.14±4.42^{c}	30.13±4.72^{c}	40.40±3.35^{c}
Aqueous	3.84±2.09^{a}	9.71±2.48^{b}	15.92±4.22^{b}	22.25±4.60^{b}
Control	2.12±1.30^{a}			

Values are represented by the mean ± SD (n = 5). In each column, figures marked by the same alphabets do not significantly differ (p = 0.005).

Table 4. Percentage antifeedant activity (percentage reduction in leaf area consumption) of different fractions isolated from the DCM leaf extracts of *A. bracteolata* against *E. Vittella*

Treatments	Concentration (ppm)			
	125	**250**	**500**	**1000**
Fraction1	4.11±3.69[a]	5.77±2.04[ab]	6.93±2.67[b]	12.09±3.81[b]
Fraction2	4.99±1.74[a]	7.70±2.34[b]	9.31±2.09[b]	17.70±3.82[b]
Fraction3	15.73±1.72[b]	22.64±1.66[c]	27.11±3.06[c]	37.52 ±2.89[c]
Fraction4	41.58±4.83[d]	54.95±4.07[e]	62.84±4.22[e]	72.92±4.32[e]
Fraction5	26.13±2.51[c]	35.04±1.78[d]	44.75±2.14[d]	51.29±2.06[d]
Fraction6	45.68±5.17[d]	58.32±4.11[e]	69.61±2.60[f]	78.32±3.30[e]
Control	1.13±0.37[a]			

Values are represented by the mean ± SD (n = 5). In each column, figures marked by the same alphabets do not significantly differ (p = 0.005).

Table 5. Larvicidal activity of different crude extracts from *A. bracteolata*against *E. vittella*

Treatments	Concentration (%)			
	0.625	**1.25**	**2.5**	**5**
Hexane	16.22±5.29[a]	22.44±4.33[a]	32.66±4.34[a]	44.88±5.01[a]
DCM	36.66±4.71[c]	44.88±5.01[c]	53.11±4.54[c]	65.33±5.05[c]
Acetone	22.44±4.33[b]	34.66±5.05[b]	42.88±4.41[b]	50.88±5.63[b]
Aqueous	10.22±0.49[a]	16.22±5.18[a]	26.44±4.93[a]	34.66±5.05[a]

Values are represented by the mean ± SD (n = 5). In each column, figures marked by the same alphabets do not significantly differ (p = 0.005).

Table 6.Larvicidalactivity of different fractions isolated from the DCM extract of of*A. bracteolata*against *E. Vittella*

Treatments	Concentration (ppm)			
	125	**250**	**500**	**1000**
Fraction 1	2.44±4.47[a]	8.00±4.47[a]	12.22±4.37[a]	20.44±0.99[a]
Fraction 2	0.00±0.00[a]	14.22±5.29[a]	26.44±4.93[b]	34.66±5.05[b]
Fraction 3	22.44±4.33[b]	30.66±1.49[b]	42.88±4.41[c]	53.11±4.54[c]
Fraction 4	40.88±1.98[c]	51.11±2.48[c]	65.33±5.05[d]	79.55±0.99[d]
Fraction 5	24.44±5.15[b]	34.66±5.05[b]	50.88±5.63[c]	59.11±1.98[c]
Fraction 6	46.88±4.54[c]	55.11±5.00[c]	69.33±1.49[d]	81.77±3.97[e]

Values are represented by the mean ± SD (n = 5).In each column, figures marked by the same alphabets do not significantly differ (p = 0.005).

4. DISCUSSION

The exploration for novel insecticide compounds derived from plants has accelerated in recent years due to the potential for producing novel pesticidal molecules from plants [37]. Herbivore attacks on plants and their products are limited by plant toxic compounds [38].Plant secondary chemical compounds are utilized in environmentally friendly insect pest management because they aggravate some physiological processes related to insect feeding, reproduction, metamorphosis, and behavior[39].

The current study found that the hexane, DCM, acetone, and aqueous extracts of *A. bracteolata* leaves demonstrated concentration-dependent larvicidal and antifeedant properties.The highest antifeedant activity against *E. vitella* has been shown by the DCM extracts, which were followed by the hexane and acetone extracts. Numerous plant preparations have been reported to be successful in combating moth pests that are significant to agriculture, which is consistent with the current findings. According to a previous study by Pavunraj [40], the dichloromethane extract of *Spilanthes acmella* (L.) Murr. leaves exhibited strong antifeedant activity against *E. vitella* larvae, showing 56.75% at 5% concentration.Therefore, the DCM extract was fractionated using solvents of increasing polarity. Based on the TLC profiles, six fractions were isolated. All the fractions were tested against the 4th instar larvae of *E. vitella*. All the fractions exhibited antifeedant activity; the maximum antifeedant activity was recorded for fraction 6. Our results coincide with earlier findings by Pavunraj et al.[41], who reported that *Catharanthus roseus*-derived fractions exhibited antifeedant activity of 84.60% against *E. vitella.* This result corroborates early findings where the ethyl acetate extract of *M. corchorifolia* showed maximum antifeedant activity against *E. Vittella* Pavunraj et al. [42].The present result also coincides with the findings of Praveena et al.[43], who reported that to screen various extracts of *Clausena dentata*, *Dodonea viscosa*, *Anacardium occidentale,* and *Nicotiana tobacum*. Among the various extracts tested, 5% concentrations of extract gave 100% FDI (Feeding Deterrence Index). Comparing the antifeedent activity of the plants mentioned above, pet ether extracts from *D. viscosa* (83.4%) and *A. occidentale* (87.4%) showed greater antifeedent activity at 3% concentration against *E. vittella*.

In our experiments, the DCM extract of *A. bracteolata* and fraction 6 from the DCM extract have proven to have the very best larvicidal activity against *E. vitella*, while fractions 1 and 2 have proven to have low larvicidal activity at 125 ppm concentrations. This outcome corroborates the studies accomplished by Pavunraj et al. [44], who demonstrated that the DCM extract of *Acalypha fruticosa* leaves exhibited strong larvicidal effects on *E. vittella*. In addition, Hanem et al.[45] pronounced that the DCM extract of *Hyptis brevipes* had 100% larvicidal activity against *S. littoralis*. Muthu et al. [46] mentioned that *C. phlomidis* extract exhibited larvicidal interest against *E. vittella* at a 50000 ppm concentration. For example, earlier findings by Baskar et al. [47] about *Atalantia monophylla* fractions confirmed precise larvicidal interest in *S. litura*. Also, Pavunraj et al. [40] reported that dichloromethane extracts from the leaves of *Spilanthes acmella* confirmed high larvicidal activity (75.11%) on *E. vitella.*

In our study, fraction 6 from the DCM extract of *A. bracteolata* confirmed better degrees of antifeedant and larvicidal activity towards *E.vitella* due to the presence of phytochemical constituents such as alkaloids, anthroquinones, diterpinoids, flavonoids, polyphenol, and saponins. Similarly, flavonoids isolated from *Cicer arietinum* showed antifeedant activity against *H. armigera, Heliothis virescens, S littoralis, S. exigua* and *S. frugiperda* [48]. In addition, coumarin, quinones and terpenoids from ninth fraction of *Atalantiamonophylla* confirmed larvicidal and antifeedant activity against *H. armigera* Baskar et al. [47].The efficacy of a rapidly growing number of plant extracts containing secondary metabolites against lepidopteran pests has been highlighted by recent research by Pavunraj et al.[49].In the current investigation, various abnormalities in larvae, pupae, and adults were also noted in the DCM extract and its fractions.

5. CONCLUSION

Various solvent crude extracts and fractions extracted from DCM extracts of *A. bracteolata* leaves showed insecticidal and antifeedant properties against the spotted bollworm, *E. vittella*. Overall, the current study found that *A. bracteolata* fraction 3 exhibited strong larvicidal and antifeedant properties as well as a notable influence on deformities in the larval, pupal, and adult stages. These findings support the potential of this cost-effective and easily accessible fraction as a sustainable source of botanical products that are effective in combating agricultural pests.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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