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Research Article

Ovicidal Activity of *Couroupita guianensis* (Aubl.) against *Spodoptera litura* (Fab.)

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Hexane, chloroform, and ethyl acetate extracts of *Couroupita guianensis* leaves were studied for ovicidal activity against *S. litura*. All the extracts showed ovicidal activity against *S. litura*. Maximum activity was noticed in hexane extract and it showed the least LC_{50} and LC_{90} values; the regression equation was also higher than the other extracts. All the analyzed values showed homogeneity variance. The active hexane extract was fractionated and eight fractions were isolated. The fractions were studied at different concentrations. Among the fractions, fraction 8 showed maximum ovicidal activity with least LC_{50} and LC_{90} values. Fraction 8 differed statistically from the other fractions; the regression equation value was higher than the other fractions. All the P values obtained from regression analysis were significant. The results of the present investigation clearly suggest that the active fraction could be purified to isolate active compound(s) and could be used to develop an insecticidal formulation to control economically important agricultural pests.

1. Introduction

India is an agricultural country and more than 80% of the population depend on agriculture [1]. Pathogenic organisms and insect pests cause crop loss of 120 billion US dollars worldwide and reduce the yield by 20–40% [2]. In India, approximately 18% of food grains are lost due to pathogens and insect pests. To control the pests and reduce the loss, different chemical pesticides are used. Application of chemical pesticides is polluting the environment, causing ill effects on nontarget organisms, developing resistance, and causing resurgence of pests [3]. These call for an alternative to chemical pesticides through natural means of pest control, including vigorous search for new sources of botanical insecticides [4]. Plant-based pesticides are highly suitable since they have low toxicity, are easily biodegradable, and have multimode of action [5]; they are suitable for organic agriculture [6].

Botanical extracts are used as insecticides for centuries and their active compounds reduce the opportunity for the development of insect resistance [7]. Plants have evolved a range of adaptations to increase their survival and reproduction by minimising the impact of phytophagous insects. Plants defend themselves from herbivores with the help of secondary metabolites produced by them and these secondary chemicals can act as repellents or toxins to herbivores and affect their behaviour, growth, or survival. Volatile plant signals attract natural enemies of the herbivore insect pests [8]. Presently, botanicals are used as insecticides which constitute only 1% of the world insecticide market [9].

Plant-derived substances have multimode of actions against different agricultural pests and act as antifeedants [10] and larvicidal [1] agents; they reduce adult emergence and increase adult abnormalities [11, 12]; they inhibit larval growth [13] and cause ovicidal and oviposition deterrent activities [14]; and they bring about cytological changes [5].

Couroupita guianensis leaves extracts showed antifeedant, larvicidal, and ovicidal activities against *Helicoverpa armigera* [15, 16] and antifeedant activity against *Spodoptera litura* [17]. *S. litura* is a major polyphagous pest attacking more than 150 host species affecting the yield [18]. It causes serious damage to young plants and the buds of different vegetable crops in Thiruvallur and Kancheepuram districts of Tamil Nadu. The present study was aimed to evaluate the ovicidal activity of

different crude extracts and fractions of *C. guianensis* against *S. litura*.

2. Materials and Methods

2.1. Plant Collection. Leaves of *C. guianensis* were collected from Loyola College Campus, Chennai, Tamil Nadu, India. The plant was identified by Dr. M. Ayyanar, Taxonomist, Entomology Research Institute, Loyola College. The voucher specimen (ERIH: 1310) was deposited at the institute herbarium. The plant material was shade-dried at room temperature and powdered coarsely. The plant materials were sequentially extracted using hexane, chloroform, and ethyl acetate. The active hexane extract was fractionated using silica gel column chromatography with increasing polarity of hexane : ethyl acetate combinations. Isolated fractions were concentrated using vacuum rotary evaporator with reduced pressure and the collected fractions were stored at 4°C in the refrigerator [15].

2.2. Insect Culture. Egg masses of *S. litura* were collected from groundnut field at Tiruttani in Thiruvallur District of Tamil Nadu. The eggs were surface-sterilized with 0.02% sodium hypochlorite solution, dried, and allowed to hatch. After hatching, the neonate larvae were reared on leaves of castor, *Ricinus communis*, till prepupal stage. Sterilized soil was provided for pupation at room temperature (27 ± 2°C) with a photoperiod of 14 : 10 (light : dark) and 75 ± 5% relative humidity in insectary. After pupation, the pupae were collected from the soil and placed inside the oviposition chamber. After adult emergence, cotton soaked with 10% (w/v) sugar solution with few drops of multivitamins was provided for adult feeding to increase the fecundity. Potted groundnut plant was kept inside adult emergence cage for egg laying. After hatching, the larvae were fed with tender castor leaves. The eggs laid by the laboratory reared insects were used for the present study [10].

2.3. Ovicidal Activity. The ovicidal activity of the crude extracts and fractions was studied by spraying them on freshly laid eggs of *S. litura*. The sprayed concentrations were 5, 10, 25 and 50 mg/mL for crude extracts and 125, 250, 500 and 1000 μg/mL for fractions. Spray solution of 0.5 mL was used per replicate. Azadirachtin was used as positive control [19]. Five replicates were maintained for each treatment with 20 eggs per replicate (total $n = 100$). The experiment was conducted at laboratory conditions (room temperature of 27 ± 2°C with 14 : 10 (light : dark) photoperiod and 75 ± 5% relative humidity). The number of eggs hatched in control and treatments was recorded up to 96 hrs. Percent of egg mortality was calculated according to Abbott [20].

2.4. Statistical Analysis. The ovicidal activity was analysed using one-way ANOVA. Significant differences between treatments were determined using Tukey's multiple-range HSD tests ($P \leq 0.05$). Analyses were performed with the original data after transformation with various approaches (the arcsin, logarithmic, and square root methods). The distribution of the fraction data did not show significant deviations from normality. Shapiro-wilk test for original crude data showed normality. Linear regression analyses were performed for all dose-response experimental data. LC_{50} and LC_{90} values were calculated using probit analysis [21].

3. Result

Ovicidal activity of different crude extracts of *C. guianensis* against *S. litura* is presented in Table 1. Maximum ovicidal activity of 67.33% was observed in hexane extract at 50 mg/mL concentration. The chloroform and ethyl acetate extracts showed ovicidal activity of 47 and 42%, respectively. Chloroform and ethyl acetate extracts showed statistically similar activity. Hexane extract was statistically different from chloroform and ethyl acetate extracts. At 25 mg/mL concentration, hexane extract exhibited 51.17% ovicidal activity against *S. litura* followed by chloroform and ethyl acetate extracts. All the three extracts statistically differed from each other at 25 and 50 mg/mL concentrations. Hexane extract exhibited 39.52% ovicidal activity at 10 mg/mL concentration against *S. litura* which was statically similar to chloroform extract that showed 31.20% ovicidal activity (P value 0.63). Lowest concentration of hexane and chloroform extracts showed statistically similar (P value 0.92) ovicidal activity. All the concentrations of ethyl acetate extracts showed minimum ovicidal activity. The homogeneity of variance was significant at all the analyses; also the ANOVA was significant (P value 0). The R^2 indicated that increasing concentration of the extracts increased the activity (Table 1). Regression ANOVA derived from all the three extracts showed significant value (P value 0).

The minimum quantity of hexane extract needed to kill 50% eggs of *S. litura* is shown in Table 1. Ethyl acetate extract required maximum quantity (55.94 mg/mL) for 50% egg mortality of *S. litura*. The obtained χ^2 values were significant for all the tested extracts. The probit analysis clearly indicates that the hexane extract has the potential to kill the eggs of *S. litura*.

Bioassay-guided fractionation of hexane extract was done and finally 8 fractions were obtained; they were screened at different concentrations. Among the fractions tested, fraction 8 showed maximum ovicidal activity of 30.46% at 125 μg/mL concentration (Table 2) followed by fractions 3 and 7 which showed ovicidal activity of 28.24 and 23.91%, respectively. Fractions 3, 7, and 8 were statistically similar (P value 0.15). Minimum ovicidal activity of 4.32% was noticed in fraction 5. Fractions 4 and 2 were statistically similar to fraction 5 (P value 0.15). At 250 μg/mL concentration, fraction 8 exhibited 51.05% ovicidal activity. Minimum ovicidal activity was noticed in fraction 4. Fractions 1, 3, and 7 exhibited more than 30% ovicidal activity. Fraction 8 showed 59.82% ovicidal activity at 500 μg/mL concentration followed by fractions 7, 3, and 1. Maximum ovicidal activity of 71.69% was noticed in fraction 8 at 1000 μg/mL concentration followed by fraction 7 which exhibited 60.93% ovicidal activity. Minimum ovicidal activity of 19.53% was noticed in fraction 5 which was statistically similar to fraction 4 (P value 1). Fractions

Table 1: Ovicidal activity and effective concentrations (mg/mL) of *Couroupita guianensis* crude extracts against *Spodoptera litura*.

Solvent extract	Concentration (mg/mL)				R	R^2	Regression equation	P value	LC_{50}	LC_{90}	χ^2
	5	10	25	50							
Hexane	21.98 ± 4.03^{b}	39.52 ± 5.94^{b}	51.17 ± 5.94^{c}	67.33 ± 4.03^{b}	0.92	0.84	26.78 ± 0.899	0.000	28.05	82.50	44.04*
Chloroform	23.16 ± 5.27^{b}	31.20 ± 5.20^{b}	41.82 ± 5.52^{b}	47.57 ± 4.70^{a}	0.84	0.70	24.60 ± 0.050	0.000	49.99	145.40	31.02*
Ethyl acetate	6.93 ± 4.77^{a}	16.16 ± 4.20^{a}	24.41 ± 2.12^{a}	42.85 ± 6.26^{a}	0.95	0.89	5.76 ± 0.75	0.000	55.94	108.28	36.31*
ANOVA	Df 2, 12, F.18, 35 P 0	Df 2, 12, F.26.15 P 0	Df 2, 12, F.39.31 P 0	Df 2, 12, F.32.54 P 0							
Homogeneity	0.67	0.52	0.13	0.38							

Means followed by the same letter do not differ significantly using Tukey's test ($P \leq 0.05$) and complete regression equations; * χ^2 values are significant.

Table 2: Ovicidal activity and effective concentrations (μg/mL) of *Couroupita guianensis* hexane fractions against *Spodoptera litura*.

Fractions	Concentration (μg/mL)				R	R^2	Regression equation	P value	LC_{50}	LC_{90}	χ^2
	125	250	500	1000							
1	17.42 ± 4.61^{cd}	32.63 ± 4.04^{cd}	43.50 ± 4.13^{de}	51.11 ± 3.10^{c}	0.87	0.76	20.11 ± 0.034	0.000	871.14	2268.09	43.11*
2	10.81 ± 3.62^{abc}	17.30 ± 4.10^{ab}	24.97 ± 4.52^{b}	32.57 ± 5.12^{b}	0.87	0.76	10.37 ± 0.024	0.000	1509.42	3137.48	27.91
3	28.24 ± 1.96^{e}	35.84 ± 4.45^{d}	42.39 ± 2.28^{d}	47.83 ± 2.30^{c}	0.88	0.78	28.99 ± 0.020	0.000	1021.37	3426.26	12.92
4	7.60 ± 2.94^{ab}	13.04 ± 2.93^{a}	15.20 ± 2.33^{a}	19.70 ± 5.79^{a}	0.81	0.65	7.53 ± 0.015	0.000	2213.23	4273.60	21.80
5	4.32 ± 4.61^{a}	11.98 ± 6.14^{a}	17.42 ± 2.71^{a}	19.53 ± 2.65^{a}	0.72	0.52	6.21 ± 0.015	0.000	2131.16	3997.78	55.51*
6	15.26 ± 4.75^{bc}	23.85 ± 4.52^{bc}	32.57 ± 3.29^{c}	42.33 ± 3.66^{c}	0.91	0.83	14.89 ± 0.020	0.000	1167.88	2688.15	25.02
7	23.91 ± 2.94^{de}	40.17 ± 5.69^{d}	50.00 ± 4.34^{e}	60.93 ± 5.31^{d}	0.89	0.79	26.09 ± 0.038	0.000	636.06	1951.39	39.33*
8	30.46 ± 3.24^{e}	51.05 ± 4.18^{e}	59.82 ± 4.30^{f}	71.69 ± 2.90^{e}	0.89	0.79	34.14 ± 0.041	0.000	384.43	1576.55	41.13*
Azadirachtin	42.33 ± 3.66^{f}	54.26 ± 4.01^{e}	65.20 ± 2.98^{f}	76.02 ± 3.50^{e}	0.92	0.85	42.76 ± 0.036	0.000	206.42	1525.75	18.92
ANOVA	Df 8, 36 F 54.93	Df 8, 36 F 59.80	Df 8, 36 F 126.85	Df 8, 36 F 111.95							
Homogeneity	0.74	0.93	0.60	0.001							

Means followed by the same letter do not differ significantly using Tukey's test ($P \leq 0.05$) and complete regression equations; * χ^2 values are significant.

1, 3, and 6 showed ovicidal activity between 42 and 51% and were statistically similar (P value 0.065). The data of all the fractions showed homogeneity variance except at 1000 μg/mL concentration while using one-way ANOVA. The R^2 value exhibited concentration dependent activity. Minimum R^2 value was observed in fraction 5 which showed less than 20% ovicidal activity at maximum concentration. Higher concentration of the fraction increased the ovicidal activity. Maximum regression coefficient was observed in fraction 8 followed by fraction 7. Minimum regression coefficient value was noticed in fraction 5 (Table 2). All these data clearly indicated concentration-dependent activity. All the analysed regression data were significant (P value 0).

Minimum LC_{50} and LC_{90} values of 384.43 and 1576.55 μg/mL, respectively, were obtained in fraction 8 (Table 2). Fraction 4 showed maximum LC_{50} and LC_{90} values of 2213.23 and 4273.60 μg/mL, respectively. Fraction 5 had lower percent of ovicidal activity than the other fractions; in case of probit analysis, fraction 4 showed lower value than fraction 5. Fractions 1 and 7 showed less than 1000 μg/mL LC_{50} values. Fractions 1, 5, 7, and 8 showed significant χ^2 values.

4. Discussion

Hexane, chloroform, and ethyl acetate extracts of *C. guianensis* showed ovicidal activity against *S. litura*. This finding corroborates with the findings of Deepa and Remadevi [22] who reported that the petroleum ether, chloroform, ethyl acetate, methanol, ethyl alcohol, and acetone extracts of *Acacia concinna* and *Butea monosperma* showed ovicidal activity against lepidopteran insect, *Hyblaea puera*. Similarly, water extract exhibited ovicidal activity against *Sambucus ebulus* and *Tribolium confusum* [23]. Crude extracts with a mixture of compounds showed strong ovicidal activity against *S. litura* in this study. Similarly, many researchers around the world have reported many plant extracts with ovicidal activity. *Myrtus communis, Melaleuca alternifolia, Pimenta dioica, Syzygium aromaticum, Eucalyptus citriodora*, and *E. globulus* exhibited ovicidal activity against *Trialeurodes vaporariorum* [24]; *E. globulus* and *Syzygium aromaticum* showed ovicidal activity against *Tribolium castaneum* [25, 26]; and *E. camaldulensis* showed ovicidal activity against *T. confusum* and *Ephestia kuehniella* [27]. Methanol extract of *Celosia argentea, Ricinus communis, Mikania micrantha,*

and *Catharanthus roseus* reduced the egg hatchability in *Brontispa longissima* and maximum reduction was observed in *M. micrantha* [28]. Similarly, citronella oil reduced the egg hatchability up to 95% against *Helicoverpa armigera* [29].

Fractions from hexane extract showed ovicidal activity against *S. litura*. Fractions exhibited maximum ovicidal activity at lower concentrations than the crude hexane extract. This result corroborates with the findings of Jeyasankar et al. [30] who reported that ethyl acetate extract, its fractions, and isolated compound showed ovicidal activity against *S. litura*. Maximum activity was noticed at lower dose in the purified compound than the higher dose treated fractions and crudes extracts. In the present study, the presence of alkaloids, coumarin, and quinone in the hexane extract could be responsible for ovicidal activity against *S. litura*. Similarly, Maciel et al. [31] reported that the presence of different phytochemicals like tannins, triterpenes, and alkaloids in the ethanol extract of leaves and seeds of *M. azedarach* is responsible for ovicidal activity. In the present study, partially purified extract (fractions) showed maximum ovicidal activity against *S. litura*. Similar results were obtained by Alouani et al. [32] against mosquito larvae.

Hexane extract and fraction 8 exhibited ovicidal activity against *S. litura* with least LC_{50} values than the other extracts and fractions. In this study, hexane extract eluted fractions using hexane : ethyl acetate or ethyl acetate showed ovicidal activity. The present findings coincide with the findings of Baskar and Ignacimuthu [16] who reported that hexane extract fractions eluted with hexane : ethyl acetate from *C. guianensis* showed maximum ovicidal activity against *H. armigera*. Hexane extracts derived ethyl acetate fractions from *Atalantia monophylla* showed maximum ovicidal activity against *H. armigera* and *S. litura* [33, 34]. Similarly, fractions eluted using hexane : ethyl acetate from chloroform extract of *Clerodendrum phlomidis* showed maximum ovicidal activity against *Earias vittella* [14].

5. Conclusion

The present study clearly indicates that the hexane extract and its active fraction showed the least LC_{50} values against the eggs of *S. litura*. Further study is necessary to identify the active principle(s) responsible for the activity and to develop a new formulation to control the agricultural pests.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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