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Evaluation of antifeedant activity of *Calotropis Gigantea* extracts against *Spodoptera litura*

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Abstract
Calotropis gigantea (Erukku), a prevalent tropical and subtropical waste land weed in Asia, possesses various poisonous elements that naturally defend crops against pest infestations. The challenge posed by *Spodoptera litura*, a nocturnal polyphagous pest notorious for its resistance to insecticides, necessitates the development of alternative control strategies that are both environmentally safe and beneficial. In this study, we assessed the antifeedant action of *C. gigantea* through the preparation of extracts from its leaves, fruit follicles, and latex using alcohol, chloroform, and methanol solvents. The lethal concentration 50 (LC_{50}) of these extracts was determined to evaluate their efficacy against *S. litura* larvae. Remarkably, the methanol extract of *C. gigantea* latex exhibited the highest antifeedant activity, with percentages of 23.98% and 80.34% observed at concentrations of 0.4% and 4.0%, respectively. Additionally, among the crude extracts tested, the methanol extracts of *C. gigantea* leaves, fruit follicles, and latex displayed LC_{50} values of 64.17±7.892, 71.56±6.639, and 99.42±8.012, respectively, indicating their significant effectiveness against *S. litura* larvae. These findings underscore the potential of *C. gigantea* extracts as promising biopesticides for managing *S. litura* infestations while highlighting the importance of exploring natural alternatives for pest control, particularly in the context of evolving insecticide resistance and environmental sustainability.

Keywords: Antifeedant activity, *Calotropis gigantea*, $LC_{50,}$ *S. litura*

Introduction
Calotropis gigantea R. Br., commonly known as milkweed or erukku, is a prevalent weed found in waste lands across tropical and subtropical regions of Asia. Aside from its ecological role, it has garnered attention for its diverse applications in medicine and industry. Notably, researchers have explored its potential as a source of pesticides against insect pests due to its inherent properties.

Various studies have documented the multifaceted efficacy of C. gigantea extracts against pests. Solunke and Deshpande (1991) [22] highlighted its insecticidal properties, while Pari *et al*. (1998) reported its antifeedant effects. Additionally, Philip *et al*. (1993) [19] and Badshah *et al*. (2004) [5] observed its nematicidal and antitermitic potentials, respectively. Furthermore, C. gigantea exhibits antibacterial and antifungal effects according to Anil Srivatsava *et al*. (2000) [2].

The effectiveness of C. gigantea extracts extends to agricultural applications. Studies by Pugalenthi *et al*. (1994) [20] and Muhammad *et al*. (2003) [17] demonstrated its efficacy against lepidopterous and sucking pests in various crops. Arulprakash and Senthilkumar (2005) [4] found that treatments with different plant parts were effective against Callosobruchus maculatus, with whole plant powder treatment showing the highest mortality rate.

Moreover, the effectiveness of C. gigantea extends to controlling other pests. Mendki *et al*. (2005) [13] observed dose-dependent mortality of C. chinensis, while Calotropis procera demonstrated antimicrobial and insecticidal activity against common microbial contaminants and insect pests of pulses (Ahmed *et al*., 2006 [1]). Kanimozhi (2006) [11] reported significant mortality rates of various pests when treated with aqueous extracts of C. gigantea plant parts.

Spodoptera litura (F), commonly known as tobacco cutworm or cotton leaf worm, poses a significant threat to agriculture, particularly in the Asiatic region. Its polyphagous nature leads to damage in a wide range of crops, including lettuce, cabbage, cotton, and eggplants, among others.

Given the escalating challenges posed by S. litura and the potential of C. gigantea extracts as natural pesticides, this study aims to evaluate the lethal concentration 50 (LC_{50}) of different solvent extracts of C. gigantea against S. litura larvae. By exploring the efficacy of C. gigantea extracts, this research seeks to contribute to the development of environmentally friendly and sustainable pest management strategies in agriculture.

Material and method
1. Collection and rearing of *Spodoptera litura*
The egg masses of *Spodoptera litura* were collected from the farm of Soyabean near Karad rural. After hatching the larvae were fed on fresh and tender leaves of castor leaves *(Ricinus communis. L*). Third instar larvae were fed on middle leaves of castor. Effect of extracts was studied on third instar larvae of *Spodoptera litura*.

Fig 1: Rearing of *Spodoptera litura* under laboratory condition

2. Collection and Extraction of plant material

2.1 Leaves and fruit follicle

The fresh leaves and fruits of *Calotropisgigantea* were collected from roadsides and barren land near Karad region. Leaves and fruits were washed with fresh water and shed dried. After drying, the leaves and fruit follicles were ground in grinder. The extraction was carried out by Soxhlets method, by using the solvent namely: Methanol, Alcohol and Chloroform.10gm of dried leaf powder and fruit follicle powder were accurately weighed and dissolved. The suspended solutions were kept in rotary shaker for 24 hour andthe supernatant was concentrated by drying.Dried extract was used for phytochemical and bioassays and stored at 4ºC until use.

2.2. Latex

A V-shaped incision was made on the plant's branches to collect the latex that was seeping out of the plant aseptically. Using a bench centrifuge, the aseptically collected latex was converted into a sterile centrifuge tube and centrifuged for five minutes at 1500 rpm. The pellet was collected in a sterile container after centrifugation, with the supernatant being discarded. The pellets were dried using a Rotary evaporatorat 100ºC, then kept at 4ºC in an airtight container or, for best preservation, by adding a little chloroform before use.

3. Lethal concentrations of solvent extracts

To determine the toxic concentration of each herbaceous plant extract, a range-finding test was conducted to establish concentrations at which 20–80% larval mortality could be achieved. Initially, a concentration of 5 µg/ml was tested for all herbaceous plant extracts, and the resulting larval mortality was observed. Based on the larval mortality observed at 5 µg/ml concentration, further adjustments were made to establish five concentrations until the targeted 20–80% larval mortality range was reached. Each treatment was replicated thrice, with ten larvae per replication. Larval mortality was recorded daily for three days, and after 72 hours, the final larval mortality was considered for analysis.

4. Larvicidal Activity

The leaf dip technique was employed to apply different concentrations of crude extract to leaf discs. Following a 24-hour treatment period, larvae were transferred to fresh cotton leaves untreated with the extract, with the diet replenished every 24 hours. After 96 hours of treatment, larval mortality was assessed. Three replicates, each containing 10 larvae, were maintained for every treatment. LC_{50} and LC_{90} values were determined using probit analysis (Finney, 1971). Larvae that survived were subsequently fed a diet comprising cotton leaves until they reached the pupal and adult stages. Pupal mortality rate was calculated by subtracting the number of emerging adults from the total number of pupae. The experiment was conducted over 32 days at a temperature of 27°C, with a photoperiod of 14:10 light: dark hours and a relative humidity of 75%

Fig 2: Larvicidal activity of *Spodoptera litura*: Setup

5. Antifeedant activity

The antifeedant activity of the crude extracts was assessed utilizing the leaf disc no-choice method, as outlined by Isman *et al*. (1990). Fresh cotton leaves were cut into discs measuring 4 cm in diameter and immersed in crude extracts at concentrations of 0.5%, 1.0%, 2.5%, and 5.0%, corresponding to 125, 250, 500, and 1000 ppm for each fraction. Azadirachtin (purchased from EID-Parry, India Ltd., Chennai) with a purity of 40.86% served as the positive control, while leaf discs treated with acetone and water represented the negative control. To prevent rapid drying of the leaf discs, each 1.5 cm × 9 cm petri dish was supplied with a third instar larva and damp filter paper. Using a leaf area meter, the amount of leaf area consumed by treated and control larvae was measured over 24 hours. The leaf area consumed during treatment was adjusted relative to the negative control. Five replicates were maintained for each treatment. Antifeedant activity was calculated using the formula described by Isman *et al*. (1990).

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

6. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC50, and other statistics at Parasitol Res (2008) 103:325–331 327 95% fiducial limits of upper confidence limit and lower confidence limit, and chi-square values were calculated by using GraphPad prism software. Results with $p<0.05$ were considered to be statisticallysignificant.

Result and discussion

1. Antifeedant activity and Lethal concentrations of solvent extracts

Throughout history, traditional insecticides have been derived from crude botanical extracts of plant leaves, roots, seeds, flowers, and bark, owing to the diverse array of active chemicals present in these extracts. Preliminary screening serves as an effective method to evaluate the larvicidal potential of commonly used plants. In our study, all extracts exhibited larvicidal effects, with methanol and alcohol extracts showing the highest levels of larval mortality. Additionally, irrespective of the solvents used, all extracts demonstrated more than 50% feeding deterrent activity. Particularly noteworthy was the methanol extract of C. gigantea latex, which exhibited high antifeedant activity at concentrations of 0.4% and 4.0%. On the other hand, chloroform extract of *C. gigantea* latex displayed lower antifeedant activity. Methanol extracts consistently exhibited prominent antifeedant activity across all studied plant parts. The larvicidal efficacy of *C. gigantea* parts in various solvent extracts was documented, with methanol extracts of leaves, fruit follicles, and latex showing significant LC_{50} values against *S. litura* larvae. Notably, after 24 hours of feeding on extract-treated castor leaves, mortality reached 100% at 5,000 ppm, indicating the potent activity of methanol extract of *C. gigantea*. This aligns with previous studies suggesting the insecticidal and growth-inhibiting activities of crude plant extracts against lepidopteran species. Moreover, Vetal and Pardeshi (2019) evaluated the insecticidal potential of *Annona squamosa* seed extracts against *Spodoptera litura* larvae, further highlighting the utilization of plant-based products as environmentally friendly pesticides.

Overall, our findings underscore the larvicidal potential of natural product extracts and warrant further investigation into the active ingredients responsible for their insecticidal properties.

Author's contribution

Both the authors contributed equally for conceptualization and designing, collection of plant, collection of insect, laboratory experiment, and datacollection, analysis of data and interpretation and preparation of manuscript of this research paper.

Conflict of Interest

Authors have declared that no competing interests exist.

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Table 1: Antifeedant activity of *C. gigantea* on *S. litura*

Extract	Solvent	Concentration (%)									
		0.4	0.8	1.2	1.6	2	2.4	2.8	3.2	3.6	4.0
Leaf	Alcohol	18.65 ± 0.65b	25.26 ± 1.25c	29.49 ± 3.14b	34.21 ± 2.26c	41.43 ± 4.33c	45.26 ± 4.27c	49.38 ± 5.24c	53.33 ± 6.25c	58.13 ± 5.43c	64.73 ± 6.13c
	Chloroform	17.02 ± 0.72b	21.96 ± 1.56c	26.21 ± 3.78b	31.06 ± 2.84c	37.43 ± 3.98c	42.06 ± 3.09c	47.68 ± 4.16c	53.69 ± 5.24c	56.72 ± 5.17c	64.97 ± 5.26c
	Methanol	19.98 ± 0.65b	30.41 ± 2.26c	34.89 ± 3.23c	40.56 ± 4.47c	47.78 ± 5.34c	55.73 ± 6.45c	58.12 ± 4.35c	60.06 ± 4.33b	68.24 ± 3.81b	79.06 ± 3.42c
Follicle	Alcohol	18. 11 ± 2.09b	26.06 ± 1.38c	29.99 ± 3.84b	32.41 ± 2.26c	39.56 ± 4.53c	43.16 ± 4.47c	47.18 ± 5.34c	49.44 ± 4.09bc	56.73 ± 6.43c	62.24 ± 3.71
	Chloroform	17.26 ± 0.45b	23. 16 ± 1.21c	27.42 ± 3.21b	33.45 ± 2.32c	39.23 ± 3.63c	43.56 ± 3.97c	47.13 ± 4.84c	52.31 ± 5.35c	55.41 ± 5.58c	62.13 ± 5.98c
	Methanol	20.19 ± 0.59b	26.31 ± 2.81c	31.81 ± 3.23c	38.05 ± 4.78c	44.21 ± 5.57c	50.45 ± 6.18c	55.02 ± 4.45c	61.31 ± 4.15b	66.36 ± 3.08b	70.56 ± 3.22c
Latex	Alcohol	16.21± 0.35b	20.76 ± 1.67c	24.06 ± 3.61b	30.02 ± 2.12c	34.16 ± 4.05c	40.22 ± 4.17c	46.08 ± 5.34c	50.93 ± 6.25c	56.56 ± 5.32c	62.83 ± 6.47c
	Chloroform	17. 31 ± 2.31b	22.28 ± 1.16c	27.32 ± 3.24b	31.04 ± 2.06c	36.156 ± 4.03c	40.23 ± 4.12c	44.28 ± 4.34c	48.64 ± 4.12bc	53.31 ± 6.03c	60.04 ± 3.01
	Methanol	23.98 ± 0.61b	34.41 ± 2.32c	36.28 ± 3.34c	43.35 ± 4.56c	49.28 ± 5.14c	58.53 ± 6.18c	61.32 ± 4.23c	64.16 ± 4.31b	69.22 ± 3.68b	80.34 ± 3.71c
	Control	3.18 ± 0.49a									

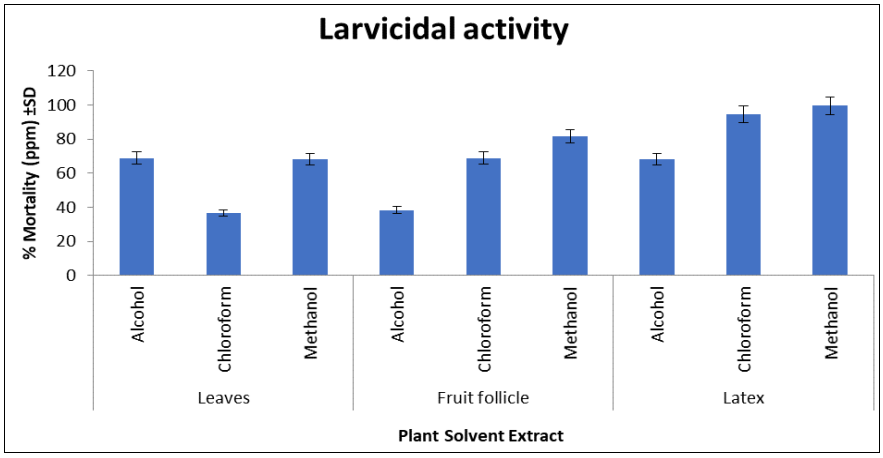

Fig 1: The larvicidal activity of crude leaf extracts against third instar larvae of a*Spodopteralitura* (F.)

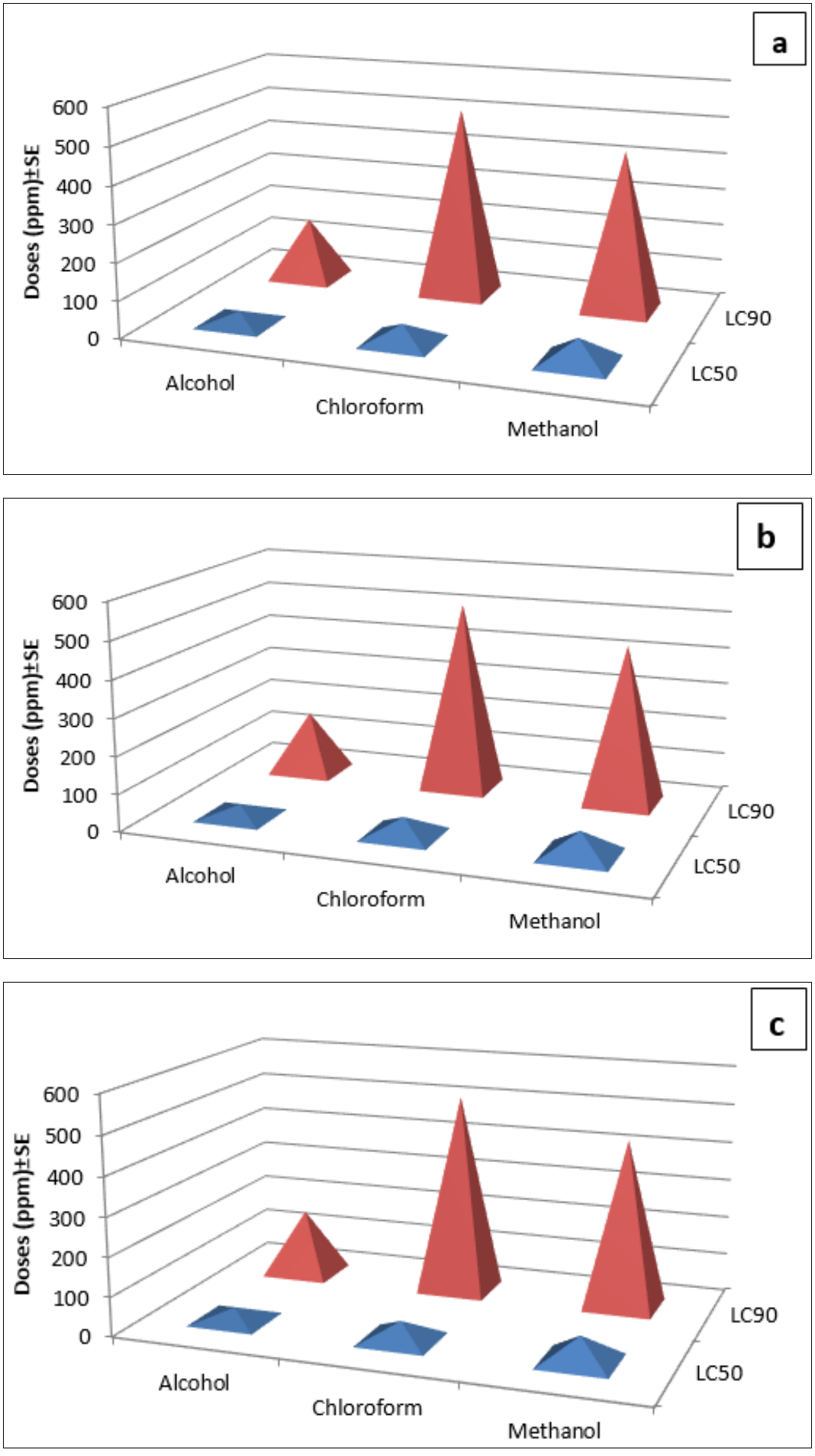

Fig 2: Graph showing the LC50 and LC90 values of pest a *S. litura* (F.) a. *C. gigantea*n leaf extract, b. fruit follicle c. latex

Table 2: Larvicidal activity of*C.gigantea* on *S. litura*

Extract	Solvent	LC_{50}	95% Confidence limit		LC_{90}	95% Confidence limit	
			Lower limit	Upper limit		Lower limit	Upper limit
Leaves	Alcohol	32.46±2.43	30.12	43.52	168.46±20.966	134.37	216.56
	Chloroform	48.26±7.196	44.83	83.04	916.38±221.977	493.30	1363.45
	Methanol	64.17±7.892	52.60	83.54	434.76±91.744	269.9	629.58
Fruit follicle	Alcohol	34.23±2.15	30.74	43.86	210.22±24.673	151.86	248.58
	Chloroform	54.84±7.196	50.83	83.04	958.38±221.977	493.30	1363.4
	Methanol	71.56±6.639	68.55	94.5	554.80±81.177	382.69	700.91
Latex	Alcohol	38.27±7.32	32.60	46.54	469.76±91.744	269.94)	629.58–
	Chloroform	74.43±8.574	68.6	110.23	367.93±54.614	237.88)	451.97
	Methanol	99.42±8.012	83.12	105.12	676.60±90.746	462.74	818.46

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