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Evaluation of Pesticidal Activity and Phytochemical Analysis of *Coscinium fenestratum* and *Careya arborea*

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Ramya D K*, Roopahree T S.

Government College of Pharmacy, Bangalore, Karnataka. India.

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ABSTRACT

Coscinium fenestratum and *Careya arborea* were analyzed for their pesticidal activity by leaf dip and diet bioassay techniques against *Spodoptera litura* and *Helicoverpa armigera*. *Spodoptera litura* and *Helicoverpa armigera* are the devastating pests of numerous wild and cultivated plants throughout the world. It has been reported to attack more than 150 species of agricultural crops including cotton, groundnut, tobacco, maize, bean, potatoes, soybean, rice, sunflower, tomato etc. Management of these insect has been largely based on insecticides, but the development of resistance to most of the synthetic insecticides and an associated environmental problem has necessitated searching for some alternative natural pesticides. New types of herbal pesticides originating from natural products, targeting *Spodoptera litura* and *Helicoverpa* could be a useful alternative for integrated pest management. Herbs were extracted successively with pet ether, chloroform, methanol, ethanol, and water.Extracts were standardized and estimated for their flavonoid content by aluminum chloride assay. The pesticidal activity of all the extracts was evaluated by leaf dip and diet bioassay techniques against *Spodoptera litura* and *Helicoverpa armigera*. Methanol and ethanol extracts of *Coscinium fenestratum* and *Careya arborea* showed optimum mortality rate at 72 hours at the concentration of 50µg/ml. The methanol extract of *Coscinium fenestratum* and *Careya arborea* showed high flavonoids content.

INTRODUCTION

Use of synthetic pesticides began in the 1930s and widespread after World War II and they were found to increase farm yield. Presently, farmers depend heavily on synthetic pesticides for crop protection from insects. The use of synthetic pesticides in agriculture comes with a cost for the environment and the health of animals and humans.

There are many classes of synthetic pesticides causing acute and chronic effects on non-target organisms like animals and humans especially in the reproductive, endocrine and CNS [1] they are also associated with problems like soil pollution, water pollution, and residual effects etc., other problems associated with synthetic pesticides is that the pests can develop resistance towards pesticides. Within a pest population, there is a genetic variation in their resistance to pesticides. Bioassay leads to spraying of pesticides frequently, with the higher dose, causing various serious effects [2].

To overcome all these problems associated with synthetic pesticides, there is a renewed interest in the use of botanicals for crop protection. Over the last few decades, there has been increasing focus on plant-derived products to fight and reduce losses caused by agricultural pests and diseases. Medicinal crops are treated with highly lethal synthetic insecticides so that harmful and harmless beneficial insects are also killed. These insecticides also leave deleterious effects on the plants [3].

Plant-derived pesticides are eco-friendly, safer for use and very effective. Apart from these, natural pesticides do not show any residual effect and organisms develop resistance gradually. Hence plant derived pesticides should be the first choice for most of the garden pest control needs [4]. Presently, the loss due to pests and diseases is about 35% on the field and 14% in storage giving a total loss of about 50% of agricultural crops annually. Hence in this regard, the plants *Coscinium fenestratum and Careya arborea* are screened for the presence of pesticide activity as they contain Flavanoids as their important active principle and have scanty or no reports for pesticide activity. Therefore it is necessary to screen these plants for the presence of pesticide activity [5].

MATERIALS AND METHODS

Collection of Plant Material

The dried Stem bark of *Coscinium fenestratum* and dried leaves *Careya arborea* were obtained from Malappuram District, Kerala. Identification and authentication of plant materials were done by Dr. Siddamallayya and Dr. Rama Rao, at National Ayurvedic Dietetics Research Institute, Bangalore. 4th instar larvae of *Helicoverpa armigera* and *Spodoptera litura* were procured from NBAIR Bangalore.

Fig.1: *Coscinium fenestratum (Bark)*

Fig.2: *Careya arborea* (leaves).

Fig.3: *Spodoptera litura (Larvae*).

Fig.4: *Helicoverpa armigera* (Larvae)

Extraction of the herbs.

Stem Bark of *Coscinium fenestratum* and Leaves of *Careya arborea* were powdered in the blender. Approximately 50 gm of powder was weighed accurately and subjected to extraction in soxhlet apparatus successively with solvents of increasing polarity; viz. Petroleum ether (60-80ºc), Chloroform, Methanol, 95% alcohol and relaxation with water for 8 hours. The extract was collected and each time before successive extraction with next solvent the powdered material was air dried. .The extract was concentrated by evaporating solvent on a Hotplate. Extracts were labeled and stored in airtight containers at room temperature for further studies [6].

Preliminary phytochemical screening of extracts.

Phytochemical analysis of the different extracts was carried out as per the standard procedures [7].

Determination of flavonoid content.

The standard stock solution of Rutin 100mg/ml was prepared. 0.5ml, 1.0ml, 1.5ml, 2.0ml and 2.5ml of above stock solution was pipetted into 10ml of volumetric flask**.**Methanol and extract of *Coscinium fenestratum* (bark) and *Careya arborea* (Leaves) were dissolved separately in methanol to get 1mg/ml concentration

0.5ml of each test extract solution was pipetted into a 25ml volumetric flask (taken in triplicate). Blank was prepared with 1ml of solvent methanol (Without test extract or standard). 4ml of distilled water was added followed by the addition of 0.3 ml of sodium nitrite (5% NaNO2, w/v) and allowed to stand for 6 min. Later 0.3 ml of aluminum trichloride (10% AlCl3) was added and incubated for 6 min, followed by the addition of 4ml of sodium hydroxide (NaOH, 4% w/v) and volume was made up to the 10ml with distilled water. After 15 min of incubation, the mixture turns to pink whose absorbance was measured at 510 nm using a colorimeter.Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of Rutin. The concentration of total flavonoid compounds in the extract was determined by using the formula:

$$T = \frac{CV}{M}$$

Where, T = Total flavanoidal content mg/g of plant extract in rutin,

C = concentration of rutin from the calibration curve,

V = volume of the extract in mL,

M = weight of the pure plant methanol extract [8].

PESTICIDAL ACTIVITY

Leaf bio-assay of *spodoptera litura*

The cotton leaf worm, *Spodoptera litura* 4th instar larvae were reared on castor bean leaves. Twenty larvae were treated in each replicate and three replicates were considered for one treatment. The test extracts were used at concentration 10, 20, 30, 40, 50µg/ml using the leaf dipping technique.The castor bean leaves were cut into equivalent circles 2.5 cm in diameter and immersed in the test solutions for 30 seconds and dried before introducing to insects in plastic Petri plates. The experiment was carried out at 25± 2^{o}C and 70% relative humidity. Control was concurrently conducted with water. After 72 hours exposure, the alive larval number was counted and LC_{50} were calculated at each concentration [9].

Diet bioassay of *Helicoverpa armigera*

Extract were taken at concentration 10, 20, 30, 40, 50µg/ml. Prepared semi-synthetic assay diet and kept in hot water bath at 60°C to prevent solidification. Poured the calculated amount of warm diet into each vial containing calculated amount of extracts and mixed thoroughly on a vortex mixer. Precaution The diluted extracts should never be exposed to hot diet above 60°C while mixing, otherwise, the extracts could get denatured. Kept the bioassay vials with the solidifying diet on the laminar air flow bench till diet gets solidified. Healthy single 4th instar larvae were picked with the help of hair brush and transferred onto the diet in each vial. Bioassay vials were covered with the cotton plug and kept in the environmental chamber (26±1°C and RH

65±5%).After 72 hours exposure, the alive larval number was counted and LC_{50} were calculated at each concentration.

STATISTICAL ANALYSIS

Leaf Bioassay and Diet Bioassay data's were subjected to Probit analysis using SPSS or POLO Statistical software to determine LC_{50} values [10].

RESULTS AND DISCUSSION

Phytochemical screening:

Indian system of medicine has a long history of use of medicinal plants but they lack adequate scientific documentation, particularly in light of modern scientific knowledge. The medicinal value of the plant lies in the bioactive phytoconstituents of the plant and which shows various physiological effects on human body. Through phytochemical investigation, one could detect the various important compounds which could be used as the base of modern drugs for curing various diseases [11]. The Preliminary phytochemical investigation of the plants is an important aspect in finding the future biological activity of the plant [12].

The phytochemical investigation was carried out using different test showed that the presence of alkaloids, carbohydrates, proteins, flavonoids, glycosides, triterpenoids, saponins, steroids and tannins. (Table No.1).

Table No.1. Preliminary Phytochemical screening of extracts.

	Coscinium fenestratum					*Careya arborea*				
Phytochemicals	**P E**	**CF**	**MT**	**AL**	**AQ**	**P E**	**CF**	**MT**	**AL**	**AQ**
Test for alkaloids										
i. Mayer's test	-	-	+	-	-	-	-	-	-	-
ii. Hager's test	-	-	+	-	-	-	-	-	-	-
iii. Wagner's test	-	-	+	-	+	-	+	-	-	-
iv. Dragendroff's test	-	+	-	-	-	-	-	-	-	-
Test for glycosides										
i. Borntrager's test	-	-	-	-	-	-	-	-	-	-
ii. Mod-Borntrager's test	-	-	-	-	-	-	-	-	-	-
iii. Legal's test	-	-	-	-		+	-	-	+	-
Test for Phytosterols and Triterpenoids										
i. Salkowaski test	-	-	-	-	-	-	-	-	-	-
ii. Libermannbuchard test	-	-	-	-	-	-	-	-	-	-
Test for Saponins										
i. Foam test	-	-	+	+	+	-	-	-	-	-
ii. Hemolysis test	-	-	-	-	-	-	-	-	-	-
Test for Tannins and phenolic compounds										
i. Ferric chloride test	-	-	-	-	-	-	+	-	-	+
ii. Gelatin test	-	-	-	-	-	-	-	-	-	-
iii. Extract+dil KMNO4	-	-	+	-	-	-	-	-	-	-
iv. Extract+K3Fe(CN)6	-	-	-	-	-	-	-	-	-	-
Test for Flavonoids										
i. Ferric chloride test	-	-	+	+	-	-	-	-	-	-
ii. Shinoda test	-	-	+	+	-	+	+	+	+	-
iii. Lead acetate test	-	-	-	-	-	+	+	+	+	+
Test for carbohydrates										
i. Molish test	-	-	+	+	+	-	-	-	-	-

ii. Feling's test	-	-	-	-	-	-	-	-	-	-
Test for proteins										
i. Biuret test	-	-	-	-	-	-	-	-	-	-
ii. Ninhydrin test	-	-	-	-	-	-	-	-	-	-
Test for gums and mucilage										
i. Ruthenium red	-	-	-	-	-	-	-	-	-	-
Test for Fixed oils										
i. Spot test	-	-	-	-	-	-	-	-	-	-

'+' indicates positive, '-' indicates negative

Total Flavonoid content

It was observed that methanol and ethanolic extracts contain flavonoids. As per the literature, flavonoids are attributed to pesticidal activity. Hence determination of Total flavonoids was carried out. Among tested extracts methanol and ethanol extracts of *Coscinium fenestratum* showed high Flavanodal content than *Careya arborea* (TableNo.2).

Table No.2 Total Flavonoid content for *Coscinium fenestratum*(stem bark) and *Careya arborea(leaves)*

Sr. No.	SOLVENTS	*COSCINIUM FENESTRATUM* (STEM BARK) (%)*	*CAREYA ARBOREA* (LEAVES)*
1	METHANOL	73.8.±1..59	70±0.52
2	ETHANOL	72.5±0.21	68±0.68

***Values are expressed in terms of Mean±SEM of results done in Triplicate**

Pesticidal activity:

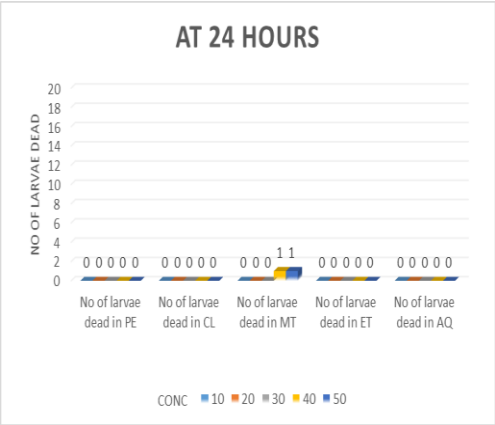
Evaluation of the Pesticidal activity of *Coscinium fenestratum* and *Careya arborea* on *Spodoptera litura* and *Helicoverpa armigera* was carried out. Leaf bioassay was employed for *Spodoptera litura* and Diet bioassay for *Helicoverpa armigera* with different extracts at the concentrations of 10,20,30,40 and 50(µg/ml). Mortality was observed at 24, 48,72hours.

Methanol and Ethanol extracts showed the high Mortality rate with the higher concentration of 50 (µg/ml) at 72 hours. Chloroform extracts showed the least activity. Pet ether and water extracts did not show any activity. (Table No.3-6, Graph No.1-4).

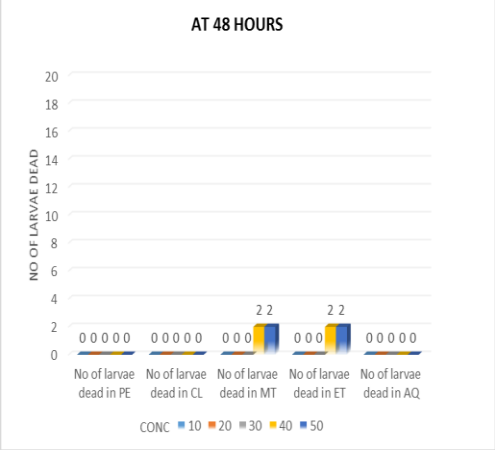
Table No.3: Leaf Bioassay of *Coscinium fenestratum* on *Spodoptera litura.*

Extract	Concentration (µg/ml)	No. of larvae tested	24 Hrs		48 hrs		72 hrs	
			No of larvae alive	No of larvae dead	No of larvae alive	No of larvae dead	No of larvae alive	No of larvae dead
Pet Ether	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	20	0
	30	20	20	0	20	0	20	0
	40	20	20	0	20	0	20	0
	50	20	20	0	20	0	20	0
Chloroform	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	19	1
	30	20	20	0	20	0	18	2
	40	20	20	0	20	0	16	4
	50	20	20	0	20	0	11	9
Methanol	10	20	20	0	20	0	19	1
	20	20	20	0	20	0	19	1
	30	20	20	0	20	0	16	4
	40	20	19	1	18	2	13	7
	50	20	19	1	18	2	6	14
Ethanol	10	20	20	0	20	0	18	2
	20	20	20	0	20	0	17	3
	30	20	20	0	20	0	14	6
	40	20	20	0	18	2	12	8
	50	20	20	0	18	2	10	10
Water	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	20	0
	30	20	20	0	20	0	20	0
	40	20	20	0	20	0	19	1
	50	20	20	0	20	0	19	1
	Control	20	20	0	20	0	20	0

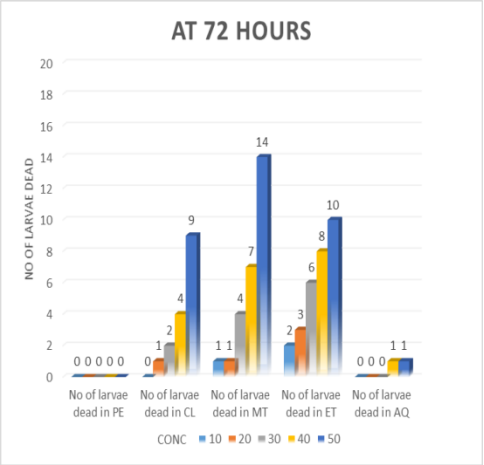
Graph No.1: Leaf Bioassay of *Coscinium fenestratum* on *Spodoptera litura*



As per the results at 24 hrs two larvae were dead at high concentration in the methanol extract of *Coscinium fenestatum.*



At 48 hours none of the larvae were dead in pet ether, chloroform, and water extracts. At the concentrations of 40 and 50 (µg/ml) two larvae were dead in methanol and ethanol extracts of Coscinium *fenestatum.*

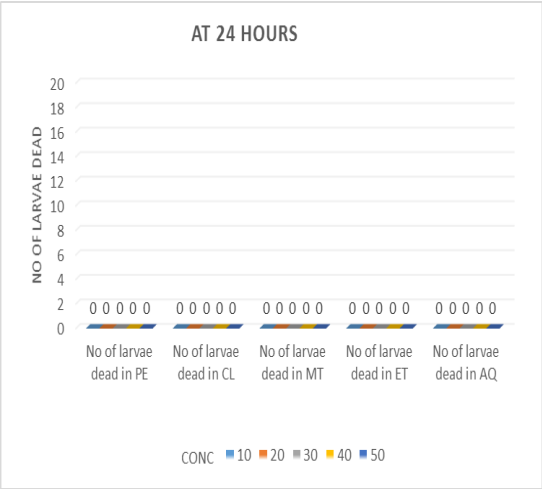


At 72 hours larval death was observed in all the extracts of *Coscinium fenestratum*

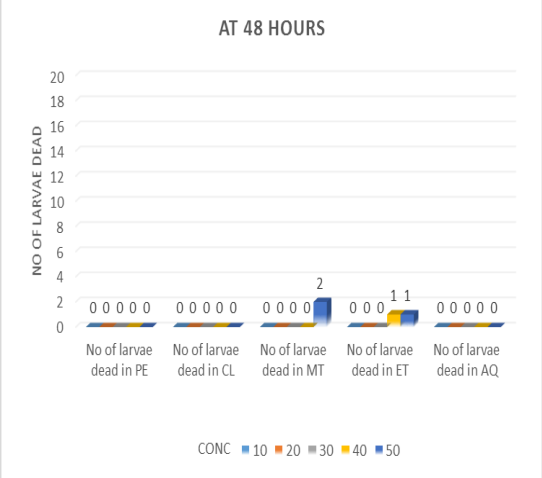
Table No.4: Diet Bioassay of *Coscinium fenestratum* on *Helicoverpa armigera*

Extract	Concentration (µg/ml)	No. of larvae tested	24 Hrs		48 hrs		72 hrs	
			No of larvae alive	No of larvae dead	No of larvae alive	No of larvae dead	No of larvae alive	No of larvae dead
Pet Ether	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	20	0
	30	20	20	0	20	0	20	0
	40	20	20	0	20	0	20	0
	50	20	20	0	20	0	20	0
Chloroform	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	20	0
	30	20	20	0	20	0	20	0
	40	20	20	0	20	0	19	1
	50	20	20	0	20	0	19	1
Methanol	10	20	20	0	20	0	17	3
	20	20	20	0	20	0	15	5
	30	20	20	0	20	0	13	7
	40	20	20	0	20	0	7	13
	50	20	20	0	18	2	6	14
Ethanol	10	20	20	0	20	0	16	4
	20	20	20	0	20	0	13	7
	30	20	20	0	20	0	13	7
	40	20	20	0	19	1	7	13
	50	20	20	0	19	1	5	15
Water	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	20	0
	30	20	20	0	20	0	19	1
	40	20	20	0	20	0	19	1
	50	20	20	0	20	0	19	1
	Control	20	20	0	20	0	20	0

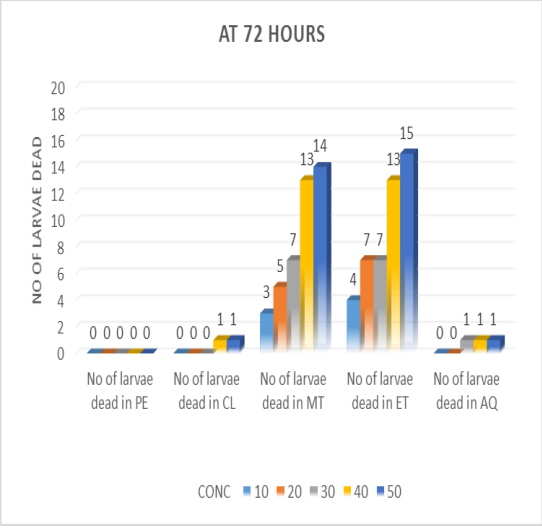
Graph No.2: Diet bio-assay of *Coscinium fenestratum* on *helicoverpa armigera*



As per the results at 24hours none of the larvae were dead in all the extracts of *Coscinium fenestatum*



At 48 hours none of the larvae were dead in pet ether, chloroform, and water extracts. At the concentrations of 50 (µg/ml) three larvae were dead in methanol and ethanol extracts of *Coscinium*

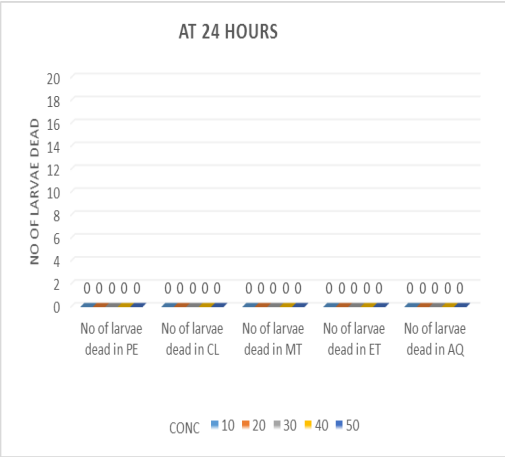


At 72 hours larval death was observed in Chloroform, methanol, ethanol and water extracts of *Coscinium fenestratum*

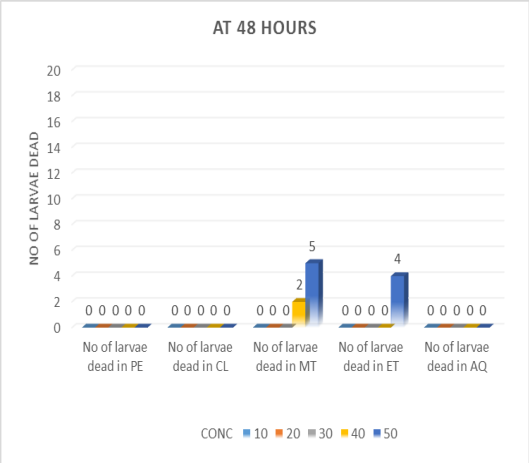
Table No.5: Leaf Bioassay of *Careya arborea* on *Spodoptera litura.*

Extract	Concentration (µg/ml)	No. of larvae tested	24 Hrs		48 hrs		72 hrs	
			No of larvae alive	No of larvae dead	No of larvae alive	No of larvae dead	No of larvae alive	No of larvae dead
Pet Ether	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	20	0
	30	20	20	0	20	0	20	0
	40	20	20	0	20	0	20	0
	50	20	20	0	20	0	20	0
Chloroform	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	20	0
	30	20	20	0	20	0	19	1
	40	20	20	0	20	0	15	5
	50	20	20	0	20	0	15	5
Methanol	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	19	1
	30	20	20	0	20	0	19	1
	40	20	20	0	18	2	15	5
	50	20	20	0	15	5	8	12
Ethanol	10	20	20	0	20	0	18	0
	20	20	20	0	20	0	17	0
	30	20	20	0	20	0	14	0
	40	20	20	0	20	0	12	4
	50	20	20	0	16	4	10	6
Water	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	20	0
	30	20	20	0	20	0	20	0
	40	20	20	0	20	0	20	0
	50	20	20	0	20	0	18	2
	Control	20	20	0	20	0	20	0

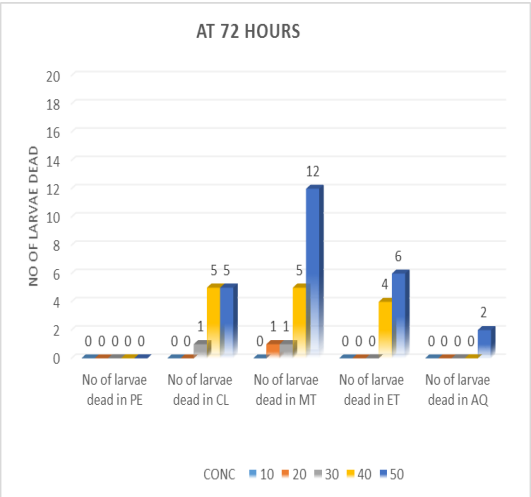
Graph No.3: Leaf Bioassay of *Careya arborea* on *Spodoptera litura*



As per the results at 24hours none of the larvae were dead



. At 48 hours five larvae were dead in methanol extract and four larvae were dead in ethanol of Careya *arborea* at the concentrations of 40 and 50 (µg/ml).

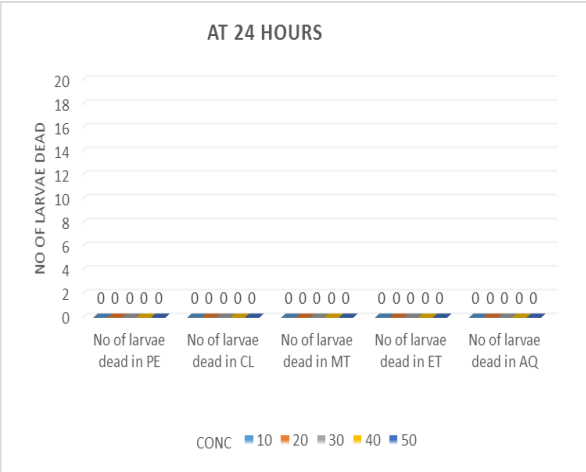


At 72 hours larval death was observed in Chloroform, methanol, ethanol and water extracts of *Careya arborea*

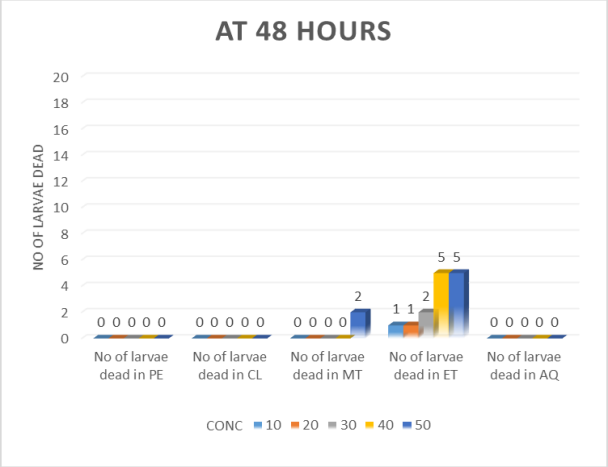
Table No.6: Diet Bioassay of *Careya arborea* on *Helicoverpa armigera*

Extract	Concentration (µg/ml)	No. of larvae released	24 Hrs		48 hrs		72 hrs	
			No of larvae alive	No of larvae dead	No of larvae alive	No of larvae dead	No of larvae alive	No of larvae dead
Pet Ether	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	20	0
	30	20	20	0	20	0	20	0
	40	20	20	0	20	0	20	0
	50	20	20	0	20	0	20	0
Chloroform	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	20	0
	30	20	20	0	20	0	19	1
	40	20	20	0	20	0	18	2
	50	20	20	0	20	0	18	2
Methanol	10	20	20	0	20	0	17	3
	20	20	20	0	20	0	15	5
	30	20	20	0	20	0	9	11
	40	20	20	0	20	0	8	12
	50	20	20	0	18	2	5	15
Ethanol	10	20	20	0	19	1	17	3
	20	20	20	0	19	1	15	5
	30	20	20	0	18	2	10	10
	40	20	20	0	15	5	8	12
	50	20	20	0	15	5	5	15
Water	10	20	20	0	20	0	20	0
	20	20	20	0	20	0	20	0
	30	20	20	0	20	0	20	0
	40	20	20	0	20	0	18	2
	50	20	20	0	20	0	18	2
	Control	20	20	0	20	0	20	0

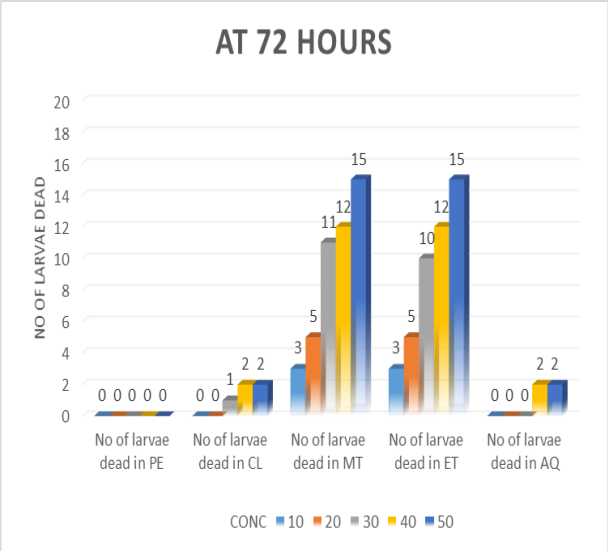
Graph No.4: Diet Bioassay of *Careya arborea* on *Helicoverpa armigera*



As per the results at 24hours none of the larvae were dead



At 48 hours larval death was observed in ethanol extract of Careya *arborea* at the concentrations of 40 and 50 (µg/ml).



At 72 hours larval death was observed in Chloroform, methanol, ethanol and water extracts of *Careya arborea.*

Statistical analysis

LC_{50} were determined by using SPSS software. (Table No.7-10, Graph No.5-8).

Table No.7: LC 50 of *Coscinium fenestratum* on *Spodoptera litura*

EXTRACT	Time (Hrs)	LC50	LOWER BOUNDQ	UPPER BOUND	Chi square (x2)
Pet ether	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
Chloroform	24	0	0	0	0
	48	0	0	0	0
	72	47.252	41.297	59.458	0.692
Methanol	24	0	0	0	0
	48	0	0	0	0
	72	37.968	33.95	44.086	4.954
Ethanol	24	0	0	0	0
	48	76.861	.	.	0.838
	72	37.462	30.946	47.378	1.68
water	24	0	0	0	0
	48	0	0	0	0
	72	79.37	0	0	0.901

Graph No.5: LC 50 of *Coscinium fenestratum* on *Spodoptera litura*

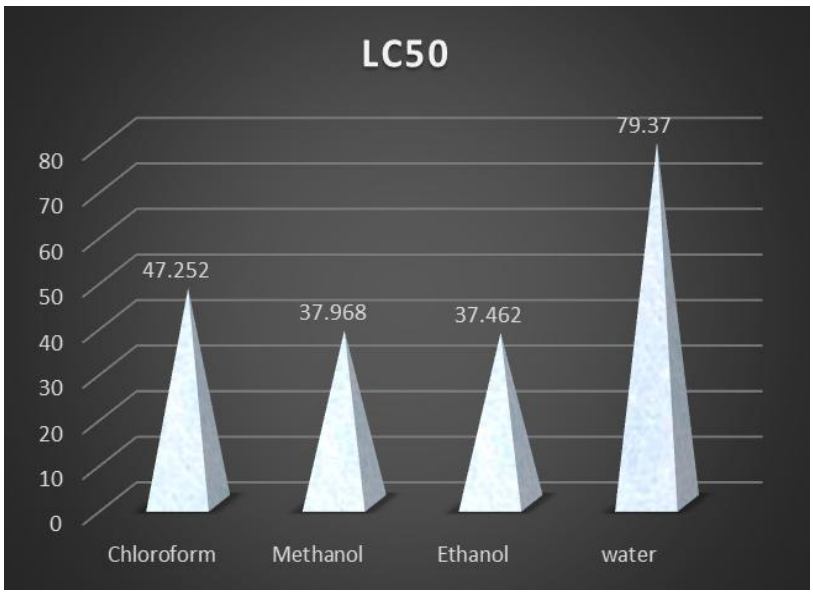


Table No.8: LC 50 of *Coscinium fenestratum* on *Helicoverpa armigera.*

EXTRACT	Time (Hrs)	LC50	LOWER BOUNDQ	UPPER BOUND	Chi square (x2)
Pet ether	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
Chloroform	24	0	0	0	0
	48	0	0	0	0
	72	65.117	.	.	0.389
Methanol	24	0	0	0	0
	48	55.122	.	.	0.008
	72	27.056	22.106	32.185	2.428
Ethanol	24	0	0	0	0
	48	76.861	.	.	0.838
	72	24.331	18.673	29.877	5.08
water	24	0	0	0	0
	48	0	0	0	0
	72	92.729	.	.	1.348

Graph No.6: LC 50 of *Coscinium fenestratum* on *Helicoverpa armigera*

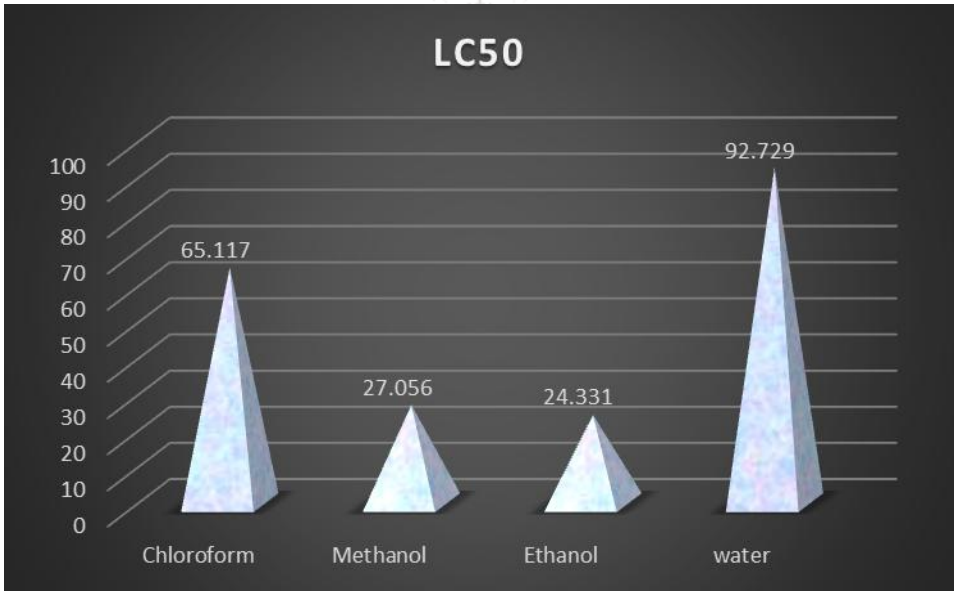


Table No.9: LC 50 of *Careya arborea* on *Spodoptera litura.*

EXTRACT	Time (Hrs)	LC50	LOWER BOUNDQ	UPPER BOUND	Chi square (x2)
Pet ether	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
Chloroform	24	0	0	0	0
	48	0	0	0	0
	72	53.019	45.73	77.081	2.348
Methanol	24	0	0	0	0
	48	59.93	49.616	130.324	2.197
	72	42.878	38.512	49.171	2.678
Ethanol	24	0	0	0	0
	48	42.878	38.512	49.171	2.678
	72	52.011	43.876	74.441	2.112
water	24	0	0	0	0
	48	0	0	0	0
	72	61.976	.	.	0.009

Graph No.7: LC 50 of *Careya arborea* on *Spodoptera litura*

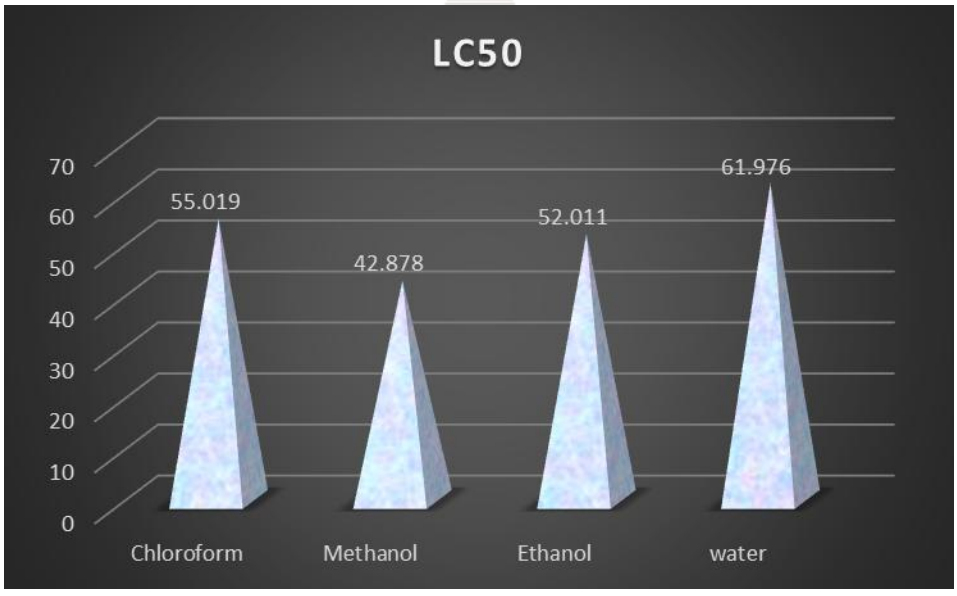
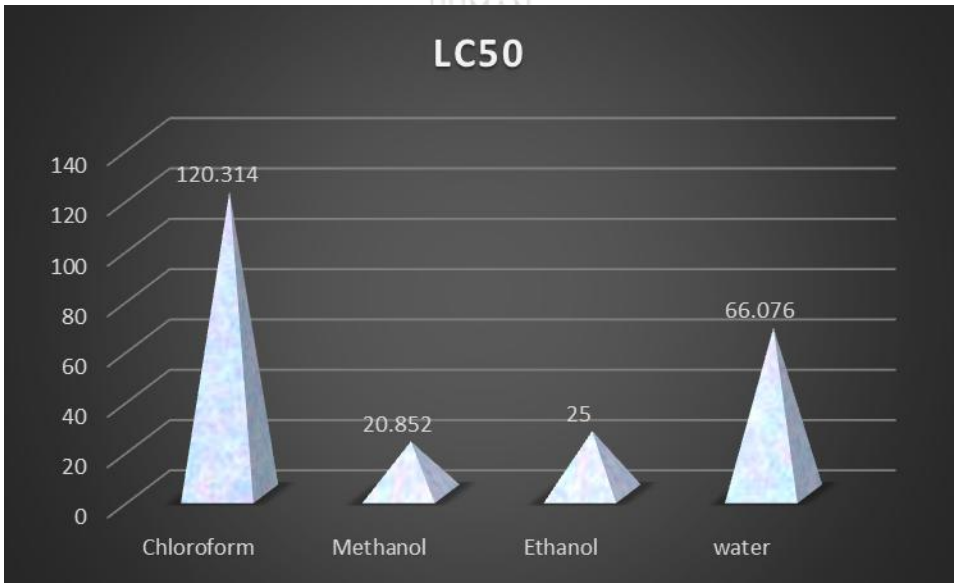


Table No.10: LC 50 of *Careya arborea* on *Helicoverpa armigera.*

EXTRACT	Time (Hrs)	LC50	LOWER BOUNDQ	UPPER BOUND	Chi square (x2)
Pet ether	24	0	0	0	0
	48	0	0	0	0
	72	0	0	0	0
Chloroform	24	0	0	0	0
	48	0	0	0	0
	72	120.314			1.143
Methanol	24	0	0	0	0
	48	55.122			0.008
	72	20.852			2.611
Ethanol	24	0	0	0	0
	48	52.61	43.079	79.44	1.728
	72	25	20.306	29.694	2.276
water	24	0	0	0	0
	48	0	0	0	0
	72	66.076			8.276

Graph No.8: LC 50 of *Careya arborea* on *Helicoverpa armigera.*



CONCLUSION

The study was successful in establishing quality standards for selected crude drugs viz., stem bark of *Coscinium fenestratum* and Leaves of *Careya arborea.* It was found that methanolic

extract of *Coscinium fenestratum and Careya arborea* have excellent pesticidal activity. Which could be due to flavonoids compounds present in these substracts. These are the common plants found in many regions of India and does not possess toxic or side effects humans, grazing animals or to the environment. Hence these extracts can be explored for their pesticidal efficacy as the alternative to synthetic pesticides. These plants may be beneficial in eradicating Pests like *Spodoptera litura* and *Helicoverpa armigera.*

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