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RESEARCH ARTICLE

Biological activity of *Cassia tora* L. Evaluated by dose-mortality, repellent activity, cytotoxicity, larvicidal activity and piscicidal activity

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Abstract

Petroleum ether (PE), chloroform ($CHCl_3$), and methanol (CH_3OH) extracts of the whole *Cassia tora* Linn. plant were evaluated by dose-mortality tests and for their repellent activity against *Callosobruchus chinensis* L., *Sitophilus oryzae* L., and *Tribolium castaneum* (Hbst.), their cytotoxicity against *Artemia salina* L. nauplii, their larvicidal activity against *Culex quinquefasciatus* Say larvae, and their piscicidal activity against the fish *Poecilia reticulata* Peters, under laboratory conditions. In the dose-mortality test, the PE and CH_3OH extracts of the tested plant showed favorable results against *C. chinensis*, the PE extract was the most active with an LD_{50} of 1.51 mg/cm^2 after 48 h of exposure. Against *S. oryzae*, the PE extract showed the highest activity with an LD_{50} of 0.62 mg/cm^2 after 48 h of exposure. And against *T. castaneum*, only the PE and CH_3OH extracts of the tested plant caused mortality, while the PE extract was more active with an LD_{50} of 0.82 mg/cm^2 after 48 h of exposure. However, the *C. tora* extract with $CHCl_3$ induced no mortality in *C. chinensis* or *T. castaneum*. In the repellent test, with the exception of the PE and $CHCl_3$ extracts against *T. castaneum*, all extracts showed significant repellent activity against *C. chinensis*, *S. oryzae*, and *T. castaneum*. Regarding the cytotoxicity test, all *C. tora* extracts reacted against *A. salina* nauplii, with the PE extract proving the most effective, with a minimum LC_{50} of 33.54 ppm after 48 h of exposure. Against *C. quinquefasciatus* larvae, the $CHCl_3$ extract exhibited the highest larvicidal activity, with an LC_{50} of 60.93 ppm after 48 h of exposure. During the piscicidal activity test, only the CH_3OH extracts of the tested plant showed promising results against the fish *P. reticulata*, with an LC_{50} of 28.85 ppm after 48 h of exposure. In contrast, the PE and $CHCl_3$ extracts of *C. tora* were not lethal to *P. reticulata.*

Keywords: *Cassia tora*, Cytotoxicity, Dose-mortality, Larvicidal and Piscicidal activity, Repellency.

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Introduction

Cassia tora Linn. is an annual subshrub that grows throughout tropical Asia and Africa, thriving in uncultivated areas. This invasive and unsightly annual herbaceous plant belongs to the legume family and grows in tropical and subtropical regions worldwide (Sarkar et al. 2012). It is commonly known as 'scicklepod'. Its leaves contain several anthraquinone glycosides, recognized for their therapeutic properties (Maity et al. 1998). The plant is harmless to animals and humans; however, its leaves and seeds are used in Ayurvedic medicine to treat coughs, leprosy, ringworm, colic, flatulence, dyspepsia, constipation, bronchitis, and heart disorders (Maity et al. 1998). The leaves and seeds are a valuable remedy for skin conditions, including ringworm and itching (Shadab et al. 2019). Extracts of *C. tora* have been shown to stimulate the activity of liver enzymes in rats with ethanol-induced hepatotoxicity, including catalase, superoxide dismutase, and glutathione peroxidase (Choi et al. 2001). Ethanolic extracts of this plant have also demonstrated lipid-lowering activity in Wistar rats (Patil et al. 2004). Furthermore, sennosides, known for their medicinal properties, have been detected in the plant's leaves (Lohar et al. 1975).

Phytochemical analysis of the leaves revealed the presence of polyphenols. The presence of phenolic compounds prompted us to evaluate their antioxidant and antiproliferative potential. The antiproliferative activity of the methanolic extract of its leaves, in the presence of cisplatin (an anticancer drug), was studied on human cervical cancer cells (Abraham et al. 2009). Furthermore, its seeds are traditionally recognized for improving visual acuity (Mar and Read 1936). In addition, it has been reported that the plant possesses nitrite-scavenging properties (Park et al. 1995) and that it stimulates the formation of medullary cross-linking (Chan et

al. 1976), decreases hypertension (Koo et al. 1976), protects against ethanol-induced hepatotoxicity (Choi et al. 2001) and lowers liver lipid levels (Ha et al. 2001).

In addition, several studies have shown that different parts of this plant are useful for treating many diseases; various parts of this plant have been used for their laxative, antigenotoxic (Wu and Yen 2004), antipsoriatic (Geetha 2014), hepatoprotective (Choi et al. 2001), antimutagenic (Wu and Yen 1999), anticancer (Rejiya and Cibin 2009), antihyperlipidemic (Patil et al. 2004), anthelmintic (Daiziel 1995), antinociceptive (Chidume 2002), antiappetitive and larvicidal (Baskar and Ignacimuthu 2012) properties. Previous studies have highlighted the need for further research on *C. tora*. The present study therefore aimed to evaluate the insecticidal, repellent, larvicidal, cytotoxic, and piscicidal effects of the plant against three common stored-food pests: *Callosobruchus chinensis* L., *Sitophilus oryzae* L., and *Tribolium castaneum*, as well as against *Artemia salina* L. and the larvae of the insect vectors *Culex quinquefasciatus* and *Poecilia reticulata*. The beetle tested, *C. chinensis*, belongs to the Chrysomelidae family and is commonly known as the legume weevil, Chinese weevil, or Cowpea weevil (Chandra and Girish 2006).

This species is one of the most damaging pests of stored pulses due to its varied diet and wide distribution (Yanagi et al. 2013). The rice weevil, *S. oryzae*, is the most devastating pest of stored cereals worldwide, as it feeds directly on the grains (Agrawal and Singh 1979, Tabasum et al. 2012). It is the main pest of stored rice in warm climate regions (Akhtar et al. 2015). Another pathogen, *T. castaneum*, commonly known as the "red flour beetle" (Coleoptera: Tenebrionidae), is not adapted to the consumption of whole grains, but almost all types of flours, crushed cereals, etc., including whole wheat flour, bran, rice flour, cornmeal, barley flour and oat flour (Islam et al. 2019). Furthermore, to test cytotoxic activities, we used *A. salina* L. (Anostraca: Artemiidae), a genus of crustaceans commonly known as brine shrimp (crayfish). This organism is typically used to assess the toxicity of substances (Ruebhart et al. 2008). *Culex* species, particularly *C. quinquefasciatus* (Vadivalagan et al. 2017), are the primary vector of Bancroftian filariasis and a potential vector of *Dirofilaria immitis* (Bhattacharya et al. 2016). Finally, the guppy, *P. reticulata*, is a typical tropical freshwater aquarium fish. Wild guppies consume a wide variety of foods, including benthic algae and mosquito larvae, which helps to limit the spread of malaria. This species of fish is used as a model organism in studies on ecology, evolution and behavior (Magurran 2005).

Materials and Methods

Collection of plant materials

Whole, vigorous, insect and disease-free plants of *C. tora* growing in the wild were collected from cultivated fields on the Rajshahi University campus. Reference specimens were identified and preserved in the herbarium of the Rajshahi University Department of Botany. The soil around the roots was removed and dried. The leaves and other parts were cut into small fragments, dried in the shade, and then ground into a powder using an electric grinder. Finally, the powder was stored in an airtight container for the duration of the study.

Preparation of extracts

The dried powder was then calibrated and placed in Erlenmeyer flasks for later use. Three organic solvents-PE, $CHCl_3$, and CH_3OH-were used at a ratio of 100 g × 300 mL twice, with each solution being stirred for 48 h. After 24 h of stirring in a similar container, each concentrate was filtered through filter paper (Fisher Scientific, Hampton, New Hampshire, USA) at 24-h intervals in the same flask, and then evaporated until the extract was obtained. The concentrates were transferred to glass bottles and stored in a refrigerator at 4°C with appropriate labeling until use.

Collection and rearing of test insects

Adult *C. chinensis*, *S. oryzae*, and *T. castaneum* were collected from rearing colonies maintained at the Crop Protection and Toxicology Laboratory. The hatching of *A. salina* nauplii from cysts, mosquito larvae from egg rafts and the rearing of guppies were also maintained in the same laboratory, under optimal temperature conditions, at the Department of Zoology of Rajshahi University, Bangladesh. Given their short life cycle and the simplicity of the rearing techniques, these insects, along with three other pathogens of similar age, were readily available and used for this study.

Hatching of nauplii from Artemia cysts

Fresh cysts in vials were purchased from the Nilkhet Pet and Aquarium Market in Dhaka, Bangladesh. *Artemia* eggs were incubated in an aquarium containing 1 liter of 1 M NaCl saline (pH 8.5). Incubation lasted 48 hours under fluorescent light, and nauplii hatched in 24 to 36 hours at a temperature of 30 to 35°C. After hatching, the nauplii were transferred to test tubes, ten nauplii per tube. 1.5 mL of NaCl solution was added to each tube.

Hatching of mosquito larvae from egg rafts

Mosquito egg rafts were collected on the Rajshahi University campus and reared in the Crop Protection and Toxicology Lab., Dept. of Zoology at the same university. This collection and rearing process was maintained throughout the experimental period to ensure a continuous supply of larvae of the same age for testing.

Rearing of guppies

Common guppies (*P. reticulata*) were purchased from a pet shop in Rajshahi and acclimated for one week prior to testing. The tests were conducted in demineralized water at room temperature. Oxygenation was adequate, and the fish were deprived of food throughout the testing and rearing periods before being placed in the aquarium. The temperature was maintained between 22 and 25°C. Five identical fish were introduced into each 500 mL beaker during the experiment.

Experiments of test agents

Dose-mortality tests on *T. castaneum*

The insecticidal activity test on *T. castaneum* differs from that performed on *C. chinensis* or *S. oryzae* due to the specificity of their feeding habits. *Ad hoc* tests were also conducted to determine the final concentrations for dose determination. For insecticidal activity tests, extracts collected using CH_3OH and PE were dissolved in their extraction solvent at different concentrations and used to determine mortality and significant doses. The final concentrations of the PE extract from *C. tora* used in this experiment were 2.546, 2.037, 1.528, 1.019, and 0.509 mg/cm^2; those of the CH_3OH extract were 3.565, 3.056, 2.546, and 2.037 mg/cm^2 against *T. castaneum*. For each study, 1 ml of solvent was deposited in a 50 mm petridish to form a uniform film. The dishes were then air-dried, leaving the concentrate. The amount of extract in 1 ml of compound was calculated by simply dividing this value by the surface area of the Petri dish, thus determining the dose per square centimeter. After drying, ten beetles (aged 3 to 4 days) were introduced into each petridish, in three replicates. A control group was established with a similar number of beetles after preparing the petridish by adding and dispersing the solvent. The treated insects were then placed in an incubator at a temperature comparable to that of the parent cultures, and the mortality rate was recorded.

Dose-mortality test on *S. oryzae* and *C. chinensis*

An *ad hoc* experiment was set up to determine the final concentrations for dose-mortality selection. The concentrations of the PE extracts were 2.546, 2.037, 1.528, 1.019, and 0.509 mg/cm^2 against *S. oryzae* and 1.782, 1.680, and 1.527 mg/cm^2 against *C. chinensis*. For the $CHCl_3$ extracts, the concentrations were 3.056, 2.546, 2.037, 1.528, and 1.019 mg/cm^2 against *S. oryzae*. The concentrations of the CH_3OH extracts were 3.056, 2.801, 2.546, 2.292, 2.037 and 1.783 mg/cm^2 against *S. oryzae* and 2.291, 2.037 and 1.782 mg/cm^2 against *C. chinensis*. However, the $CHCl_3$ extracts showed no activity against *C. chinensis*. In the experiment, 1 ml of the ready-to-use solution was mixed with the respective grains and allowed to dry. The volatile solvent evaporated rapidly after application. Ten insects of similar age were introduced onto the treated feed in three replicates. A control group was also prepared, consisting of a similar number of insects introduced onto grains treated only with the solvent and dried. The preparations were incubated at a temperature similar to that of the reared insects. Insect mortality was recorded after 6h of exposure and monitored for 48h at 6h intervals.

Cytotoxicity test

Samples at different concentrations were prepared in test tubes by adding a calculated amount of DMSO (dimethyl sulfoxide). Water was then pipetted to a volume of 10 mL in the pre-labeled tubes. Nauplii were visually counted and then transferred to tubes containing 10 mL of water. These tubes were stored at room temperature with a control group. Mortality was observed after 6, 12, 18, 24, 30, 36, and 42h of exposure. The plant extract

solutions at different concentrations for the three *C. tora* extracts in PE were 400, 300, 200, 100, and 50 ppm, in $CHCl_3$ 400, 300, 200, 100 ppm and in CH_3OH 300, 200, 100 and 50 ppm in artificial seawater containing 1% DMSO (v/v) used for this test. Ten nauplii were used in each test tube, and three replicates were performed for each concentration. The test tubes were then filled with 1 mL of NaCl solution. After 6, 12, 18, 24, 30, 36, and 42 h of incubation, the number of survivors and the number of dead nauplii were determined using a stereomicroscope, and the percentage mortality (%M) for each dose was calculated relative to the control. DMSO (1%) served as the negative control, and its final concentration in the test volume was always kept below 1% to avoid potential adverse effects due to its toxicity. The LC_{50} corresponds to the sample concentration required to kill 50% of the *Artemia* nauplii population. It was calculated from the inhibition curve (%) as a function of the logarithm of the extract concentration. According to Meyer et al. (1982), an LC_{50} value below 1 mg/ml is considered toxic, while an LC_{50} value above 1 mg/ml is considered non-toxic.

Larvicidal activity test

Mosquito egg masses were collected on the Rajshahi University campus and reared at the Crop Protection and Toxicology Laboratory of the Zoology Department at the same university. This collection and rearing process was maintained throughout the experimental period to ensure a continuous supply of larvae of the same age for testing. One-day-old mosquito larvae were used to test leaf extracts of the plants under investigation. Dosages were determined through ad hoc experiments. Samples at different concentrations were prepared in test tubes by adding a calculated amount of DMSO to make them hydrophilic before adding water to each tube. Water was then pipetted to fill the pre-labeled test tubes (up to 10 ml). Ten freshly hatched (one-day-old) larvae were added to each test tube containing different selected doses and to a control tube (containing only water and DMSO). Dead larvae were counted 6 h after application and continued for up to 42 h, by visual observation at 6 h intervals. The selected doses of *C. tora* extract against mosquito larvae were 250, 200, 150, 100, and 50 ppm in PE; 350, 250, 150, and 50 ppm in $CHCl_3$; and 300, 250, 200, 150, and 100 ppm in CH_3OH.

Piscicide activity test

The following concentrations of *C. tora* extract were used as final concentrations for the piscicidal activity test on guppies in CH_3OH extract: 66.667, 53.333, 43.333, 33.333, and 23.333 ppm, respectively. To prepare the piscicidal activity test against *P. reticulata*, the plant extract was placed in a 500 mL beaker, and a low concentration of DMSO was used to make it hydrophilic. The pre-labeled beakers (up to 300 mL) were then filled with water, and five guppies of the same size were placed in each beaker and exposed for 48h. Finally, after 6h of application, dead fish were visually examined every 6h for the following 48h. The results were then compared to the control to draw conclusions.

Repellent activity test

The repellency test was performed according to the method of McDonald et al. (1970), with some modifications. An initial concentration for each extract (PE, $CHCl_3$, and CH_3OH) was chosen as the baseline dose for the repellency test applied to adult *C. chinensis* and *S. oryzae*. Successive dilutions were then performed to obtain doses of 0.628, 0.314, 0.157, 0.078, and 0.039 mg/cm^2. For *T. castaneum*, the initial dose was fixed. Petri dishes were divided into three sections, separated by two narrow strips of adhesive tape. The two sections containing the food were placed, one with the treated food and the other with the untreated food, with the middle section positioned away from the center. Ten adult insects were then placed in the center of each Petri dish. During the *T. castaneum* infestation, paper half-discs (Whatman No. 40, 9 cm in diameter) were arranged, and specific doses of concentrates were applied individually to each one. The half-discs were then air-dried for 20 minutes. Each prepared half-disc was then taped edge-to-edge to a control half-disc and placed in a Petri dish. Three replicates were performed for each sample. Because the solvent was volatile, it evaporated within minutes. Ten insects were then placed in each half-disc.

The repellent effect was observed at hourly intervals, for up to five successive exposure periods for each of the three insect species. For *C. chinensis* and *S. oryzae*, insect counts were performed on the untreated portion and the central part of the Petri dish base. For *T. castaneum*, the count was performed on the untreated portion of the Petri dish. The recorded values were then used to calculate the percentage of repulsion, which underwent an arcsine transformation for the analysis of variance (ANOVA). The mean count was converted to the percentage of

repulsion (PR) using the formula of Talukder and Howse (1993, 1995): PR = (Nc - 5) × 20, where Nc represents the number of insects observed every hour on the untreated portion of the disc.

Statistical analysis

The mortality and lethality rates of selected plant extracts on insects, brine shrimp nauplii, mosquito larvae, and guppies were corrected using Abbott's formula: Pr = (Po - Pc / 100 - Pc) × 100, where: Pr = corrected mortality (%), Po = observed mortality (%), and Pc = control mortality (%) (Abbott 1925). The data were subjected to probit analysis according to Finney (1947) and Busvine (1971). The lethality relationship was expressed as median lethal concentration (LC_{50}) and median lethal dose (LD_{50}) for the tested agents.

Results

Dose mortality effects on *C. chinensis*, *S. oryzae* and *T. castaneum*

The results of dose-mortality tests of PE, $CHCl_3$ and CH_3OH extracts of *C. tora* against the test beetles *C. chinensis*, *S. oryzae* and *T. castaneum* are presented in Table 1. In the dose-mortality tests, against *C. chinensis* [Fig. 1 (a-d): Regression lines], the PE extract of *C. tora* showed LD_{50} values of 2.37, 1.88, 1.73, 1.63, 1.58, 1.54, 1.51, and 1.50 mg/cm^2; French CH_3OH extract showed 3.25, 2.87, 2.27, 2.01, 1.89, 1.83, 1.77 and 1.59 mg/cm^2 after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposure respectively. Against *S. oryzae* [Fig. 2 (a-f): Regression lines] PE extract gave LD_{50} values of 4.45, 2.94, 2.04, 1.39, 0.94, 0.79 and 0.62 mg/cm^2 after 12, 18, 24, 30, 36, 42 and 48 h of exposure; The $CHCl_3$ extract gave 3.91, 3.68, 3.29, 2.67, 2.18, 1.77, 1.56 and 1.33 mg/cm^2 and the CH_3OH extract gave 4.20, 3.53, 3.31, 2.83, 2.52, 2.29, 2.11 and 2.00 mg/cm^2 after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposures respectively. Against *T. castaneum* [Fig. 3 (a-d): Regression lines] the PE extract gave LD_{50} values of 3.47, 3.10, 2.60, 1.99, 1.68, 1.28, 0.93 and 0.82 mg/cm^2 after 6, 12, 18, 24, 30, 36, 42 and 48 h of exposure; the CH_3OH extract gave 5.08, 4.63, 3.43, 3.05, 2.62, 2.29 and 2.12 mg/cm^2 after 12, 18, 24, 30, 36, 42 and 48 h of exposure respectively. However, the $CHCl_3$ extract of *C. tora* showed no mortality in adult *C. chinensis* and *T. castaneum*. Based on the intensity of activity of the *C. tora* extracts, they can be ranked in descending order as follows: PE extract against *S. oryzae* > PE extract against *T. castaneum* > $CHCl_3$ extract against *S. oryzae* > PE extract against *C. chinensis* > CH_3OH extract against *C. chinensis* > CH_3OH extract against *S. oryzae* > CH_3OH extract against adult *T. castaneum*.

Table 1: LD_{50} values of *C. tora* extracts against adults of *C. chinensis*, *S. oryzae* and *T. castaneum*.

Plant name	Name of test agents	Solvent of extraction	LD_{50} (mg/cm^2) after hours (h) of exposure							
			6	12	18	24	30	36	42	48
C. tora (Whole plant)	*C. chinensis*	PE	2.37	1.88	1.73	1.63	1.58	1.54	1.51	1.50
		$CHCl_3$	Not active							
		CH_3OH	3.25	2.87	2.27	2.01	1.89	1.83	1.77	1.59
	S. oryzae	PE	-	4.45	2.94	2.04	1.39	0.94	0.79	0.62
		$CHCl_3$	3.91	3.68	3.29	2.67	2.18	1.77	1.56	1.33
		CH_3OH	4.20	3.53	3.31	2.83	2.52	2.29	2.11	2.00
	T. castaneum	PE	3.47	3.10	2.60	1.99	1.68	1.28	0.93	0.82
		$CHCl_3$	Not active							
		CH_3OH	-	5.08	4.63	3.43	3.05	2.62	2.29	2.12

- = No LD_{50} achieved.

The respective regression lines of the LD_{50} values at the minimum and maximum exposure times in Table 1 are given in Fig. 1 (a-d), 2 (a-f) and 3 (a-d) below:

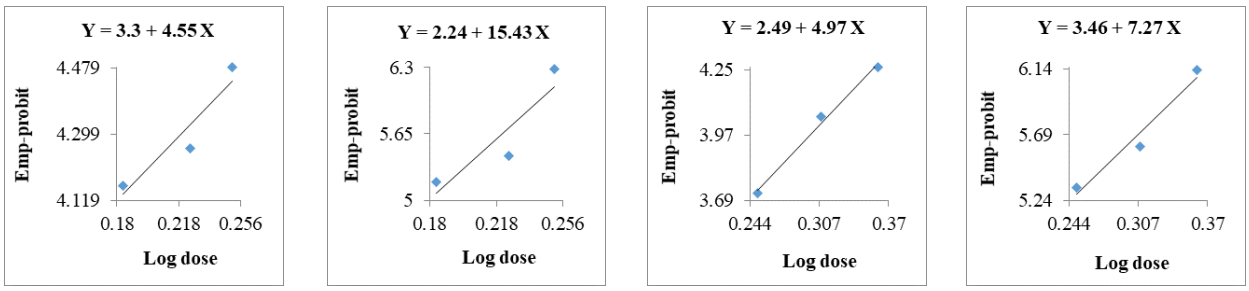
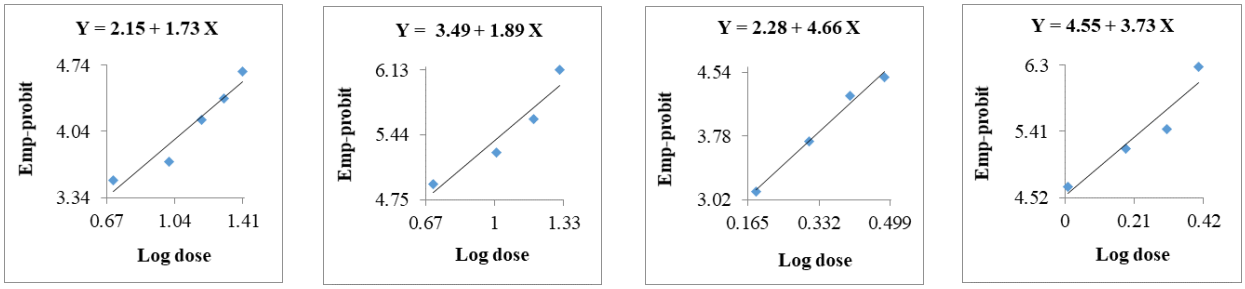


Fig. 1(a-d): Regression lines of *C. tora* extracts versus *C. chinensis* at minimum and maximum exposure times.



a) PE extract after 12 h of exposure.

b) PE extract after 48 h of exposure.

c) $CHCl_3$ extract after 6 h of exposure.

d) $CHCl_3$ extract after 48 h of exposure.

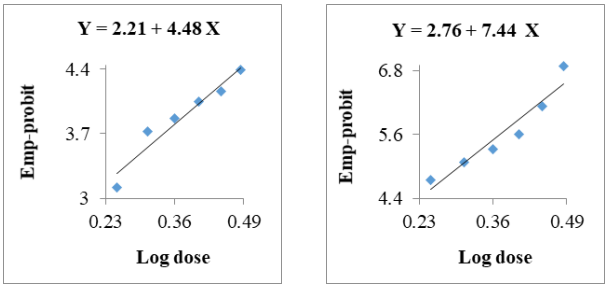
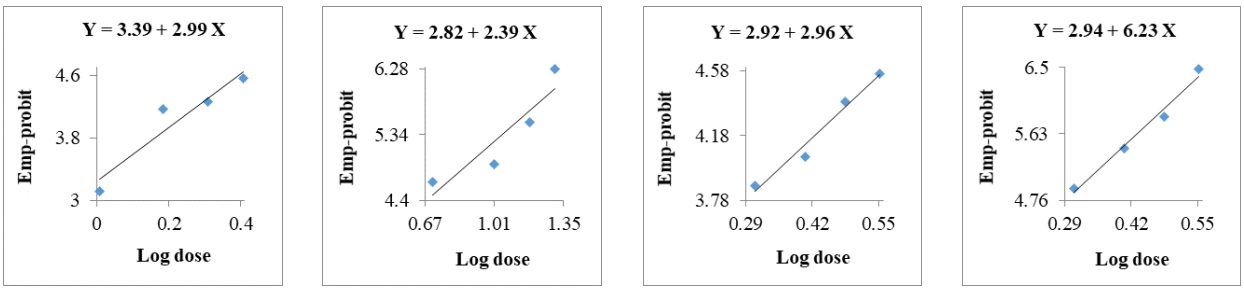


Fig. 2 (a-f): Regression lines of *C. tora* extracts against *S. oryzae* at minimum and maximum exposure times.



a) PE extract after 6 h of exposure.

b) PE extract after 48 h of exposure.

c) CH_3OH extract after 12 h of exposure.

d) CH_3OH extract after 48 h of exposure.

Fig. 3(a-d): Regression lines of *C. tora* extracts versus *T. castaneum* at minimum and maximum exposure times.

Repellent effects on adults of *C. chinensis*, *S. oryzae* and *T. castaneum*

For the repellent activity test, extracts of *C. tora* (PE, $CHCl_3$, and CH_3OH) were tested on the beetles *C. chinensis*, *S. oryzae*, and *T. castaneum*. All extracts of this plant showed repellent activity against *C. chinensis* and *S. oryzae*. Against *C. chinensis*, the PE and CH_3OH extracts showed significant repellent activity at 1% ($p<0.01$); the $CHCl_3$ extract showed significant repellent activity at 0.1% ($p<0.001$). Against *S. oryzae*, the PE, $CHCl_3$, and

CH_3OH extracts showed significant repellent activity at 1% ($p<0.01$). Against *T. castaneum*, only the CH_3OH extract showed significant repellent activity at 1% ($p<0.01$). However, the PE and $CHCl_3$ extracts of *C. tora* showed no repulsion towards *T. castaneum*. Detailed results of the analysis of variance are presented in Table 2.

Table 2: Repulsion of *C. tora* extracts against *C. chinensis*, *S. oryzae* and *T. castaneum*.

Test plant	Solvent used	Test agent	Source of variation	SS	df	MS	F	P-value
C. tora (Whole plant)	PE	*C. chinensis*	Between doses	45287.7	4	11322	45.50**	1.52E-08
		S. oryzae	Do	23088.2	4	5772.10	39.28**	4.4E-08
		T. castaneum	Do	1161.33	4	290.33	3.86	0.02
	$CHCl_3$	*C. chinensis*	Do	41056.5	4	10264.13	80.51***	2.17E-10
		S. oryzae	Do	39334.4	4	9833.60	44.04**	1.93E-08
		T. castaneum	Do	1641.87	4	410.47	3.21	0.04
	CH_3OH	*C. chinensis*	Do	18046.3	4	4511.60	33.03**	1.51E-07
		S. oryzae	Do	26887.7	4	6721.90	32.49**	1.7E-07
		T. castaneum	Do	5541.33	4	1385.33	27.29**	6E-07

* = Significant at 5% level; ** = Significant at 1% level and *** = Significant at 0.1% level.

Cytotoxicity of *A. salina* nauplii

The cytotoxic activity of the *C. tora* extract from the tested plant is shown in Table 3. This extract demonstrated promising cytotoxic potential against *A. salina* nauplii [Fig. 4 (a-f): regression lines] for the three extraction solvents used. The PE extract of *C. tora* gave LC_{50} values of 888.86, 761.87, 326.41, 177.97, 106.20, 80.91, 57.77, and 33.54 ppm; the $CHCl_3$ extract gave LC_{50} values of 1785.66, 1258.26, 704.60, 276.67, 193.41, 158.16, 124.29, and 92.74 ppm; and the CH_3OH extract yielded LC_{50} values of 1507.89, 978.98, 311.95, 157.50, 92.36, 59.73, 52.64, and 39.70 ppm after 6, 12, 18, 24, 30, 36, 42, and 48 h of exposure, respectively. The plant extracts tested against *A. salina* can be ranked in descending order of activity as follows: PE extract >CH_3OH extract >$CHCl_3$ extract.

Table 3: LC_{50} values of *C. tora* extract on *A. salina* nauplii, *C. quinquefasciatus* larvae and *P. reticulata* fish.

Plant name	Name of test agents	Solvent of extraction	LC_{50} values (ppm) at different exposure hours (h)							
				12	18	24	30	36	42	48
C. tora (Whole plant)	*A. salina* nauplii	PE	888.86	761.87	326.41	177.97	106.20	80.91	57.77	33.54
		$CHCl_3$	1785.66	1258.26	704.60	276.67	193.41	158.16	124.29	92.74
		CH_3OH	1507.89	978.98	311.95	157.50	92.36	59.73	52.64	39.70
	C. quinquefasciatus larvae	PE	686.91	500.59	430.41	262.64	159.46	109.76	84.53	62.16
		$CHCl_3$	1612.96	1592.95	1275.64	391.57	235.56	131.21	86.05	60.93
		CH_3OH	462.91	405.94	355.77	302.87	242.02	160.32	135.58	125.26
	P. reticulata fish	PE	Not active							
		$CHCl_3$	Not active							
		CH_3OH	120.15	95.48	81.48	63.96	58.21	38.07	32.08	28.85

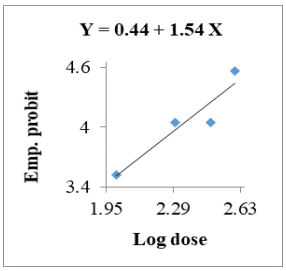
Larvicidal activity

The larvicidal activity of the *C. tora* extract is presented in Table 2. The tested extract of this plant showed promising lethal effects against *C. quinquefasciatus* larvae [Fig. 5 (a-f): Regression lines] in three different solvents. The *C. tora* extract in PE showed LC_{50} values of 686.91, 500.59, 430.41, 262.64, 159.46, 109.76, 84.53, and 62.16 ppm; in $CHCl_3$, of 1612.96, 1592.95, 1275.64, 391.57, 235.56, 131.21, 86.05, and 60.93 ppm. and in CH_3OH, the concentrations were respectively 462.91, 405.94, 355.77, 302.87, 242.02, 160.32, 135.58, and 125.26 ppm after 6, 12, 18, 24, 30, 36, 42, and 48 h of exposure. Based on the intensity of the plant's sensitivity to mosquito larvae, the extracts were ranked in descending order as follows: $CHCl_3$ extract >PE extract >CH_3OH extract.

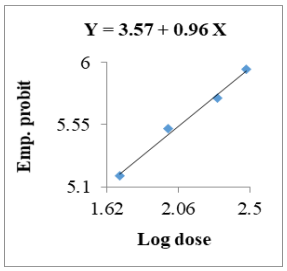
Piscicidal activity

The piscicidal activity of *C. tora* is also presented in Table 2. Only the CH_3OH extract of the tested plant showed promising toxicity potential against *P. reticulata* [Fig. 6 (a-b): Regression lines]. The *C. tora* extract in CH_3OH yielded LC_{50} values of 120.15, 95.48, 81.48, 63.96, 58.21, 38.07, 32.08, and 28.85 ppm after 6, 12, 18, 24, 30, 36, 42, and 48 h of exposure, respectively. However, the PE and $CHCl_3$ extracts of *C. tora* showed no toxicity potential against *P. reticulata*.

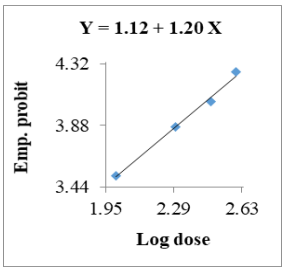
The respective regression lines of the LC_{50} values at the minimum and maximum exposure times in Table 2 are given in Fig. 4 (a-f), 5 (a-f) and 6 (a-b) below:



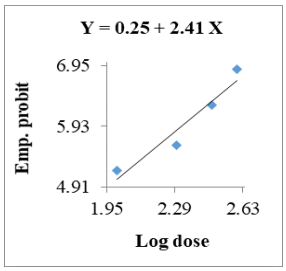
a) PE extract after 6h of exposure.



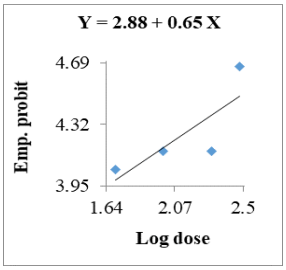
b) PE extract after 48h of exposure.



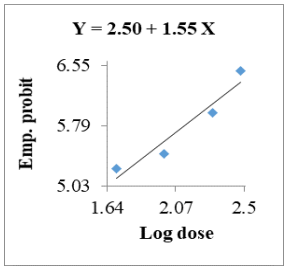
c) $CHCl_3$ extract after 6h of exposure.



d) $CHCl_3$ extract after 48h of exposure.

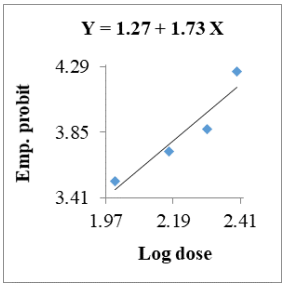


e) CH_3OH extract after 6h of exposure.

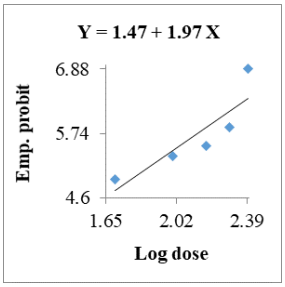


f) CH_3OH extract after 48h of exposure.

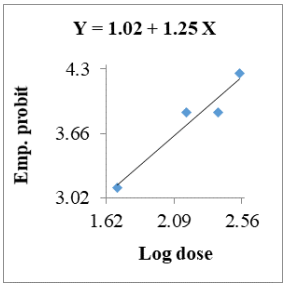
Fig. 4 (a-f): Regression lines of *C. tora* extracts versus *A. salina* at minimum and maximum exposure times.



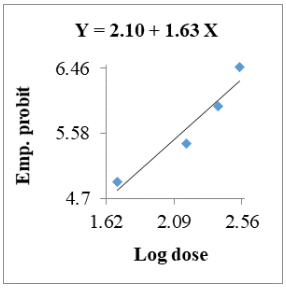
a) PE extract after 6h of exposure.



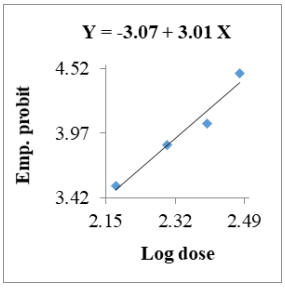
b) PE extract after 48h of exposure.



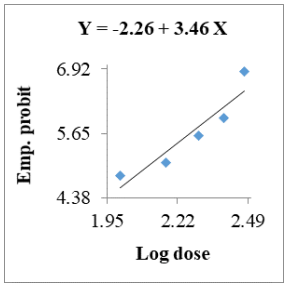
c) $CHCl_3$ extract after 6h of exposure.



d) $CHCl_3$ extract after 48h of exposure.

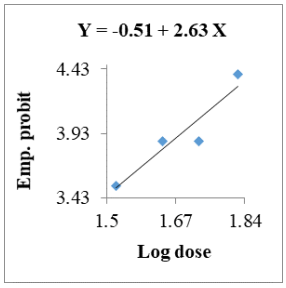


e) CH_3OH extract after 6h of exposure.

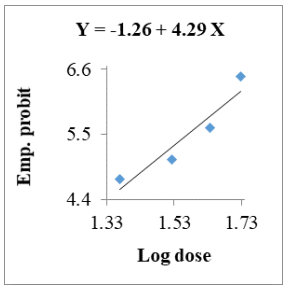


f) CH_3OH extract after 48h of exposure.

Fig. 5 (a-f): Regression lines of *C. tora* extracts against *C. quinquefasciatus* larvae at minimum and maximum exposure times.



a) CH_3OH extract after 6 h of exposure.



b) CH_3OH extract after 48 h of exposure.

Fig. 6 (a-b): Regression lines of *C. tora* extracts against *P. reticulata* at minimum and maximum exposure times.

Discussion

This study aimed to evaluate the efficacy and bioactive potential of the plant *C. tora* and built upon previous research. Leaf extracts of this plant have demonstrated purgative activity (Pal et al. 1977) and significant antifungal activity (Mukherjee et al. 1996). Furthermore, nanoparticles synthesized from *C. tora* have shown significant antibacterial activity against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria (Saravanakumar et al. 2015). The ethanolic extract and its ether-soluble and water-soluble fractions were evaluated for their lipid-lowering activity in a newt-induced hyperlipidemia model. The leaf extract of the tested plant (200 mg/100 mg/kg body weight) showed maximal antifertility activity in rats (Pawar and D'Mello 2011). Furthermore, emodin, an anthraquinone found in the root and bark of the plant, possesses antitumor activity (Choi et al. 1998).

The methanolic extract of the plant's leaves demonstrated a significant anti-inflammatory effect against carrageenan, histamine, serotonin, and dextran-induced hindleg edema in rats (Maity et al. 1997). Furthermore, this extract exhibited significant antifungal activity, inhibiting the growth of *Candida albicans*, *Aspergillus niger*, *Saccharomyces cerevisiae*, and *Trichophyton mentagrophytes* (Lemli and Cuveela 1974). One study found that both the alcoholic and crude leaf extracts were toxic to mice (Sharma et al. 2005). Previous scientific work has shown that methanolic extracts of *C. tora* L. (MECT) and *C. occidentalis* L. (MECO) exhibited greater antioxidant activity (Yen et al. 1998). The ethanolic extract (0.15 mg) and the aqueous extract (0.31 mg) of the *C. tora* plant exhibit antimicrobial activity against various bacteria, with the aqueous extract showing the greatest activity against *Staphylococcus aureus* and *Lactobacillus* (Sharma et al. 2010). Furthermore, a study revealed that ononitol monohydrate extracted from *C. tora* possesses larvicidal activity against *H. armiger* and *S. litura*.

Similarly, the leaf extract of the tested plant demonstrated insecticidal activity against *Aspidimorpha miliaris* and *Zonabris pustulata* (Baskar and Ignacimuthu 2012). A study on this plant showed that the ethanolic extract of its leaves exhibits significant antipsoriatic activity (Geetha 2014). Furthermore, a study suggests that fibers extracted from *C. tora* are safe and provide an additional source of dietary fiber, necessary for maintaining an optimal lipid profile in individuals with type 2 diabetes and mild hypercholesterolemia (Cho et al. 2005). In addition, an analysis revealed that the crude seed extract and anthraquinones isolated from the edible legume *C. tora* possess larvicidal activity against the malaria vector (Mbatchou et al. 2017). Thus, the results of this study and previous research suggest that a thorough exploration of the biological activities of the tested plant *C. tora*, followed by the identification and synthesis of its bioactive chemical components, could reveal potential applications.

Conclusion

The study of the results of lethality, repellency, cytotoxicity, larvicidal and piscicidal activity tests against *C. chinensis*, *S. oryzae*, *T. castaneum*, *A. salina*, *C. quinquefasciatus* and *P. reticulata* concluded that *C. tora* contains potent bioactive compounds that could be successfully used in pest control in agriculture, the pharmaceutical industry, for larvicidal applications and for the protection of aquatic environments. Furthermore, since these plant components are of natural origin, they are potentially biodegradable, making them environmentally friendly and commercially viable.

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Author's contribution: TM, KMM and AI collected samples and data, conducted experiments, writing the draft, analysis was done statistically. NI and MN designed the conceptualization and supervised the study, writing review and editing the manuscript. All authors have read and approved the final manuscript.

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