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Biological activity of *Abrus precatorius* L. through Dose-mortality, repellent activity and Brine shrimp lethality tests

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Abstract
Petroleum ether (Pet. ether), chloroform ($CHCl_3$) and methanol (CH_3OH) extracts of the aerial part, seed, seed pod and roots of *Abrus precatorius* L. have thoroughly been screened for their insecticidal and repellent activity against the red flour beetle, *Tribolium castaneum* (Herbst) adults and cytotoxicity against *Artemia salina* L. nauplii. The CH_3OH extracts of seed pod found most effective giving LD_{50} values 0.781, 0.587, 0.386 and 0.314mg/cm^2, followed by the CH_3OH extracts of roots giving LD_{50} values 1.277, 0.889, 0.632 and 0.411mg/cm^2, and the aerial part giving LD_{50} values 0.584, 0.511, 0.458 and 0.417 mg/cm^2 all the three for 12, 24, 36 and 48h of exposure respectively; while the seed extracts of Pet. ether and CH_3OH showed the lowest mortality and gave LD_{50} 0.578 and 0.597 mg/cm^2 both after 48h of exposure respectively. In case of cytotoxicity, all the extracts responded through brine shrimp lethality assay and the CH_3OH extract of aerial part found most effective giving LC_{50} values 59.382, 25.836, 12.360 and 1.554ppm after 12, 18, 24 and 30h of exposure respectively. The $CHCl_3$ extracts of seed and roots showed repellent activity against *T. castaneum* adults both at 1% ($P< 0.01$) level of significance, while the other extracts did not show any activity. The results revealed the potentials of *A. precatorius* to be used in the pest management sector.

Keywords: Dose mortality, repellency, *Abrus precatorius*, *Tribolium castaneum*, *Artemia salina*

1. Introduction
Abrus precatorius L. is a creeper with many branches [1] of the family Fabaceae [2-3] found all throughout the plains from Himalayan down to Southern India and Ceylon [2]. Flowers are pink, bluish and appear in clusters. Legumes are 1.5 - 3.5cm long containing red, white and black colored seeds. Red colored seeds have black spot on their tips. Roots and leaves are sweet like that of *G. glabra* [1]. It is used medically in China, Indo China, Pacific Islands, West Indies, Guinea, Brazil, Sudan, South Africa, Madagascar and India [3]. Flowering season for this plant is mainly winter, fruits ripen in summer [3]. The roots, leaves and seeds of the plant are used medicinally [2, 4]. *A. precatorius* leaves are used as aphrodisiac, tonic, removes biliousness, useful in eye diseases, cures leukoderma, itching, skin diseases and wounds [3]. The leaves are also used as diuretic, and in diarrhoea, gastritis, heart diseases, kidney diseases, insomnia, Cancer and as CNS sedative [5]. Powdered leaves mix with sugar given in case of leukoderma and menorrhagia [6]. Roots of this plant are taken for sore throat and rheumatism [3]. The seeds are used as purgative, but in large doses are acrid poison, given rise to symptoms resembling those of cholera, taken internally by women, the seed disturbs the uterine functions and prevents conception. Water decoction of the seeds of this plant reduced to a paste are used for contusion and inflammation [3]. The present investigation was carried out to find out its insecticidal and insect repellent potentials against the red flour beetle, *Tribolium castaneum* (Hbst.) [7, 8, 9], and lethality against the brine shrimp, *Artemia salina* L. [10, 11, 12] nauplii. The red flour beetle is reddish-brown in color and its antennae end in a three-segmented club [13]. Although small beetles, about ¼ of an inch long, the adults are long-lived and may live for more than three years [14], and thus became a suitable lab insect. The *A. salina* belongs to a genus of very primordial crustacean (crawfish-crayfish) the *Anostraca* (Fairy Shrimps). Crawfish of this genus just have a divided exoskeleton made of chitin enhanced protein, no usual crust of chitin (escutcheon) as the other crawfish have. There are many species within the genus of *Anostraca*, but the *A. salina* is very nice to grow, and since the rate of successful hatches is very high this species is used as a test agent in the toxicological laboratory.

2. Materials and Methods

2.1 Collection and preparation of test Materials: Different parts of *Abrus precatorius viz*. aerial part, seed, seed pod and roots were collected from the campus of the University of Rajshahi, Bangladesh and identified by comparison with the voucher specimen preserved in the herbarium of the Department of Botany, University of Rajshahi. After collection the aerial part, seed pod and roots were chopped into small pieces separately, dried under shade and powdered using a hand grinder. Dried seeds also ground to powder, and all the powdered materials were weighed and placed in separate conical flasks. Solvent was added in a ratio of 300ml for 100gm of dust and filtered twice within 48h and after complete removal of the previous solvent the next solvent was added in the same manner and continued until collection of extracts in Pet. ether, $CHCl_3$ and CH_3OH. Filtration was done by Whatman filter paper. The filtrates were set for evaporation of the solvents until the extracts were left. The extracts were then removed to glass vials and preserved in a refrigerator at 4°C with proper labeling.

2.2 Collection and culture of the test organisms: Adults of *T. castaneum* were reared in glass beakers (500ml) in a standard mixture of whole-wheat flour with powdered dry yeast (19:1) in an incubator at 30 ±0.5°C without light and humidity control for a continuous supply of adults during experimentation.

2.3 Dose-mortality test: The dose-mortality responses of *A. precatorius* were observed by residual film method [13]. The concentrations used were 2.540, 2.040, 1.520, 1.020, 0.520, 0.250 and 0.120mg cm^{-2} for the CH_3OH extract of the aerial part followed by 2.040, 1.520, 1.020, 0.520, 0.250 and 0.120mg cm^{-2} and 2.040, 1.520, 1.020, 0.520, 0.250 and 0.120mg cm^{-2} for the Pet. ether and CH3OH extracts seeds; and 2.040, 1.520, 1.020, 0.520, 0.250 and 0.120mg cm^{-2} and 2.540, 2.040, 1.520, 1.020, 0.520, 0.250 and 0.120mg cm^{-2} for the CH_3OH extracts of seed pod and roots against *T. castaneum* in the dose mortality experiments. Each of the doses were diluted in 1ml of solvent, poured into each of the Petri dishes and allowed to dry out. Ten adult beetles were released in each of the treated Petri dishes, and the experiment of all the doses for each of the extracts were replicated in thrice. The mortality was counted after 12, 24, 36, and 48h of exposure.

2.4 Statistical analysis: The mortality (%) was corrected using Abbott's formula [14]. The data were then subjected to probit analysis according to Finney [15] and Busvine [13].

2.5 Repellent activity test: The repellent activity test was adopted from the method (No. 3) of McDonald *et al*. [16] with some modifications. Half filter paper discs (Whatman No. 40, 9 cm diam.) were treated with the selected doses of 0.629, 0.314, 0.157, 0.0786, 0.0393mg cm^{-2} for all the extracts and were then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in the Petri dishes. The orientation was changed in the two remaining replicates to avoid the effects of any external directional stimulus affecting the distribution of the test insects. Ten adult insects were released in the middle of each of the filter paper circles.
Each of the concentrations for each of the extracts collected in three different solvents was tested for 3 times. Insects that settled on each of the non-treated half of the filter paper discs were counted after 1h and then observed repeatedly at hourly intervals for five hours. The average of the counts was converted to percent repulsion (*PR*) using the formula of Talukder and Howse [17-18]: $PR = (Nc - 5) \times 20$, where, Nc is the percentage of insects on the untreated half of the disc.

2.6 Brine shrimp Nauplii lethality test: Brine shrimp cysts were purchased from Kalabagan, Dhaka and kept in an aerated seawater at room (25 - 30ºC) temperature and took 30-48h to give nauplii. The series of concentrations of *A. precatorius* were 200, 100, 50, 25, 12.5 and 6.25ppm for all the extracts *viz*. aerial part, seed, seed pod and roots. Ten freshly hatched nauplii were released to each of the test tubes with different concentrations mentioned earlier and the mortality was observed after 6, 12, 18, 24 and 30h of exposures. The data was then subjected to probit analysis.

3. Results and Discussion

3.1 Dose mortality effects: The CH_3OH extracts of aerial part, seed, seed pod and roots as well as the Pet. ether extract of seeds of *A. precatorius* are represented in Table 1. The CH_3OH extracts were found effective against *T. castaneum* adults and according to the intensity of activity the source materials could be arranged in the order of: seed pod> root> aerial part> seeds and the Pet. ether extract of seeds gave LD_{50} value 0.578mg cm^{-2} after 48h of exposure and could be placed before the CH_3OH extract of seeds for its activity.

Table 1: LD_{50} values of the test extracts of *A. precatorius* established through residual film assay against *T. castaneum* adults.

Test plant	Plant part used	Solvent used	LD_{50} value (mg cm^{-2})			
			Duration of exposures (in hours)			
			12h	24h	36h	48h
A. precatorius	Aerial part	CH_3OH	0.584	0.511	0.458	0.417
	Seed	Pet. ether	9.309	2.224*	0.895*	0.578*
		CH_3OH	1.070	0.845	0.723	0.597
	Seed Pod	CH_3OH	0.781	0.587	0.386	0.314
	Root	CH_3OH	1.277	0.889	0.632	0.411

***** Variance has been adjusted for heterogeneity

3.2 Repellent effects: The $CHCl_3$ extracts of seed and root offered moderate repellent activity at 1% level of significance (P<0.01) and the Pet. ether extract of seed offered mild repellent activity at 5% level of significance (P<0.05), while the other extracts didn't show any significant repellency at all (Table 2).

Table 2: ANOVA results of the repellency against *T. castaneum* by the Pet. ether, $CHCl_3$ and CH_3OH extracts of the aerial part, seed, seed pod and roots *A. precatorius*.

Plant part used	Solvent of extraction	Sources of variation			F-ratio with level of significance		P- value	
		Between doses	Between time interval	Error	Between doses	Between time interval	Between doses	Between time interval
Aerial Part	Pet. ether	4	4	16	6.295	1.100	0.003	0.390
	$CHCl_3$	4	4	16	2.129	6.148	0.124	0.003
	CH_3OH	4	4	16	7.968	2.505	0.001	0.083
Seed	Pet. ether	4	4	16	6.387	14.769*	0.003	3.1E-05
	$CHCl_3$	4	4	16	20.324**	6.092	4.1E-06	0.004
	CH_3OH	4	4	16	7.966	2.505	0.001	0.083
Seed Pod	Pet. ether	4	4	16	1.868	2.515	0.165	0.082
	$CHCl_3$	4	4	16	1.587	1.290	0.226	0.315
	CH_3OH	4	4	16	1.520	1.424	0.243	0.271
Root	Pet. ether	4	4	16	1.868	2.515	0.165	0.083
	$CHCl_3$	4	4	16	54.991**	4.635	3.8E-09	0.011
	CH_3OH	4	4	16	1.520	1.424	0.243	0.271

** = Significant at 1% level (P<0.01); * = Significant at 5% level (P<0.05)

3.3 Brine Shrimp lethality effect: The brine shrimp lethality results for Pet. ether, $CHCl_3$ and CH_3OH extracts of *A. precatorius* represented in Table 3. The highest lethality was observed in case of CH_3OH extract of the aerial part that gave LC_{50} value 1.55ppm followed by the CH_3OH extracts of seed pod that gave LC_{50} value 1.63ppm and $CHCl_3$ extracts of seed with LC_{50} value 1.79ppm as well as the $CHCl_3$ extracts of aerial part and CH_3OH extracts of the root with LC_{50} 6.04 ppm after 30 hours of exposure against the Brine shrimp nauplii.

Table 3: LC_{50} values of the test extracts of *A. precatorius* established through Brine shrimp Lethality assay against *A. salina* nauplii.

Test plant	Plant part used	Solvent of extraction	LC_{50} value (ppm) Duration of exposures			
			12h	18h	24h	30h
A. precatorius	Aerial part	Pet. ether	83.00	44.20	8.68	6.55
		$CHCl_3$	-	434.10	43.98	2.79
		CH_3OH	59.38	25.84	12.36	1.55
	Seed	Pet. ether	611.86	208.36	18.34	9.15
		$CHCl_3$	*	73.03	35.71	1.79
		CH_3OH	12.58	20.83	26.48	33.40
	Seed pod	Pet. ether	*	780.53	39.88	15.61
		$CHCl_3$	*	784.18	198.80	14.19
		CH_3OH	2.67	15.95	2.82	1.63
	Root	Pet. ether	*	420.20	12.42	14.81
		$CHCl_3$	17.79	19.20	12.85	6.04
		CH_3OH	17.79	19.20	12.85	6.04

4. Discussion

Findings of this investigation receive support from the previous researchers' achievements. Though the research on insecticidal properties is scanty but the plant and its extracts have been long used in tribal communities as a toxin for various purposes. Fresh bark used in India for skin diseases [19]. Dried entire plant is used in preparing medicated oils [19]; fresh leaf is used in Thailand as an anti-inflammatory [20]; ethanol and aqueous extracts of dried seeds of this Indian plant were reported to have antifungal activity against *Cryptococcus neoformans* [21]. Chloroform – methanol extract of *A. precatorius* has shown to have some antidiabetic properties. [22]. The protein content isolated from the seeds of *A. precatorius* had cytotoxic effect on the tumor cells, and exhibited activity against Yoshida sarcoma in rats and a fibrosarcoma in mice [23-24]. Dimetry and his team found that hypaphorine alkaloid isolated from *A. precatorius* seeds was the most efficient component against the two-spotted spider mite where the pre-oviposition period was relatively elongated and the oviposition period was significantly shortened [25]. He also concluded that the fecundity of the females was drastically reduced in all treatments in comparison to the control which is very much similar to this investigations [25]. The repellency and mortality found in this investigation could take place because of the presence of Abrin according to a research finding; who found that Abrin is a ribosome-inactivating protein which blocks protein synthesis and is one of the deadliest plant toxins known [26]. It also receives support that Abrin and Abrin agglutinin are type IV ribosome inactivating proteins that inhibit protein synthesis in eukaryotes and induce apoptosis [27]. Seeds have the potential to be a good insecticide [28], which is very much relevant and strong. As some findings of this investigation were against the stored product pests, however it receives support from previous investigators works [29] that stored products cover a major portion of agricultural produces but several species of insects infest those in storage and cause a huge damage. However, aerial part, seed, seed pod and root extracts of *A. precatorius* using Pet. ether, $CHCl_3$ and CH_3OH were found effective against the brine shrimp nauplii was previously approved [30].

5. Conclusion

The findings of the present study indicate the effects of *A. precatorius* extracts on the adults of *T. castaneum* and *A. salina* nauplii. Being natural in origin these plant materials might be biodegradable and thus safe and sustainable to the environment as well as it will be economically sustainable.

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