JSR Publications

Available Online

J. Sci. Res. **9** (4), 367-373 (2017)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Dose-mortality, Cytotoxicity and Repellent Activity of *Abutilon hirtum* (Lam.) Sweet against *Callosobruchus chinensis* (L.), *Artemia salina* L. and *Tribolium castaneum* (Hbst.)

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Received 15 May 2017, accepted in final revised form 30 September 2017

Abstract

Petroleum ether (Pet. ether), chloroform ($CHCl_3$) and methanol (CH_3OH) extracts of the aerial parts of *Abutilon hirtum* (Lam.) Sweet were subjected to dose-mortality against the stored grain pest *Callosobruchus chinensis* (L.), cytotoxicity against brine Shrimp *Artemia salina* L. nauplii and repellent activity against adult beetles of *Tribolium castaneum* (Hbst.). Against *C. chinensis* only CH_3OH extract showed promising mortality and provided LD_{50} values 1.344, 1.294, 1.243 and 1.152 mg/cm^2 after 6, 12, 18 and 24 h of exposure respectively, however, Pet. ether and $CHCl_3$ extracts didn't show mortality. Against *A. salina* nauplii Pet. ether, $CHCl_3$ and CH_3OH extracts showed cytotoxic effects; while Pet. ether extract gave LC_{50} values 2461.031, 642, 191.233, 94.618 ppm after 6, 12, 18 and 24 h of exposure respectively, and $CHCl_3$ extract provided LC_{50} values 1336.124, 679.387, 276.961 and 199.988 ppm; and CH_3OH offered 531.896, 212.840, 91.499 and 72.975 ppm after 6, 12, 18 and 24 h of exposure respectively. For repellency against *T. castaneum* adults $CHCl_3$ extract showed significant result at 1% level of significance ($P < 0.01$), while the CH_3OH extract showed moderate repellency at 5% level of significance ($P < 0.05$), but the Pet. ether extract didn't show any significant repellent activity.

Keywords: Dose-mortality; Cytotoxicity; Repellent activity; *Abutilon hirtum; C. chinensis*; *A. salina*; *T. castaneum.*

doi: http://dx.doi.org/10.3329/jsr.v9i4.32590 J. Sci. Res. **9** (4), 367-373 (2017)

1. Introduction

As traditional medicine has remained the most inexpensive and easily approachable source of treatment in the primary health care system; and thus the local therapy is the only means of medical treatment for these communities [1]. So, an increasing interest has been noticed in the study of medicinal plants and their traditional use in different parts of the world during the last few decades [2]. The present investigation was attempted on *Abutilon*

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hirtum (Lam.) Sweet, is a perennial herb or shrub, 0.5-2.5 m in height. It is commonly known as "Indian mallow" or "Florida Keys Indian Mallow" and in Bengali it is known as "Jhampi"or "Belabenda" and distributed in tropical regions [3]. Traditionally, the leaves used as demulcent, diuretic and in diarrhea [4]. The decoction of the leaves used as a mouth wash and in bladder inflammations, wounds and treatment of ulcers, in addition to the fruits are eaten raw and the water extract of the bark is given to ease childbirth in Kenya [5,6]. In Malaysia, *A. hirtum* is used as a poultice to ease the pain of kidney gravel and often mixed with glutinous rice and applied to ulcers. The leaves or flowers are applied to abscesses [7]. The strategy for the present investigation was designed to carry on screening of the crude extracts of the test plant species on Brine shrimp *A. salina* L. nauplii and stored grain pest *C. chinensis* (L.) and adult flour beetles of *T. castaneum* (Hbst.) for the detection of biological activity and keeping an option to show the extent of activity by analyzing the data statistically that read on various parameters during the course of the investigation. *C. chinensis* (Coleoptera: Chryssomelidae) commonly known as pulse beetle found as pest to many stored legumes [8]. The eggs are cemented to the surface of pulses and are smooth, domed structures with oval, flat bases. The larvae and pupae are only found in cells bored within the seeds of pulses [9]. It takes 20-25 days for being adult from eggs [10,11]. *A. salina* (Anostraca: Artemiidae) commonly known as Brine shrimp belongs to a genus of very primordial crustacean (Crayfish); *Artemia* is one of the standard organisms for testing the toxicity of chemicals [12]. The females can produce eggs either as a result of mating or via parthenogenesis. Eggs hatch into nauplii that are about 0.5 mm in length. Eggs remain in a dormant state as cysts. These cysts can last for a number of years, and will hatch when they are placed in saltwater [13]. *T. castaneum* (Family: Tenebrionidae) is a worldwide pest of stored products and of Indo-Australian in origin [14]. The red flour beetle may produce an allergic response [15]. Eggs are microscopic and slender larvae are creamy yellow to light brown in colour, while the adult is a small reddish-brown. Total life cycle contains subsequently for egg incubation 8.8 days, larval development 22-100 days depending on temperature, pupal development 4.5 days, and for reproductive maturation 4-5 days [16].

2. Materials and Methods

2.1. *Collection and preparation of test materials*

The fresh materials of *A. hirtum* were collected from the bushy area of Station Bazaar (from both the sides of the railway lines), which is located just near the Rajshahi University Campus, Bangladesh and identified by the Department of Botany, Rajshahi University, where a voucher specimen is kept in the herbarium. The areal parts of the plants (leaves, stem, fruit and flower) were collected and chopped into small pieces, dried under shade and powered with the help of a hand grinder separately, weighed and placed in conical flasks to add solvents. The solvents Pet. ether, $CHCl_3$ and CH_3OH (Merck, Germany) were used (200 g × 600 mL × 3 times) successively each of which kept for 48 h on a shaker. For each

of the extracts filtration was done by filter paper at 24 h of time interval in the same flask followed by evaporation until the extract was left as a scum. The extracts was then removed to glass vials and preserved in a refrigerator at 4°C with proper labeling.

2.2. *Collection and culture of test insects*

The test insects of *C. chinensis* and *T. castaneum* of same age were used in this investigation which were received from the stock cultures of the Crop Protection Laboratory, Department of Zoology, Rajshahi University, Bangladesh. *A. salina* is a marine crustacean which is not easy to culture like *C. chinensis* and *T. castaneum* under laboratory conditions, but, they can be reared in a short edition. These nauplii are very easy to grow from its marketed cysts to carry on toxicity tests of certain materials.

2.3. *Dose-mortality test*

2.3.1. *Dose-mortality tests on C. chinensis*

The concentrations used in this experiment were 1.070, 1.120, 1.171, 1.222 and 1.273 mg/cm^2 for Pet. ether, $CHCl_3$ and CH_3OH extracts. For each of the doses 1 mL was dropped on a Petri dish (50 mm) containing pulse grains. Then the Petri dishes were air-dried leaving the extract on it. After drying 10 beetles (3-5 days old) were released in each of the Petri dishes in 3 replicates. A control batch was also maintained with the same number of insects after preparing the Petri dish by applying and evaporating the solvent only. The treated beetles were placed in an incubator at the same temperature as reared in stock cultures and the mortality of the beetles was counted after 6, 12, 18 and 24 h of exposure.

2.3.2. *Lethality test*

Brine shrimp lethality bioassay is a recent development in the bioassay techniques for the detection of biological activity like cytotoxicity, as well as, a wide range of pharmacological activities (e.g. anticancer, antiviral, pesticidal, anti-AIDS, etc.) of the compounds. To carry on toxicity tests nauplii were grown from its marketed cysts. Test samples at different concentrations considered as doses were prepared in test tubes by addition of calculated amount of DMSO (dimethylsulfoxide) to make them hydrophilic before adding half of the required amount of water in each. Then additional amount of water were added to fill the pre-marked test-tubes with the help of a pipette. The nauplii were counted by visual inspection and were released in test-tubes containing 10 mL of water and the test-tubes along with a control batch were left for 30 h. Observation of mortality was made after 6, 12, 18, 24, 30 and 36 h of exposure. Doses for all three extracts Pet. ether, $CHCl_3$, CH_3OH were 200, 400, 800 ppm respectively.

2.4. *Repellent activity*

The method of repellency test used in this investigation was adopted from the method (No.3) of McDonald [17]. The average of the counts was converted to percent repulsion (PR) using the formula, $PR = (Nc\text{-}5) \times 20$; where, *Nc* was the average hourly observation of insects on the untreated half of the disc [18,19]. A general concentration for each of the extracts (Pet. ether, $CHCl_3$ and CH_3OH) was selected as a stock dose applied against *T. castaneum* adults and other successive doses were prepared by serial dilution to give 0.1571, 0.0785, 0.0392, 0.0196 and 0.0098 mg/cm^2. Half filter paper discs (Whatman No. 40, 9cm diam.) were prepared and selected doses of all the extracts separately applied onto each of the half-discs and allowed to dry out as exposed in the air for 20 min (approx.). Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in a Petri dish (9cm diam.). For each of the test samples three replicates were maintained. Being volatile the solvent was evaporated out within a few minutes. Then ten insects were released in the middle of each filter paper circles. Repellency was observed for one-hour interval and up to five successive hours of exposure, just by counting the number of insects from the non-treated part of the filter paper spread on the floor of the 90 mm Petri dish. The values in the recorded data were then calculated for percent repellency, which was again developed by arcsine transformation for the calculation of analysis of variance (ANOVA).

2.5. *Statistical analysis*

The mortality (%) was corrected using Abbott's formula [20]. The formula is $Pr = (Po - Pc)/(100 - Pc) \times 100$; Here, *Pr* = corrected mortality (%), *Po* = observed mortality (%) and *Pc* = mortality in the control (%). The data were then subjected to probit analysis [21,22].

3. Results

3.1. *Dose mortality effect on C. chinensis*

The results of dose mortality effects of CH_3OH extracts of *A. hirtum* against *C. chinensis* are represented in Table 1. The CH_3OH extract offered mortality by giving LD_{50} values ranged between 1.152 to 1.344 mg/cm^2 against *C. chinensis* for 24 and 6 h respectively.

Table 1. LD_{50} values of CH_3OH extracts of *A. hirtum* against *C. chinensis.*

Extract	LD_{50} (mg/cm^2)			
	6 h	12 h	18 h	24 h
CH_3OH	1.344	1.294	1.243	1.152

3.2. *Cytotoxicity test on A. salina*

The results of the lethality bioassay of Pet. ether, $CHCl_3$ and CH_3OH extract of *A. hirtum* against the brine Shrimp *A. salina* L. nauplii are represented in Table 2. All the extracts offered promising cytotoxic activity. The LC_{50} values for Pet. ether extract were 2461.031, 642, 191.233 and 94.618 ppm; for $CHCl_3$ extract were 1336.124, 679.387, 276.961 and 199.988 ppm; for CH_3OH extract were 531.896, 212.840, 91.499 and 72.975 ppm all after 6, 12, 18 and 24 h of exposures respectively.

Table 2. LC_{50} values of Pet. ether, $CHCl_3$ and CH_3OH extracts of *A. hirtum* against *A. salina*.

Selected plant	Name of the test organism	Extracts	LC_{50} (ppm)			
			6 h	12 h	18 h	24 h
A. hirtum	*A. salina*	Pet. ether	2461.031	642	191.233	94.618
		$CHCl_3$	1336.124	679.387	276.961	199.988
		CH_3OH	531.896	212.840	91.499	72.975

3.3. *Repellent effect on T. castaneum*

The $CHCl_3$ extract showed the significant repellent activity at 1% level of significance ($P < 0.01$) and the CH_3OH extracts showed the significant repellent activity at 5% level of significance ($P < 0.05$), while the Pet. ether extracts did not show any significant repellent activity against the adult beetles of *T. castaneum* (Table 3 and 4).

Table 3. ANOVA result of repellency of *T. castaneum* by *A. hirtum* extract.

Selected plant	Insect pest used	Extracts	Source 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
A. hirtum	*T. castaneum*	Pet. ether	4	4	16	1.879	1.472	0.164	0.257
		$CHCl_3$	4	4	16	31.602**	2.774	2.06E-07	0.064
		CH_3OH	4	4	16	11.872*	0.745	0.001	0.575

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

Table 4. Repellent activity of the Pet. ether, $CHCl_3$ and CH_3OH extracts of *A. hirtum* against *T. castaneum.*

Solvents	Between doses (df = 4)		Between time interval	
	F- values	level of significance	F- values	Level of significance
Pet. ether	1.879	-	1.472	-
$CHCl_3$	31.602**	$P < 0.01$	2.774	-
CH_3OH	11.872*	$P < 0.05$	0.745	-

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

4. Discussion

Now the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable [23]. The findings of the present investigation receive supports from the experiment done on *A. hirtum* and its related species by previous researchers. Works on *A. hirtum* extracts for evaluating cytotoxicity, insect mortality and repellency is scanty, and however there are some evidences of research that have been done to find out the medicinal, phytochemical, pharmacognostical and structural properties. It has been already proved that *A. hirtum* is highly effective in medicinal and traditional usage for the treatment of various human ailments [24]. The leaf extract of *A. hirtum* (Lam.) Sweet can positively be used in the treatments provided by the Herbal Medicine Industry [25]. Also leaf extract of *A. hirtum* possesses significant hepatoprotective activity [26]. Its related species *A. indicum* was found to be effective as the anti-snake venom and anti-ulcer agents [27]. And many important phytoconstituents like *β*-sitosterol and tocopherol oil were isolated from this plant [28,29]. Methanolic petroleum ether, diethyl ether and ethyl acetate extract of *A. hirtum* showed potent antiarthritic activity [30]. An another study suggested that the hydro ethanolic extract of *A. hirtum* possessed antioxidant and anticataract activity which might be due to the presence of phytochemicals such as tannins, phenols, flavonoids and alkaloids [31]. After studying the previous works and analyzing the present outcome of this investigation it is clear that *A. hirtum* contains compounds that have insect mortality, cytotoxicity and repellent activity.

5. Conclusion

By analyzing the results of dose mortality, cytotoxicity and repellent activity tests of *A. hirtum* extracts against *A. salina*, *C. chinensis* and *T. castaneum* it is possible to come to a conclusion that *A. hirtum* has some bioactive potentials could be used in strategic control of certain pest population in the field, in the storage and in the aquatic media.

6. Acknowledgment

The authors are grateful to acknowledge the University Grant Commission (UGC) of Bangladesh for financial support and the chairman, Department of Zoology, Rajshahi University, for providing laboratory facilities.

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