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EVALUATION OF BIOLOGICAL ACTIVITIES OF *EVOLVULUS NUMMULARIUS* L. THROUGH INSECTICIDAL, INSECT REPELLENCY AND BRINE SHRIMP LETHALITY TESTS

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Abstract: Assessment of *Evolvulus nummularius* L. through whole plant extracts (collected in petroleum ether [Pet.E.], chloroform [$CHCl_3$] and methanol [MeOH]) were made against *Tribolium castaneum* (Hbst.) for insecticidal and insect repellency and against *Artemia salina* L. for brine shrimp lethality, and the results were promising. The LC_{50} values against *T. castaneum* adults for Pet.E. extract were 1.280, 1.220, 1.096 and 1.030µg cm^{-2}; for $CHCl_3$ extract 3.249, 2.990, 2.526 and 2.204µg cm^{-2}; and for MeOH extract 2.925, 1.899, 1.875 and 1.789µg cm^{-2}, respectively for 12, 24, 36 and 48h of exposure. The intensity of activity could be arranged in a descending order: Pet.E. extract> MeOH extract> $CHCl_3$ extract. For repellency, $CHCl_3$ and MeOH extracts offered moderate activity ($P < 0.01$), but the Pet.E. extract did not show efficacy against the beetles. The same extracts responded positively in brine shrimp lethality assay where the LC_{50} values for Pet.E. extract were 141.553, 98.941, 24.684 and 8.560ppm; for $CHCl_3$ extracts 699.048, 366.663, 326.0039 and 40.114ppm; and for MeOH extracts 2336.982, 1258.605, 355.962 and 137.0589ppm respectively for 12, 18, 24 and 30h of exposure. However, the intensity of activity could be arranged in a descending order: Pet.E. extract> $CHCl_3$ extract> MeOH extract.

Keywords: *Evolvulus nummularius*; dose-mortality, repellency, brine shrimp lethality, *Tribolium castaneum, Artemia salina.*

সারাংশঃ *Evolvulus nummularius* L. সম্পূর্ণ গাছের পেট্রোলিয়াম ইথার [Pet.E.], ক্লোরোফোর্ম [$CHCl_3$] এবং মিথানল [MeOH] নির্যাসের পোকামাকড় দমন, পোকামাকড় বিতাড়ন কার্যকারিতা যাচাইয়ের জন্য গমের কেড়িপোকা *Tribolium castaneum* (Herbst) এর পূর্ণাঙ্গ দশার উপর ও মারণ কার্যকারিতা যাচাইয়ের জন্য *Artemia salina* L. এর উপর গবেষণাগার ব্যবস্থায় পরীক্ষা চালান হয়, এবং আশাপ্রদ ফলাফল পাওয়া যায়। এক্ষেত্রে Pet.E. নির্যাসটির বিপরীতে LC_{50} ছিল যথাক্রমে ১.২৮০, ১.২২০, ১.০৯৬ এবং ১.০৩০ মাইক্রোগ্রাম প্রতি বর্গসেন্টিমিটারে আর $CHCl_3$ নির্যাসটির বিপরীতে LC_{50} ছিল যথাক্রমে ৩.২৪৯, ২.৯৯০, ২.৫২৬ এবং ২.২০৪ মাইক্রোগ্রাম প্রতি বর্গসেন্টিমিটারে; অবশ্য MeOH নির্যাসটির বিপরীতে ছিল যথাক্রমে ২.৯২৫, ১.৮৯৯, ১.৮৭৫ ও ১.৭৮৯ মাইক্রোগ্রাম প্রতি বর্গসেন্টিমিটারে এবং সবক্ষেত্রে পাঠ নেয়া হয়েছে ১২, ২৪, ৩৬ ও ৪৮ ঘন্টায়। নিম্নক্রমানুসারে সাজালে কার্যকারিতা ফলাফল দাঁড়ায় Pet.E. নির্যাস> MeOH নির্যাস> $CHCl_3$ নির্যাস। পূর্ণাঙ্গ *T. castaneum* এর উপর $CHCl_3$ ও MeOH নির্যাস বিতাড়ন কার্যকারিতা প্রদর্শন করেছে ১% তাৎপর্যতায় ($P<০.০১$), আর Pet.E. নির্যাস কোনরূপ কার্যকারিতা দেখায়নি। একই নির্যাসগুলি brine shrimp lethality পরীক্ষণে বিষক্রিয়া দেখিয়েছে। এক্ষেত্রে Pet.E. নির্যাসটির বিপরীতে LC_{50} ছিল যথাক্রমে ১৪১.৫৫৩, ৯৮.৯৪১, ২৪.৬৮৪ ও ৮.৫৬০ পিপিএম; $CHCl_3$ নির্যাসটির বিপরীতে যথাক্রমে ৬৯৯.০৪৮, ৩৬৬.৬৬৩, ৩২৬.০০৩৯ ও ৪০.১১৪ পিপিএম; এবং MeOH নির্যাসটির বিপরীতে যথাক্রমে ২৩৩৬.৯৮২, ১২৫৮.৬০৫, ৩৫৫.৯৬২ ও ১৩৭.০৫৮৯ পিপিএম এবং সবগুলির ক্ষেত্রে পাঠ নেয়া হয়েছে ১২, ১৮, ২৪ ও ৩০ ঘন্টায়। নিম্নক্রমানুসারে সাজালে কার্যকারিতা ফলাফল দাঁড়ায় Pet.E. নির্যাস> $CHCl_3$ নির্যাস> MeOH নির্যাস।

Introduction

Evolvulus nummularius L. (=*Convolvulus nummularius* L.; Roundleaf Bindweed; ভুঁই আকড়া) (F. Convolvulaceae). It was taken into consideration for screening of its biological activities. This plant is found in Africa, Bangladesh, India, Malaysia and Myanmar. In Bangladesh it is found in all the districts. Medicinal plants are the most important source of lifesaving drugs for the majority of the world population. These plants are moving from fringe to mainstream use, as a greater number of people endeavour to opt for herbal formulations over the allopathic compounds, since these are devoid of side effects and cost effective (Dubey et al., 2004). Popular demand and scientific interest in complementary or alternative medicine, particularly medicinal botanicals, has increased considerably since mid-90's (Borchers et al., 1997). Screening and indexing of many plants, which have been used in traditional Indian and Chinese medicines since time immemorial, resulted in novel therapeutics, useful for the treatment of various ailments of man, such as rheumatism, kapha, pitha, blood pressure and cancer (Anon, 1948; Anon, 1959). In this investigation *E. nummularius* L. had been selected against the red flour beetle, *Tribolium castaneum* (Hbst.) for its toxic and repellent activity, and *Artemia salina* L. nauplii for lethality test for the detection of its biological activities.

Materials and Methods

Collection and preparation of test materials: Fresh plants were collected from the Rajshahi University Campus, Bangladesh, identified and a voucher specimen (No. 60, 10-05-1983) was kept in the herbarium of the Department of Botany, University of Rajshahi. After collection fresh plants were chopped into small pieces to dry under shade in a well-ventilated room. Dried materials were then powdered using a hand grinder, weighed and placed in a conical flask (500ml) to add petroleum ether (Pet.E.) (100gm × 300ml × 2times) for 48h. Filtration was done by Whatman filter paper at 24h interval in the same flask followed by evaporation until the extract ware left. The same material was then placed in another conical flask to add

chloroform ($CHCl_3$) and the same done for methanol (MeOH) extraction. The extracts were then transferred to glass vials and preserved in a refrigerator at 4°C with proper labeling.

Collection and culture of test insects: *T. castaneum* beetles 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°C ±0.5°C without light and humidity control, for a continuous supply of adults during experimentation.

Collection and culture of brine shrimp nauplii: Eggs (cyst) of *A. salina* were purchased from aquarium shops of Kalabagan, Dhaka and kept in aerated seawater (with 3.8% sodium chloride or 38gm salt/1000ml of pond water) at room (25-30°C) temperature. It normally takes 30-48h to give nauplii under these conditions. Freshlyhatched nauplii were used in this experiment.

Dose-mortality test against *T. castaneum*: For dose-mortality responses by surface film method, different doses of the extracts of Pet.E., $CHCl_3$ and MeOH of *E. nummularius* (whole plant) were selected through *ad hoc* experiments by diluting 50mg of each of the extracts separately in 1ml of solvent to apply in 50mm Petri dishes and by increasing or decreasing the amount of extracts in repeated manner until a suitable mortality range was obtained. For the Pet.E. extract a general concentration was selected to produce other successive doses by serial dilution to give 1.529, 1.274, 1.019, 0.764, 0.510 and 0.255µg cm^{-2}. For the $CHCl_3$ extract, a general concentration was selected to give 4.076, 3.567, 3.057, 2.548 and 2.038µg cm^{-2}. For the MeOH extract, a general concentration to give 3.567, 3.057, 2.548, 2.038 and 1.529µg cm^{-2}. Each of the doses were diluted in 1ml of the respective solvent poured into Petri dishes and allowed to dry. Ten adult beetles were released in each Petri dish, and the experiment of all the doses for each of the extracts were set in 3 replicates. The mortality of the beetles was assessed at 12, 24, 36 and 48h of exposures.

Statistical analysis of the dose-mortality data: The mortality of the beetles was recorded, while an instant observation was made just after 30 min. of application for the detection of acute toxicity, if any. The mortality (%) was corrected by Abbott's formula (1925). The statistical analyses were done according to Finney (1947) and Busvine (1971) to calculate the LC_{50} values by using a 'software' developed in the Department of Agricultural and Environmental Science, University of Newcastle upon Tyne, U.K.

Repellent activity against *T. castaneum* adults: The methodology for repellency test used in this experiment was adopted from the method (No. 3) of McDonald et al. (1970) with some modifications by Talukder and Howse (1993, 1994). Half filter paper discs (Whatman No. 40, diameter 9cm) were treated with the selected doses of 10, 5, 2.5, 1.25 and 0.625mg cm^{-2} of Pet.E. extract 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. The same was then done for the $CHCl_3$ and MeOH extracts.

Analysis of repellency data: Each concentration was tested for five 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 5 hours. The average of the counts was converted to percent repulsion (*PR*) using the formula of Talukder and Howse (1995): $PR = (Nc - 5) \times 20$, where, Nc is the percentage of insects on the untreated half of the disc.

Lethality against brine shrimp nauplii: For Pet.E. extract a 5mg sample was initially dissolved in 250µl of dimethyl sulfoxide (DMSO) to make it hydrophilic before adding 5ml of water and diluted up to 10ml with brine water. A similar dose was set to obtain a series of concentrations, *viz.* 475.9, 237.5, 118.7, 59.3 and 29.6ppm as other doses. In this way a general concentration of $CHCl_3$ extract was selected to give 499.5, 249.7, 124.8, 62.5 and 31.2ppm and for MeOH extract a general concentration was selected to give 572.5, 286.6, 143.5, 71.5 and 35.7ppm. Each concentration was tested in triplicate. Ten freshly hatched nauplii were added to each of the test tubes with different concentrations mentioned above. The lethality of the nauplii to the extracts was observed after 12, 18, 24 and 30h of exposure by counting the number died in each of the test tubes. The data was then subjected to probit analysis to get LC_{50} values.

Results and Discussion

Dose-mortality against *T. castaneum* adults: The results of the dose-mortality assay and replellency of Pet. E., $CHCl_3$ and MeOH extracts against *T. castaneum* adults are represented in Table 1. The LC_{50} values for the Pet.E. extract ranged between 1.280 to 1.030µg cm^{-2}; for the $CHCl_3$ extract it ranged between 3.249 to 2.204µg cm^{-2} and for the MeOH extract it was 2.925 to 1.789µg cm^{-2}.

Table 1 LC_{50} values of Pet.E., $CHCl_3$ and MeOH extracts of *E. nummularius* whole plant against *T. castaneum* adults.

Type of extract	Time exposed	LC_{50} value (µg cm^{-2})	95% Confidence limits		χ^2 Value (df)	F-values	
			Lower limit	Upper limit		Duration	Doses
Pet.E.	12	1.280	0.788	2.077	58.52 (4)	0.814ns	0.449ns
	24	1.220	0.691	2.153	92.39 (4)		
	36	1.096	0.799	1.502	35.83 (4)		
	48	1.030	0.779	1.364	28.80 (4)		
$CHCl_3$	12	3.249	2.839	3.717	1.41 (3)	0.825ns	45.102**
	24	2.990	2.663	3.358	0.75 (3)		
	36	2.526	2.313	2.759	3.28 (4)		
	48	2.204	2.000	2.429	5.28 (4)		
MeOH	12	2.925	2.590	3.305	0.27 (2)	0.738ns	36.402**
	24	1.899	1.645	2.191	12.19 (3)		
	36	1.875	1.772	1.984	2.92 (1)		
	48	1.789	1.706	1.876	-		

All F-values are at 4df; ns= not significant; **= P<0.01.

Repellent effects: The repellency results are presented in Table 1 and 2. For *E. nummularius* ($CHCl_3$ and MeOH) extracts mild activity (P <0.01) was established, while the Pet.E. extract did not show repellency at all (Table 1).

Table 2 Percent repulsion (PR) values and the arcsin transformed data of the Pet.E., $CHCl_3$ and MeOH extracts of *E. nummularius* (whole plant) against *T. castaneum* adults.

Type of extracts	Dose mg cm^{-2}	Observation at regular intervals in hrs				
		1	2	3	4	5
		Percent repulsion PR = (Nc – 5) × 20 (Arcsin transformed values for ANOVA)				
Pet.E	10	100 (90)	100 (90)	100 (90)	80 (63.43)	100 (90)
	5	100 (90)	100 (90)	93.4 (75.11)	100 (90)	100 (90)
	2.5	93.4 (75.11)	100 (90)	93.4 (75.11)	100 (90)	100 (90)
	1.25	93.4 (75.11)	100 (90)	100 (90)	100 (90)	100 (90)
	0.625	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
$CHCl_3$	10	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
	5	53.4 (46.95)	66.6 (54.70)	60 (50.77)	66.6 (54.70)	46.6 (53.05)
	2.5	93.4 (75.11)	93.4 (75.11)	73.4 (58.95)	93.5 (75.11)	80 (63.43)
	1.25	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
	0.625	60 (50.77)	66.6 (54.70)	100 (90)	46.6 (43.05)	33.4 (35.30)
MeOH	10	93.4 (75.11)	93.4 (75.11)	93.4 (75.11)	93.4 (75.11)	93.4 (75.11)
	5	60 (50.77)	46.6 (43.05)	46.6 (43.05)	46.6 (43.05)	46.6 (43.05)
	2.5	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
	1.25	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
	0.625	60 (50.77)	100 (90)	73.4 (58.95)	80 (58.95)	73.4 (58.95)

Note: Higher percent repulsion for lower doses in comparison to corresponding higher doses was observed even in repetition of the same experiments, however the reason is yet to be investigated.

Lethal effect: The results of the brine shrimp lethality test for Pet.E., $CHCl_3$ and MeOH extracts of *E. nummularius* (whole plant) are represented in Table 3. For Pet.E. extract the LC_{50} values ranged between 141.553 to 8.560ppm; for the $CHCl_3$ extract it was

between 699.048 to 40.114ppm, and for the MeOH extract the LC_{50} values ranged between 2336.982 to 137.0589ppm. The intensity of activity could be arranged in a descending order: Pet.E. extract> $CHCl_3$ extract> MeOH extract.

Table 3 LC_{50} values (in ppm) of Pet.E., $CHCl_3$ and MeOH extracts of *E. nummularius* against *A. salina* nauplii.

Type of extract	Duration of exposure (in hrs)			
	12	18	24	30
Pet.E	141.553	98.941	24.684	8.560
$CHCl_3$	699.048	366.663	326.0039	40.114
MeOH	2336.982	1258.605	355.962	137.0589

The record for biological activity of *E. nummularius* is very scanty. However, these findings receive supports from Pavithra et al., (2009), who studied antibacterial properties of *E. nummularius*. They found the susceptible organisms to the MeOH extract were *Escherichia coli* (MIC=12.50 mg/ml) and *Bacillus subtilus* (MIC=3.125 mg/ml) and the most resistant strains were *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The same extracts exhibited radical scavenging activity with IC=50 of 350 mg/ml. Dash et al., (2003) evaluated the aqueous and hydroalcoholic extracts of *E. nummularius* that showed anthelmintic activity against adults of the Indian earthworm *Pheretima posthuma.* Ethnomedicinal uses of *Evolvulus* species have also been reported by many authors (poor sedative and anticonvulsant properties were reported by Chitralekha et al., 1964; reports for hysteria, to cure burns, cuts, wounds and scorpion stings have been made by Jain, 1991; activity to treat scabies mentioned by Manandhar and Manandhar, 2002; and the wound healing activity was reported by Saini et al., 2007). Medicinal use of *E. nummularius* indicates the potentially of this plant to have activity against certain causal agents or pathogens (microorganisms) and even functions on the components of human body for the cure of certain diseases.

Conclusion: The results from the study show that petroleum ether, chloroform and methanol extracts of *E. nummularius* have insecticidal activity, insect repellency and brine shrimp lethality; while from the previous researchers antibacterial and antioxidant activities have been reported which can be corroborated to the usage of this plant in traditional medicine. And thus, a comprehensive phytochemical analysis of the plant for its insecticidal and repellent components, as well as the physiological studies of the active ingredients are very much to be solicited for their effective use in the future pest control and pharmaceutical endeavours.

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