

Bioactive Potentials of *Cleome viscosa* L. Extracts: Dose-mortality, Insect Repellency and Brine Shrimp Lethality

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Abstract

Petroleum ether (Pet. ether), chloroform (CHCl₃) and methanol (CH₃OH) extracts of *Cleome viscosa* have thoroughly screened against *Tribolium castaneum* (Hbst.) for repellency and dose mortality, against *Callosobruchus chinensis* for dose mortality activities, and against *Artemia salina* nauplii for lethality. In case of repellency of *T. castaneum*, Pet. ether extract found significantly active at 1% level of significance (P < 0.01), CHCl₃ extract offered activity at 5% level of significance (P < 0.05) and CH₃OH extract did not show any activity at all. In case of mortality against *T. castaneum* the LD₅₀ values of Pet. ether, CHCl₃, and CH₃OH extracts were 2.380, 2.188, 1.867, 1.658 and 1.429 mg/cm²; 4.950, 2.906, 2.664, 2.658 and 2.609 mg/cm²; and 4.950 and 2.791, 2.609, 2.552 and 2.474 mg/cm² after 0.5, 6, 12, 18 and 24 h of exposure respectively. Against *C. chinensis* the LD₅₀ values of the same extracts were 0.781, 0.670, 0.642, 0.614, 0.560 and 0.494 mg/cm²; 1.964, 1.050, 0.867, 0.725, 0.635 and 0.590 mg/cm²; and 2.631, 1.851, 1.471, 1.703, 1.524 and 1.457 mg/cm² after 6, 12, 18, 24, 30 and 36 h of exposure respectively. In case of cytotoxicity test against *A. salina*, the extracts offered LC₅₀ values in a descending order CH₃OH > CHCl₃ > Pet. ether extracts.

Keywords: Repellency; dose-mortality; cytotoxicity; *C. viscosa*; *T. castaneum*; *C. chinensis*; *A. salina*.

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1. Introduction

C. viscosa L. (Family: Capparacae or Cappardaceae) is an annual sticky herb found as a common weed [1], which is commonly known as ‘Asian spider flower’, ‘Tickweed’, ‘wild or dog mustard’ and in Bengali it is called “Hul hul” [2,3]. This plant is found in all over the world and widely used as a popular medicinal plant in herbal industry. Also found in

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the plains of Arabia, Egypt, Pakistan, and also in Asia and Africa [4]. This herb grows very well in rainy season with sticky shoot and yellow flowers. The plant is collected from the wild area and used it locally as a food and medicine which is occasionally cultivated in India, where it is achieving popularity as a substitute for cumin [5]. It tests bitter and possesses a sharp mustard-like flavor [6]. The pungent seed can be pickled or used as a mustard substitute. A condiment is made by the juice of this plant [7]; oil obtained from the seeds is used for cooking and also used as a stimulant in food in order to improve the appetite. In Ayurveda, it is used as an anthelmintic, carminative, stimulant and vesicant. The leaves are used as an external application to treat wounds and ulcers. A paste of the root is applied externally in the treatment of earaches [8]. The present investigation was designed to find out the repellent potential of the crude extracts of the test plant species on test organisms, *T. castaneum*, *C. chinensis* and *A. salina* nauplii. *T. castaneum* (Coleoptera: Tenebrionidae) is a worldwide pest of stored products and of Indo-Australian in origin [9]. It is generally found in granaries, mills, warehouses and live on cracked grain on breakfast food or meal, rice, dried fruit, bleached and unbleached wheat flour, cornmeal, barley flour and oatmeal [10]. The eggs are microscopic and the slender larvae are creamy yellow to light brown in color. The adult is a small reddish-brown beetle. Total life cycle contains subsequently for egg incubation 8.8 days, larval development takes 22-100 days, pupal development 4.5 days, and for reproductive maturation 5-4 days, but these are highly dependent on variation of temperature [11]. *C. chinensis* (Coleoptera: Chrysomelidae) commonly known as pulse beetle found as pest to many stored legumes [12]. Adults are brown in colour with black and grey patches over the body. It possesses egg, larva, pupa and adult stage in its life cycle. Pulse beetle is one of the most damaging to the stored legumes industry due to their generalized legume diets and wide distribution [13]. *A. salina* is a species of aquatic crustaceans also known as brine shrimp. An Iranian geographer firstly reported it in Urmia Lake in 982 AD [14]. *Artemia* populations are found worldwide in inland saltwater lakes, but not in oceans. *Artemia* are able to avoid cohabiting with most types of predators, such as fish, as they have the ability to live in waters of very high salinity (up to 25%) [15]. A female uterus of *A. salina* may contain up to 200 eggs [16]. Eggs are hatched into nauplii that are about 0.5-0.6 mm in length. Eggs remain in a dormant state as cyst. These cysts can last for a couple of years, and will hatch when they are placed in saltwater [17].

2. Materials and Methods

2.1. Collection and preparation of test materials

C. viscosa plants were collected from the University of Rajshahi Campus (Bangladesh) and identified by the Department of Botany, University of Rajshahi where a voucher specimen is kept in the Herbarium. The plant parts (leaves, stem, root, fruit and flower) were collected separately and chopped into small pieces, dried under shade and powered with the help of a hand grinder, weighed and placed in conical flasks to add solvents. Pet.

ether, CHCl_3 and CH_3OH (Merck, Germany) were used ($200 \text{ g} \times 600 \text{ mL} \times 2$ times) successively each of which kept 48 h on a shaker. For each of the extract filtration was done by filter paper at 24 h interval in the same flask followed by evaporation until the extract was left as a scum. The extracts were 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 *T. castaneum* and *C. chinensis* of same age (3-5 days) used in this investigation were collected from the stock cultures of the Crop Protection Laboratory, Department of Zoology, University of Rajshahi, Bangladesh. *A. salina* nauplii were emerged from the cysts released in a container of salty water and were used within a period of 24 -30 h of age.

2.3. Repellent activity

The method of repellency test used in this investigation was adopted from the method (No. 3) of McDonald *et al.* [18]. The average of the counts was converted to percent repulsion (PR) using the formula, $PR = (Nc - 5) \times 20$; where, Nc is the average hourly observation of insects on the untreated half of the disc [19,20]. A general concentration for each of the extracts (Pet. ether, CHCl_3 and CH_3OH) was selected as stock dose for repellency 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 . For the application of *T. castaneum* half filter paper discs (Whatman No. 40, 9 cm diam.) were prepared and selected doses of all the extracts separately applied onto each of the half-disc and allowed to dry out as exposed in the air for 20 min (approximately). 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 (9 cm 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 of the 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).

2.4. Dose-mortality test

2.4.1. Dose-mortality tests on *T. castaneum*

For insecticidal activity test each of the three extracts was dissolved in its solvent of extraction at different concentrations to go through the *Ad Hoc* experiment to get

considerable mortality and that were considered as doses. The concentrations used in this experiment were 2.037, 1.782, 1.527, 1.273 and 1.032 mg/cm² for Pet. ether extract; 2.291, 2.164, 2.037, 1.909 and 1.782 mg/cm² or CHCl₃ extract; and 2.419, 2.291, 2.164, 2.037 and 1.909 mg/cm² for the CH₃OH extract. For each of the doses 1 mL was dropped on a Petri dish (50mm) in such a way that it made a uniform film over the Petri dish. Then the Petri dishes were air-dried leaving the extract on it. The actual extract present in 1ml mixture was calculated just dividing the value by the area of the Petri dish and thus the dose per square centimeter was calculated. After drying 10 beetles (3-5 day 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 0.5, 6, 12, 18 and 24 h of exposure.

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

The concentrations used in this experiment were 0.763, 0.612, 0.509, 0.407 and 0.305 mg/cm² for Pet. ether extract; 1.018, 0.763, 0.509, 0.254, and 0.152 mg/cm² for CHCl₃ and CH₃OH extracts. For each dose 1 mL was dropped on a Petri dish (50 mm) where a few pulse grains were kept. 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 and the whole experiment was set in 3 replicates. A control batch was also maintained with the same number of insects after preparing the Petri dish in the same way as done for *T. castaneum* with only solvent. The mortality of the beetles was counted after 6, 12, 18, 24, 30 and 36 h of exposure.

2.4.3. *Brine shrimp 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 was 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 was 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, CHCl₃, CH₃OH were 200, 400 and 800 ppm.

2.4.4. Statistical analysis

The mortality (%) was corrected using Abbott's formula, $Pr = (Po - Pc)/(100 - Pc) \times 100$; 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. Repellent activity of *T. castaneum*

The Pet. ether and CHCl_3 extracts found to be active against *T. castaneum* adults with repellency at 1% and 5% levels of significance $**=(P < 0.01)$ and $*=(P < 0.05)$. While, the methanol extract of *C. viscosa* did not show any activity at all (Table 1).

Table 1. ANOVA results of the repellency of *T. castaneum* by the Pet. ether, CHCl_3 and CH_3OH extracts of *C. viscosa* (whole Plant).

| Test organism | Extracts of <i>C. viscosa</i> | 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 |
| <i>T. castaneum</i> | Pet. ether | 2751.967 | 459.015 | 16 | 48.517** | 8.092 | 9.5E-09 | 0.0009 |
| | CHCl_3 | 1316.798 | 753.716 | 16 | 12.698* | 7.268 | 7.68E-05 | 0.0015 |
| | CH_3OH | 188.272 | 2865.556 | 16 | 0.290 | 4.410 | 0.8803 | 0.0137 |

3.2. Dose mortality effects on *T. castaneum* and *C. chinensis*

The results of the dose mortality assays of Pet. ether, CHCl_3 and CH_3OH extracts of *C. viscosa* against adult beetles of *T. castaneum* are represented in Table 2. The LD_{50} values were 2.380, 2.188, 1.867, 1.658 and 1.429 mg/cm^2 ; 4.950, 2.906, 2.664, 2.658 and 2.609 mg/cm^2 ; and 4.950, 2.791, 2.609, 2.552 and 2.474 mg/cm^2 all after 0.5, 6, 12, 18 and 24 h of exposure respectively. Against *C. chinensis* Pet. ether, CHCl_3 and CH_3OH extracts are represented in Table 3. The LD_{50} values were 0.781, 0.670, 0.642, 0.614, 0.560 and 0.494 mg/cm^2 ; 1.964, 1.050, 0.867, 0.725, 0.635 and 0.590 mg/cm^2 ; and 2.631, 1.851, 1.471, 1.703, 1.524 and 1.457 mg/cm^2 all after 6, 12, 18, 24, 30 and 36 h of exposure respectively. According to the intensity of activity the extracts could be arranged in a descending order of Pet. ether > CH_3OH > CHCl_3 extracts in case of *T. castaneum*; and against *C. chinensis* Pet. ether > CHCl_3 > CH_3OH extracts as well (Tables 2, 3).

Table 2. LD₅₀ values of Pet. ether, CHCl₃ and CH₃OH extracts of *C. viscosa* against *T. castaneum*.

| Extracts | LD ₅₀ at different exposures (mg/cm ²) | | | | |
|--------------------|---|--------|--------|--------|--------|
| | 0.5h | 6h | 12h | 18h | 24h |
| Pet. ether | 2.3807 | 2.1883 | 1.8677 | 1.6589 | 1.4292 |
| CHCl ₃ | 4.9502 | 2.9060 | 2.6643 | 2.6586 | 2.6098 |
| CH ₃ OH | 4.9502 | 2.7914 | 2.6098 | 2.5528 | 2.4747 |

Table 3. LD₅₀ values of Pet. ether, CHCl₃ and CH₃OH extracts of *C. viscosa* against *C. chinensis*

| Extracts | LD ₅₀ at different exposures (mg/cm ²) | | | | | |
|--------------------|---|--------|--------|--------|--------|--------|
| | 6 h | 12 h | 18 h | 24 h | 30 h | 36 h |
| Pet. ether | 0.7817 | 0.6701 | 0.6420 | 0.6140 | 0.5607 | 0.4942 |
| CHCl ₃ | 1.9647 | 1.0501 | 0.8678 | 0.7252 | 0.6350 | 0.5907 |
| CH ₃ OH | 2.6315 | 1.8518 | 1.4712 | 1.7033 | 1.5248 | 1.4578 |

3.3. Lethal effect of *A. salina*

Pet. ether, CHCl₃, CH₃OH extracts offered cytotoxicity and gave LC₅₀ values 3071.54, 1741.01, 1122.06 and 918.11 ppm; 101.534, 29.366, 18.078 and 3.103 ppm; and 73.368, 38.989, 24.735 and 23.299 ppm after 12, 18, 24 and 30 h of exposure respectively. Against *A. salina* the extracts could be arranged in a descending order of CH₃OH > CHCl₃ > Pet. ether extracts (Table 4).

Table 4. LC₅₀ values of Pet. ether, CHCl₃ and CH₃OH extracts of *C. viscosa* against *A. salina*.

| Extracts | LC ₅₀ at different exposures (ppm) | | | |
|--------------------|---|----------|----------|---------|
| | 12 h | 18 h | 24 h | 30 h |
| Pet. ether | 3071.548 | 1741.013 | 1122.066 | 918.115 |
| CHCl ₃ | 101.534 | 29.366 | 18.078 | 3.103 |
| CH ₃ OH | 73.368 | 38.989 | 24.735 | 23.299 |

4. Discussion

The aforementioned data receive supports from the previous researchers' findings. A recent study has been conducted on the contact toxicity effects of three *Cleome* species's methanol extracts against *Sitophilus oryzae* and found much promising toxicity with recording the least median lethal concentration (LC₅₀). This work also confirmed ovicidal activities and oviposition deterrent of *C. viscosa* against *S. oryzae* [23]. Another study has also been found to show antifeedant and chronic toxic properties of seed extracts of *C. viscosa* against *Hispa armigera* [24]. Moreover, another investigation on toxicity on albino rats has been stipulated the methanolic extracts of *Cleome viscosa* to be significant in wound healing activity as evidenced by the reduction in the number of days required, and the wound contraction compared to the standard [25]. *C. viscosa* seed oil has peripheral analgesic and antiemetic activities [26]. Potent contact and stomach activity of *C. viscosa* extracts against first instars of *Clavigralla tomentosicollis* also confirmed the insecticidal activity of this plant [27]. Its related species *Cleome rupicola* plant extract is

applied traditionally as eye-drops [28], *Cleome Arabica*, a desert plant widely distributed in the North Africa is used as a sedative for rheumatic and abdominal aches [29] and *C. gynandra* is used to relieve pain, cure skin disease on leprosy, earache [30]. Acetone, methanol, benzene and hexane extracts of *C. viscosa* were found active against larvicidal and ovicidal activity in *Aedes aegypti* [31]. This present investigation has also supported with the result of another research, where *C. viscosa* offered promising insecticidal activity against *Callosobruchus chinensis*. Various solvents viz.; acetone, chloroform, petroleum ether, methanol, hexane, and water extracts of *C. viscosa* have shown much insecticidal potential as LD₅₀ obtained in each was very low. These also have significantly repelled large number of insects at a very low dose [32].

5. Conclusion

After analyses of data it is revealed that extracts of *C. viscosa* possesses insect killing properties, along with repellent and cytotoxic activities. Recently, the use of environment friendly and biodegradable natural insecticides of plant origin have received renewed attention because they are rich with bioactive compounds active against specific target insects. So, further investigation is needed to isolate the exact compound which could be a great lead of pharmaceutical and agricultural potentials.

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