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CYTOTOXICITY ASSESSMENT OF *HERITIERA LITTORALIS* (AITON), *MADHUCA LONGIFOLIA* (KÖNIG) MACBR., *NERIUM INDICUM* MILL. AND *SAPIUM INDICUM* (WILLD.) LEAVES ON *ARTEMIA SALINA* (L.)

N Fatema[1], K Hasan[2], A Islam[1], N Yasmin[3], M Nesa[3] and N Islam[3*]

[1]Institute of Environmental Science, University of Rajshahi, Rajshahi-6205, Bangladesh
[2]Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
[3]Department of Zoology, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract

Petroleum ether, $CHCl_3$ and CH_3OH extracts of leaves of *Heritiera littoralis* (Aiton), *Madhuca longifolia* (König) Macbr., *Nerium indicum* Mill. and *Sapium indicum* (Willd.) were subjected to assess cytotoxicity against *Artemia salina* (L.) nauplii. The petroleum ether extract of *H. littoralis* leaves showed LC_{50} values 273.77, 97.27, 51.60, 37.12, 14.60 and 12.59 ppm after 12, 18, 24, 30, 36 and 42 h; the $CHCl_3$ extract showed LC_{50} values 733.25, 105.51, 40.72 and 18.20 ppm after 6, 12, 18 and 24 h whereas CH_3OH extract showed 73.05, 30.62, 24.56, 20.85, 16.21 and 6.71 ppm after 6, 12, 18, 24, 30 and 36 h of exposure respectively. The petroleum ether extract of *M. longifolia* leaves possess LC_{50} values 259.35, 115.17, 56.84 and 8.73 ppm after 12, 18, 24 and 30 h; the $CHCl_3$ extract possess LC_{50} values 585.43, 205.86, 112.74, 75.62, 52.84and 47.34 ppm after 12, 18, 24, 30, 36 and 42 h but CH_3OH extract possess LC_{50} values 185.87, 60.70, 30.11 and 15.39 ppm after 12, 18, 24 and 30 h of exposure respectively. The petroleum ether extract of *N. Indicum* leaves recorded LC_{50} values 249.82, 146.07, 80.23, 54.21 and 40.19 ppm after 18, 24, 30, 36 and 42 h; the $CHCl_3$ extract gave LC_{50} values 36.13, 21.72, 19.03, 16.81 and 16.34 ppm after 12, 18, 24, 30 and 36 h but CH_3OH extract recorded LC_{50} values 394.90, 129.69, 81.50, 73.10 and 37.51 ppm after 18, 24, 30, 36 and 42 h of exposure respectively. Similarly, the petroleum ether extract of *S. indicum* leaves showed LC_{50} values 24.79, 13.18 and 4.61 ppm after 12, 18 and 24 h; the $CHCl_3$ extract were 50.45, 42.64, 21.20 and 14.93 ppm after 18, 24, 30 and 36 h of exposure and the CH_3OH extract showed LC_{50} values 306.37, 217.18, 149.38, 73.52, 54.45 and 22.91 ppm after 12, 18, 24, 30, 36 and 42 h of exposure respectively. The intensity of efficacy of the extracts could be arranged in the following descending order of *S. indicum* (petroleum etroleum ether extract) >*H. littoralis* (CH_3OH extract) >*M. longifolia* (petroleum ether extract) >*N. indicum* ($CHCl_3$ extract).

Key words: *Artemia salina*, Cytotoxicity, *Heritiera littoralis*, *Madhuca longifolia*, *Nerium indicum*, *Sapium indicum*, Plant extracts

Introduction

The plant *Heritiera littoralis* (Aiton) (Malvaceae) has medicinal properties and various traditional uses. It has anti-cancer (Ioannou et al. 2009), anti-inflammatory (Tewtrakul et al. 2010), antifungal (Bandaranayake 2002), antibacterial (Islam 2017) and larvicidal (Ali et al. 2012) properties. It has also been used to control mosquitoes and as a piscicide (Pattanaik et al. 2008, Bandaranayake 2002). *Madhuca longifolia* (König) Macbr. (Sapotaceae) has many beneficial uses and ethnomedicinal importance. It has antidiabetic and anti-

*Author for correspondence: n_islamm@yahoo.com

inflammatory properties (Ghosh et al. 2009, Dahake et al. 2010), antifungal and antioxidant properties (Prashanth et al. 2010). It also has good larvicidal and ovicidal activities (Banerji et al. 1985). *Nerium indicum* Mill. (Apocynaceae) leaves have been applied externally in the treatment of scabies and to reduce swellings. The leaves and the flowers are cardiotonic, diaphoretic, diuretic, emetic, expectorant and sternutatory (Jawarkar et al. 2012). Leaves and bark are treated as insecticide, rat poison and parasitic (Dey and Chaudhuri 2014, Url 1. 2019). The leaves also exhibited antioxidant (Vinayagam and Sudha 2011), analgesic (Shah et al. 2011) and antiviral (Rajbhandari et al. 2001) activities. *Sapium indicum* (Willd.) is an evergreen tree of Euphorbiaceae. Fruits of this plant have a significant antimicrobial (Chumkaew et al. 2003, Silprasit et al. 2011), insecticidal (Khanam et al. 2008), pesticidal (Khalil 1984, Chowdhury 1996) and antifungal (Miah et al. 1990) activities. *Artemia salina* (L.) (Anostraca: Artemiidae) is commonly known as Brine shrimp belongs to a genus of crustaceans (Crayfish). It is one of the standard organisms for testing the toxicity of chemicals (Ruebhart et al. 2008). The females of this crustacean 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 can remain in a dormant state as cysts. These cysts can last for several years and will hatch when they are placed in saltwater (Sara 2012). The present investigation was designed to the screening of the crude extracts of the above plants on cytotoxicity of *A. salina.*

Materials and Methods

Collection and preparation of the tested plants

The leaves of the selected plants *H. littoralis*, *M. longifolia*, *N. indicum* and *S. indicum* were collected from Khulna, Bagerhat and Rajshahi Districts of Bangladesh. The leaves of the collected plant were cut into small pieces, spread out in wooden trays and kept in a well-ventilated room to dry them at room temperature. Well dried leaves were ground to powder with a blender, weighed, kept in separate conical flasks and then extracted with a sufficient amount of solvent (petroleum ether, $CHCl_3$ or CH_3OH) (100 g × 300 ml × 2 times) for 48 h. Filtration was done by Whatman No. 40 filter paper and after evaporation, the extracts were collected in glass vials and kept in a cool place at 4ºC with appropriate labeling.

Hatching of Brine shrimp cysts

Fresh cysts in vials were purchased from the Pet and Aquarium market of Nilkhet, Dhaka, Bangladesh. Eggs of Brine shrimp were hatched in an aquarium using 1 liter of 1 M NaCl brine solution (pH 8.5). The eggs were incubated for 48 h under fluorescent light and the nauplii were hatched within 24-36 h at 30-35°C. After hatching, the nauplii were transferred to test tubes. Ten nauplii were transferred to each tube. 1.5 ml NaCl solution was added to each test tube.

Test sample preparation and Brine shrimp lethality test

Test samples at different concentrations were considered as doses prepared in test tubes by addition of a calculated amount of DMSO (dimethylsulfoxide). Then water was added to fill the pre-marked (up to 10 ml) 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 were kept at room temperature along with a control batch. Observation of mortality was made after 6, 12, 18, 24, 30, 36 and 42 h of exposure.

Plant extract solutions at different concentrations (for all three extracts of *H. littoralis* leaves in petroleum ether 90.67, 64.67, 53.33, 34.33 and 21.00 ppm; in $CHCl_3$ 168.67, 101.00, 53.33, 23.33 and 12.50 ppm and in CH_3OH were 200.00, 100.00, 50.00, 25.00 and 12.50 ppm. For *M. longifolia* in Petroleum ether were 169.00, 127.00, 101.00, 85.00 and 43.00 ppm; in $CHCl_3$ 179.00, 119.00, 89.00, 59.00 and 44.00 ppm and in CH_3OH were 200.00, 100.00, 50.00, 25.00 and 12.50 ppm. For *N. indicum* in Petroleum ether 200.00, 100.00, 50.00, 25.00 and 12.50 ppm; in $CHCl_3$ 180, 90, 45, 22.5 and 11.25 and in CH_3OH were 100.00, 50.00, 25.00, 12.50 and 6.25 ppm. The doses for all the three extracts of *S. indicum* leaves were in Petroleum ether 100.00, 50.00, 25.00, 12.50 and 6.25 ppm; in $CHCl_3$ 174.00, 87.00, 43.50, 21.75 and 10.86 ppm and CH_3OH 100.00, 50.00, 25.00, 12.50 and 6.25 ppm in artificial seawater containing 1% DMSO (v/v) used for this assay. Ten nauplii were used in each of the test tubes and three replicates were used for each concentration. Test tubes later had 1 ml of NaCl solution added to them. After 6, 12, 18, 24, 30, 36 and 42 h incubation, the number of survivors and count of the dead nauplii was done using a dissection microscope and the percentage of the mortality (%M) for each of the doses was calculated as compared with the control. DMSO (1%) served as the negative control and the final concentration of DMSO in the assay volume was kept always below 1% to prevent possible false effects originating from DMSO toxicity. LC_{50} value is the concentration of the sample required to kill 50% of the Brine shrimp population and this was calculated from the plot of % inhibition against the log concentration of sample extract. According to Meyer et al. (1982), an LC_{50} value of less than 1 mg/ml is considered toxic while an LC_{50} value greater than 1 mg/ml is deemed to be non-toxic.

Statistical analysis

In this study, recorded values were expressed as mean ± standard error of the mean (SEM). In the cytotoxicity study, LC_{50}-values and 95% of confidence intervals were determined. The mortality (%) was observed using Abbott's formula (Abbott 1925) [$P_r = (P_o - P_c) / (100 - P_c) \times 100$; Here, P_r = corrected mortality (%), P_0 = observed mortality (%) and P_c = mortality in the control (%)] and subjected to probit analysis (Finney 1947, Busvine 1971).

Results

Brine shrimp cytotoxicity

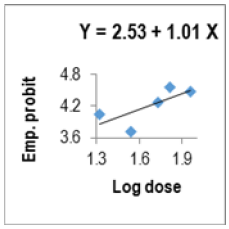
The cytotoxic activity of the test plants had been shown in Table 1. All the tested plants showed promising cytotoxic potentiality against *A. salina* nauplii of the three extracts for each of the plants in different solvents. For *H. littoralis* [Fig. 1 (a-p): Regression lines] the highest and lowest activity were observed in CH_3OH (LC_{50} = 6.71 ppm) after 36 h and in $CHCl_3$ (LC_{50} = 733.25 ppm) after 6 h of exposure. For the extracts of *M. longifolia* [Fig. 2 (a-n): Regression lines] the highest and lowest activities were observed in case of petroleum ether (LC_{50} = 8.73 ppm) after 30 h and in $CHCl_3$ (LC_{50} = 585.43 ppm) after 12 h of exposure. In case of *N. indicum* [Fig. 3 (a-o): Regression lines] the highest and lowest activity were observed in $CHCl_3$ (LC_{50} = 16.34 ppm) after 36 h and in CH_3OH (LC_{50} = 394.90 ppm) after 18 h of exposure. Finally, the highest and lowest efficacies were observed in petroleum ether (LC_{50} = 4.61 ppm) after 24 h and in CH_3OH (LC_{50} = 306.37 ppm) after 12 h of exposure in case of *S. indicum* [Fig. 4 (a-m): Regression lines].

According to the intensity of activity, the extracts of the test plants could be arranged in the following descending order: *S. indicum* (petroleum ether extract) >*H. littoralis* (CH_3OH extract) >*M. longifolia* (petroleum ether extract) >*N. indicum* ($CHCl_3$ extract).

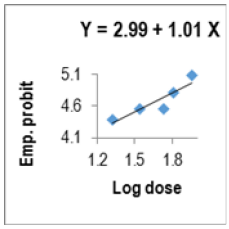
Table 1. LC_{50} values of the leaf extracts of *H. littoralis*, *M. longifolia*, *N. indicum* and *S. indicum* on Brine shrimp *A. salina* nauplii

Plant name	Solvent	LC_{50} (ppm) at different exposure						
		6 h	12 h	18 h	24 h	30 h	36 h	42 h
H. littoralis	Petroleum ether	-	273.77(a)	97.27(b)	51.60(c)	37.12(d)	14.60(e)	12.59(f)
	$CHCl_3$	733.25(g)	105.51(h)	40.72(i)	18.20(j)	-	-	-
	CH_3OH	73.05(k)	30.62(l)	24.56(m)	20.85(n)	16.21(o)	6.71(p)	-
M. longifolia	Petroleum ether	-	259.35(a)	115.17(b)	56.84(c)	8.73(d)	-	-
	$CHCl_3$	-	585.43(e)	205.86(f)	112.74(g)	75.62(h)	52.84(i)	47.34(j)
	CH_3OH	-	185.87(k)	60.70(l)	30.11(m)	15.39(n)	-	-
N. indicum	Petroleum ether	-	-	249.81(a)	146.07(b)	80.23(c)	54.21(d)	40.19(e)
	$CHCl_3$	-	36.13(f)	21.72(g)	19.03(h)	16.81(i)	16.34(j)	-
	CH_3OH	-	-	394.90(k)	129.69(l)	81.50(m)	73.10(n)	37.51(o)
S. indicum	Petroleum ether	-	24.79(a)	13.18(b)	4.61(c)	-	-	-
	$CHCl_3$	-	-	50.45(d)	42.64(e)	21.20(f)	14.93(g)	-
	CH_3OH	-	306.37(h)	217.18(i)	149.38(j)	73.52(k)	54.45(l)	22.91(m)

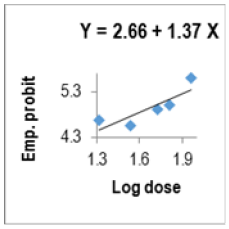
Respective regression lines of the LC_{50} values in Table 1 are given in Fig. 1 (a-p), Fig. 2 (a-n), Fig. 3 (a-o) and Fig. 4 (a-m) below.



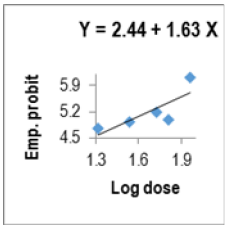
a) Pet. ether extract after 12 h of exposure.



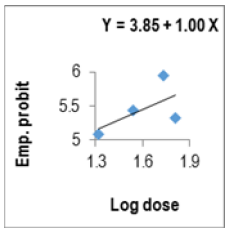
b) Pet. ether extract after 18 h of exposure.



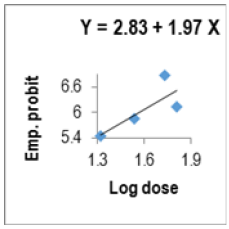
c) Pet. ether extract after 24 h of exposure.



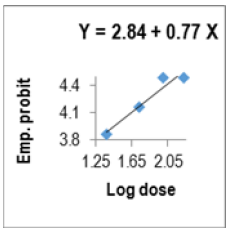
d) Pet. ether extract after 30 h of exposure.



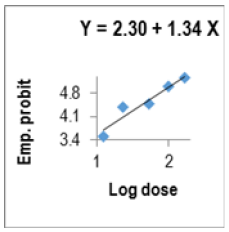
e) Pet. ether extract after 36 h of exposure.



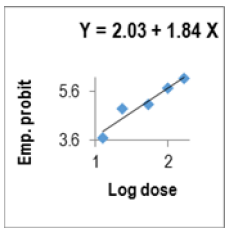
f) Pet. ether extract after 42 h of exposure.



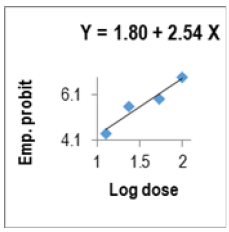
g) $CHCl_3$ extract after 6 h of exposure.



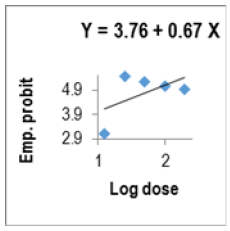
h) $CHCl_3$ extract after 12 h of exposure.



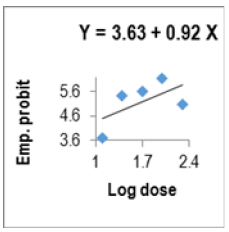
i) $CHCl_3$ extract after 18 h of exposure.



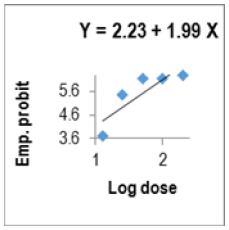
j) $CHCl_3$ extract after 24 h of exposure.



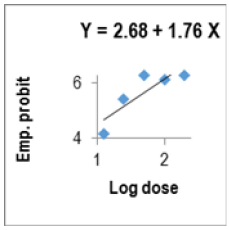
k) CH_3OH extract after 6 h of exposure.



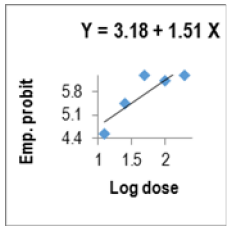
l) CH_3OH extract after 12 h of exposure.



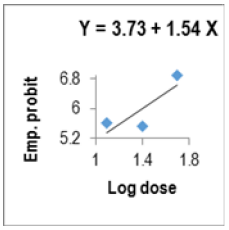
m) CH_3OH extract after 18 h of exposure.



n) CH_3OH extract after 24 h of exposure.

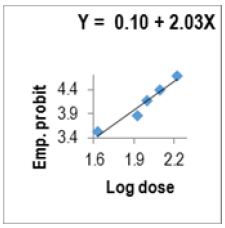


o) CH_3OH extract after 30 h of exposure.

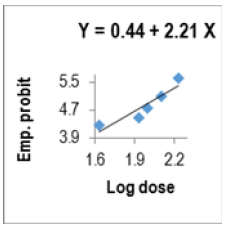


p) CH_3OH extract after 36 h of exposure.

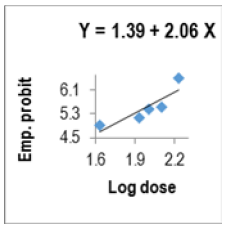
Fig. 1 (a-p): Regression lines of *H. littoralis* extracts against *A. salina* nauplii at different hours of exposure.



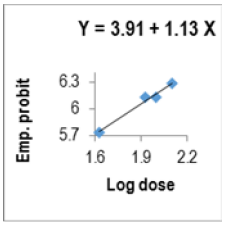
a) Pet. ether extract after 12 h of exposure.



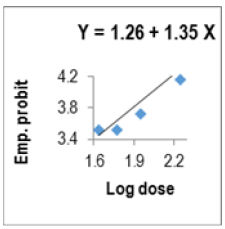
b) Pet. ether extract after 18 h of exposure.



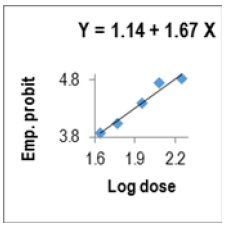
c) Pet. ether extract after 24 h of exposure.



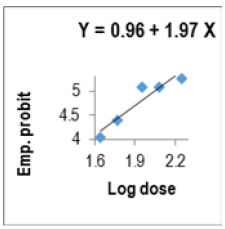
d) Pet. ether extract after 30 h of exposure.



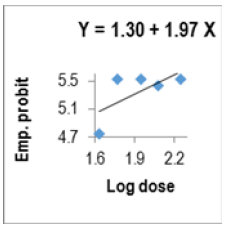
e) $CHCl_3$ extract after 12 h of exposure.



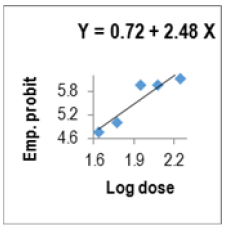
f) $CHCl_3$ extract after 18 h of exposure.



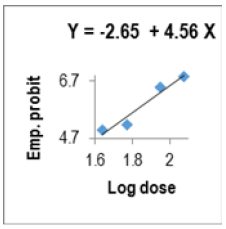
g) $CHCl_3$ extract after 24 h of exposure.



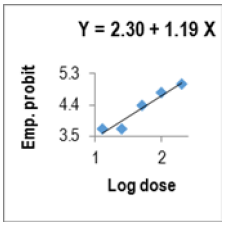
h) $CHCl_3$ extract after 30 h of exposure.



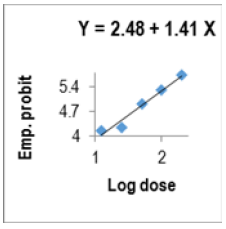
i) $CHCl_3$ extract after 36 h of exposure.



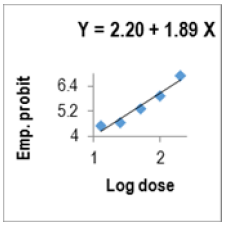
j) $CHCl_3$ extract after 42 h of exposure.



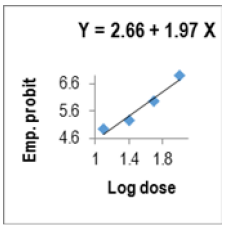
k) CH_3OH extract after 12 h of exposure.



l) CH_3OH extract after 18 h of exposure.

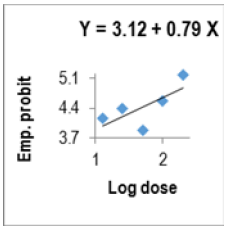


m) CH_3OH extract after 24 h of exposure.

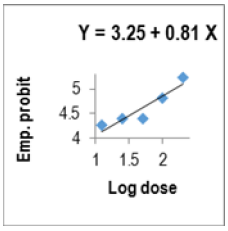


n) CH_3OH extract after 30 h of exposure.

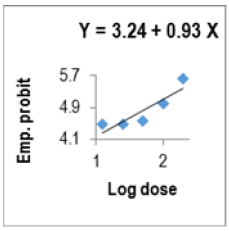
Fig. 2 (a-n): Regression lines of *M. longifolia* extracts against *A. salina* nauplii at different hours of exposure.



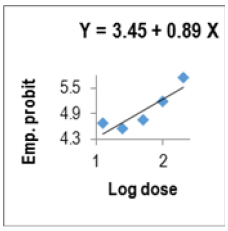
a) Pet. ether extract after 18 h of exposure.



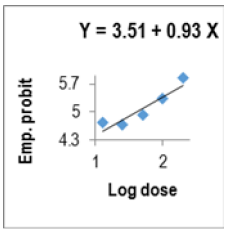
b) Pet. ether extract after 24 h of exposure.



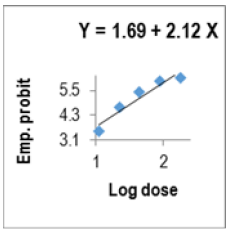
c) Pet. ether extract after 30 h of exposure.



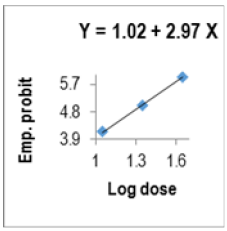
d) Pet. ether extract after 36 h of exposure.



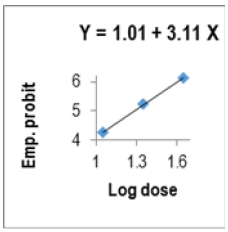
e) Pet. ether extract after 42 h of exposure.



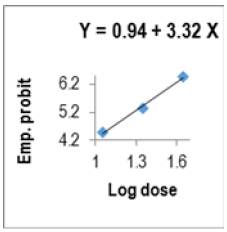
f) $CHCl_3$ extract after 12 h of exposure.



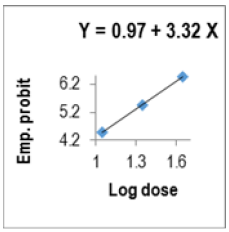
g) $CHCl_3$ extract after 18 h of exposure.



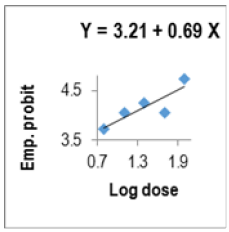
h) $CHCl_3$ extract after 24 h of exposure.



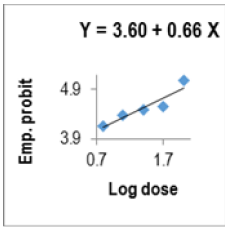
i) $CHCl_3$ extract after 30 h of exposure.



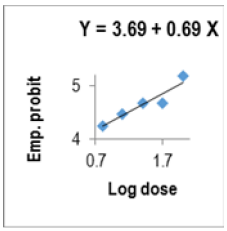
j) $CHCl_3$ extract after 36 h of exposure.



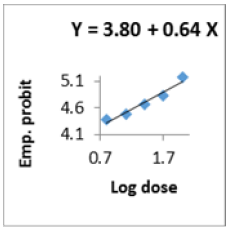
k) CH_3OH extract after 18 of exposure.



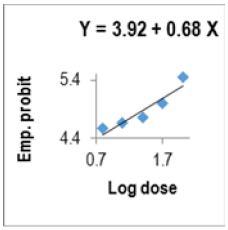
l) CH_3OH extract after 24 h of exposure.



m) CH_3OH extract after 30 h of exposure.

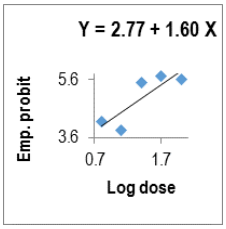


n) CH_3OH extract after 36 h of exposure.

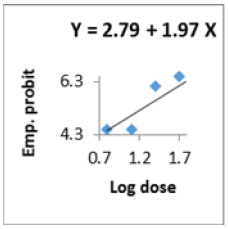


o) CH_3OH extract after 42 h of exposure.

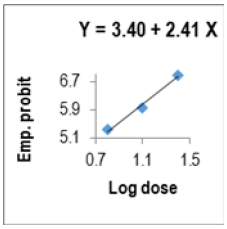
Fig. 3 (a-o): Regression lines of *N. indicum* extracts against *A. salina* nauplii at different hours of exposure.



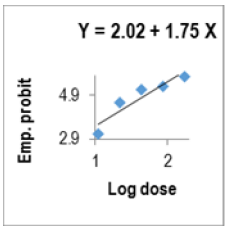
a) Pet. ether extract after 12 h of exposure.



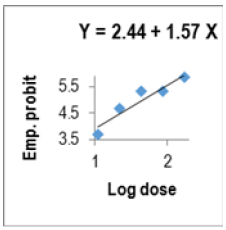
b) Pet. ether extract after 18 h of exposure.



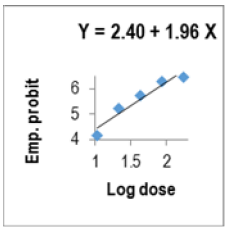
c) Pet. ether extract after 24 h of exposure.



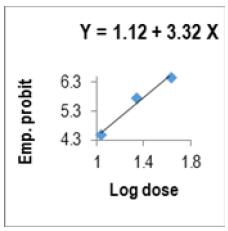
d) $CHCl_3$ extract after 18 h of exposure.



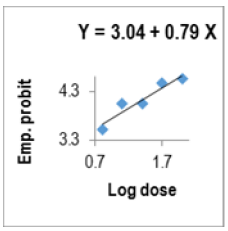
e) $CHCl_3$ extract after 24 h of exposure.



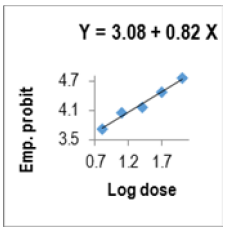
f) $CHCl_3$ extract after 30 h of exposure.



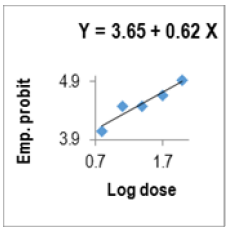
g) $CHCl_3$ extract after 36 h of exposure.



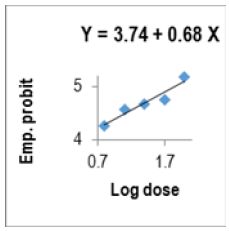
h) CH_3OH extract after 12 h of exposure.



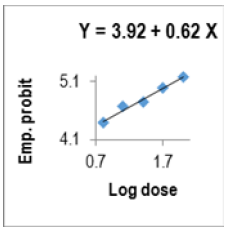
i) CH_3OH extract after 18 h of exposure.



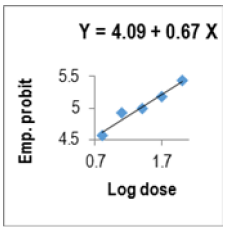
j) CH_3OH extract after 24 h of exposure.



k) CH_3OH extract after 30 h of exposure.



l) CH_3OH extract after 36 h of exposure.



m) CH_3OH extract after 42 h of exposure.

Fig. 4 (a-m): Regression lines of *S. indicum* extracts against *A. salina* nauplii at different hours of exposure.

Discussion

The findings of this investigation on the four medicinal plants *H. littoralis*, *M. longifolia*, *N. indicum* and *S. indicum* have got support from the works of previous researchers. Patra and Mohanta (2014) reported the antimicrobial activities of the mangrove plant *H. littoralis.* Salini (2015) found this plant used as a mosquito control agent, cure for diarrhoea and as a fish toxicant. Bark, leaves, roots and stems are used by rural people for the treatment of diabetes and goitre, gastrointestinal disorders, skin diseases and hepatic disorders (Ali et al. 2011, Hossain et al. 2013, Patra and Thatoi 2013). The potentiality of the alcoholic extract of leaves and flowers of *M. longifolia* are revealed significant antibacterial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal activities against *Aspergillus oryzae* and *Aspergillus niger* (Kalaivani and Jagadeesan 2013). Purohit et al. (2012) reported the immunomodulatory activity of *M. longifolia* and reported ethanolic extract enhances the humoral and cell-mediated immune response in Swiss albino mice. Chetwani et al. (2017) reported the leaves extracts of *N. indicum* were found to be active against the bacteria *Pseudomonas aeruginosa*. Reddy (2010) reported the alcoholic extract of *N. indicum* leaves inhibited the growth rate of some bacterial spp. *Staphylococcus aureus*, *Candida albicans* and some fungi *Aspergillus niger*, Mucor., *Rhizopus* and *Penicillium* even at lower concentrations. Leaves of this plant exhibited antioxidant (Vinayagam and Sudha 2011), antiviral (Rajbhandari et al. 2001) and analgesic (Shah et al. 2011) properties. Very strong support was reported from previous researchers on the potentiality of the plant *S. indicum*. Azis et al. (2015) described that *S. indicum* has good wound healing properties. They also mentioned the cytotoxic effects of *S. indicum*. The findings of Rahman and Monowar (2014) revealed that the petroleum ether extract of the fruit is more toxic than organochlorine compounds. The intravenous and oral administration of the extract kills laboratory animals, rats and mice that can be used as an alternative source of bio-pesticides (Rahman and Monowar 2014). Thus, the test plants *H. littoralis*, *M. longifolia*, *N. indicum* and *S. indicum* need further investigations to be attempted to reveal their total usefulness in the field of health and pest control technology.

Conclusion

By analyzing the results of cytotoxicity tests of *H. littoralis*, *M. longifolia, N. indicum* and *S. indicum* leaf extracts in petroleum ether, $CHCl_3$ and CH_3OH against *A. salina* it could be concluded that the plants have some bioactive potentials and that could be used in controlling pest organisms in aquatic media. Further studies on isolation and identification of the biologically active compounds of these plants are necessary.

Acknowledgements

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