Research Article

Cytotoxic and Anthelmintic Activity of Three Selected Medicinal Plants Used in Bangladesh

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ABSTRACT

Ficus hispida (Moraceae), *Hemigraphis alternata* (Acanthaceae) and *Senna sophera* (Fabaceae) are traditionally used as anthelmintic, pain killer, antidiarrhoeal, anti-pyretic etc. The present communication attempts to evaluate the cytotoxicity and anthelmintic activities of crude methanol extracts of three Bangladeshi medicinal plants. The high cost of conventional anthelmintic drugs and the development of anthelmintic resistance led to the evaluation of medicinal plants as an alternative source of anthelmintics. In addition, brine shrimp lethality assay was used to investigate the cytotoxicity effect and in vitro anthelmintic assay was used to evaluate the anthelmintic activity of these plant extracts. In cytotoxic study, all extracts were showed a moderate toxic effect and among them, MFHL was showed the highest cytotoxicity activity. Moreover, the leaves extracts (MFHL, MHAL, and MSSL) were found to have significant anthelmintic activity at the dose of 50 mg/mL and 100 mg/mL in the tested earthworms. Among them, 100mg/mL MFHL extract was showed the highest significant result. So, the results indicate that *F. hispida*, *H. alternata* and *S. sophera* leaves extract are moderately toxic (active) and may provide a potential source of anthelmintic effect.

Keywords: Cytotoxicity, *F. hispida*, *H. alternata*, *S. sophera*, brine shrimp, anthelmintic activity.

INTRODUCTION

For ages nature has gifted us plenty of herbs and plants which form the main source of traditional medicines used to help in relief from illness and are still widely used all over the world. Herbal treatment is still used for many health problems. Herbs are safe, less toxic, economical and a reliable key natural resource of drugs all over the world [1].

Based on different biological models, various assays are applied for the research of potential toxicity of herbal extracts. Now-a-days brine shrimp assay has been widely used to attempt the toxicity of a great variety of plant products. Brine shrimp lethality assay is a momentous tool for the preliminary cytotoxicity assay of crude extract and others based on the capability to kill a laboratory cultured larvae (nauplii) [2-3]. Previous research history ensured that the brine shrimp assay has a good correlation with cytotoxic activity in some human solid tumors and with pesticidal activity. In addition, according to National Cancer Institute (NCI, USA), there is a meaningful correlation between in vitro growth inhibition of solid tumor cell lines in human with brine shrimp bioassay because it is regarded as a prescreening tool for the research of antitumor drugs [4]. For the detection of bioactive compound from either natural or synthetic origin, brine shrimp (*Artemia salina,* fairy shrimp or sea monkeys) lethality assay is a rapid and comprehensive test. Moreover, it is also an inexpensive and simple test as no aseptic techniques are required and easily utilizes a large number of organisms for statistical validation and requires no extraordinary equipment and relatively small amount of crude sample (2-20 mg or less) is necessary [3, 5].

Helminth infections are among the commonest infections in human, affecting a large proportion of the world's population. Various species of stomach as well as intestinal worms comprising of mixed infection cause parasitic gastroenteritis which lead to weakness, reduced weight gain, loss appetite, decreased productivity and decreased feed efficiency [6]. In developing countries it is one of the most important group of parasitic diseases resulting in heavy production losses in livestock. It contributes to the prevalence of malnutrition, anemia, eosinophilia, and pneumonia and poses a major threat to public health. Although a wide variety of anthelmintics is used for the treatment of helminths in people, the development of resistance in helminths against commonly used anthelmintics has always been a challenge faced by the public health care professionals. In addition, some parasites are also retaining after administered of albendazole an anthelmintic marketed drug. As a result, helminthiasis is rarely fatal, but is a major cause of morbidity in our countries [7-9].

These factors paved the way for herbal remedies as alternative anthelmintics. Screening and proper assessment of the claimed medicinal plants could offer possible alternatives that may be sustainable, affordable and environmentally acceptable.

So, the investigation was carried out to screen the cytotoxic and anthelmintic activity of three medicinal plants used for herbal treatment by local communities against some parasite.

MATERIALS AND METHODS

Drugs and chemicals

Methanol and dimethyl sulfoxide (DMSO) were obtained from Merck, Germany. Sodium chloride salt was provided by ACI Ltd., Bangladesh. Criston 2 (Vincristine Sulphate) was purchased from Beacon Pharmaceuticals Ltd, Bangladesh. The solvent used in the experiments were of analytical grade. In addition, the eggs of the brine shrimp were collected from an aquarium shop (Khulna, Bangladesh).

Collection and identification of plant material

Middle aged freshly green leaves of *F. hispida*, *H. alterneta*, *and S. sophera* plants were collected from Ambottola region of Jashore University of Science & Technology and Godkhali flower garden, Jashore, Bangladesh, in March, 2018. The collected leaves were identified and confirmed by National Herbarium, Bangladesh.

Preparation and extraction of plant material

For methanolic extraction, 200 gm. of powdered leaves of each plant were taken. First, the leaves of *F. hispida*, *H. alterneta*, *S. sophera* were separated from plant and thoroughly washed with fresh water to remove all dirt and contaminants and dried in shade at room temperature (25±2°C) for 10-12 days. The materials were grinded into coarse powder and cold extraction method was used to extract the active components. The ground leaves (200 gm. per plant) were soaked in sufficient amount (approximately 1.5 L) of methanol for 12-14 days at room temperature with periodical shaking and stirring. The whole mixtures were primarily filtered through cotton and then through Whatman No.1 filters. The solvent was evaporated with a rotary evaporator under reduced pressure at 40°C temperature to yield semisolid crude extract. The percentage yields of the extract were 3.16 % (w/w), 3.19 % (w/w) and 2.99 % (w/w) for *F. hispida*, *H. alterneta*, *S. sophera* respectively. The extracts were then preserved in a refrigerator till further use.

Cytotoxic activity test

Brine shrimp lethality bioassay

The Method described by Firdous et al with slight modification was applied to detect cytotoxicity activity of MFHL, MHAL and MSSL extracts in vitro [10]. A total of 38 g of sea salt was weighed accurately, dissolved in 1L distilled water and then filtered off to get a clear solution. Artificial sea water was taken in the beaker and brine shrimp eggs (*Artemia salina*) were added and incubated at 28 °C in front of a lamp. The shrimps were allowed for 48 h to hatch and mature as nauplii (larvae).

Different concentrations including 1600µg/ml, 800µg/ml, 400µg/ml, 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml and 12.5µg/ml were prepared using simulated sea water along with DMSO (Dimethyl sulfoxide) for MFHL, MHAL, MSSL and standard respectively. The concentration of DMSO in these test tubes did not exceed 10 µL/ml. 10 nauplii were taken carefully by micropipette. Then the diluted solutions were added to the 5 ml of sea water containing 10 Nauplii. After 24 h, the mortality of the brine shrimp for the respective treatments was measured using a magnifying glass and the number of survived nauplii in each tube was counted.

From this, the percentage of lethality of brine shrimp nauplii was calculated at each concentration for each sample by dividing the number of dead nauplii by the total number, and then multiplied by 100%.

Anthelmintic activity

The Anthelmintic assay was carried as per the method followed by Islam et al with minor modifications [11]. The assay was performed on adult Bangladeshi earthworms, *Pheretima posthuma* due to its anatomical and physiological resemblance with that of intestinal round worm parasite of human beings [12]. The earthworms were collected from moist soil and washed with normal saline to remove all faecal matter and were used for the anthelmintic study. The earthworms of 6-8 cm in length and 0.2-0.3 cm in width were used for all experimental protocol. The earthworms were divided into ten groups containing six earthworms in each group. All the extracts and standard drug solution were freshly prepared in normal saline before starting the experiments. Different extracts and standard drug solutions were poured in different petri plates. All the earthworms were released into 10ml of formulation as follows: MFHL, MHAL, MSSL extract and Albendazole in three different concentrations. Observations were made for the time taken to paralysis and death of worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility when dipped in warm water (50ºC) followed with fading away of their body colors.

Various concentrations (25, 50 and 100 mg/ml) of each extract were tested by bioassay, which involved determination of time of paralysis and time of death of the worms. Albendazole was used as standard reference and saline water as control.

Statistical Analysis

The experimental results are expressed as mean ± SEM (Standard Error of mean). Statistical analyses graphical representations of results for anthelmintic study were evaluated by one-way ANOVA following Dunnett's test through the SPSS software (version 16; IBM Corporation, New York, USA). Microsoft Excel (Office 2010 version) was used to calculate the LC_{50} value of extracts. The obtained results were compared with the standard group. The $p<0.05$ was considered to be statistically significant.

RESULTS

Brine shrimp lethality bioassay

As a dose dependent manner, the mortality rate of brine shrimp was found to be increased with increasing concentration of the sample. The plot of percent mortality versus concentration on the graph paper produced an approximate linear correlation between them (Figure 1, 2, 3, 4). The median lethal concentrations at which 50% lethality (LC_{50}) of brine shrimp nauplii occurred were found to be 657.08 µg/ml, 895.28 µg/ml and 778.48 µg/ml for the crude extract of *F. hispida*, *H. alterneta*, *S. sophera* respectively (Table 1). Here, lethal concentration (LC_{50}) of Vincristine Sulphate (standard) was 2.05µg/ml (Table 2).

Table 1: Cytotoxic effect of *F. hispida*, *H. alterneta* and *S. sophera* extracts on brine shrimp

Conc. (µg/ml)	Log C	% mortality			LC_{50} (µg/ml)		
		MFHL	MHAL	MSSL	MFHL	MHAL	MSSL
1600	3.204	80	70	80			
800	2.903	60	50	60			
400	2.602	50	40	40			
100	2.000	40	30	30	657.08	895.28	778.48
50	1.699	30	20	20			
25	1.398	20	20	10	(Toxic)	(Toxic)	(Toxic)
12.5	1.099	10	10	0			

Table 2: Cytotoxic effect of Vincristine Sulphate

Conc. (µg/ml) LC_{50} (µg/ml)	Log C	% mortality
10	1.000	100
5	0.699	90
1	0.000	70
0.5	-0.301	50
2.05 (Toxic)		
0.25	-0.602	30
0.125	-0.903	20
0.06	-1.221	10

Table 3: Effect of standard and *F. hispida* leaves extract on anthelmintic test

Treatment groups	Conc. (mg/mL)	Paralysis time (min)	Death time (min)
Normal saline	-	-	-
Albendazole	50 mg/mL	3.40±0.68	11.00±0.71
MFHL	25 mg/mL	10.00±1.08	31.50±1.85
MFHL	50 mg/mL	6.75±1.11	19.00±0.82
MFHL	100 mg/mL	4.75±0.85	11.75±0.85

Paralysis and death time are denoted as mean ± SEM; n=5 earthworms.

Table 4: Effect of standard and *H. alternata* leaves extract on anthelmintic test

Treatment groups	Conc. (mg/mL)	Paralysis time (min)	Death time (min)
Normal saline	-	-	-
Albendazole	50 mg/mL	3.40±0.68	11.00±0.71
MHAL	25 mg/mL	11.75±1.38	30.50±2.22
MHAL	50 mg/mL	8.50±1.44	21.00±2.12
MHAL	100 mg/mL	5.00±0.71	14.00±1.47

Paralysis and death time are denoted as mean ± SEM; n=5 earthworms.

Table 5: Effect of standard and *S. sophera* leaves extract on anthelmintic test

Treatment groups	Conc. (mg/mL)	Paralysis time (min)	Death time (min)
Normal saline	-	-	-
Albendazole	50 mg/mL	3.40±0.68	11.00±0.71
MSSL	25 mg/mL	14.50±1.71	29.50±2.50
MSSL	50 mg/mL	12.00±1.08	22.25±1.25
MSSL	100 mg/mL	6.50±0.65	13.75±0.95

Paralysis and death time are denoted as mean ± SEM; n=5 earthworms.

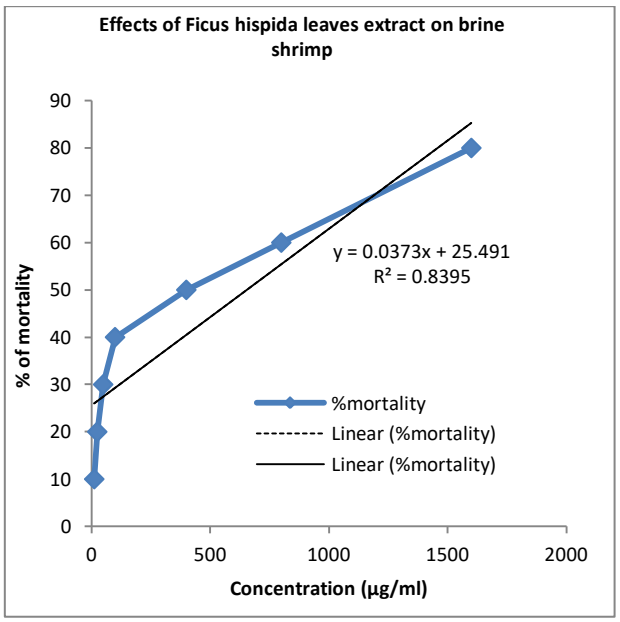

Figure 1: Plot of log concentration of methanolic extract of *Ficus hispida* leaves versus percent shrimp mortality after 24 h of exposure.

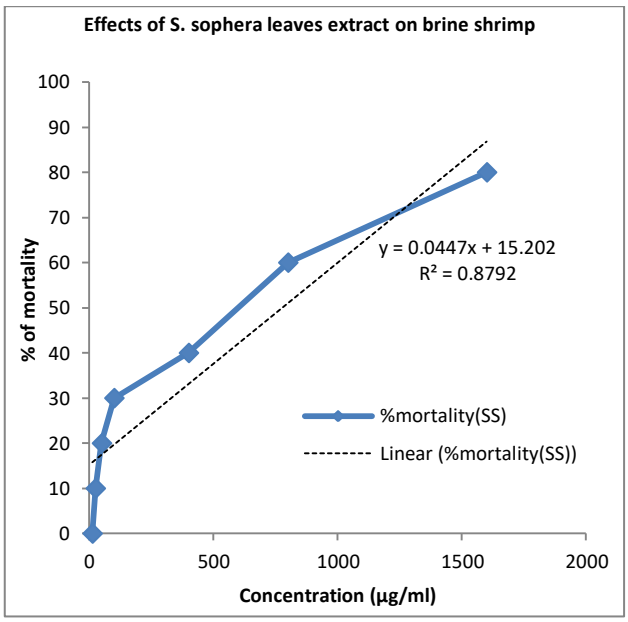

Figure 3: Plot of log concentration of methanolic extract of *Senna sophera* leaves versus percent shrimp mortality after 24 h of exposure.

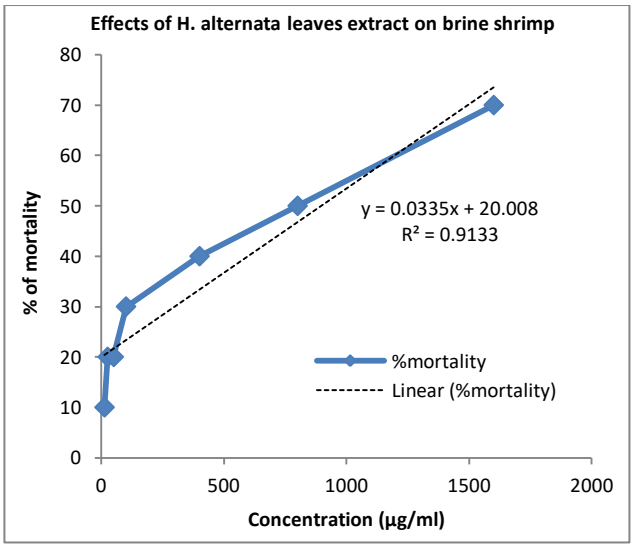

Figure 2: Plot of log concentration of methanolic extract of *Hemigraphis alternata* leaves versus percent shrimp mortality after 24 h of exposure.

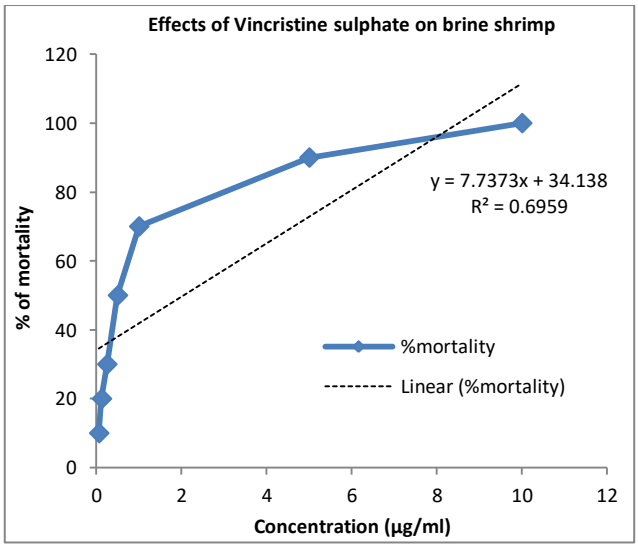

Figure 4: Plot of log concentration of methanolic extract of Vincristine Sulphate leaves versus percent shrimp mortality after 24 h of exposure.

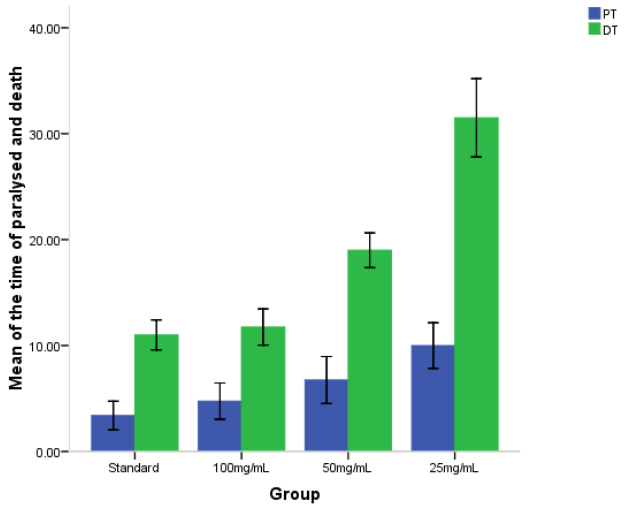

Figure 5: Effect of *Ficus hispida* leaves methanolic extract treatment on helminthiasis

PT= Paralyzed Time; DT= Death Time.

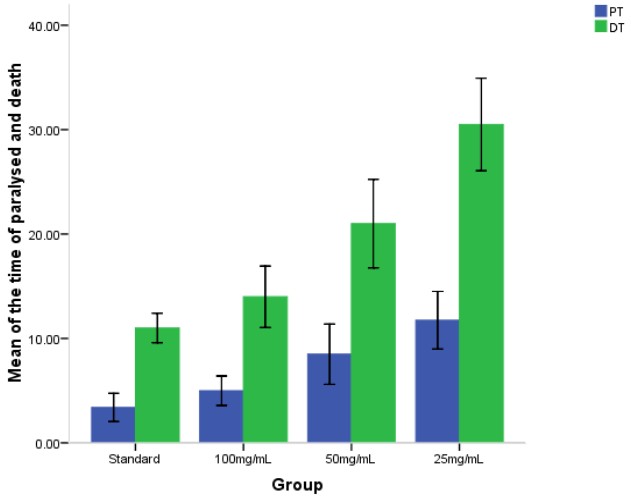

Figure 6: Effect of *H. alternata* leaves methanolic extract treatment on helminthiasis

PT= Paralyzed Time; DT= Death Time.

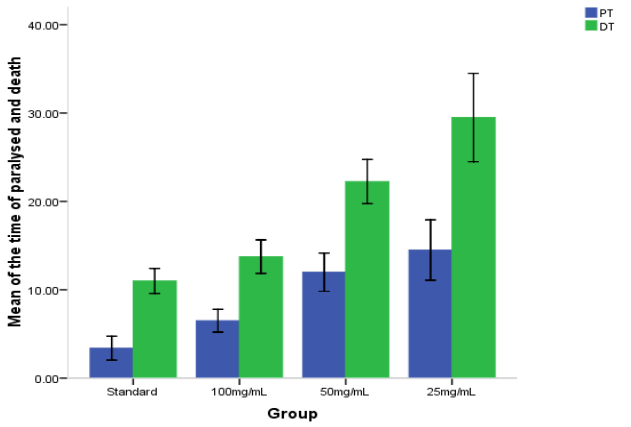

Figure 7: Effect of *S. sophera* leaves methanolic extract treatment on helminthiasis

PT= Paralyzed Time; DT= Death Time.

Anthelmintic activity

The results in table 3, 4 & 5 indicate the anthelmintic activities of methanolic extract of *F. hispida*, *H. alterneta* and *S. sophera* against the *Pheretima posthuma* earthworms. All extracts at a concentration of 25mg/ml, 50mg/ml and 100mg/ml was produced paralysis ranging from loss of motility to loss of response to external stimuli, which gradually progressed to death. *F. hispida*, *H. alterneta* and *S. sophera* at high concentration 50mg and 100mg were showed good anthelmintic activity compared with the effect produced by the reference standard drug, Albendazole (50mg/ml). Graphical representation has been shown in Figure 5, 6 & 7.

DISCUSSION

The leaves extract of MFHL, MHAL and MSSL exhibits cytotoxic activity against the brine shrimp and considered as containing active and potent component. The results showed that the brine shrimp lethality of the three selected plant extracts was found to be concentration-dependent in manner. According to Meyer et al. if it has an LC_{50} value of less than 1000 µg/mL then the crude extract is considered as toxic (active) while non-toxic (inactive) if it is greater than 1000 µg/mL [13]. The *F. hispida*, *H. alterneta* and *S. sophera* leaves extract LC_{50} was 657.08 µg/ml 895.28 µg/ml and 778.48 µg/ml respectively. And it possessed a moderate toxicity effect. The extract MFHL was found to be potent cytotoxic agent to the brine shrimps as it produced highest LC_{50}. The MHLA and MSSL extract also produced a moderate cytotoxic activity in comparison to the standard, vincristine sulfate.

The anthelmintic activity of methanolic extract of *F. hispida*, *H. alterneta* and *S. sophera* leaves and albendazole in earthworms were performed in the laboratory conditions. *Pheretima posthuma* worms can be used successfully for the anthelmintic activity study as it is easy, prominent, an adaptable to laboratory conditions, and reproducible method in all aspects such as equal age, size and weight of the worms [14]. In the present anthelmintic activity study, when the time of paralysis and time of death of earthworms were compared between plants extract and standard, the results showed that the time taken for paralysis and death is more closely to standard. Both 50 mg/ml and 100 mg/ml concentration of three extracts were showed significant results close to standard. Among three plants extract *F. hispida* (50 mg/ml and 100 mg/ml) was showed higher significant performance (Table 3) compared to *H. alternata* and *S. sophera* (50 mg/ml and 100 mg/ml) (Table 4 & 5).

Tannins (phytoconstituents) are responsible to produce anthelmintic activities. Tannins can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite (earthworms) and may cause death [15].

These findings support the use of *F. hispida*, *H. alterneta* and *S. sophera* as anthelmintics in the traditional

medicine. The present study reveals that the methanolic extract was endowed with anthelmintic activity.

CONCLUSION

From the effective study, it could be suggested that the methanolic extracts of *F. hispida*, *H. alterneta* and *S. sophera* leaves might possess cytotoxicity and anthelmintic activities. Nevertheless, further quantitative chemical studies are now under way to isolate and determine the structure of the active constituents. In case of brine shrimp lethality assay, the valuable data suggests that each plant extract has a high amount of bioactive substances and may contain compounds that possess cytotoxicity effects. Moreover, the anthelmintic activity of methanolic extract of three plants against earthworms suggests that it is workable against parasitic infections of humans. Further studies are under process to identify the possible phytoconstituents responsible for cytotoxic and anthelmintic activity.

Abbreviation

MFHL= Methanolic extract of *Ficus hispida* Leaves, MHAL= Methanolic extract of *Hemigraphis alternata* Leaves, MSSL= Methanolic extract *Senna sophera* Leaves, DMSO=Dimethyl sulfoxide, VS= Vincristine Sulphate. LC= Lethal Concentration.

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