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EVALUATION OF PHYTOCHEMICAL SCREENING AND CYTOTOXIC ACTIVITY OF DIFFERENT EXTRACTS OF MONOCHORIA HASTATA LEAVES

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ABSTRACT

Aims: The aim of this study was to find out the phytochemicals, cytotoxicity activity of the different extracts of. *Monochoria hastata* leaves. **Place and Duration of Study:** The study was carried out in September 2017 in the Department of Pharmacy, Jagannath University, Dhaka, Bangladesh. **Materials & Methods:** Phytochemicals screening performed by different types of tests such as Braymer's test, Salkowski test, Precipitate test, Molisch's test,etc. Cytotoxicity activity was determined against brine shrimp nauplii by using the brine shrimp lethality bioassay. **Results & Discussion:** In phytochemical screening, leaves contain different types of compound such as glycosides, alkaloids, flavonoids, tannin etc. In Brine Shrimp lethality bioassay, after 18hrs later the LC_{50} of *n*-hexane extract of the *M. hastata* was 19.05µg/ml, after 2hours later 6.76 µg/ml and after 24hours later 0.99 µg/ml. After 18hours later the LC_{50} of $CHCL_3$ extract was 22.90µg/ml, after 20hours later 12.58 µg/ml, after 24hours later 1.89 µg/ml. After 18hours later the LC_{50} of ethyl acetate extract was 3.28µg/ml, after 20hours later 1.43 µg/ml and after 24hours later 0.148 µg/ml respectively. **Conclusion:** The plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important drug candidates.

KEYWORDS: Monochoria hastata, Cytotoxicity, Brine shrimp nauplii bioassay

INTRODUCTION

Medicinal plants have been associated with the human health care system from time immemorial, particularly among tribal.[1] Medicinal plants has assumed massive importance in present days, due to the tremendous developments in the field of allopathic medicines during the 20th century.[2] At present people are being bombarded with thousands of unhealthy products, the level of sensibility in front of diseases is very high.[3] The use of medicinal plants can represent the best solution.[3] *Monochoria hastata* (Family *Pontederiaceae*) commonly Bengali name as Boronokha is a very common plant which is used in our country for several purposes. *M. hastata* is an important medicinal plant, found in the region of South India, Australia, and Southeast Asia.[5] Leaves of mature *M. hastata* have a hastate base and acuminate tips. They have long sheath like petioles. The blue to purple flowers of *M. hastata* are held on spike like racemes. There can be from 15-60 flowers per raceme. Flowers are basically a cluster of six petals small flowers. Each flower from the cluster is having an erect stigma surrounded by 4/5 stamens having yellow filaments. Fruits are ellipsoid 3-valved capsules about 0.4 in. (1 cm) long. The numerous oblong seeds have 8-12 longitudinal ribs. *M. hastata* is listed as a Federal Noxious Weed.[6] It grows in rice fields and other shallow bodies of water. They are grown at the watery lands, swamp area, beside ponds, in open wet lands etc at low and medium altitudes. *M. hastata* Plant is considered alterative, tonic and cooling. Rhizomes are powdered with charcoal and used for scurf. The leaves are used for poulticing boils after they have burst. Juice of roots used for stomach pains, asthma, toothache.[8] Juice of leaves used for cough. Root bark used for asthma. Juice of leaves applied to boils. In India, it used in an herbal mixture to strengthen uterine tone.[9] In Bangladesh, Mostly it is used against diarrhea and dysentery.

MATERIALS AND METHODS

Collection, identification and authentication of selected plant

The fresh leaves of were collected at February-2015 from Dhaka district, Bangladesh this plant leaves was identified by expert taxonomist. It was authenticated at Bangladesh National Herbarium, where a voucher specimen (No. DACB-41971) for had been deposited.

Extraction of plants

Plants were washed properly to remove dirty materials and shade dried for several days with sun drying. These were dried in an oven for 24 hours at considerably low temperature for better grinding. The dried plants were ground into coarse powder by a grinding machine.

Powdered plant materials that having a weight of about 350 gm were taken in three amber colored reagent bottle and soaked in 1 liter of ethanol solvent. The bottle with its contents were sealed and kept for a period of about 7 days with occasional shaking and stirring. The whole mixture was then filtered through cotton and then through Whatman No.1 filters paper and was concentrated with a rotary evaporator under reduced pressure at 50°C temperature to afford crude extracts.

Phytochemical screening

Test for tannins (Braymer's Test): 2 ml of the extract was stirred with 2 ml of distilled water and 2-3 drops of $FeCl_3$ (5%) solution were added. The formation of green color precipitate indicates the presence of tannins. Observation: Green color precipitate indicates the presence of tannins.

Test for flavonoids: 1 ml of the extract, 1ml of 10% lead acetate $(Pb(OAc)_4)$ solution is added.[9] Observation: Yellow color precipitate was taken as a positive result for flavonoids.

Test for terpenoids: 2 ml of the extract was dissolved in 2 ml of chloroform $(CH3CO)_2O$ and evaporated to dryness. 2 ml of concentrated sulphuric acid (H_2SO_4) was added and heated for about 2 min. Observation: Deep red color indicates the presence of terpenoids.

Test for steroids (Salkowski Test): 2 ml of the extract was mixed with a few drops of acetic anhydride, boiled and colored. 2 ml concentrated Sulphuric acid was then added from the sides of the test tube. Observation: The formation of reddish brown ring at the junction of two layers and green color of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids.

Test for phlobatannins (Precipitate Test): 2 ml of the extract was added to 2 ml of HCl (1%) and the extract was boiled. Observation: Deposition of a Red precipitate was taken as an indication of presence of phlobatannins.

Test for carbohydrates (Molisch's Test): 2 ml of the extract was treated with 2 drops of ethanolic αnaphthol (20%) solution in a test tube. Observation: Formation of the reddish violet ring at the junction indicates the presence of carbohydrate.

Test for coumarins: 2 ml of the extract was added to 3 ml of NaOH (10%). Observation: Formation of the yellow color indicates the presence of coumarins.

Test for alkaloids (Hager's Test): 3 ml of the extract solution was treated with a few drops of Hager's reagent (saturated picric acid solution). Observation: Presence of alkaloids confirmed by the formation of a yellow colored precipitate.

Test for proteins (Xanthoproteic Test): The extracts were treated with a few drops of conc. nitric acid. Observation: Formation of yellow color indicates the presence of proteins.

Test for anthraquinones (Borntrager's Test): 3 ml of the extract was treated with 3 ml of benzene and then 5 ml aqueous NH_3 (10%) was added in a test tube. Observation: After shaking, change in color of the aqueous layer was observed. Pink, violet or red color in the aqueous layer indicated the presence of anthraquinones.

Test for Anthocyanins: 2 ml of the extract was treated with 2 ml of HCl(2N) and then added NH_3 in a test tube. Observation: formation of pinkish red to bluish violet color indicates the presence of anthocyanins.

Test for glycosides (Keller Killiani Test): Test solution was treated with a few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added. Observation: Formation of two layers. Lower reddish brown layer and upper acetic acid layer which turn a bluish green would indicate a positive test for glycosides.

Test for saponins (Foam Test): 5 ml of the extract was shaken vigorously with an equal volume of distilled water in a test tube and the mixture was warmed. Observation: The formation of emulsion forms or stable foam was taken as an indication of the presence of saponins.

Test for phenols: To 1 ml of the extracts of sample, 2 ml of distilled water, followed by a few drops of 10% aqueous ferric chloride solution was added. Observation: Formation of blue or green color indicated the presence of phenols.

Evaluation of Cytotoxicity

The brine shrimp lethality bioassay is considered a useful tool for preliminary assessment of toxicity.[10] It has also been suggested for screening pharmacological activities in plant extracts. It is a recent development in the assay procedure for the bioactive compounds and natural product extracts, which indicates cytotoxicity as well as a wide range of

pharmacological activities e.g. anticancer, antiviral, and pharmacological activities of natural products etc.[11]

4 mg of each of the extract were dissolved in DMSO and solutions of various concentrations such as 400µg/ml to 0.781µg/ml were obtained by the serial dilution technique. Standard Vincristine Sulfate was used as the positive control and DMSO was used as the control, respectively. Next ten matured shrimps were taken to each of the experimental vials and the control vial. The number of the nauplii that died after 24 hrs was counted and the LC_{50} was calculated from the regression equation, obtained from the logarithm of sample concentration versus percentage mortality of the shrimp nauplii. The experiment was repeated three times at each concentration.

RESULTS

Here (+) means the component is present and (-) means the component is absent. The presence of Tannins, Flavonoids, Terpenoids, Carbohydrates, Coumarins, Alkaloids, Proteins, Phenols, Glycosides, Saponins and the absences of Quinones, Anthocyanins, Anthraquinones, and Steroids has been shown qualitatively in the Table

Table: Phytochemical test results of crude different extract of the leaves of *Monochoria hastata*.

Phytochemical tests	Different types of extract
Tannins	+++
Flavonoids	++
Terpenoids	+
Steroids	-
Phlobatannins	-
Carbohydrates	++
Coumarins	++
Alkaloids	+
Proteins	++
Anthraquinones	-
Anthocyanins	-
Glycosides	+
Saponins	++
Phenols	++

Table 1: After 18 hours later result of Brine shrimp lethality bioassay of distilled *n*-hexane of the leaves extract of *Monochoria hastata*.

Conc. of extract(µg/ml)	No. of brine shrimp inserted	No. of alive brine shrimp	No. of dead brine shrimp	% of mortality	Log conc. (µg/ml)	LC_{50} (µg/ml)
10	10	6	4	40	1	19.05
20	10	5	5	50	1.301	
40	10	4	6	60	1.602	
80	10	3	7	70	1.903	
160	10	2	8	80	2.204	
320	10	2	8	80	2.505	

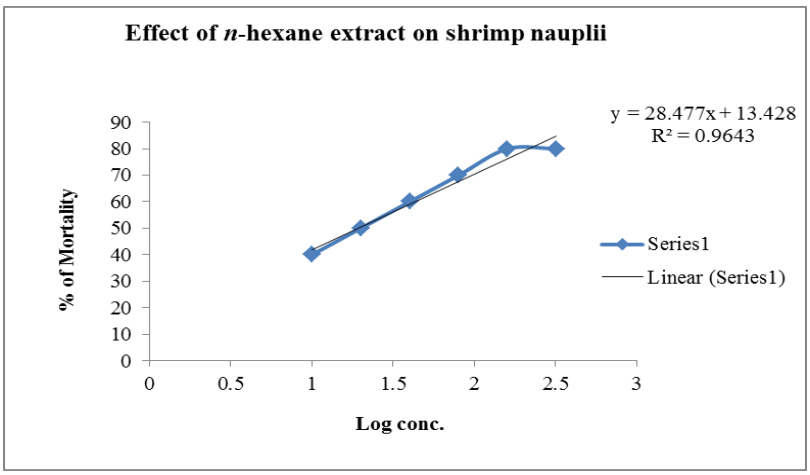


Table 2: After 18 hours later result of Brine shrimp lethality bioassay of distilled $CHCL_3$ extracts of the leaves of *Monochoria hastata.*

Conc. of Extract (µg/ml)	No of Brine shrimp inserted	No of Alive Brine Shrimp	No of death Brine shrimp	% Mortality	Log Conc. (µg/ml)	LC_{50} (µg/ml)
10	10	6	4	40	1	22.90
20	10	5	5	50	1.301	
40	10	5	5	50	1.602	
80	10	3	7	70	1.903	
160	10	2	8	80	2.204	
320	10	1	9	90	2.505	

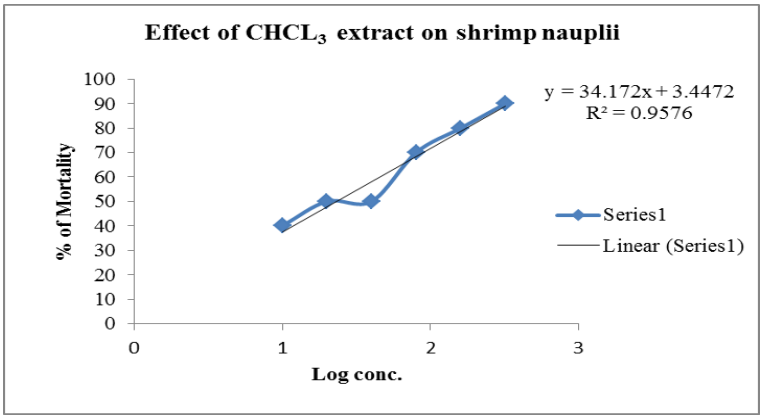


Table 3: After 18 hours later result of Brine shrimp lethality bioassay of ethyl acetate extracts of the leaves *Monochoria hastata.*

Conc. of Extract (µg/ml)	No of Brine shrimp inserted	No of Alive Brine Shrimp	No of death Brine shrimp	% Mortality	Log Conc. (µg/ml)	LC_{50} (µg/ml)
10	10	4	6	60	1	3.28
20	10	3	7	70	1.301	
40	10	2	8	80	1.602	
80	10	1	9	90	1.903	
160	10	1	9	90	2.204	
320	10	0	10	100	2.505	

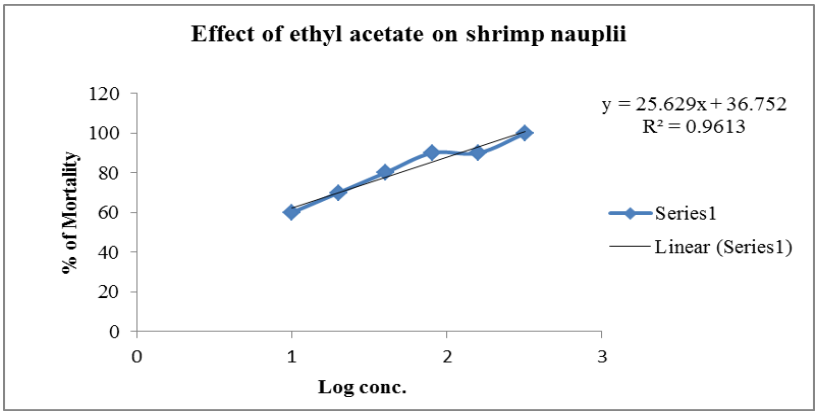


Table 4: After 20 hours later result of Brine shrimp lethality bioassay of distilled *n*-hexane extracts of the leaves of *Monochoria hastata.*

Conc. of Extract (µg/ml)	No of Brine shrimp inserted	No of Alive Brine Shrimp	No of death Brine shrimp	% Mortality	Log Conc. (µg/ml)	LC_{50} (µg/ml)
10	10	5	5	50	1	6.76
20	10	4	6	60	1.301	
40	10	3	7	70	1.602	
80	10	3	7	70	1.903	
160	10	2	8	80	2.204	
320	10	2	8	80	2.505	

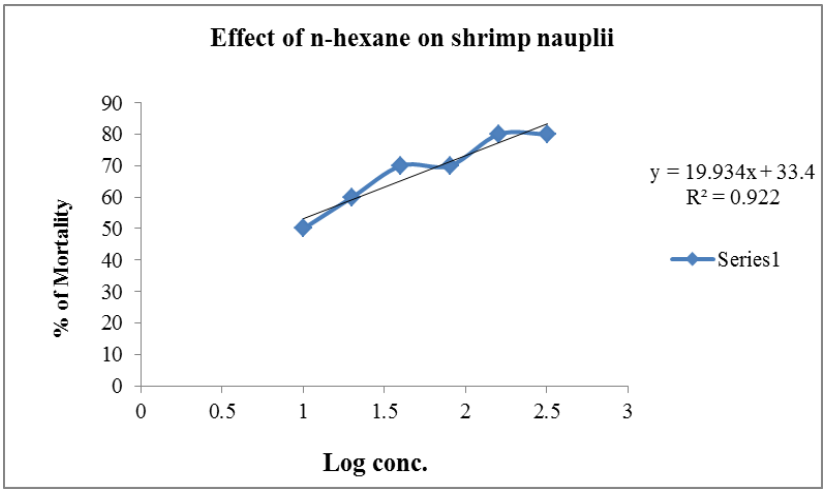


Table 5: After 20 hours later result of Brine shrimp lethality bioassay of distilled $CHCL_3$ extracts of the leaves *Monochoria hastata.*

Conc. of Extract (µg/ml)	No. of Brine shrimp inserted	No. of Alive Brine Shrimp	No. of death Brine shrimp	% Mortality	Log Conc. (µg/ml)	LC_{50} (µg/ml)
10	10	5	5	50	1	12.58
20	10	5	5	50	1.301	
40	10	3	7	70	1.602	
80	10	2	8	80	1.903	
160	10	2	8	80	2.204	
320	10	0	10	100	2.505	

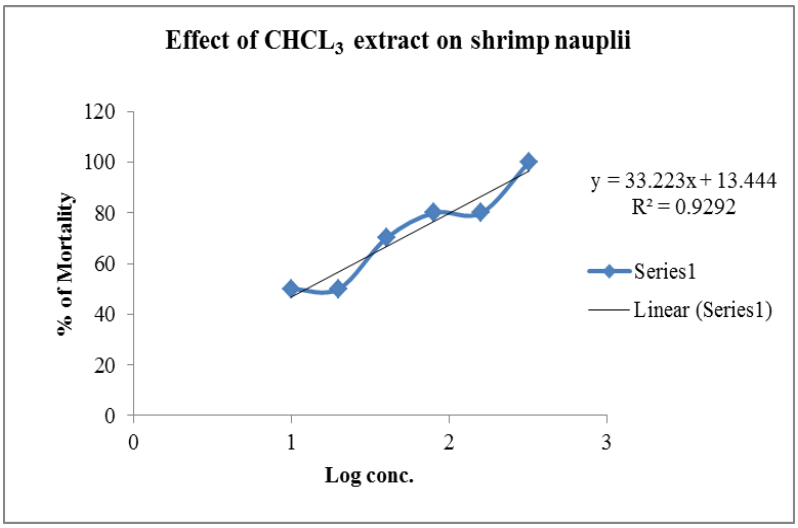


Table 6: After 20 hours later result of Brine shrimp lethality bioassay of ethyl acetate extracts of the leaves *Monochoria hastata.*

Conc. of Extract (µg/ml)	No. of Brine shrimp inserted	No. of Alive Brine Shrimp	No. of death Brine shrimp	% Mortality	Log Conc. (µg/ml)	LC_{50} (µg/ml)
10	10	3	7	70	1	1.43
20	10	3	7	70	1.301	
40	10	2	8	80	1.602	
80	10	1	9	90	1.903	
160	10	1	9	90	2.204	
320	10	0	10	100	2.505	

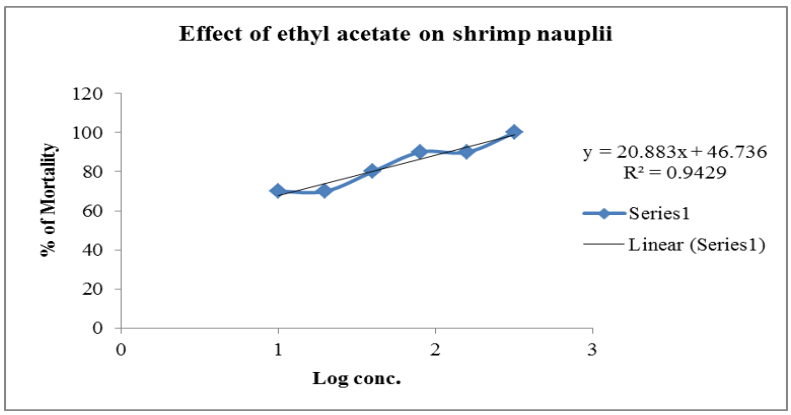


Table 8: After 24 hours later result of Brine shrimp lethality bioassay of distilled *n*-hexane extracts of the leaves *Monochoria hastata.*

Conc. of Extract (µg/ml)	No. of Brine shrimp inserted	No. of Alive Brine Shrimp	No. of death Brine shrimp	% Mortality	Log Conc. (µg/ml)	LC_{50} (µg/ml)
10	10	3	7	70	1	0.99
20	10	2	8	80	1.301	
40	10	2	8	80	1.602	
80	10	1	9	90	1.903	
160	10	0	10	100	2.204	
320	10	0	10	100	2.505	

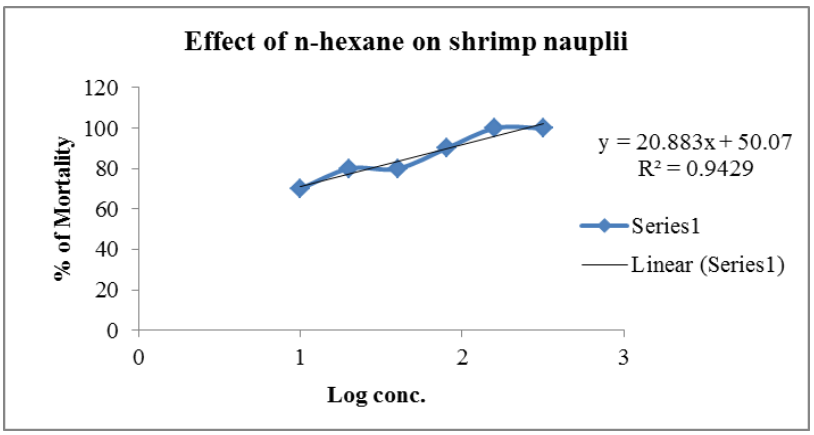


Table 9: After 24 hours later result of Brine shrimp lethality bioassay of ethyl acetate extracts of the leaves *Monochoria hastata.*

Conc. of Extract (µg/ml)	No of Brine shrimp inserted	No of Alive Brine Shrimp	No of death Brine shrimp	% Mortality	Log Conc. (µg/ml)	LC_{50} (µg/ml)
10	10	2	8	80	1	0.148
20	10	2	8	80	1.301	
40	10	1	9	90	1.602	
80	10	0	10	100	1.903	
160	10	0	10	100	2.204	
320	10	0	10	100	2.505	

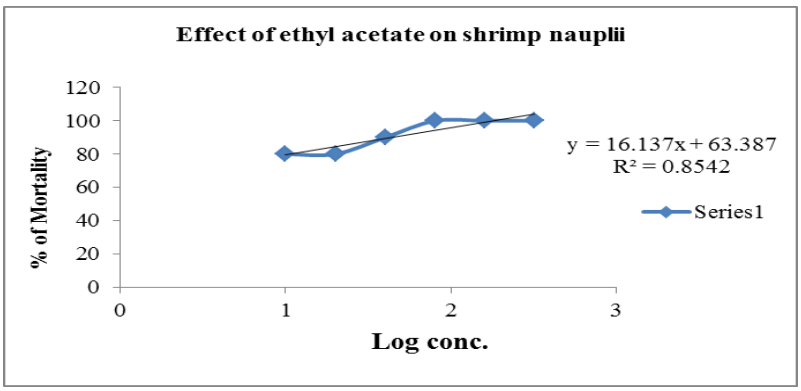
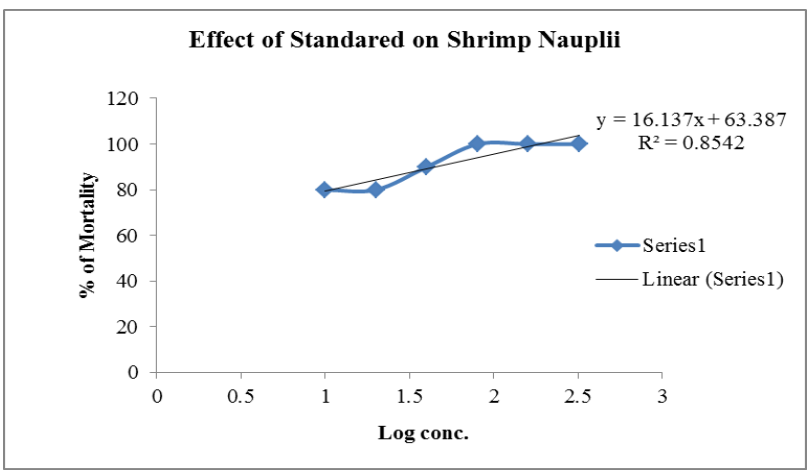


Table 10: Brine shrimp lethality bioassay on Standard of the leaves of *Monochoria hastata.*

Conc. of extract(µg/ml)	No. of brine shrimp inserted	No. of alive brine shrimp	No. of dead brine shrimp	% mortality of aqueous sample	Log conc. (µg/ml)	LC_{50} (µg/ml)
10	10	4	6	60	1	1.36
20	10	3	7	70	1.301	
40	10	3	7	70	1.602	
80	10	0	10	100	1.903	
160	10	0	10	100	2.204	
320	10	0	10	100	2.505	



DISCUSSION

The brine shrimp lethality bioassay is a convenient and rapid method for screening of natural products for finding leads for anticancer, antimicrobial, anti HIV drugs and was reasonably include in the present study.[12,13] Here the different extracts of *Monochoria hastata* have shown to have some lethal effect against the brine shrimp nauplii.[14] In Brine Shrimp Lethality Bioassay, after 18hrs later the LC_{50} of *n*-hexane extract of the *M. hastata* was 19.05µg/ml, after 20hours later 6.76 µg/ml and after 24hours later 0.99 µg/ml. After 18hours later the LC_{50} of $CHCL_3$ extract was 22.90µg/ml, after 20hours later 12.58 µg/ml, after 24hours later 1.89 µg/ml. After 18hours later the LC_{50} of ethyl acetate extract was 3.28µg/ml, after 20hours later 1.43 µg/ml and after 24hours later 0.148 µg/ml respectively.[15]

CONCLUSION

Successive chromatographic separation and the *n*-hexane, ethyl acetate, chloroform soluble fractions of ethanolic extract of *Monochoria hastata* leaves showed significant cytotoxic activities. The medicinal values of the fruits of this plant may be due to the presence of phytochemical constituents like tannins, saponins, phenols, flavonoids, alkaloid and terpenoids. So, further investigations are needed to isolate and identify the active compounds present in the plant extract and its various fractions and their efficacy need to be tested. It will help in the development of new, novel and safe drugs for the treatment of various diseases.

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REFERENCES

1. Tippo O., stern W.L., Humanistic Botany. New Yourk: W.W. Norton., 1977.
2. Schultes R.E., The future of plants as sources of new biodynamic compounds. Plants in the Development of modern Medicine (swain T, ed). Cambridge, MA:Harvard university Press, 1972; 103-124.
3. Ahmad and Beg, 2001; Arthur, 1954; Deininger, 1984
4. Verpoorte R., Pharmacognosy in the new millennium: lead finding and biotechnology. J pharm Pharmacol 2000; 52: 253-262.
5. Vagelos P.R., Are prescription drug prices high? Science, 1991; 252: 1080-1084.
6. www.uonbi.ac.ke/faculties/ids/cvpdf/kaendi_munguti_cv.pdf
7. http://icwow.blogspot.com/2012/08/boronokha-ful-monochoria-hastata.html

8. http://www.itis.gov/servlet/SingleRpt/SingleRpt?search_topic=TSN&search_value=503870.
9. http://wiki.bugwood.org/Monochoria_hastata
10. http://www.google.com.bd/search?q=present+reseach+in+biological+avtivity+of+monochoria+hastata & ie=utf-8&oe=utf-8&aq=t&rls=org.mozilla:en-US:official&client=firefox-a&channel=fflb
11. Ghani, A., Practical Phytochemistry, Jahangirnagar University, Savar, Dhaka, 1997; 84-89.
12. Lee HS. How safe is the re-administration of streptokinase. Drug saf., 1995; 13: 76-80. Do: 10.2165/00002018-199513020-00002.
13. Meyer B.N., Ferringni N.R., puam J.E., Lacobsen L.B., Nichols D.E. Drug info journal, 31: 516-554.
14. .Austin et al., 1999. Austin DJ, Meyers MB; proceedings of the national Academy of science of the united states of America, Phytochemistry, 1965; 4: 245.
15. FantasticoE. B.B. and Mercado, B. L. Dormancy and germination of *C.rutidosperma* DC.*Phil. Agr.*, 1985; 68: 130-138.