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In vitro cytotoxic and membrane stabilizing activities of *Alternanthera versicolor* L.

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Abstract
The crude methanolic extract of leaves of Alternanthera *versicolor* L. as well as their pet-ether, carbon tetrachloride, chloroform and aqueous soluble fractions were tested to investigate the membrane stabilizing and cytotoxic activity. Among all extractives, dichloromethane soluble fraction (DCMSF) of crude extract of leaves of *Alternanthera versicolor* L. exhibited lowest cytotoxicity effect with LC_{50} value 30.63 µg/ml. From investigation results it can also be seen that mortality increased gradually with an increase in concentration of the test samples and maximum mortalities took place at the highest concentration of 400µg/ml. In addition, aqueous soluble fraction (AQSF) shows highest % inhibition of hypotonic solution induces hemolysis of erythrocyte membrane and dichloromethane soluble fraction (DCMSF) shows highest % inhibition of heat induces hemolysis of erythrocyte membrane. Therefore, considering the potential bioactivity, the plant can further be studied extensively to find out their unexplored efficacy and to rationalize their uses of traditional medicine.

Keywords: *Alternanthera versicolor* L., extraction, cytotoxic, membrane stabilizing

Introduction
Medicinal plants are continuously exceptionally promising for the advancement of modern drugs. Adequate scientific screening is essential to distinguish any plant with medicinal quality. It is known that traditionally different plants have different effectiveness in treating different sorts of diseases. In case the proper plant is known for mending a specific malady, the bioactive lead molecule(s) should be isolated from the plant (Kumara *et al*. 2001, Li *et al*. 2003) [1].

Nature has been the primary source of medicinal agents for a long time and a large number of contemporary drugs have been developed from natural source. Medicinal plants have been used to cure illness all over the world for thousands of years and, also used as a medical source. In developing countries like Bangladesh, rural people depend on conventional medicine to treat a wide range of illnesses.

Alternanthera is a genus of approximately 200 low herbaceous plant species in Amaranthaceae, the amaranth family. Alternanthera plants are known to produce allelopathic compounds due to presence of Alkaloids and phenols that inhibit the germination and early seedling growth of crops and vegetables (Sánchez-Del Pino *et al*., 2012; Tanveer *et al*. 2013) [6, 9]. *Alternanthera versicolor* L. is a valuable herb known for its application in the folk medicine in many parts of the world. *Alternanthera versicolor* L. is an annual herb, belongs to the family *Amaranthaceae*. Leaves, bark, root etc. of Lal Bishari (local name) has been used traditionally in Ayurveda for the treatment of various diseases. Since this plant shows excellent medicinal properties, the present study reports the results of preliminary studies of membrane stabilizing and cytotoxic activities of leaves of *Alternanthera versicolor* L. This could explore this plant's remedial potential to a large extent.

Materials and Methods
Collection of plant materials: The fresh leaves of *Alternanthera versicolor* L ware collected from Jessore, Bangladesh in the month of July 2018, and a voucher specimen (accession number -44951) for this plant sample has been deposited in Bangladesh National Herbarium (BNH) for future reference.

Extraction: To facilitate grinding, the plants' leaves were gathered, washed thoroughly, and then sun dried for several days. Then it was powdered. For 15 days, 300 g powdered *Alternanthera versicolor* L. leaves were soaked in 1600 ml methanol with occasional shaking. After that, the extract was filtered through a new cotton plug and Whatman 1 filter paper.

A rotary evaporator was used to concentrate the whole mixture at a lower temperature (40-45^0) and pressure.
The concentrated methanol extract (ME) was partitioned by modified Kupchan method (Van Wagenen *et al*., 1993) [10] into pet ether soluble fractions (PESF), carbon tetrachloride soluble fractions (CTSF), chloroform soluble fractions (CLSF) and aqueous soluble fractions (AQSF). These fractions were used for different biological screenings.

Cytotoxic screening: Using existing protocols (Meyer *et al*., 1982; McLaughlin *et al*., 1998) [4, 3] against *Artemina salina* in a 1-day in vivo assay, this approach was used to determine the general toxic property of the plant extractives. The norm was vincristine sulphate.

Membrane stabilizing activity: The capacity of the extractives to inhibit hypotonic solution and heat induced hemolysis of human erythrocytes was determined using the method developed by Shindhe *et al*. (1999) [8], Omale *et al*. (2008) [5], and Sikder *et al*. (2013) [7]. The norm was acetyl salicylic acid.

Result and Discussion

Cytotoxic activity: The methanol extracts of leaves of *Alternanthera versicolor* L. and its different partitionates were tested for brine shrimp lethality bioassay using the methodology developed by Meyer *et al.* (1982) [4]. Regression analysis was utilized to find the lethal concentration, LC_{50} of the test samples. It can be seen from the regression analysis that it showed best-fit line between the concentration of sample in logarithmic scale and the percentage of shrimp died. Vincristine sulfate (VS) was used as positive control and the LC_{50} was found 0.451µg/ml. The LC_{50} values of MESF, PESF, DCMSF, and CFSF were found to be 308.40 µg/ml, 106.84 µg/ml, 30.63 µg/ml, and 133.03 µg/ml, respectively (Table 1). It can be clearly seen from Fig. 1 that among all the partitionates of crude methanol extract of *Alternanthera versicolor* L, methanolic soluble fraction displayed highest cytotoxic activity.

Table 1: LC50 values of standard and different partitionates of *Alternanthera versicolor* L. in brine shrimp lethality bioassay.

Test samples	Regression line	R^2	LC_{50} (µg/ml)
VS	y = 30.8x + 60.64	0.972	0.451
MESF	y = 24.16x - 10.137	0.8392	308.4
PESF	y = 46.507x + 0.9859	0.8652	106.84
DCMSF	y = 41.877x - 12.238	0.9104	30.63
CLSF	y = 27.381x - 8.1555	0.928	133.03
AQSF	y = 25.704x - 5.5892	0.9037	145.50

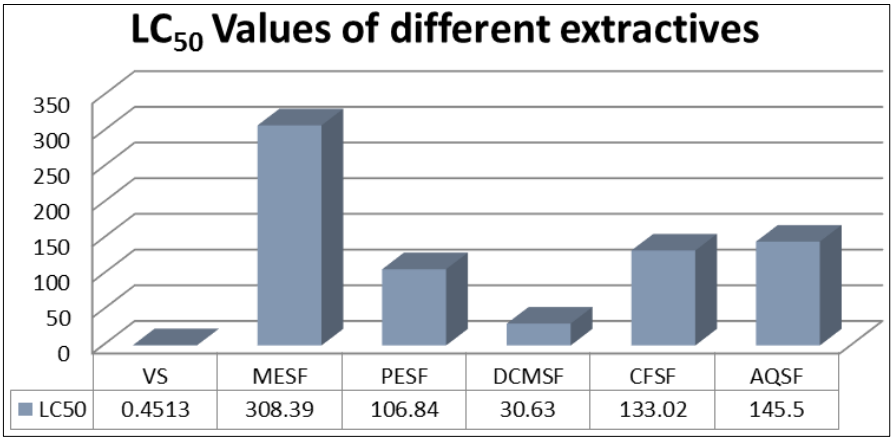

Fig 1: LC_{50} values of the different extractives of *Alternanthera versicolor* L. & VS

Membrane stabilizing activity

The various extracts of leaves of *Alternanthera versicolor* L. were tested and compared to the standard Acetyl Salicylic Acid in Table 2 to know the activity against lysis of human erythrocyte membrane induced by hypotonic solution as well as induced by heat at concentration 1.0 mg/mL. In hypotonic solution induced condition aqueous soluble fraction (AQSF) revealed 57.96% followed by the Dichloromethane soluble fraction (DCMSF), Chloroform soluble fraction (CLSF), Pet ether soluble fraction (PESF), and Methanolic soluble fraction (MESF) which shows 46.67%, 40.43%, 38.04% and 18.63% inhibition of haemolysis of RBC, respectively as compared to standard acetyl salicylic acid (82.27%). All the fraction of *Alternanthera versicolor* L. have been showed mild to high inhibition of hemolysis of RBC in hypotonic medium. From Fig. 2, AQSF shows highest % inhibition of hypotonic solution induces hemolysis of erythrocyte membrane. It can also be seen from Fig. 3 that dichloromethane soluble fraction (DCMSF) shows highest % inhibition of heat induces hemolysis of erythrocyte membrane where DCMSF is 87.57% and standard ASA is 42.12%.

Table 2: Effect of different extractives of *Alternanthera versicolor* L. on membrane stabilization.

Samples	% Inhibition of haemolysis (Hypotonic solution)	
	Heat induced	Hypotonic solution induced
Hypotonic medium		
MESF	61.92	18.63
PESF	25.93	38.04
DCMSF	87.57	46.67

CLSF	45.84	40.43
AQSF	24.04	57.96
ASA	42.12	82.27

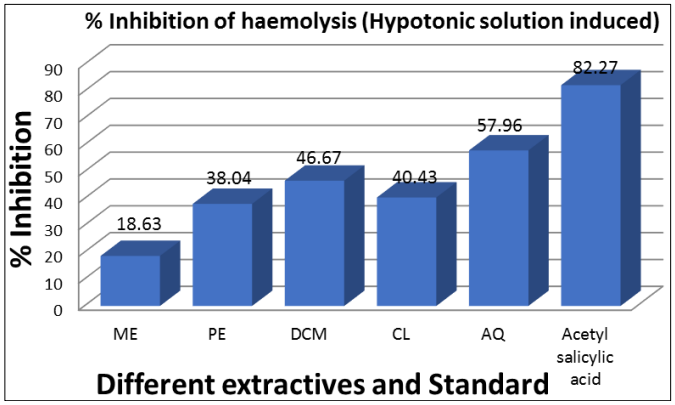

Fig 2: % inhibition of haemolysis of different extractives of *Alternanthera versicolor* (Lem.) on hypotonic solution induced condition

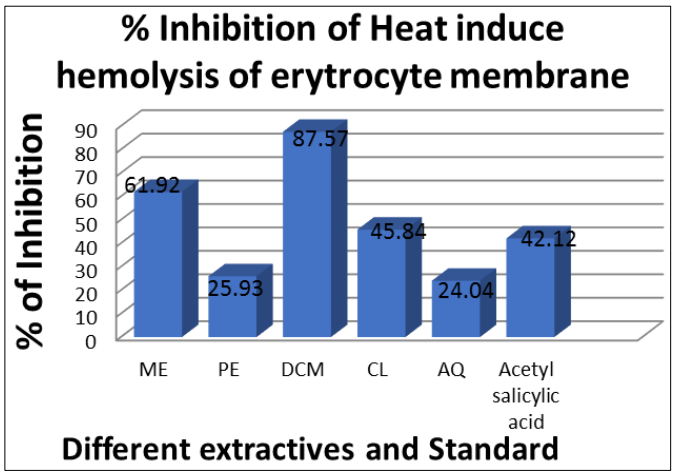

Fig 3: % inhibition of haemolysis of different extractives of *Alternanthera versicolor* (Lem.) on Heat-induced condition

Conclusion

In conclusion, *Alternanthera versicolor* L. extractives were evaluated to investigate their membrane stabilizing, and cytotoxic activity. The result shows that incase of hypotonic solution induced haemolysis, aqueous soluble fraction (AQSF) of crude extract of leaves of *Alternanthera versicolor* L. exhibited highest membrane stabilizing activity. In addition, in case of heat induces hemolysis of erythrocyte membrane, dichloromethane soluble fraction (DCMSF) shows highest membrane stabilizing activity. From the test result, it is also found that methanolic soluble fraction (MESF) exhibits highest cytotoxic activity. However, further investigations related to *in vivo* effects of the plant are required to confirm of its pharmacological actions.

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