The cytotoxic properties of ethanolic extract of Leucas aspera (Family-Lamiaceae) has been investigated in the present study. The cytotoxic potential of the L. aspera ethanolic extract was assessed by brine shrimp lethality bioassay method. Phytochemical study was done through conducting preliminary phytochemical group tests. In brine shrimp lethality bioassay, LC50 value of L. aspera ethanolic extract was found 181.68 µg/ml with 95% confidence limit where the lower and upper limits were 125.12 and 265.96 µg/ml respectively, which indicated that the extract has promising cytotoxic properties.

Key words: Leucas aspera, Cytotoxicity, Lamiaceae, Brine shrimp lethality bioassay.

INTRODUCTION
Cytotoxicity is the quality of being toxic to cells. Among many recent advances in cancer chemotherapy, phytochemicals play an important role as cancer chemotherapeutic drugs. A search for new anticancer drugs has taken many different approaches. The brine shrimp lethality bioassay is efficient, rapid and inexpensive tests that require only a relatively small amount of samples. The technique is also easily mastered and costs little (Meyer et al 1982); therefore, had been successively employed for in vivo lethality bioassay-guide fractionation of active cytotoxic and antitumor agents such as trilobacin from the bark of Asimina triloba (Zhao et al 1992) and ent-kaur-16-en-19-oic acid from Elaeoselinum foetidum (Mongelli et al 2002).

Leucas aspera locally known as Thumbarai, Goma madhupati or Dronapushpi is a common aromatic annual herb with opposite decussate, linear lanceolate leaves and white ligulate flowers and belongs to family Lamiaceae (Figure 1). It is found in Africa, Asia, Pacific Islands, South America and China. In Bangladesh, it grows in Dhaka, Sylhet, Comilla, Chittagong and Chittagong Hill Tracts. Leucas aspera is an important medicinal plant in indigenous system of medicine.
chronic skin eruptions. Chloroform and ether extracts of the plant possess anti-fungal activity. Flowers are used in cold (Ghani, 1998). Keeping in view the medicinal properties of plants and natural products (Nakanishi, 1982; Hohtola, 2010; Dahija and Gautam, 2011; Zahid Hosen et al. 2011; Jain et al. 2011; Emran et al. 2012; Chowdhury et al. 2012), the present investigation was directed toward evaluating cytotoxic properties of the ethanolic extract of Leucas aspera plant using brine shrimp lethality assay.

MATERIALS AND METHODS

Collection of plant
The plant Leucas aspera was collected from the hilly areas adjacent to the University of Chittagong, Bangladesh. The plant was taxonomically classified and identified scientifically by Dr. Saikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Bangladesh.

Preparation of plant extract
The fresh plant of Leucas aspera was washed with water immediately after collection. The collected plants were chopped into small pieces, air dried at room temperature for about 10 days, ground into powder form and stored in an airtight container. About 900 g powder was macerated in 2.5 litre pure ethanol for 5 days at room temperature with occasional stirring. After 5 days, ethanol extract was filtered with Whatman No. 1 filter paper. The extract was concentrated under reduced pressure below 50°C through cyclone vacuum evaporator. The concentrated extract was collected in a petri dish and allowed to air dry for complete evaporation of ethanol. The whole process was repeated three times and finally, 50 g blackish-green colored, concentrated plant extract was obtained (yield 5.55% w/w) which was kept in refrigerator at 4°C.

Assay for cytotoxicity
Cytotoxic activity of plant extract was determined by brine shrimp lethality bioassay (Meyer et al. 1982). Shrimp eggs were added to the artificial "sea water" (38 g sea salt pure NaCl was weighed, dissolved in 1 litre of distilled water adjusted to pH 8.5 using 1 N NaOH and was filtered off to get clear solution) in the larger compartment of an unequally dividend tank which was darkened by covering it with aluminum foil. The chamber was kept under illumination using a table lamp for 48 h for the eggs to hatch into shrimp larvae. The illuminated compartment attracts shrimp larvae (nauplii) through perforations in the dam. Ten shrimp larvae were added to 10 ml of sea water in 10 test tubes and 1000, 800, 600, 400, 200, 100, 80, 60, 40 and 20 µg/ml solutions of extracts, prepared from 10 mg/ml of crude through serial dilution, were added to these test tubes. Each concentration was tested in triplicate. A control containing 10 ml of DMSO solvent was used for each solvent. The test tubes were maintained under illumination. After 24 hours, survivors were counted with the aid of a 3x magnifying glass. From the % lethality of brine shrimp, the probits were calculated for each concentration by using computer software "BioStat-2009". Probits were then plotted against corresponding log concentration of stem extract to get LC50 (lethal concentration 50) value through regression analysis.

Plant sample was also subjected to qualitative secondary metabolite tests. Results showed that L. aspera possessed alkaloid, terpenoids, steroids and flavonoids. Presence of such alkaloid and flavonoids might have some role in showing antioxidant and cytotoxic properties of the Leucas aspera.

RESULTS AND DISCUSSION

Cytotoxic activity of Leucas aspera ethanolic extract was determined by brine shrimp lethality bioassay. Percentage lethality of brine shrimp at ten different concentrations (20 to 1000 µg/ml) of Leucas aspera ethanolic extract was studied. Plant extract showed lethality in a dose dependent manner. More specifically, 2.5, 10, 20, 30, 40, 50, 60, 80, 90 and 97.25% mortality of brine shrimp was observed at 20, 40, 60, 80 100, 200, 400, 600, 800 and 1000 µg/ml concentrations respectively (Table 1).

From the % lethality of brine shrimp, the probits were calculated for each concentration by using computer program "BioStat-2009". Response (%) or lethality (%) was then plotted against corresponding log concentration of plant extract. From this plot, LC50 (lethal concentration 50) value was found by regression analysis using computer program "BioStat-2009".

LC50 value of Leucas aspera ethanolic extract was found 181.68 µg/ml with 95% confidence limit where the lower and the upper limits were 125.12 and 265.96 µg/ml respectively (Table 2, Figure 1).
Table 1. Brine shrimp cytotoxicity of L. aspera ethanolic extract

<table>
<thead>
<tr>
<th>Dose (µg/ml)</th>
<th>Log dose</th>
<th>Total (n)</th>
<th>Alive</th>
<th>Death</th>
<th>% Lethality</th>
<th>Actual %**</th>
<th>Probit Y*</th>
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<tr>
<td>60</td>
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<td>2</td>
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<td>20</td>
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<td>0</td>
<td>10</td>
<td>100</td>
<td>100</td>
<td>6.9604</td>
</tr>
</tbody>
</table>

*Probit Y were calculated using statistical software "Biostat 2009"; **Actual % = Actual formulas (n is the number of animals in a group):
For the 0% dead, 100 (0.25/n), for the 100% dead, 100 (n-0.25)/n.

Table 2. Summary of parameters by probit analysis

<table>
<thead>
<tr>
<th>Log10 LC50</th>
<th>LC50 (µg/ml)</th>
<th>95% confidence limit (µg/ml)</th>
<th>Regression equation</th>
<th>Chi square</th>
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</thead>
<tbody>
<tr>
<td>2.2593</td>
<td>181.6772</td>
<td>125.12-265.96</td>
<td>Y = 56.288*X - 76.735</td>
<td>0.7637</td>
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</tbody>
</table>

Fig. 1. Regression line for determining the LC50 value of ethanolic extract of L. aspera

CONCLUSION
The results of the study demonstrated that ethanolic extract of L. aspera plant exhibited very significant cytotoxic effect in experimental models which supported the claims by traditional medicine practitioners. The phytochemical screening showed that the plant Leucas aspera contained alkaloids, glycosides, steroids, flavonoids, tannins, phlobatannins and saponins which were known to show medicinal activity, but further studies are still necessary to elucidate a mechanistic way how the plant contributes in cytotoxic and other pharmacologic properties.

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