INTRODUCTION
Herbal medicines are the staple of medical treatment in many developing countries. The use of plant constituents as remedy for diseases is very promising. The herb, Euphorbia hirta L. (Family: Euphorbiaceae) grows as a low growing common weed in waste places and roadsides throughout Bangladesh. It is very much used as an astringent in chronic diarrhoeas and dysenteries [1]. Sedative, anxiolytic, analgesic, antipyretic and anti-inflammatory properties of Euphorbia hirta L. has been reported in literature [2, 3]. Euphorbia hirta extract also showed antidiarrhoeic activity and a flavonoid, quercitrin, with antidiarrhoeic activity was isolated from this plant [4]. Leaf extracts of Euphorbia hirta increase urine output and electrolytes in rats [5]. Six compounds have been isolated from the leaves of this plant and identified as gallic acid, quercitrin, myricitrin, 3,4-di-O-galloylquinic acid, 2,4,6-tri-O-galloyl-D-glucose and 1,2,3,4,6-penta-O-galloyl-beta-D-glucose [6]. Despite these biological properties and the various chemical constituents reported in Euphorbia hirta, no data on the antibacterial and antineoplastic activities of root of this plant could be found in the available literature. In this study we prepare methanolic extract from root of Euphorbia hirta and with TLC screening of this extract, we evaluate its antibacterial and antineoplastic activities against some pathogenic bacteria and Ehrlich’s Ascites Carcinoma (EAC) cell line. 

ABSTRACT
This dissertation describes the antibacterial and antineoplastic effect (against Ehrlich’s Ascites Carcinoma; EAC) of methanol extract of root of Euphorbia hirta L. Methanol extract showed moderate activity against both Gram positive (Staphylococcus aureus, Bacillus subtilis, and Bacillus megaterium) and Gram negative (Escherchia coli, Shigella dysenteriae and Shigella sonnei) bacteria. In vivo the root extract resulted 45% and 54.4% Ehrlich’s Ascites Carcinoma (EAC) cell growth inhibition at the dose of 10 and 20 mg/kg body weight, respectively. The root extract also showed moderate cytotoxic effect (LC₅₀ 37.07 µg.ml⁻¹) against Artemia salina (brine shrimp nauplii) in respect to ampicillin trihydrate (LC₅₀ 16.87 µg.ml⁻¹).

Keywords: Euphorbia hirta, Antimicrobial, Antineoplastic, Ehrlich’s Ascites Carcinoma.

MATERIALS AND METHOD

Plant Materials
Plants Euphoria hirta and its root were collected during the month of March-April 2009 from relevant area of Rajshahi University campus and were taxonomically identified by Professor A.T.M Naderuzzaman, Department of Botany, Rajshahi University, Bangladesh. Voucher specimen (23DU- Herborium, collection date 24-02-09) of this plant was kept in the Department of Botany, Dhaka University, Bangladesh.

Extraction and TLC screening
The collected roots were shade dried and reduced to coarse powder. Then the powder was immersed in methanol at room temperature for 7 days. The solvent was completely removed by rotary vacuum evaporator and the crude methanol extract was stored in a vacuum desiccator for further use. Methanol extract was run on pre-coated silica gel plate using n-hexane and ethyl acetate (9:1) as the mobile phase and vanillin-H₂SO₄ reagent was used as spray reagent. Methanol extract of root gave positive test for glycosides, terpenoids and flavonoids [7, 8].

Microorganisms
Three Gram positive (Staphylococcus aureus ATCC25923, Bacillus subtilis QL40 and Bacillus megaterium QL38), three Gram
negative (Escherichia coli ATCC27853, Shigella sonnei C182, Shigella shiga C180 and Shigella dysenteriae ATCC26131) pathogenic bacterial strains were collected from the Institute of Biological Science (IBS), University of Rajshahi, Bangladesh.

**Antibacterial study**

The methanol extract was tested for antibacterial by disc diffusion assay method [9]. Kanamycin disc (30 µg/disc) and blank disc impregnated with the respective solvent were used as positive and negative control, respectively. The antibacterial activity of the sample was tested against each bacterium at concentrations of 30 µg/disc, 200 µg/disc and 400 µg/disc. Each experiment was carried out in triplicates and diameter of the zone of inhibition surrounding each disc was recorded.

**Brine Shrimp Lethality Bioassay**

The experiment was carried out using the method described by Meyer [10]. In brief, Artemia salina Leach (brine shrimp eggs) was allowed to hatch and mature as nauplii (Larvae) in seawater for 48h at 25°C. Serially diluted test solutions (80 µL in DMSO from a stock solution of 5 mg/mL DMSO) were added to the seawater (5 mL), containing 10 nauplii. After incubation for 24h at 25°C, the number of survivors was counted. The LC$_{50}$ was determined using probit analysis as described by Finney (1971) [11]. Ampicillin trihydrate was used as positive control.

**Animals**

Adult Swiss Albino male mice (25-30g) were used throughout the studies. They were obtained from International Center for Diarrheal Diseases Research, Bangladesh (ICDDR’B), Animals were housed in well-ventilated room at temperature 24±2°C and fed with standard mouse-pellet (collected from ICDDR’B) and adequate water.

**Tumor Cells**

Ehrlich Ascites Carcinoma (EAC) cells were obtained by the courtesy of Indian Institute for Chemical Biology, (IICB), Kolkata, India and were maintained by weekly intraperitoneal (i.p.) inoculation of 10⁵ cells/mouse.

**Acute Toxicity Study (LD$_{50}$)**

The acute toxicity study was conducted by the method of Lorke [12] to determine the LD$_{50}$ value of methanol extract of Euphorbia hirta root in mice. This method was carried out by a single intraperitoneal injection in twenty animals (4 in each group) at different doses (100, 200, 400, 800 and 1600 mg/kg body weight). LD$_{50}$ was evaluated by recording mortality after 24 hours.

**Cell growth inhibition**

In vivo tumour cell growth inhibition was carried out by the method as described by Sur et al [13]. For this study, the mice were divided into four groups (6 mice in each group). For therapeutic evaluation, 2 x 10⁵ cells/mouse were inoculated into each group of mice on the first day. Treatment was started after 24 hours of tumour inoculation and continued for 5 days. Group 1 was treated with the vehicle (2% v/v Dimethylsulfoxide; DMSO) and was considered as untreated tumour control. Methanol extract (20 and 40 mg/kg body weight), was administered intraperitoneal (i.p) in groups 2 and 3, respectively. Group 4 received bleomycin (0.3 mg/kg i.p.). In each case the volume of the test solution injected were 0.1 ml/day/mouse. The mice were sacrificed on the 6th day after transplantation and tumour cells were collected by repeated intraperitoneal wash with normal saline (0.9 % NaCl). Viable tumor cells per mouse of the treated group were compared with those of control.

**Statistical Analysis**

All values were expressed as mean ± SEM (Standard Error of Mean). Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett’s t’ test using SPSS statistical software of 10 version. P<0.05 were considered to be statistically significant when compared with control.

**RESULTS AND DISCUSSION**

Infection-causing bacteria are rapidly becoming resistant to conventional drugs for example Methicillin- and Vancomycin-resistant Staphylococcus aureus (MRSA/VRSA) [14]. Scientists are now working to explore alternative drugs from plant sources to explore new and potent antibacterial principles. In the continuation of new antibacterial drug discovery, methanol extract of root of Euphorbia hirta was investigated. In vitro antibacterial activity study, methanol extract showed moderate antibacterial activity against both Gram positive (Staphylococcus aureus, Bacillus subtilis, and Bacillus megaterium) and Gram negative (Escherchia coli, Shigella dysenteriae and Shigella sonnei) bacteria, with inhibition zones in the range of 09-15 mm (Table 1). The standard Doxycyclin was found to have pronounced effect (zones of inhibitions 23-29 mm) at the concentration of 30 µg/disc.

**Table 1. In vitro antibacterial activity of methanol extract of Euphorbia hirta root.**

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Methanol extract (diameter in mm)</th>
<th>Doxycycline (30 µg/disc)</th>
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<tbody>
<tr>
<td></td>
<td>30 µg/disc</td>
<td>200 µg/disc</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>R</td>
<td>10 ± 1.1</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>R</td>
<td>12 ± 0.6</td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>R</td>
<td>10 ± 0.6</td>
</tr>
<tr>
<td>Escherchia coli</td>
<td>R</td>
<td>09 ± 0.8</td>
</tr>
<tr>
<td>Shigella dysenteriae</td>
<td>R</td>
<td>12 ± 0.5</td>
</tr>
<tr>
<td>Shigella sonnei</td>
<td>R</td>
<td>11 ± 1.0</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SEM, R = Resistance*
In brine shrimp lethality bioassay, the crude methanol extract of *Euphorbia hirta* root showed positive result indicating that it is biologically active. The LC_{50} value of methanol extract was 37.07 µg.ml^{-1} whereas ampicillin trihydrate have a LC_{50} value of 16.87 µg.ml^{-1} (Table 2). The mortality rates of brine shrimp were found to be increased with increasing concentrations of the sample.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LC_{50} (µg.ml^{-1})</th>
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<tbody>
<tr>
<td>Ampicillin trihydrate</td>
<td>16.87 ± 0.89</td>
</tr>
<tr>
<td>Methanol extract</td>
<td>37.07 ± 0.74</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SEM*

Intraperitoneal administration of graded doses of methanol extract in mice produced a LD_{50} of 348.6 ± 1.73 mg/kg body weight. Methanol extract resulted in 45.1% and 54.8% EAC cell growth inhibition at the dose of 10 and 20 mg/Kg body weight, respectively (Table 3). On the other hand, the established antitumour drug bleomycin showed 93.7% inhibition at 0.3 mg/kg body weight. The percentage of EAC cell growth inhibition by methanolic root extract of *Euphorbia hirta* was increased dose dependently.

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug used with EAC treated mice</th>
<th>Dose mg/Kg (i.p)</th>
<th>Number of EAC cells/mouse on day 5 after tumour cell inoculation (x10^7)</th>
<th>% Cell growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (EAC) without drug</td>
<td>-</td>
<td>5.45 ± 1.25</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Methanol extract</td>
<td>10</td>
<td>2.99 ± 0.36</td>
<td>45.1</td>
</tr>
<tr>
<td>3</td>
<td>Methanol extract</td>
<td>20</td>
<td>2.48 ± 0.42</td>
<td>54.4</td>
</tr>
<tr>
<td>4</td>
<td>Bleomycin</td>
<td>0.3</td>
<td>0.34 ± 0.19</td>
<td>93.7</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SEM, *Significantly different from group 1; P<0.001, *Significantly different from group 1; P<0.01

The overall findings of this study form a good basis to select this plant for further phytochemical and pharmacological investigation and suggest that the methanol extract contain certain constituents with antibacterial and antineoplastic properties that can be used for the therapy of infectious diseases and cancer. Our future studies to isolate these active phytochemicals and determine their activities against microorganisms and different cancer cell lines, are in progress.

### REFERENCES

12. Lorke DA, A new approach to practical acute toxicity testing, Archives of Toxicology 54, 1983, 275-287.

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