ORIGINAL ARTICLE

Assessment Of Bioactivity Of Bangladeshi Medicinal Plants Using Brine Shrimp Lethality Assay


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ABSTRACT

Twenty six Bangladeshi medicinal plants used in traditional medicines were evaluated for brine shrimp lethality toxicity. Different solvent extracts of Trachyspermum ammi, Cissampelos pareira, Vetiveria zizanioides, Cassia angustifolia, Woodfordia fruticosa, Cinnamomum tamala, Neolomarkicia cadamba, Amaranthus viridis, Amaranthus tricolor, Brassica juncea, Brassica oleracea, Raphanus sativus, Curcuma longa, Curcuma zedoaria, Elettaria cardamomum, Ficus religiosa, Ficus benghalensis, Prunus cerasoides, Chenopodium album, Spinacia oleracea, Smplocos racemosa, Terminalia chebula, Tinospora cordifolia, Cyperus rotundus, Pterocarpus santalinus, and Lagenaria siceraria were used in the study. Of the 26 plants tested, 20 plants (76.9%) were toxic to brine shrimp (LC50 < 30 microg/ml). Among the extracts screened, the ethanolic extract of Spinacia oleracea leaves and methanolic extract of Amaranthus viridis whole plants had the highest toxicity to brine shrimp (LC50 = 0.06 microg/ml). The drug vincristine sulfate was considered as reference standard.

Key words: Cytotoxicity, brine shrimp, Bangladesh, medicinal plants

Introduction

A large percentage of the population of developing countries depends on traditional medicines for their primary health-care needs (FAO, 2004). Bangladesh has a rich heritage of herbal medicines among the South Asian countries. The poor and ethnic peoples of Bangladesh rely on medicinal plants as prescribed by traditional medicinal practitioners for treatment against various diseases. Although a large number of medicinal plants are used in the traditional medicinal system of Bangladesh, the majority of these plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactivity (Ghani, 2003). Traditional records and ecological diversity indicate that Bangladeshi plants represent an exciting resource for possible lead compounds in drug design and development (Uddin et al., 2011).

Twenty six Bangladeshi medicinal plants (Trachyspermum ammi, Cissampelos pareira, Vetiveria zizanioides, Cassia angustifolia, Woodfordia fruticosa, Cinnamomum tamala, Neolomarkicia cadamba, Amaranthus viridis, Amaranthus tricolor, Brassica juncea, Brassica oleracea, Raphanus sativus, Curcuma longa, Curcuma zedoaria, Elettaria cardamomum, Ficus religiosa, Ficus benghalensis, Prunus cerasoides, Chenopodium album, Spinacia oleracea, Symlocos racemosa, Terminalia chebula, Tinospora cordifolia, Cyperus rotundus, Pterocarpus santalinus, and Lagenaria siceraria) were collected from various regions of Bangladesh following accounts of their medicinal uses (Ghani, 2003; Yusuf et al., 1994) or when our ongoing ethnomedicinal surveys (Nawaz et al., 2009; Rahmatullah et al., 2009a-c; Chowdhury et al., 2010; Hasan et al., 2010; Hossen et al., 2010; Mollik et al., 2010a, b; Rahmatullah et al., 2010a-g; Haque et al., 2011; Islam et al., 2011; Jahan et al., 2011; Rahmatullah et al., 2011a, b; Sarker et al., 2011, 2012; Rahmatullah et al., 2012a-d) indicated that they were used by traditional medicinal practitioners for treatment of various ailments. Extracts of various plant parts were screened for their cytotoxic activities.

Cytotoxic potential of whole plant or plant part extracts were measured by the brine shrimp (Artemia salina) nauplii lethality assays. This assay is a useful tool for isolation of bioactive compounds from plant extracts (Sam, 1993). The assay was first proposed by Michael et al. (1956), and later developed by Vanhaecke
et al. (1981) and Sleet and Brendel (1983). We used this assay for determination of the cytotoxic potential of the 26 Bangladeshi medicinal plants in the present study.

Materials and Methods

Plant materials:

The plant samples were collected from various regions of Bangladesh based on their ethnobotanical uses. To determine whether a plant has any ethnobotanical use, surveys were conducted among folk and tribal medicinal practitioners as described before in the Introduction. All plants were identified at the Bangladesh National Herbarium at Dhaka where voucher specimens were also deposited. The various plant parts were cut into small pieces, air-dried under shade and grounded using a laboratory mill and blender.

Extraction:

A known amount of dried powdered sample (usually 100g) was extracted by maceration with methanol or chloroform at a ratio of plant material to solvent of 1:5. Collected extracts were then filtered and concentrated in vacuo at 40°C. The solvent-free extracts were kept refrigerated at 4°C and used for cytotoxicity assays.

Toxicity testing against brine shrimp:

Hatching shrimp. Brine shrimp eggs, *Artemia salina* leach were hatched in artificial seawater prepared by dissolving 38g of sea salt in 1L of distilled water. The pH of the solution was adjusted to 8.5. After 48h incubation at room temperature (26-30°C) under constant aeration, the larvae (nauplii) were attracted to one side of the vessel with a light source and collected with a pipette. Nauplii were separated from eggs by aliquoting them three times in small beakers containing seawater.

Brine shrimp assay. The bioactivity of the extracts was monitored by the brine shrimp lethality test (Meyer et al., 1982). Samples were dissolved in dimethylsulfoxide (DMSO) and diluted with artificial sea salt water so that final DMSO concentration did not exceed 0.05%. 50 l of 2000 g of the plant extract was placed in one sample tube and a two-fold dilution carried out down the column of sample tubes. The last sample tube was left with sea salt water and DMSO only to serve as the drug-free control. The total volume was adjusted to 5 ml with sea salt water. 100 l of suspension of nauplii containing 10 larvae was added into each tube and incubated for 24h. The tubes were then examined under a magnifying glass and the number of dead nauplii in each tube counted. Experiments were conducted with control (vehicle treated), and different concentrations of the test substances in a set of three tubes per dose. Vincristine sulfate was used as a positive control in all experiments.

Statistical analysis:

Lethality assays were evaluated by Finney computer statistical program to determine the LC50 values and 95% confidence intervals. All data were expressed as mean (Microg/ml) (Zhao et al., 1992; McLaughlin, 1991).

Results and Discussion

The cytotoxic activity of different plant parts or whole plants of 26 Bangladeshi traditionally used medicinal plants were investigated against brine shrimp (*Artemia salina*) in vitro. The results are shown in Table 1. All the crude extracts of different plant species resulting in LC50 values of less than 200 microg/ml were considered active against brine shrimp. McLaughlin (1991) has reported that the results obtained with *Artemia salina* are quantitative and reproducible, and the activities parallel cytotoxicities. As a general observation, ED50 values for cytotoxicities will fall one order of magnitude (10 times) lower than LC50 values for brine shrimp. According to the standards of the American National Cancer Institute (NCI), ED50 values of ≤ 20 microg/ml for not pure compounds are considered active (Cordell et al., 1993), so we can take a level for the median lethal concentration (LC50) as 200 microg/ml. About 80% of the plant parts or whole plants in different solvents used in the present study exhibited toxic effects to brine shrimp at LC50 values of lower than 30 microg/ml. The Amaranthaceae and Chenopodiaceae family plants exhibited the most cytotoxicity in these brine shrimp lethality assays. It is interesting that of the two Chenopodiaceae family plants, *Chenopodium album* reportedly prevented progression of cell growth and enhanced cell toxicity in human breast cancer cell lines (Khoobchandani et al., 2009). Possible anti-tumor promoters have also been reported in *Spinacia oleracea* (Wang et al., 2002). The results suggest that brine shrimp lethality assays can be a good indicator for locating plants with anti-cancer activities.
In the present study, DMSO was used as the solvent and as negative control. This is in accordance with previous report that brine shrimp nauplii can tolerate up to 11% of DMSO (Sam, 1993). Further studies are being conducted to isolate and purify the bioactive constituents for further evaluation in human cell line cultures for cytotoxic effects.

Table 1: The LC50 values of different Bangladeshi medicinal plant extracts against *Artemia salina*.

<table>
<thead>
<tr>
<th>Serial number</th>
<th>Scientific name of plant</th>
<th>Family</th>
<th>Part extracted</th>
<th>Solvent used for extraction</th>
<th>Probit Analysis (Mean)</th>
<th>Vincristine sulfate (standard)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Trachyspermum ammi</em> (L.) Sprague ex Turrill</td>
<td>Apiaceae</td>
<td>Fruit</td>
<td>Ethanol</td>
<td>11.11</td>
<td>0.62</td>
</tr>
<tr>
<td>2</td>
<td><em>Cissampelos pareira</em> L.</td>
<td>Menispermaceae</td>
<td>Root</td>
<td>Ethanol</td>
<td>40.10</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Vetiveria zizinoidea</em> (L.) Nash.</td>
<td>Poaceae</td>
<td>Root</td>
<td>Ethanol</td>
<td>67.48</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Cassia angustifolia</em> Vahl. (standard)</td>
<td>Fabaceae</td>
<td>Leaf</td>
<td>Ethanol</td>
<td>109.35</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Woodfordia fruticosa</em> (L.) Kurz.</td>
<td>Myrtaceae</td>
<td>Flower</td>
<td>Ethanol</td>
<td>140.89</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Cinnamomum tamala</em> (Ham.) Nees &amp; Eberm.</td>
<td>Lauraceae</td>
<td>Leaf</td>
<td>Ethanol</td>
<td>142.50</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Neolomarckia cadamba</em> (Roxb.) Bosser</td>
<td>Rubiaceae</td>
<td>Leaf</td>
<td>Methanol</td>
<td>36.72</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Amaranthus viridis</em> L.</td>
<td>Amaranthaceae</td>
<td>Whole plant</td>
<td>Methanol</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><em>Amaranthus tricolor</em> L.</td>
<td>Amaranthaceae</td>
<td>Whole plant</td>
<td>Methanol</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td><em>Brassica juncea</em> (L.) Czerm.</td>
<td>Cruciferae</td>
<td>Leaf</td>
<td>Ethanol</td>
<td>17.04</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td><em>Brassica oleracea</em> L.</td>
<td>Cruciferae</td>
<td>Leaf</td>
<td>Ethanol</td>
<td>7.81</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td><em>Raphanus sativus</em> L.</td>
<td>Cruciferae</td>
<td>Leaf</td>
<td>Ethanol</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td><em>Curcuma longa</em> L.</td>
<td>Zingiberaceae</td>
<td>Rhizome</td>
<td>Ethanol</td>
<td>3.88</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td><em>Curcuma zedoaria</em> Roscoe</td>
<td>Zingiberaceae</td>
<td>Rhizome</td>
<td>Ethanol</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td><em>Elettaria cardamomum</em> Maton</td>
<td>Zingiberaceae</td>
<td>Fruit</td>
<td>Ethanol</td>
<td>18.51</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td><em>Ficus religiosa</em> L.</td>
<td>Moraceae</td>
<td>Bark</td>
<td>Ethanol</td>
<td>13.84</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td><em>Ficus benghalensis</em> L.</td>
<td>Moraceae</td>
<td>Bark</td>
<td>Ethanol</td>
<td>6.82</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td><em>Prunus cerasoides</em> D. Don.</td>
<td>Rosaceae</td>
<td>Stem</td>
<td>Ethanol</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td><em>Chenopodium album</em> L.</td>
<td>Chenopodiaceae</td>
<td>Whole plant</td>
<td>Methanol</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td><em>Spinacia oleracea</em> L.</td>
<td>Chenopodiaceae</td>
<td>Leaf</td>
<td>Ethanol</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td><em>Symplocos racemosa</em> Roxb.</td>
<td>Symplocaceae</td>
<td>Stem</td>
<td>Ethanol</td>
<td>2.90</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td><em>Terminalia chebula</em> (Gaertn.) Retz.</td>
<td>Combretaceae</td>
<td>Fruit</td>
<td>Ethanol</td>
<td>1.53</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td><em>Tinospora cordifolia</em> (Willd.) Hook. f. &amp; Thomson</td>
<td>Menispermaceae</td>
<td>Stem</td>
<td>Ethanol</td>
<td>15.29</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td><em>Cyperus rotundus</em> L.</td>
<td>Cyperaceae</td>
<td>Root</td>
<td>Ethanol</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td><em>Pterocarpus santalinus</em> L.</td>
<td>Fabaceae</td>
<td>Stem</td>
<td>Ethanol</td>
<td>22.03</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td><em>Lagenaria siceraria</em> (Molina) Standl.</td>
<td>Cucurbitaceae</td>
<td>Leaf</td>
<td>Ethanol</td>
<td>23.26</td>
<td></td>
</tr>
</tbody>
</table>

The results are presented as LC50 values (microg/ml).

References


