Comparative Anti-microbial activity and brine shrimp lethality bioassay of different parts of the plant *Moringa oleifera* lam

Kaniz Fatima Urmi¹, Nurul Huda Md. Masum², Abu Hasanat Md. Zulfiker², Md. Kamal Hossain³ and Kaiser Hamid*⁴

¹Department of Pharmacy, Jahangirnagar University, Dhaka, Bangladesh.
²Department of Pharmacy, Southeast University, Dhaka, Bangladesh.
³Vetafarm Manufacturing Pty. Ltd Wagga Wagga, NSW, Australia.
⁴Lecturer (on leave), Department of Pharmacy, East West University, Dhaka, Bangladesh.

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**ABSTRACT**

The aim of the present study was to determine the antimicrobial and cytotoxic activity of different parts of the plant *Moringa oleifera* Lam. Disc diffusion method and brine shrimp lethality bioassay were used for antimicrobial activity and cytotoxic activity respectively. Chloroform fractions of leaf and fruit part have shown moderate antimicrobial activity with zone of inhibition (ZOI) ranging from 9-28 mm against all the experimental microbes. Ethyl acetate fraction of bark and fruit found to have highest antimicrobial activity with zone of inhibition (ZOI) 36 mm against *Shigella dysphoria*. Pet ether fraction of bark but not leaf showed activity against *Bacillus megaterium*. Pet ether fraction of bark showed highest activity against *Candida albicans* with zone of inhibition (ZOI) of 35 mm while chloroform fraction of the leaf showed highest activity against *Bacillus megaterium* with zone of inhibition (ZOI) of 25 mm. All the fractions were found to have potential cytotoxic activity having LC₅₀ values ranging from 0.43-1.18 µg/ml in comparison with vincristine sulphate having LC₅₀ value of 0.53 µg/ml. Ethyl acetate fraction of fruit showed highest cytotoxic activity with LC₅₀ value of 0.43 µg/ml while pet ether fraction of bark showed lowest cytotoxic activity with LC₅₀ value of 1.18 µg/ml.

**INTRODUCTION**

Resistant strains of bacteria are the causes of numerous clinical problems worldwide. The development and increase resistance among pathogens causing nosocomial and community-acquired infections are known to be associated with the widespread utilization (and sometimes overutilization) of antibiotics. Increased healthcare costs, high rate of morbidity and mortality, in developing countries are due to the infectious diseases from resistant microorganisms (Pfaller et al., 1997).

The worldwide concern of multiresistant bacteria justifies the investments in the search for alternative forms of treatment of infections. As a result, a number of medicinal plants used in indigenous medicine have been tested and found to possess bactericidal properties (Chea et al., 2007; More et al., 2008; Oliveira et al., 2007; Soberón et al., 2007; Zuo et al., 2008). The traditional systems of medicine have become increasingly important in view of their safety during the past decade. It has been suggested that in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs.

Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons (Farnsworth et al., 1991). Due to the multiple uses and well-known bactericidal potential, the moringa plant (*Moringa oleifera*) has been the object of much research. (Cáceres et al., 1991; Ghebremichael et al., 2005; Suarez et al., 2003; Suarez et al., 2005). *Moringa oleifera* Lam belonging to the family Moringaceae is known as ‘sajna’ in Bangladesh. It is also known as the horseradish tree, drumstick tree, saijhan, or Ben oil tree. *Moringa oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range

* Corresponding Author

Kaiser Hamid, Department of Pharmacy, East West University, Dhaka, Bangladesh, Cell: +8801926759309
of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, β-carotene, amino acids and various phenolics (Faroq et al., 2007). The seeds of *M. oleifera* have been reported for analgesic (Sutar et al., 2008) and antipyretic activities (Hukkeri et al., 2006). Its leaves have shown wound healing (Hukkeri et al., 2006), analgesic (Rao et al., 2003), hepatoprotective (Selvakumar et al., 2008; Nadro et al., 2006), antiulcer (Pal et al., 1995), hypotensive (Faizi et al., 1995) and diuretic activities (Armando et al., 1992). Roots have shown antifertility activity (Shukla et al., 1988). In an earlier study, it has been found that lectins isolated from the leaves of this plant possess both antimicrobial and cytotoxic activity (Khatun et al., 2009).

The present study was undertaken to explore as well as to compare the antimicrobial and brine shrimp lethality bioassay of different parts (leaf, bark and root) of the plant *M. oleifera*.

**MATERIALS AND METHODS**

**Plant materials**

Different parts of the test plant were collected during the month of January, 2010 from Ramnagar, Comilla, Bangladesh and identified from the Bangladesh National Herbarium, Dhaka where a voucher specimen was deposited having the accession no. 35199.

**Preparation of Crude Plant Extract**

About 200 g of dried, ground separate parts of the plant were soaked in 1.5 L of 98% methanol for 5-7 days, stirring every 18 h using a sterilized glass rod, separately. The final extracts were passed through No. 1 Whatman filter paper (Whatman Ltd., UK) that is followed by solvent-solvent partitioning with petroleum ether, chloroform and ethyl acetate. The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40 °C and stored at 4°C for further use.

**Antimicrobial Activity Measurement:**

For antimicrobial assay, 4 mg plant extract was dissolved in 10 ml of methanol to give solutions of concentration 400µg/ml. Then sterile filter paper discs (5 mm in diameter) were impregnated with known amount of the test substances and dried. The impregnated discs were placed on plates (petri dishes) containing suitable medium (nutrient agar) seeded with the test organisms. The plates were then kept at low temperature (4°C) for 24 hours to allow maximum diffusion. The plates were then kept in an incubator (37°C) for 12-18 hours to allow the growth of microorganism. If the test material has antimicrobial activity, it will inhibit the growth of the microorganism, giving a clear, distinct zone called “Zone of Inhibition”.

The antimicrobial activity of the test agent was determined in term of millimeter by measuring the diameter of the zone of inhibition. The greater the zone of inhibition, the greater the activity of the test material against the test organism. (Barry, 1976). Kanamycin 30 µg/ml was used as standard antibiotic.

**Cytotoxic Activity Test**

Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds (Meyer et al., 1982; Zhao et al., 1992). Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of Brine shrimp (*Artemia salina* Leach) were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in a tank at a temperature around 37 °C with constant oxygen supply. Two days were allowed to hatch and mature the nauplii. Stock solution of the sample was prepared by dissolving required amount of extract in specific volume of pure dimethyl sulfoxide (DMSO). Four milliliter of seawater was given to each of the vials. Then specific volumes of sample were transferred from the stock solution to the vials to get final sample concentrations of 0.78125, 1.5625, 3.125, 6.25, 12.5, 25, 50, 100, 200 and 400µg/ml. In the control vials same volumes of DMSO (as in the sample vials) were taken. With the help of a Pasteur pipette 10 living nauplii were put to each of the vials. After 24 h the vials were observed and the number of nauplii survived in each vial was counted. From this, the percentage of lethality of brine Shrimp nauplii was calculated for each concentration of the extract. Vincristine sulphate was used as standard cytotoxic agent.

**Statistical Analysis**

The lethal concentrations of plant extract resulting in 50% mortality of the brine shrimp (LC₅₀) from the 24 h counts and the dose-response data were transformed into a straight line by means of a trend line fit linear regression analysis (MS Excel version 7); the LC₅₀ was derived from the best-fit line obtained.

**RESULTS AND DISCUSSION**

**Antimicrobial assay**

Chloroform fraction of leaf and fruit part has shown moderate antimicrobial activity with zone of inhibition (ZOI) ranging from 9-28 mm against all the experimental microbes. While ethyl acetate fraction of bark and fruit has shown highest activity with zone of inhibition (ZOI) 36 mm against *Shigella dysphoria*. Pet ether fraction of bark showed greater activity than pet ether fraction of leaf and fruit. Pet ether fraction of bark showed highest activity against *Candida albicans* with zone of inhibition (ZOI) of 35 mm while chloroform fraction of the leaf showed highest activity against *Bacillus megaterium* with zone of inhibition (ZOI) of 25 mm (Table 1).

The antimicrobial activity of the leaves was congruent with the previous studies done by Busani et al., 2012, Aktar et al., 2006; Foidl et al., 2001) who reported on the antibacterial properties of *M. oleifera* seed and leaf. The leaves of *M. oleifera* have been known to contain a number of phytochemicals including flavonoids, saponins, tannins and other phenolic compounds that have antimicrobial activities (Sato et al., 2004; Cushine and Lamb, 2005; Mboto et al., 2009). This would suggest that the antimicrobial activities observed in this study could be attributed to such compounds. The mechanisms of actions of these compounds are not yet well understood.
compounds have been proven to be via cell membranes perturbations (Esimone et al., 2006).

Compounds like pterygospermin, benzyl glucosinolate and benzyl isothiocyanate have been isolated from M. oleifera leaves and these compounds have been reported to have antimicrobial properties against a wide range of bacteria which could partly explain the observed bacteriostatic and bactericidal activity (Fahey, 2005).

It has also been reported that crushed seed extract of M. oleifera had bactericidal activity against Staphylococcus pyogenes and Pseudomonas aeruginosa (Suarez et al., 2005). It has also been reported that Pterygospermin, a bactericidal and fungicidal compound contained in an aqueous extract from seed of M. oleifera was effective against Staphylococcus aureus as the antibiotic neomycin (Harvey, 2005).

The antimicrobial activity of the extract also might be due to the presence of lipophilic compounds that might bind within or internal to the cytoplasmic membrane of the organisms (Boyd and Beveridge, 1979; 1981).

### Brine shrimp lethality bioassay

The brine shrimp lethality bioassay (BSLA) has been used routinely in the primary screening of the crude extracts to assess the toxicity towards the brine shrimp, which could also provide possible indication of toxicity of the test materials. A number of novel antitumor and pesticidal natural products have been isolated using this method (Kumar et al., 2011).

All the plant extracts has shown potential cytotoxic activity having LC50 values ranging from 0.43-1.18 µg/ml in comparison with vincristine sulphate having LC50 value of 0.53 µg/ml. Ethyl acetate fraction of fruit showed highest cytotoxic activity having LC50 of 0.43 while pet ether fraction of bark showed lowest cytotoxic activity with LC50 value of 1.18 (Table 2).

### CONCLUSION

The present study deduces that the plant M. oleifera can be a good source of novel antimicrobial and cytotoxic agent. The next steps would be the isolation, purification, characterization, and testing of individual compound.

### REFERENCES


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### Table 1: Zone of Inhibition (ZOI) in millimeters (mm) of pet ether, ethyl acetate and chloroform fraction of methanolic extract of leaf, bark and fruit part of Moringa oleifera on different microorganisms.

<table>
<thead>
<tr>
<th>Name of Microorganisms</th>
<th>Leaf</th>
<th>Bark</th>
<th>Fruit</th>
<th>Kanamycin 30µg/disc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PE</td>
<td>EA</td>
<td>CF</td>
<td>PE</td>
</tr>
<tr>
<td>Salmonella paratyphi</td>
<td>-</td>
<td>07</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Shigella boydii</td>
<td>-</td>
<td>12</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>-</td>
<td>09</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>-</td>
<td>10</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Azpergillus niger</td>
<td>-</td>
<td>10</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>05</td>
<td>-</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
<td>09</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>-</td>
<td>20</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>10</td>
<td>-</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>Vibrio mimicus</td>
<td>-</td>
<td>17</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Sarcina lutea</td>
<td>-</td>
<td>12</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>Shigella dysphoria</td>
<td>-</td>
<td>16</td>
<td>14</td>
<td>27</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>-</td>
<td>12</td>
<td>09</td>
<td>11</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
<td>12</td>
<td>09</td>
<td>17</td>
</tr>
</tbody>
</table>

- : No inhibition, PE: Petroleum ether, EA: Ethyl acetate, CF: Chloroform

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### Table 2: Results of the brine shrimp lethality bioassay of Moringa oleifera

<table>
<thead>
<tr>
<th>Sample</th>
<th>LC50 values(µg/ml)</th>
<th>Regression equation</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>0.86</td>
<td>y = 23.73x + 29.36</td>
<td>0.972</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>0.76</td>
<td>y = 22.34x + 33.12</td>
<td>0.880</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>0.54</td>
<td>y = 18.32x + 40.14</td>
<td>0.893</td>
</tr>
<tr>
<td>Bark</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>1.18</td>
<td>y = 30.60x + 13.82</td>
<td>0.926</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>0.81</td>
<td>y = 29.39x + 26.33</td>
<td>0.871</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>0.65</td>
<td>y = 27.68x + 31.96</td>
<td>0.954</td>
</tr>
<tr>
<td>Fruit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>0.63</td>
<td>y = 27.58x + 32.59</td>
<td>0.887</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>0.43</td>
<td>y = 20.23x + 41.26</td>
<td>0.838</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>0.55</td>
<td>y = 25.77x + 35.85</td>
<td>0.971</td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vincristine sulfate</td>
<td>0.53</td>
<td>y = 24.96x + 36.85</td>
<td>0.978</td>
</tr>
</tbody>
</table>


Harvey M. Moringa leaf powder- The world’s greatest unknown supplement. 2005; 23-34.


