Research Paper

Antibacterial and Cytotoxic Activities of the Fruit Extract of Averrhoa carambola

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Abstract

The investigation was conducted with methanolic fruit extract of Averrhoa carambola for its antibacterial and cytotoxic activities. Antibacterial activity of the extract was evaluated against various Gram-positive and Gram-negative bacteria using disk diffusion technique. For cytotoxic activity, brine shrimp lethality bioassay was performed to estimate LC50 values. In our preliminary screening, the n-hexane, water and dichloromethane soluble fractions of the crude methanolic extract of Averrhoa carambola were subjected to antibacterial activity and brine shrimp lethality bioassay. The water and dichloromethane soluble partitionate of the methanol extract of Averrhoa carambola exhibited mild to moderate antibacterial activity. Water extract of the said plant showed antibacterial activity against Staphylococcus aureus and Bacillus cereus (Gram positive) while dichloromethane its extract showed antibacterial activity against Escherichia coli and Salmonella typhi (Gram-negative). Dichloromethane soluble partitionate exhibited strong cytotoxicity against the brine shrimp, Artemia salina having LC50 of 1.180 µg/mL.

Keywords: Averrhoa carambola, Oxalidaceae, Antibacterial Activity, Cytotoxic Activity, Brine Shrimp Lethality Bioassay

1. Introduction

Many plants are used as folk medicines for infectious diseases such as urinary tract infections, diarrhea, cutaneous abscesses, bronchitis and parasitic diseases (Ahmad et al, 1998). Due to the indiscriminate use of antibacterial drugs, the microorganisms have developed resistance to many commercial antibiotics. Therefore, investigation of the chemical compounds within medicinal plants has become desirable (Ahmad et al, 1998).

Averrhoa carambola (Oxalidaceae) (Figure 1) is grown in Malaysia and Taiwan, with smaller concentrations in Thailand, Israel, Florida, Brazil, Philippines, China, Australia, Indonesia, in the warmer parts of India, Bangladesh and other areas of the world with the same climate (Ghani, 2003). This tree is also known as the star fruit tree and is commonly used to treat headaches, vomiting, coughing and hangovers (Carolina et al, 2005). Furthermore, it is used as an appetite stimulant, a diuretic and as an anti diarrheal and febrifugal agent. A. carambola has also been used in the treatment of eczemas (P.M. Corr’e, 1984). In addition, the extract obtained through decocting the leaves of A. carambola has been used in the treatment of diabetes (Provasi et al, 2001).

Phytochemistry studies have shown that the fruit of A. carambola is rich in antioxidants, especially polyphenolic compounds, which act against reactive oxygen species. Polyphenols and ascorbic acid content were determined as indicators for the presence of antioxidant compounds in fruits of Averrhoa carambola (Tiwari et al, 1979).

Furthermore, the insoluble fibers of the star fruit make slow the absorption of carbohydrates which significantly
reducing blood glucose levels. The fiber can also act to prevent cardiovascular disease by reducing serum triglyceride and total cholesterol levels (Chau et al, 2004a; 2004b and Shui & Leong, 2006). In Ayurveda (a Hindu system of traditional medicine), many medicinal uses of ripe fruits are also discussed (Ahmad et al, 1998).

The purpose of the present study was to evaluate Averrhoa carambola fruits as potential source of natural antibacterial agents. As a part of our continuing study on chemical and biological characterization of different plants, attempt was made this time to investigate the antibacterial activity of A. carambola against different Gram-positive and Gram-negative bacteria (Mothana & Lindequist, 2005). The cytotoxic activity of the plant materials was performed by using brine shrimp lethality bioassay which was proposed by Michael et al (1956) and modified by Solis et al (1993).

2. Materials and Methods

2.1. Plant Material

The fruit of the plant A. carambola was collected from Gazipur, Bangladesh in March 2009. The specimens of the plant were submitted to the Herbarium of Botany Department, University of Dhaka and taxonomically identified and authenticated by the experts (Voucher number: 0324).

2.2. Extraction and Isolation

The fruit (2000 g) of A. carambola was extracted with 2.5 L of methanol for 7 days and filtered through a cotton plug followed by Whatman filter paper No. 1. The extract was later concentrated with a rotary evaporator. An aliquot (5.0 g) of the concentrated aqueous methanol extract was fractionated by the modified Kupchan portioning protocol (Van-Wagenen et al, 1993) into n-hexane, water, and dichloromethane followed by subsequent evaporation of solvent afforded n-hexane (1.0 g), aqueous (1.20 g), dichloromethane (0.60 g).

2.3. Antibacterial Screening

The antibacterial activity of the extractives was determined by the disc diffusion method (Bauer et al, 1966). The antibacterial strains used for the experiment (Table 1) were collected as pure cultures from the Institute of Nutrition and Food Sciences (INFS), University of Dhaka. The extractives were dissolved separately in chloroform and methanol as required and applied to sterile filter paper discs at 300 g/disc. It was then carefully dried to evaporate the residual solvent. Standard Kanamycin (30 g/disc) discs were used as positive control.

2.4. Cytotoxicity Evaluation

For cytotoxicity screening, the n-hexane, aqueous and dichloromethane soluble materials of crude methanol extract were separately dissolved in DMSO. The test samples were then applied against Artemia salina in a 1-day in vitro assay (Culkin, 1965 and McLaughlin & Rogers, 1998). Artificial sea water was prepared as described by Culkin (1965) with slight modification of chemical composition. Four mg of each of the extractives was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.78125 µg/mL were obtained by serial dilution technique. Vincristine sulfate and DMSO were used as the positive control and negative control respectively. The median lethal concentration (LC50) of the test samples after 24 hours of exposure were determined from a plot of % of the dead shrimps against the logarithm of the sample concentration.

2.5. Statistical Analysis

Regression analysis was carried out for analyzing the data obtained from different samples to study the relationship between cytotoxic activity and vinblastine. For each of the extractive, three samples were prepared for each of the bioassay and the data were taken as mean±SD. Differences at P value of less than 0.05 were considered as statistically significant.
3. Results and Discussion

3.1. Antibacterial Activity of A. carambola

Different partitionates of methanol extract of A. carambola were tested for antibacterial activities against a number of gram-positive and gram-negative bacteria. Among the partitionates, water and dichloromethane soluble fraction of the methanol extract exhibited mild to moderate antibacterial activity (Table 1). The water soluble fraction demonstrated moderate antibacterial activity against Staphylococcus aureus and Bacillus cereus having the diameter of zone of inhibition of 23 mm and 21 mm respectively.

3.2. Brine Shrimp Lethality Bioassay of A. carambola

Table 2 shows the results of the brine shrimp lethality assay after 24 hours exposure to the samples and the positive control vincristine sulfate. The positive control, compared with the negative control (sea water) was lethal, depicting significant mortality to the shrimp. The median lethal concentration (LC50) of the test samples after 24 hours was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the graph by means of regression analysis.

The n-hexane and water soluble partitionates showed less cytotoxicity than dichloromethane fraction. Comparison with positive control, vincristine sulfate indicated that cytotoxicity exhibited by dichloromethane fraction was promising, which might be due to the presence of cytotoxic compounds in this fraction.

Further, bioactivity guided investigation should be conducted to find out the antitumor and pesticidal compounds.

4. Conclusion

The Water and dichloromethane soluble fractions of crude methanol extract of A. carambola showed moderate antibacterial activity whereas the dichloromethane soluble fractions demonstrated potent cytotoxic activity.

Our results support the traditional uses of this plant in various infectious diseases. The plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important drug candidates.
Acknowledgements
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References


Table 2. Results of Cytotoxicity Screening of A. carambola

<table>
<thead>
<tr>
<th>Sample</th>
<th>LC50 (µg/mL)</th>
<th>Regression Equation</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vincristine Sulphate</td>
<td>0.812</td>
<td>y = 33.219x + 52.781</td>
<td>0.9717</td>
</tr>
<tr>
<td>HSF</td>
<td>15.25</td>
<td>y = 28.381x + 16.745</td>
<td>0.9503</td>
</tr>
<tr>
<td>WSF</td>
<td>10.832</td>
<td>y = 22.344x + 52.665</td>
<td>0.9260</td>
</tr>
<tr>
<td>DCMSF</td>
<td>1.180</td>
<td>y = 24.371x + 37.745</td>
<td>0.9816</td>
</tr>
</tbody>
</table>

HSF = Hexane soluble fraction of methanol extract, WSF = Water soluble fraction of methanol extract, DCMSF = Dichloromethane soluble fraction of methanol extract