INTRODUCTION

Annona squamosa Linn. (Fam: Annonaceae) is a small tree, about 3 to 6 meters high, with oblong to lanceolate leaves, greenish flowers and warty skinned sweet fruits, planted as a cash crop throughout Bangladesh. Leaves, bark and unripe fruits of the plant are used to treat infections and night blindness, and also to relieve cerebral congestion. Roots of the plant induce permanent sterility in women and extract of its leaves exerts anti-tumor activity in carcinogenesis, and suppresses mutagenic and carcinogenic actions of tobacco specific nitrosamines.

Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand in the present world for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. We, therefore, report here in the results of preliminary antimicrobial and phytochemical screening of the ethanolic crude extracts of the leaves of the above mentioned three Bangladeshi medicinal plants, which have much folklore reputation.

MATERIALS AND METHODS

Collection and Preparation of the Plant Materials

Annona squamosa Linn. And Calotropis procera R. Br. leaves were collected from Jahangirnagar University campus, Dhaka, Bangladesh while Piper betel Linn. was collected from the local market of Dhaka, Bangladesh in February 2011, of which voucher specimens (nos. 39316, 37537 and 37536, respectively) have been deposited in the Bangladesh National Herbarium for future reference. Leaves of the above mentioned plants were shed dried for several days after washing. The plant materials were then ground to a coarse powder.

Antimicrobial and Phytochemical Screening of Three Reputed Bangladeshi Medicinal Plants

Keywords: Antimicrobial activity, Disc diffusion, Annona squamosa, Calotropis procera, Piper betel, Phytochemical constituents.

ABSTRACT

Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand in the present world for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc. We, therefore, report here in the results of preliminary antimicrobial and phytochemical screening of the ethanolic crude extracts of the leaves of the above mentioned three Bangladeshi medicinal plants, which have much folklore reputation.
Experimental Procedures

Antibacterial screening: The antimicrobial study was carried out by the disc diffusion technique for bacteria. Standard kanamycin disc (30 μg/disc) and discs containing the test materials (500 μg/disc) and the respective solvents were used as the positive control, test sample and the negative control, respectively. According to this method, the antimicrobial potencies of the test samples were measured by determining the diameter of the zones of inhibition in millimeter.

Phytochemical screening

The phytochemical screening of the crude ethanol extracts of three plants were carried out by using standard chromogenic reagents—lead acetate, potassium dichromate, ferric chloride, hydrochloric acid, sulphuric acid, Mayer’s reagent, Dragendorff’s reagent, Wagner’s reagent, Hager’s reagent, Molisch reagent, Benedict’s reagent and Fehling’s solutions were used to detect steroids, alkaloids, gums, flavonoids, saponins, tannins, and reducing sugars using standard protocol.

The extracts of three plants were subjected for this screening using for its different chemical groups as alkaloids, phenols, steroids, tannins, flavonoids, saponins, glycosides and volatile oils as the different chemical groups are reported for specific biologic activities. The colour intensity or the precipitate formation was used as analytical responses to these tests. In each test 10% (w/v) solution of the extract in ethanol was taken.

Table 1: In vitro anti-microbial activity of ethanolic extract of leaves of piper betel linn, calotropis procera r. br and anonna squamosa linn

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Piper betel Linn.</th>
<th>Calotropis procera R. Br.</th>
<th>Anonna squamosa Linn.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kanamycin (50μg/disc)</td>
<td>Ethanol extract (500μg/disc)</td>
<td>Kanamycin (50μg/disc)</td>
</tr>
<tr>
<td>Gram +ve B. subtilis</td>
<td>25</td>
<td>13</td>
<td>25</td>
</tr>
<tr>
<td>Gram +ve S. aureus</td>
<td>20</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Gram +ve P. aeruginosa</td>
<td>20</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Gram -ve E. coli</td>
<td>15</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 2: Status of phytoconstituents in the ethanolic extract of leaves of anonna squamosa linn, calotropis procera r. br. and piper betel linn

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Anonna squamosa Linn.</th>
<th>Leaves extract of Plants</th>
<th>Calotropis procera R. Br.</th>
<th>Piper betel Linn.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Volatile oils</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+= present; - = absent

RESULTS AND DISCUSSION

The results of the in vitro antimicrobial screening assay of the ethanolic crude extracts of the leaves of Anonna squamosa Linn., Calotropis procera R. Br. and Piper betel Linn. against the tested Gram positive and Gram negative strains are shown in the TABLE 1. Among all, the ethanolic extract of leaves of Piper betel Linn. at a concentration of 500 μg/disc showed the maximum antimicrobial activity against Bacillus subtilis with zone of inhibition 13 mm (TABLE 1) followed by Staphylococcus aureus (12 mm) and Pseudomonas aeruginosa (12 mm) and Escherichia coli (10 mm).

The ethanolic extract of the leaves of Calotropis procera R. Br. at the same concentration showed the maximum antimicrobial activity against E.coli with zone of inhibition 10 mm (TABLE 1) followed by Pseudomonas aeruginosa (9 mm), Bacillus subtilis (9 mm) and Staphylococcus aureus (7 mm). Similarly, the ethanolic extract of Anonna squamosa Linn. leaves showed the maximum antimicrobial activity against Escherichia coli having zone of inhibition 9 mm (TABLE 1). It was followed by Pseudomonas aeruginosa (9 mm), Bacillus subtilis (5 mm) and Staphylococcus aureus (5 mm). Finally, as a whole, the maximum zone of inhibition was found for Piper betel Linn. followed by Calotropis procera R. Br. and Anonna squamosa Linn. irrespective of the microorganisms used (TABLE 1). On the other hand, the zones of inhibition in case of the standard kanamycin with B subtilis, S aureus, P aeruginosa and E coli were 25, 20, 20 and 15 mm, respectively (TABLE 1). From the above stated data for both of the standard and test samples, it is clear that the zone of inhibition showed by the extracts of the above mentioned plants leaves are significantly comparable with that of the kanamycin standard. So the present investigation provides evidence for the antimicrobial nature of the ethanolic extracts of the leaves of Piper betel Linn., Calotropis procera R. Br. and Anonna squamosa Linn. and the probable presence of antimicrobial phytoconstituents in their ethanolic extracts. Overall antimicrobial activity of the ethanolic extract of the studied plants is Piper betel Linn. > Calotropis procera R. Br. > Anonna squamosa Linn. Bioassay guided separation of the extracts may led to the isolation of compounds responsible for antimicrobial activity.

In phytochemical screening (TABLE 2), various tests were performed for the determination of the presence or absence of alkaloids, phenols, steroids, tannins, flavonoids, saponins, glycosides and volatile oils in the prepared extracts. Steroids, tannins, saponins, glycosides and volatile oils are found to be present in the leaf extract of the A. squamosa Linn. (TABLE 2) but alkaloids, phenols and flavonoids are found to be absent in the same extract (TABLE 2). On the other hand, all the constituents for which the tests were carried out are found to be present in the Calotropis procera R. Br. leaves extract (TABLE 2).

The Piper betel Linn leaves extract is found to contain alkaloids, phenols, steroids, tannins and volatile oils, whereas the tests for flavonoids, saponins and glycoside are negative, indicating their absence in the extract of the Piper betel Linn. leaves (TABLE 2). Traditional practitioners use widely and effectively the plants, that are investigated in our present study, for the treatment of various infectious diseases. The phytoconstituents that are found to be present in the investigated plants from the present study and also that are reported in the literatures, could be responsible for the remedies results in from the use of the plants and hence for the folklore reputation. However, these plants need to be subjected for further research to explore their extensive pharmacological and other relevant activities.
CONCLUSION

Anonna squamosa Linn, Calotropis procera R. Br. and Piper betel Linn. etc. are medicinally important plants and are used in the treatment of various diseases in traditional systems of medicine. This report provides valuable antimicrobial and phytochemical information about the plants. Such information may serve as a base for new phytochemical, pharmacological, toxicological and clinical research. The future prospects of the present research work include isolation and purification of antimicrobials from the active extracts and their further pharmacological evaluation and clinical trials.

ACKNOWLEDGEMENTS

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REFERENCES

5. Ghani A. Medicinal Plants of Bangladesh with chemical constituents and uses 2003.