EVALUATION OF ANTIMICROBIAL ACTIVITIES OF ARISTOLOCHIA INDICA (LINN)

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ABSTRACT
The present study was designated to evaluate the antimicrobial activities of ethanolic extract of Aristolochia indica L. which was a creeper used as traditional folk medicine for the treatment of different infectious diseases and disorders. The antimicrobial activities of the extract against 12 strains belong to bacterial and fungi species were tested by using agar diffusion method. The results showed that ethanolic extract of Aristolochia indica had moderately significant antibacterial and significant antifungal activity. It inhibited the growth of both bacterial and fungal species dose dependently. The inhibition of growth was highest at 100mg/ml as compared to the controls. Ethanolic extract showed stronger antimicrobial activity against the fungi than that of the bacteria’s. Thus we can conclude that Aristolochia indica Linn was a potent antimicrobial agent which can be tried as a Novel anti-fungal agent.

Keywords: Antimicrobial, Aristolochia indica and Agar diffusion assay.

INTRODUCTION
Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents. The first step towards this goal is the screening of plants used in popular medicine. Thus, antimicrobial research is geared towards the discovery and development of novel antibacterial and antifungal agents1. Herbal medicine represents one of the most important fields of traditional medicine in India. These herbal medicines have been utilized in developing countries not only as a way to rescue the traditional medicine but also an alternative solution to health problems. The world health organization (WHO) also reports that 80% of the world population depend mainly on traditional medicine and the traditional treatment involve mainly the use of plant extracts2.

Plant based antimicrobial agents represent a vast unexploited source for medicines and further exploration of plant antimicrobials needs to occur3. Aristolochia indica, commonly known as Ishwari, Nakuli and Linn was a potent antimicrobial agent which can be tried as a Novel anti-fungal agent. The plant is widely distributed throughout the tropical and subtropical regions of India. This paper reports the antimicrobial activity of Aristolochia indica. This particular plant and its leaves were selected on the basis of its traditional and medicinal uses and it is found to be very effective against microbes.

MATERIAL AND METHODS

Plant Collection
The plant materials (leaves) were freshly collected in various parts of trichy in the beginning of September month and were officially authenticated by Dr. N. Ravichandran. The voucher specimen of the same was submitted to the herbarium of CARISM, SASTRA University and Thanjavur.

Preparation and selection of plant extract
The collected leaves were shade dried, coarsely powdered and extracted by using hot continuous extraction technique in a soxhlet extractor using various solvents such as ethanol, petroleum ether, ethyl acetate, chloroform and acetone until the extracts were colourless in the siphon tube. The extracts were concentrated and dried under vacuum. Preliminary phytochemical studies revealed that the ethanolic extract was found to possess higher number of phytoconstituents than any other extracts. So, for the present study ethanolic extract was selected for the antimicrobial evaluation.

Test microorganisms
The following bacterial strains and fungi strains were used for screening the antimicrobial activity on a broad spectrum basis. All microbial human pathogens were procured from IMT, Chandigarh. Gram negative bacteria such as Escherichia coli (MTCC 724), Pseudomonas aeruginosa (MTCC 741), Salmonella typhi (MTCC 733), Vibrio vulnificus (MTCC 1145), Proteus vulgaris (MTCC 426), Gram Positive bacteria’s such as Bacillus subtilis (MTCC 441), Staphylococcus aureus (MTCC 96), Streptococcus pneumoniae (MTCC 655), and Fungi such as Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus (MTCC 1344) and Candida albicans (MTCC 227) were utilized for the study.

Media used
Nutrient agar (NA) and potato Dextrose agar (PDA) were used respectively for testing the antibacterial and antifungal activity and their compositions were given below.

Composition of Nutrient Agar: (gm/litre)

- Peptone : 5.0
- Beef extract : 3.0
- Sodium chloride : 3.0
- Agar : 15 g
- Distilled water : 1 litre
- pH : 5.6 ± 0.2

Composition of potato Dextrose Agar (PDA) (gm/l)

- Infusion from potatoes : 200 g
- Dextrose : 20 g
- Agar : 15 g
- Distilled water : 1 litre
- pH : 5.6 ± 0.2

Inoculation
Inoculation of each bacterial and fungi strain were suspended in nutrient broth and incubated for 8 hrs at 37°C.

Determination of anti-microbial activity
Agar well diffusion method4-11 was followed to determine the anti-microbial activity. Nutrient Agar (NA) and potato dextrose agar (PDA) were inoculated with the organisms and allowed to solidify. Four wells of 10mm diameter were made in each of this plate using sterile cork borer. About 0.3 ml of different concentration of plant extract were added using sterilized dropping pipette into the wells and allowed to diffuse at room temperature for 2 hrs. The
plates were incubated at 37°C for 18 – 24 hrs for bacteria pathogens and 3 days for fungal pathogens. Diameter of inhibition zones were recorded for determining the anti-microbial activity.

**RESULTS AND DISCUSSION**

The results of antimicrobial activity of alcoholic extract of *Aristolochia indica* Linn against various human pathogens were given in the Table 01.

In the present study, the effect of ethanolic extract of *Aristolochia indica* Linn against various microbes and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameters. The results were tabulated in table 01. The results showed that the ethanolic extract of *Aristolochia indica* dose dependently inhibit the growth of microorganisms such as bacteria’s and fungi. The extract showed poor antimicrobial activity against, *Salmonella typhi* (MTCC 733), *Staphylococcus aureus* (MTCC 96), *Streptococcus pneumoniae* (MTCC 655), *Escherichia coli* (MTCC 724), *Vibrio vulnificus* (MTCC 1145) and *Candida albicans* (MTCC 227). The extract exhibited moderate antimicrobial activity against *Bacillus subtilis* (MTCC 441), *Proteus vulgaris* (MTCC 426) and *Aspergillus flavus* (MTCC 1344). *Aristolochia indica* exhibited significant antimicrobial activity against *Pseudomonas aeruginosa* (MTCC 741), *Aspergillus niger* (MTCC 1344) and *Aspergillus fumigatus* (MTCC 1344).

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Inhibition zones in mm</th>
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<tbody>
<tr>
<td></td>
<td>Std 10µg/ml</td>
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<tr>
<td><strong>Bacteria</strong></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella typhi</em> (MTCC 733)</td>
<td>Clotrimazolone</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> (MTCC 441)</td>
<td>Ampicillin</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (MTCC 96)</td>
<td>38</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> (MTCC 655)</td>
<td>39</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> (MTCC 741)</td>
<td>25</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em> (MTCC 426)</td>
<td>32</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (MTCC 724)</td>
<td>Kanamycin</td>
</tr>
<tr>
<td><em>Vibrio vulnificus</em> (MTCC 1145)</td>
<td>32</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em> (MTCC 1344)</td>
<td>Penicillin</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em> (MTCC 1344)</td>
<td>36</td>
</tr>
<tr>
<td><em>Aspergillus fumigatus</em> (MTCC 1344)</td>
<td>25</td>
</tr>
<tr>
<td><em>Candida albicans</em> (MTCC 227)</td>
<td>37</td>
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The present results showed that medicinal plants which were used in traditional medicine against infections may have some antimicrobial activity. This is true for *Aristolochia indica* Linn ethanolic extract. These results were consistent with traditional uses of the plant leaves *Aristolochia indica* Linn and pharmacological actions of its leaves and essential oil. The concentrations of ethanolic extract needed for bacteriostasis were 10,000 times higher than the concentrations of usual antibiotics. However, the Soskht extracts were very crude preparations, and further purifications may yield more potent compounds. Furthermore, the detection of antimicrobial activities—albeit to varying extents—indicates that the plants may be sources for bacteriological and fungicidal drugs. The present results and similar results in the past showed that there may be plenty of unexploited natural sources of compounds in higher plants, which can be used to control microorganisms. Bioassay-guided research could reveal new, renewable and more potent compounds in these plants.

**REFERENCES**