Antibacterial Activity of Root of *Aristolochia Indica* on *Bacillus Subtilis*

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**ABSTRACT**

Indiscriminate uses of commercial antimicrobial drugs are creating lot of side effects and pathogenic microbes are posing multiresistant and chemical systems are creating lot of side effect, so a search for new anti-infectious agent in the field of ethnopharmacology is paving way. India has been using plants as medicine since Vedic period and major population in developing countries still use traditional folk medicine obtained from plant resources which are cost effective and ecofriendly. Many workers have dealt with antibacterial activities of *A. indica* and other medicinal plants against plant and clinical pathogens. The present study aimed at evaluating in vitro antibacterial activity of root of *A. indica* against gram positive *B. subtilis*, a food poisoning pathogen. *Aristolochia indica*, a twinning paleoherb belongs to family Aristolochiaceae and is native of India and eastward. It is a creeper and reported to treat cholera, leprosy, skin disease, menstrual problems and snakebites. The plant is also used as abortifacient, antiseptic, antipyretic and antifertility agent. *Bacillus subtilis* a gram positive bacterium which has the ability to tolerate extreme environmental conditions by forming endospore was tested for antibacterial assay. Though it is not considered as human pathogen, it causes food poisoning by producing extracellular toxin, proteolytic enzyme subtilisin. Subtilisin results in allergic reactions.

**INTRODUCTION**

In current scenario, number and types of infectious diseases are increasing at alarming rate. Worldwide, in developing countries, most human die due to infectious bacterial disease. Microorganisms are posing multiresistant and chemical systems are creating lot of side effect, so a search for new anti-infectious agent in the field of ethnopharmacology is paving way. India has been using plants as medicine since Vedic period and major population in developing countries still use traditional folk medicine obtained from plant resources which are cost effective and ecofriendly. Many workers have dealt with antibacterial activities of *Aristolochia indica* and other medicinal plants against plant and clinical pathogens. The present study aimed at evaluating in vitro antibacterial activity of root of *A. indica* against gram positive *B. subtilis*, a food poisoning pathogen.

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MATERIALS AND METHODS

Collection of plant materials
Fresh *Aristolochia* plants were collected from nearby scrubby forest of Bangalore. The root part of the plant was selected for antibacterial assay because of its aromaticity and bitter quality. Undertaking ethnomedicinal investigations, the species was identified and authenticated by the taxonomist, Department of Studies in Botany, Manasagangotri, University of Mysore, Mysore, as *Aristolochia indica* L. The roots were thoroughly washed, sun dried and powdered in a blender.

Solvents used for extraction
Acetone, Butanol, DMF, Distilled water and Ether (Reachem).

Preparation of root extracts
Ten grams of root powder was homogenated in five different solvents separately. All the five root extracts were filtered using Buckner funnel with Whatman No. 1 filter paper and stored in an air tight bottle at 4°C for further analysis.

Bacterial strain
The test organism *B. subtilis* was obtained from the Department of Microbiology, University of Mysore, India. The bacteria was cultured on nutrient agar (Hi media) for 24 h at 37°C and suspension was prepared using sterile double distilled water and homogenized.

Standard antibiotics
Five different antibiotics of different concentrations were used as standards and tested against *B. subtilis*. The antibiotics were namely Ciprofloxacin (30 µg/disc), Nitrofurantoin (300 µg/disc), Ofloxacin (5 µg/disc), Pefloxacin (5 µg/disc) and Sparfloxacin (5 µg/disc).

Antibacterial assay
The antibacterial activity assay of root extracts was performed by agar disc diffusion method. Sterilized solidified nutrient agar medium (Hi Media) was seeded with 0.5 ml of inoculum by spread plate technique. All five standard antibiotic discs impregnated separately with five different solvents and discs of root extracts of five different pure solvents were placed on the agar medium seeded with test organism. Two discs were placed in each plate for duplication. The plates were incubated at 37°C for 24 h. The antibacterial activity of the test samples was determined by measuring the diameter of zone of inhibition expressed in centimeter.

Here an attempt was made to compare the antibacterial efficiency of root extracts along with solvents and standard antibiotics activity.

Statistical analysis
The values were statistically analyzed and compared by Post Hoc Scheffe test.

RESULTS AND DISCUSSION
The antibacterial activity of five standard antibiotics of different concentrations, solvents and root extracts of *A. indica* are furnished in Table 1–3. As per observations, among the five standard antibiotics (Table 1) Ciprofloxacin (5.3 cm) showed highest activity followed by Sparfloxacin, Ofloxacin, Pefloxacin and Nitrofurantoin (1.3 cm) being least. Among the five solvents (Table 2), butanol (1.0 cm) showed highest activity, no activity was observed in distilled water and the rest of the solvents activities were in between. All the five root extracts (Table 3) exhibited different degrees of antibacterial activity of which butanol extract (2.4 cm) and ether extract (2.0 cm) showed maximum activity, acetone, DMF and distilled water exhibited minimum activity. The results revealed that all the extracts are potent antimicrobials
against the test organism. The antibacterial activity was observed from the zone of inhibition. The diameter of inhibition zones for each of the standards, solvents and root extracts were compared and data was subjected statistically to Post Hoc Scheffe test.

Comparison of antibacterial assays of standard antibiotic against all five root extracts

All the five standard activity of antibiotics was compared individually with all five different root extracts of *A. indica* by Scheffe test (Table 4). Except Nitrofurantoin, the rest Ciprofloxacin, Ofloxacin, Pefloxacin and Sparfloxacin exhibited maximum activity than all the five root extracts. Butanol extract activity was much closer to the standard antibiotics followed by ether and aqueous extracts. Activities of the above three extracts exceeded the Nitrofurantoin activity. Acetone and DMF extracts showed minimum activity as that of Nitrofurantoin.

### Table 4: Comparison of antibacterial activities of standard antibiotics and root extracts - *Bacillus subtilis*

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Butanol root extract</th>
<th>Ether root extract</th>
<th>Dist. water root extract</th>
<th>DMF root extract</th>
<th>Acetone root extract</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sparfloxacin</strong></td>
<td>2.3750</td>
<td>1.9750</td>
<td>1.9750</td>
<td>2.3750</td>
<td>2.3750</td>
<td>5.3250</td>
</tr>
<tr>
<td><strong>Nitrofurantoin</strong></td>
<td>1.9750</td>
<td>1.9750</td>
<td>2.3750</td>
<td>3.5000</td>
<td>2.3750</td>
<td>5.3250</td>
</tr>
<tr>
<td><strong>Ofloxacin</strong></td>
<td>1.9750</td>
<td>1.9750</td>
<td>2.3750</td>
<td>3.5000</td>
<td>2.3750</td>
<td>5.3250</td>
</tr>
<tr>
<td><strong>Pefloxacin</strong></td>
<td>1.9750</td>
<td>1.9750</td>
<td>2.3750</td>
<td>3.5000</td>
<td>2.3750</td>
<td>5.3250</td>
</tr>
<tr>
<td><strong>Ciprofloxacin</strong></td>
<td>1.9750</td>
<td>1.9750</td>
<td>2.3750</td>
<td>3.5000</td>
<td>2.3750</td>
<td>5.3250</td>
</tr>
</tbody>
</table>

Comparison of antibacterial assays of solvents alone against all five root extracts

All the four solvents except distilled water exhibited minimal antibacterial activity (Table 2). So each solvent was compared to all five different root extracts by Scheffe test (Table 5). The root extracts exhibited double times the activity than solvents.

From past thousands of years plant derived medicines have been part of traditional health care in most parts of the world and yet there is increasing interest in them as sources of agents to fight microbial disease. Bioactive plant extracts are promising source of majority of drugs.

The study was a preliminary assessment of easily available medications of plant origin which can be effective for bacterial infections. According to the findings of this study, the root extracts showed relatively high antibacterial

### Table 5: Comparison of antibacterial activities of solvents and root extract - *Bacillus subtilis*

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Butanol root extract</th>
<th>Ether root extract</th>
<th>Dist. water root extract</th>
<th>DMF root extract</th>
<th>Acetone root extract</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acetone solvent</strong></td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>1.0000</td>
<td>2.0000</td>
<td>5.3250</td>
</tr>
<tr>
<td><strong>DMF solvent</strong></td>
<td>0.5250</td>
<td>0.5250</td>
<td>0.5250</td>
<td>0.5250</td>
<td>0.5250</td>
<td>5.3250</td>
</tr>
<tr>
<td><strong>Distilled water</strong></td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>5.3250</td>
</tr>
<tr>
<td><strong>Ether solvent</strong></td>
<td>0.6250</td>
<td>0.6250</td>
<td>0.6250</td>
<td>0.6250</td>
<td>0.6250</td>
<td>5.3250</td>
</tr>
</tbody>
</table>
activity against gram positive obligate aerobic rod *B. subtilis*. The growth of *B. subtilis* was controlled by all the root extracts indicating that root had a potency to fight against food poisoning agent. The same root with different solvent extraction exhibited varied antibacterial activity. Aqueous root extract exhibiting antibacterial activity proves to be promising and economical.

Nitrofurantoin is known to cause several side effects like stomach upset, diarrhea, loss of appetite, nausea, dizziness and headache. Compared to butanol, acetone and ether root extracts, the reduced Nitrofurantoin activity against *B. subtilis* promises the efficacy as well as the use of natural source components in treating infectious diseases or disorders without side effects. This preliminary screening emphasizes further work to describe the bioactive compound involved in antibacterial activities and their activity in vivo trials before application. So that natural extracts can replace the synthetic chemicals which cause side effects.

**CONCLUSION**

The demonstration of antibacterial activity against gram-positive bacteria is an indication that *Aristolochia indica* is a potential source for the production of drugs with a broad spectrum of activity. The results of the study also supports the traditional application of the plant and suggests that plant extracts possess compounds with antibacterial properties which can be used as antibacterial agents in novel drugs for the treatment of several ailments and infections. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibacterial from this plant are the future challenges.

Combination of traditional knowledge and scientific findings can design drugs which could act as strong dose against multiple drug resistance organisms. These natural therapeutant can meet challenges and can serve as an efficient community-based healthcare system.

**REFERENCES**