ANTI-MICROBIAL ACTIVITY OF TUBERS OF ARISAEMA LESCHENAUTII

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ABSTRACT

This study was carried out with an objective to investigate the antimicrobial activity of tubers of Arisaema leschenaultii. The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on some bacterial and fungal strains. In the present study, the microbial activity of Ethanolic and Aqueous extract of Arisaema Leschenaultii was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using MIC and zone of inhibition method. The antibacterial and antifungal activities of ethanolic and aqueous extracts of Arisaema Leschenaultii were tested against Gram-positive, Gram-negative fungal strains—Aspergillus niger, Aspergillus flavus, Candida albicans. MIC and Zone of inhibition of extracts were compared with that of different standards like ciprofloxacin, for antibacterial activity and ketoconazole for antifungal activity. The results showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms. The phytochemical analyses of the plants were carried out. The antimicrobial activity of the Cassia fistula was due to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

KEYWORDS: Anti-microbial activity, Arisaema leschenaultia.
INTRODUCTION
Arisaema leschenaultii (B.) AL. (Family Araceae) is commonly known as Dhei or Cobra Lilly. It is widely distributed over the greater part of India on the hills of Assam, Karnataka, Kerala and Tamilnadu. Different parts of plant are traditionally used in Ayurveda for the treatment of urinary diseases, colitis, eczema, purging, gonorrhea, piles, haemorrhoids, syphilis, roundworm, fistula and sinus.[1] The whole plant of this species has been reported to show antiseptic property in buffaloes. Kumari et al., studied that AL is used as abortifacient and contraceptives for pig and cattle and also reported the 2method of preparing contraceptives from this plant.[2] Satyanarayana reported the fructosans is present in AL.[3]

EXTRACTION OF DRUG MATERIAL
Plant material of A. leschenaultii was collected from different regions, thoroughly washed, and dried at 55°C in an air dryer for 48 h. Dried plant parts were powdered with Wiley Mill (Model 4276-M, Thomas Scientific, USA) to pass 20 mesh sieve and stored in sealed plastic bags. About 200 g of powdered material was taken and extracted with successive soxhlet extraction method using different solvents (petroleum ether, benzene, chloroform, acetone, ethanol, methanol and water). Process was repeated thrice for complete extraction. After extraction, extracts were combined and evaporated to dryness in vacuo.

MATERIAL AND METHODS
Anti-microbial activity
a. Bacterial strains used and culture conditions
The microbial strains used as test organism were Gram positive: Staphylococcus aureus (ATCC No. 27853), Bacillus subtilis (ATCC No. 6633), Sarcina lutea (ATCC No. 14241), Bacillus cereus (ATCC No. 11778), Gram negative: Escherichia coli (ATCC No. 25922), Pseudomonas aeruginosa (ATCC No. 19429), Klebsiella pneumoniae (ATCC No. 9261) and Fungi: Aspergillus flavus (ATCC No. 11492), Aspergillus niger (ATCC No. 16404) and Candida albicans (ATCC No. 10231) obtained from DRDE, Gwalior.

b. Culture conditions
All bacteria were cultured aerobically overnight at 37°C in nutrient agar medium. Fungi were cultured 3-5 days at 30°C in Sabouraud’s dextrose agar medium.
Nutrient agar medium
Beef extract 1.5 g
Peptone 5.0 g
Agar 17.0 g
Yeast 1.5 g
Sodium chloride 5.0 g
Distilled water 1000 mL
Final pH 7.4±0.2

Sabouraud’s agar medium
Dextrose 40 g
Peptone 10 g
Agar 15 g
Distilled water 1000 mL

c. Sterilization
The sterilization of media, culture tubes, pipettes and other materials was done by autoclaving at 15 lb/sq inch pressures for 30 min.
d. Bacterial stock culture
A loopful of bacterial strain was transferred to nutrient medium and incubated overnight at 37ºC. The number of colony forming unit was found to be $10^3$ per mL.
e. Fungal stock culture
Fungi were cultured 3-5 days at 30ºC in Sabouraud’s dextrose agar medium. The number of spores was found to be $10^4$ per mL.
f. Preparation of drug solution
Stock solutions of all standard drugs were prepared in DMSO at concentration 1000 μg/mL. The samples were dissolved in DMSO at different concentration ranging from 10-50 mg/mL.

Agar diffusion method
The agar diffusion test was used to investigate antimicrobial effects of different fractions of A. leschenaulltii. In this method plates, containing 10 mL of nutrient agar media for bacteria and Sabouraud’s agar medium for fungi, were overlaid with 10 mL of inoculated stock solution of bacteria and fungi. Equidistant holes were made in the agar. A 1 mL volume of
each sample (10-50 μg/mL) was pipetted into the agar wells. Standard compound (100 μg/mL) was used as positive control and the negative control was ethanol. After 24 h (for bacteria) and 3-5 days (for fungi) incubation the diameter of inhibition zones (no growth) around the holes in the bacterial lawn was measured. A positive result was defined as zone of inhibition of 9 mm or more around the holes, indicates the presence of antibacterial substance in the extracts tested (Smania et al., 2006).

**Minimum inhibition concentration (MIC)**

The antimicrobial activity of the extracts was evaluated through the determination of minimum inhibition concentration (MIC) by the microdilution method in culture broth. The extracts were dissolved in 5 mL DMSO and the solution added to 90 mL of nutrient broth for the bacteria growth and Sabouraud’s broth for fungi. Later, a series of dilutions with concentration varying from 10-100 μg/mL of sample was distributed in culture tubes having 1 mL of bacterial and fungal cultures. The culture medium plus DMSO was the growth control and the test dilution was used as sterilized control. All experiments were performed in triplicate. The plates were incubated at 37ºC overnight for bacteria and 3-5 days for fungi. The concentration of sample showing minimum turbidity at highest dilution was considered MIC. The MIC was considered the lowest concentration of the substance that inhibited the bacterial or fungal growth, after incubation. The results were expressed in μg/mL (Zacchinoz et al., 2001).

**RESULTS AND DISCUSSION**

**Anti-microbial activity**

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Okoli et al., 2009).

In recent times, the rapid development of multi-resistant bacterial and fungal strains of clinically important pathogens fetches the interest of scientist to develop newer broad spectrum antimicrobial agents. The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for the development of novel drugs because of the great diversity in their chemical structure. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Dewanjee et al., 2007).
Many researchers have reported the antimicrobial activity of various plants. Phytoconstituents present in plants namely flavonoids, alkaloids, tannins and triterpenoids are producing exciting opportunity for the expansion of modern chemotherapies against wide range of microorganisms (Cowan, 1999).

The main active components in ethanolic extracts of A. leschenaultii responsible for antimicrobial activity are carbohydrates, phenolics, tannins and terpenoids.

The results of minimum inhibitory concentration (MIC) studies are presented in Table 1. The result indicates that ethanolic extract of Blume (EEAL) showed maximum activity (minimum MIC values) against all micro-organisms. Among fungi A. niger (10 μg/mL), C. albicans (10 μg/mL) and among bacteria B. subtilis and S. lutea (20 μg/mL) was most sensitive to EEAL. Among the plant parts ethanolic extract of blume showed better activity.

Table 1: Minimum inhibitory concentration of A. leschenaultii.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Minimum inhibitory concentration (μg/mL)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>EEAL</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>20.0</td>
</tr>
<tr>
<td>B. cereus</td>
<td>20.0</td>
</tr>
<tr>
<td>S. aureus</td>
<td>30.0</td>
</tr>
<tr>
<td>S. lutea</td>
<td>20.0</td>
</tr>
<tr>
<td>E. coli</td>
<td>30.0</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>20.0</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>30.0</td>
</tr>
<tr>
<td>A. niger</td>
<td>10.0</td>
</tr>
<tr>
<td>A. flavus</td>
<td>20.0</td>
</tr>
<tr>
<td>C. albicans</td>
<td>10.0</td>
</tr>
</tbody>
</table>

**Bacteria:** B. subtilis: Bacillus subtilis; B. cereus: Bacillus cereus; S. aureus: Staphylococcus aureus; S. lutea: Sarcina lutea; E. coli: Escherichia coli; K. pneumoniae: Klebsiella pneumoniae; P. aeruginosa: Pseudomonas aeruginosa.

**Fungi:** A. niger: Aspergillus niger; A. flavus: Aspergillus flavus; C. albicans: Candida albicans.

**AEAL:** Aqueous extract of blume; **EEAL:** Ethanolic extract of blume.; **CPX:** Ciprofloxacin;

**KTZ:** Ketoconazole.

Table 2 shows zone of inhibition of A. leschenaultii blume extracts. Both in vitro grown material and extracts of blume was active against gram +ve, gram –ve bacteria and fungi.
Among the extracts EEAL was most active against all microbes followed by aqueous extract of blume. Among bacteria *B. subtilis* and among fungi *A. niger* was most sensitive to EEAL with zone of inhibition (5.5 cm) and (4.8 cm) respectively. Figure 13 shows antimicrobial activity of *A. leschenaultii* extracts by zone of inhibition method.

**Table 2: Zone of inhibition of *A. leschenaultii*.**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of inhibition (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EEAL</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>5.5</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>5.0</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>4.4</td>
</tr>
<tr>
<td><em>S. lutea</em></td>
<td>4.5</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>4.2</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>4.9</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>4.7</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>4.8</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>3.7</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>3.3</td>
</tr>
</tbody>
</table>

**Bacteria:** *B. subtilis: Bacillus subtilis; B. cereus: Bacillus cereus; S. aureus: Staphylococcus aureus; S. lutea: Sarcina lutea; E. coli: Escherichia coli; K. pneumoniae: Klebsiella pneumoniae; P. aeruginosa: Pseudomonas aeruginosa.*

**Fungi:** *A. niger: Aspergillus niger; A. flavus: Aspergillus flavus; C. albicans: Candida albicans.*

**CPX:** Ciprofloxacin; **KTZ:** Ketoconazole; **EEAL:** Ethanollic extract of blume; **AEAL:** Aqueous extract of blume;
Figure 23: Anti-microbial studies of A. leschenaultii extracts.
CONCLUSION

A. leschanaultii were effective against gram positive, gram negative microorganism and fungi. The best activity was associated with ethanolic extract of blume. Gram positive bacteria were more sensitive to drug than gram negative bacteria and fungi.

REFERENCES


