Anti-Microbial Activity of Azima tetracantha Against the Pathogens Causing Diabetic Foot Infections

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Abstract: Diabetes mellitus is a threatening as well as a complicated disorder in the current scenario. It makes the patient susceptible to various sorts of infections of which the foot infection is more dangerous as it has a very delayed wound healing process. A study aimed at testing the antimicrobial activity of an indigenous plant Azima tetracantha against those pathogens causing diabetic foot infections has been carried out. In order to carry out the study, Ethanolic extract of A.tetracantha has been prepared and tested against the selected pathogens such as S.aureus, S.saprophyticus, E.coli, P.mirabilis, B.cereus. The zone of inhibition for each microbe is measured and recorded. The results of the analysis of antimicrobial activity of A.tetracantha have been found to support the fact that the plant extract can act as a potential source of a drug that could cure diabetic foot infections. The experimental results of the analysis have been tabulated with suitable graphical representations in this paper.

1. Introduction

Azima tetracantha (Salvadoraceae) is a well known herb, termed ‘Mulsangu’ in Tamil which has been found to possess many therapeutic properties. The common names of the plant are Uppimullu, Mulchangan, Needle bush, Yasanku and Kundali in Ayurvedic medicine. Root, root bark and leaves of A. tetracantha are used with food as a remedy for rheumatism, diuretic and as stimulant. Several medicinal properties are attributed to this plant in the Indian system of medicine and included in the check list of traded medicinal plants. Traditionally Indian medical practitioners use A.tetracantha in inflammatory conditions, cough, asthma, small pox and diarrhoea. The major phytoconstituents reported in A. tetracantha are azimine, azecarpin, carpine, isorhamnitine-3-O-rutinoside, friedelin, lupeol, glutinol and β-sitosterol. A.tetracantha is reported to have anti-fungal, anti-tumour, anti-diabetic, anti-diarrhoeal and hepato-protective activities. Diabetes mellitus has an increase susceptibility to infections due to the complications developed in various physiological systems and thereby weaken the immune system. Infections are the important contributing factor to the morbidity of diabetic patients with foot problems. Prevention and treatment of such wound is of great importance, as they can lead to foot amputation. The most important characteristics of the diabetic foot infection are often poly-microbial in nature and frequently harbours anaerobic organisms synergistically present along with the aerobes. The most common aerobic organisms encountered are the Gram positive cocci, including Staphylococcus aureus, coagulase-negative Staphylococcus and Streptococcus species. The Gram negative pathogens frequently cultured include Proteus species, Escherichia coli and various other species of Enterobacteriaceae family. Fungal infections of the toe nails are the most common skin condition affecting the diabetic patients. Since A. tetracantha leaf extract is documented as anti-diabetic, it is worthwhile to evaluate the plant against the causative microbial agents for diabetic associated foot infections.

2. Materials and Methods

2.1. Collection of Plant Materials

The medicinal plant Azima tetracantha were collected from the regions of Sri Rangam and its surroundings, Trichy, South India. The collected plant parts were separated and washed thoroughly with running water to remove dirt and dust prior to the drying process. The separated plant parts
include the leaves, stem and the root. Of these parts the leaves are selected for further processing. The leaves of Azima tetracantha were shade dried at room temperature for about two weeks to remove the water and moisture content. After complete drying the leaves were powdered using a mixer grinder. The powdered leaves are now weighed and packed to avoid contamination and were stored under suitable conditions for future use.

### 2.2 Preparation of plant extract

A weighed quantity of the powdered sample was taken and packed using filter paper and was placed in the extraction thimble of the Soxhlet apparatus. Ethanol was used as the solvent using which the phytoconstituents of the sample are extracted. The extract was kept in an hot air oven at a constant temperature for 24-48 hours during which the excess solvent evaporated and the extract was concentrated into a paste like consistency. This was stored in an air tight glass container and stored in refrigerator.

### 2.3 Collection of test organisms

The five important and most commonly found clinical pathogens of diabetic foot infection are selected for the analysis anti-microbial activity of *A. tetracantha*. The selected pathogens include *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Escherichia coli*, *Proteus mirabilis*, *Bacillus cereus* which are collected from the Department of Botany, St. Joseph's College, Trichy, India. These microbes were first cultured in agar slants. After 48 hours the colonies that are present in the slant were isolated and sub-cultured in the nutrient broth and were incubated at 37°C. This inoculum is used for the determination of the antimicrobial activity of *Azima tetracantha*.

### 2.4 Antimicrobial activity Assay

#### 2.4.1 Media Preparation

For determining the anti-microbial activity of the plant extract microbial culture media were prepared with the use of Nutrient Agar. A weighed quantity of Nutrient agar was dissolved in double distilled water and autoclaved at 121°C for 15 min. After autoclaving the media was poured onto the Petri dishes in lukewarm condition and was allowed to solidify. The microbes from the inoculum were transferred to the media using spread plate method.

#### 2.4.2 Preparation of Discs

Whatman No.1 filter paper was used to make the discs which were 8mm in diameter and can hold 50µl of the extract solution. The discs were first sterilized with the use of autoclave and UV radiation. The concentrated extract was diluted and made into three different concentrations which includes 1mg/ml, 2mg/ml and 3mg/ml. Now 50µl of each concentration was poured into the discs of required quantity. This forms discs of the following concentration 50µg/50µl, 100µg/50µl and 150µg/50µl. The discs were dried and were allowed to evaporate the solvent.

#### 2.4.3 Incorporation of Discs onto the Media

The discs were then placed on the surface of the media, according to their respective markings under sterile conditions. After the incorporation of discs the petri plates were incubated at room temperature for 24 - 48 hours in an inverted position. After the incubation period a zone of inhibition was observed which was measured using a metric ruler.

### 3. Results

The ethanolic extract of the leaves of *Azima tetracantha* (EET) exhibit potent antimicrobial activity against the pathogens causing diabetic foot infections. The measured zones of inhibition are tabulated (Table. 1). It reveals that *Bacillus cereus* has the maximum zone of inhibition with 10 mm for the extract concentration of 150µg /50µl. The results of the anti-microbial activity are shown in the figures below.

**Figure 1. Anti-microbial activity of *A.tetracantha* against *S. saprophyticus*. (1- 50µg/50µl; 2- 100µg/50µl; 3- 150µg/50µl; 4- Streptomycin; 5- Ethanol)**
Figure 2. Anti-microbial activity of A. tetracantha against S. aureus. (1- 50μg/50μl; 2- 100μg/50μl; 3- 150μg/50μl; 4- Streptomycin; 5- Ethanol)

Figure 3. Anti-microbial activity of A. tetracantha against E. coli (1- 50μg/50μl; 2- 100μg/50μl; 3- 150μg/50μl; 4- Streptomycin; 5- Ethanol)

Figure 4. Anti-microbial activity of A. tetracantha against P. mirabilis (1- 50μg/50μl; 2- 100μg/50μl; 3- 150μg/50μl; 4- Streptomycin; 5- Ethanol)

Figure 5. Anti-microbial activity of A. tetracantha against B. cereus. (1- 50μg/50μl; 2- 100μg/50μl; 3- 150μg/50μl; 4- Streptomycin; 5- Ethanol)

From the above observed microbial culture results, the zone of inhibition formed for each of the discs was measured using metric scale and was recorded as shown in the tables below (Table.1 & Table.2) and each concentration of EEAT and the positive control Streptomycin were compared for their activity as represented in Graph. 1

Table 1: Zone of Inhibition (mm) formed by the action of various concentrations of Ethanolic extract of Azima tetracantha (EEAT) on various species of Microbes that causes diabetic foot infection.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Strains Tested</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50μg/50μl</td>
</tr>
<tr>
<td>1.</td>
<td>S. saprophyticus</td>
<td>3mm</td>
</tr>
<tr>
<td>2.</td>
<td>S. aureus</td>
<td>2mm</td>
</tr>
<tr>
<td>3.</td>
<td>E. coli</td>
<td>----</td>
</tr>
<tr>
<td>4.</td>
<td>P. mirabilis</td>
<td>----</td>
</tr>
<tr>
<td>5.</td>
<td>B. cereus</td>
<td>3mm</td>
</tr>
</tbody>
</table>

Table.2: Antibiotic sensitivity test for Streptomycin 10mcg (Positive control)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Microbial Strain</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>S. saprophyticus</td>
<td>11</td>
</tr>
<tr>
<td>2.</td>
<td>S. aureus</td>
<td>12</td>
</tr>
<tr>
<td>3.</td>
<td>E. coli</td>
<td>11</td>
</tr>
<tr>
<td>4.</td>
<td>P. mirabilis</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td>B. cereus</td>
<td>17</td>
</tr>
</tbody>
</table>

Graph. 1: Comparison of Anti-Microbial activity on the basis of Zones of inhibition formed for each of the discs placed on the microbial culture plate including the ethanolic extracts of Azima tetracantha and Streptomycin as positive control.
From the observations made it is evident that in almost all the cases the size of the zone of inhibition increased with the increase in the concentration of the plant extract. Hence, the plant extract contains compounds that can resist and inhibit the growth and development of the micro-organisms that are acting as potential causatives of the diabetic foot infection.

4. Discussion

_Azima tetracantha_ has been traditionally used to treat several diseases. Diabetes mellitus is a widespread disorder which arises due to the deficiency of insulin hormone which plays a major role in reducing the level of glucose in the blood leading to several severe complications while _A.tetracantha_ extract is found to lower the blood glucose by a pancreato-trophic action and thus exhibit anti-diabetic activity. Injuries and trauma in the epidermal layer allows colonization of microbes of a wider range causing infections. In case of diabetic foot infection the colonization is commonly poly-microbial and hence requires a poly-antimicrobial agent for its treatment. Current study demonstrated the antimicrobial effect of the ethanolic extract of _A.tetracantha_ leaf on selected pathogens which are the common causative agents for diabetic foot infections. The results showed promising effects regarding the inhibition of selected micro-organisms.

5. Conclusion

From the results observed in this study, it may be concluded that the ethanolic extract of _A.tetracantha_ possess antimicrobial activity which can be very effective in the prophylaxis and treatment of the Diabetic foot infections.

6. References


