AZIMA TETRACANTHA LAM. AGAINST CAUSATIVE AGENTS IN DIABETIC FOOT INFECTIONS

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ABSTRACT

Objective: Azima tetracantha Lam. (Salvadoraceae), a widely growing herb has been used in the traditional medicine for treating many ailments. The objective of the present study was to evaluate the antimicrobial activity of aqueous extract of A. tetracantha leaves (AEAT) against the causative agents in diabetic foot infections and to understand the major phyto-constituents.

Methods: Susceptibility analysis was carried out by disc diffusion method at two different concentrations of AEAT (500 & 1000 µg/ml). UHPLC-ESI MS/MS was carried over with C18 RP column using gradient mobile system and the spectrum obtained was interpreted.

Results: All the strains tested were more susceptible to AEAT with maximum of 15mm inhibition observed for Aspergillus niger and Klebsiella pneumoniae. UHPLC-ESI MS/MS study confirmed the presence of Isorhamnetin-3-O-rutinoside, Myricetin and Friedelin in AEAT.

Conclusion: To conclude AEAT is found to be a good source of lead compounds in diabetic foot infections.

Keywords: Azima tetracantha, UHPLC ESI-MS/MS, antimicrobial

INTRODUCTION

Azima tetracantha (Salvadoraceae) is a well known medicinal herb, termed 'Mulasangini' in Tamil and 'Kundail' in Sanskrit. Root, root bark and leaves of A. tetracantha are used with food as a remedy for rheumatism, diuretic and as stimulant [1,2]. Traditionally Indian medical practitioners use A. tetracantha in inflammatory conditions, cough, asthma, small pox and diarrhoea [3,4]. The major phyto-constituents reported in A. tetracantha are azimeine, azecarpin, carpine, isorhamnatin-3-O-rutinoside, friedelin, klopein, glutinol and β-sitosterol [5,6]. A. tetracantha is reported to have antitumour [7] and antidiabetic [9], antidiarrhoal [10] and hepatoprotective [11] activities.

Diabetes mellitus has an increase susceptibility to infections due to the complications developed in various physiological systems and thereby weaken the immune system [12]. Infections are the important contributing factor to the morbidity of diabetic patients with foot problems [13]. 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 polymicrobial in nature and frequently harbour anaerobic organisms synergestically 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 [14]. 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.

MATERIALS AND METHODS

Preparation of Plant Extract

The aerial part (leaves) of A. tetracantha was collected from the Panayur area of Madurai, Tamilnadu as raw material, during the second week of February and a voucher specimen is stored in Ulula College of Pharmacy (015/UCP) and the plant material was authenticated by a renowned scientist. About 100 g of coarse powdered leaf in 1.5 L water is boiled, cooled and filtered. The filtrate is evaporated to dryness in desiccator and stored in refrigerator.

Susceptibility analysis was carried out by disc diffusion method at two different concentrations of AEAT (500 & 1000 µg/ml). UHPLC-ESI MS/MS was carried over with C18 RP column using gradient mobile system and the spectrum obtained was interpreted. All the strains tested were more susceptible to AEAT with maximum of 15mm inhibition observed for Aspergillus niger and Klebsiella pneumoniae. UHPLC-ESI MS/MS study confirmed the presence of Isorhamnetin-3-O-rutinoside, Myricetin and Friedelin in AEAT.

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appropriate temperature and time in an inverted position. After incubation the zone of inhibition was measured using a metric ruler.

**RESULTS**

**Phytochemical analysis**

Preliminary phytochemical screening of AEAT showed the presence of terpenoids, alkaloids, saponins, tannins, phenolic compounds, flavonoids and steroids. The characterisations of the compounds using UHPLC ESI MS/MS analysis in negative ion mode are furnished in Table 1.

<table>
<thead>
<tr>
<th>[M-H] m/z (g mol⁻¹)</th>
<th>Retention Time (min)</th>
<th>Fragmentation in MS</th>
<th>Identity</th>
<th>Molecular Formula</th>
<th>Structural Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>425.5</td>
<td>22.2-22.6</td>
<td>125.2, 205.3, 273.4</td>
<td>Friedelin</td>
<td>C₃₀H₅₀O</td>
<td><img src="image" alt="Friedelin" /></td>
</tr>
<tr>
<td>622.6</td>
<td>14.4-14.5</td>
<td>315</td>
<td>Isorhamnetin-3-O-rutinoside</td>
<td>C₂₈H₃₂O₁₆</td>
<td><img src="image" alt="Isorhamnetin" /></td>
</tr>
<tr>
<td>317.3</td>
<td>24.5-24.7</td>
<td>151.2, 179.2, 317.3</td>
<td>Myricetin</td>
<td>C₁₅H₁₀O₈</td>
<td><img src="image" alt="Myricetin" /></td>
</tr>
</tbody>
</table>

The mass fragmentation pattern shows a molecular ion peak at m/z 425.5 (Fig 1). The loss of a methyl group was indicated by the presence of a peak at m/z 411. The peak recorded in between Retention time 22.2 to 22.6 min showed other significant ions at m/z 125.2, 205.3 and 273.4 were attributed to the fragmentation of A, B, C and D rings, respectively. To the molecular weight 425.5g mol⁻¹, the corresponding molecular formula extracted from mass data bank is found to be C₃₀H₅₀O. These results were confirmed with small molecular data base and from previous studies [17] the identified compound is Friedelin.

![Fig: 1 Mass spectra of Friedelin from AEAT](image)

The peak recorded in between Retention time 14.4 to 14.5 min showed one major signal in the mass spectra (Fig 2). This correlates with Isorhamnetin which exhibits specific fragmentation with the loss of methyl radical, thus giving m/z 315. To the molecular weight 622.6 g mol⁻¹, the corresponding molecular formula is found to be C₂₈H₃₂O₁₆, from mass data bank. These results were confirmed with small molecular data base and from previous studies [18] and the identified compound is Isorhamnetin-3-O-rutinoside.

![Fig: 2 Mass spectra of Isorhamnetin-3-O-rutinoside from AEAT](image)

The peak recorded in between Retention time 24.5 to 24.7 min showed three major signals in the mass spectra (Fig 3). The peak corresponding to the electron spray ionization at m/z 151.2, m/z 179.2 and the fragment ion at m/z 317.3 and having molecular weight of 317.3 g mol⁻¹ corresponds to the molecular formula C₁₅H₁₀O₈. These results were confirmed with small molecular data base and from previous studies [19] and the identified compound is Myricetin.

![Fig: 3 Mass spectra of Myricetin from AEAT](image)
Antimicrobial Study

Obtained crude extracts after pooled together was subjected to antimicrobial screening using disc diffusion method. AEAT exhibit potent antimicrobial activity against all the tested strains. Its inhibitory effect is proportional to the concentration gradient which is evident from the results. The susceptibility analysis of A. tetracantha is briefly expressed in Table 2.

Table 2 Antimicrobial activity of AEAT

<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>AEAT 500 µg/ml</td>
</tr>
<tr>
<td>1</td>
<td>Candida albicans *</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>Aspergillus niger MTCC</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>Bacillus subtilis MTCC 619</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>Klebsiella pneumonia *</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas aeruginosa *</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Proteus vulgaris MTCC</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>Staphylococcus aureus *</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>Pseudomonas fluorescens</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>*</td>
<td>7</td>
</tr>
</tbody>
</table>


DISCUSSIONS

Traditionally A. tetracantha has been used to treat many diseases. Diabetes mellitus (DM) is a chronic disorder caused by partial or complete insulin deficiency which produces inadequate glucose control and leads to acute and chronic complications while A. tetracantha extract lowers blood glucose by a pancreatotrophic action and thereby exhibit potent antidiabetic activity [9]. The skin is colonized with an indigenous microbial flora. The normal flora may act as a competitive inhibitor of pathogenic microbes. Breaks in the skin, such as leg ulcers, burns and surgical or traumatic wounds and infections like Diabetic foot infections allow colonization with a broader range of bacteria [20]. As the Diabetic foot infections are often polymicrobial, a combination of antimicrobial agents would be more effective, than a single agent alone [14].

Current study demonstrates the antimicrobial effect of A. tetracantha leaf aqueous extract on selected pathogens which are the causative agents for diabetic foot infections. The results showed promising effects regarding the inhibition of selected pathogens. Also an antimicrobial activity of Hexane, ethyl acetate, and methanol leaf extracts of A. tetracantha were reported to be potent against clinical pathogens and fungi [21].

AEAT led to the confirmation of three compounds viz’ Friedelin, Isoharmetin-3-O-rutinoside and Myricetin using LC-MS technique. Myricetin is a novel compound that has an excellent antidiabetic effect at cellular level and being under process in management of prevention and treatment of Diabetes Mellitus [22]. Myricetin also proved to be a potent antibacterial agent against six different strains of both Gram positive and negative [23].

Friedelin from A. tetracantha is a strong antifungal agent [24] and also able to stimulate glucose uptake up to 1.8 fold compared with insulin-treated cells by mimick insulin action and that would be useful in the treatment of diabetes type-2 [25]. Isoharmetin-3-O-rutinoside, may acts as insulin regulator to promote regeneration of beta-cells of the islets of Langerhans [9]. 3-O-rutinosides of isoharmetin, quer cetin and Kaempferol etc. from Calotropis procera showed antimicrobial activity against the test microorganisms including the Gram Positive bacteria (Staphylococcus aureus and Bacillus subtilis) were more susceptible than the Gram negative (Pseudomonas aeruginosa and Salmonella enteritidis) and the yeast species were more susceptible than the filamentous fungi [26].

Myricetin has been reported very well for its antibacterial activity. The triterpenoid, friedelin and flavonoid glycoside Isoharmetin-3-0-rutinoside reported to possess excellent antifungal activity. These three compounds possess very good antidiabetic activity also. So the activity reported here may be attributed to the presence and combined efficiency in AEAT.

CONCLUSION

From the outcome of this study, it may be concluded AEAT possesses antimicrobial and hypoglycaemic property which are very well effective in the prophylaxis and treatment of the Diabetic foot infections.

REFERENCES


