ABSTRACT

The objective of the present study was to assess the antibacterial activity of Acacia catechu willd (AC). AC is a moderate sized deciduous tree growing in tropical countries commonly called “Karungali” in Tamil. It is a potent medicinal plant in the traditional Indian medicinal systems. Aqueous and ethanolic extract of Heartwood of Acacia catechu is tested for antibacterial activity against Staphylococcus aureus, Bacillus subtilis, E.coli, Pseudomonas aeruginosa, Klebsiella pneumoniae. Disc diffusion technique was followed for screening antibacterial activity. The discs were loaded with 50µl of aqueous and ethanolic extracts at different concentrations. Positive controls used were Amoxycillin (30 µg/disc) and Ciprofloxacin (5 µg/disc). After incubation at 37ºc over night, the zone of inhibition was measured. Ethanolic extract showed significant activity than Aqueous extract against the micro organism compared to standard.
KEY WORDS

Acacia catechu willd, Anti bacterial evaluation, Mac Farland’s standard, Zone of inhibition.

INTRODUCTION

Acacia catechu Willd. (AC) (Family: Fabaceae and subfamily: Mimosoideae) known as Black cutch. AC, a deciduous thorn like tree mainly found in India and also found in deciduous forests around the world. The leaves, bark, heartwood has many nutritional and medicinal uses. The foliage is softly textured, light green and oval-shaped. The branches are thin and spike like because tiny thorns grow around the exterior. AC typically reaches heights of up to 50 feet. The sap wood of AC is large and yellowish white and heart wood is small and red in colour. The chief constituents of the heartwood are catechin and catechuttanic acid. The wood contains epicatechin\(^2\), Atzelchin, catechin tetramer, dicatechin, gallochin, gossypetin, phlobatannin, kaempferol, quercitrin, quercitin\(^17\). The catechin content varies from 4 to 7 per cent and is distributed throughout the heartwood from the root to the branches. Another important constituent is taxifolin. Catechin is biologically highly active. It is used as a haemostatic. Taxifolin has antibacterial\(^1\), anti-fungal\(^13\), antiviral, anti-inflammatory, and antioxidant activity\(^15,16\). The extract of Acacia catechu have been reported to have various pharmacological effects like immuno modulatory\(^14\), anti pyretic, hypoglycaemic\(^7\), anti diarrhoeal\(^8\), hepatoprotective activity\(^3,6\). Heartwood is used to yield concentrated aqueous extract i.e. cutch. Cutch (extract) is astrigent, cooling, and digestive. It is useful in cold and cough\(^1,4,5,8\) ulcers, boils and eruptions of the skin, bleeding piles, uterine haemorrhages, atonic dyspepsia, chronic bronchitis etc. An antibacterial mouthwash made from the extract treats gingivitis and mouth sores.

MATERIALS AND METHODS

plant material

The ethanolic and aqueous extract of heartwood of Acacia catechu willd was obtained from Green Chem Herbal Extract & Formulations, Bangalore.

Test microorganisms

Bacterial strains used were Staphylococcus aureus [Gram positive GP], Bacillus subtilis[GP], E. coli[Gram negative GN], Psuedomonas aeruginosa[GN] and Klebsiella pneumonia [GN]. The organisms were obtained from department of microbiology, Saveetha Dental College and maintained in nutrient agar slope at 4°C.

methodology

The extracts were prepared in the following concentrations in sterile water. 2mg/ml, 3mg/ml and 4mg/ml. 50µl of extract of different concentrations were loaded on sterile filter paper discs measuring 6mm in diameter, so that the concentration of the extract on each disc was 100µg, 150µg and 200 µg respectively. The discs were dried and kept aseptically.

Screening of antibacterial activity [disc diffusion technique]

Over night broth culture of the bacterial strains compared to Mac Farland’s standard\(^9-12\) 0.5 were prepared. Ten plates of Muller Hinton agar [MHA-Hi media M1084] was taken and divided into two sets of 5 each for testing ethanolic and aqueous extracts respectively. Lawn culture of the test organisms were made on the MHA plates using sterile cotton swab and the plates were dried for 15 minutes. Filter paper discs loaded with different concentrations of the extract were placed on the respective plates. The plates were incubated at 37°C overnight and the zone of inhibition of growth was measured in
millimeters. Standard antibiotic discs of amoxicillin (30mcg/disc) and Ciprofloxacin (5mcg/disc) were used as positive control. All the tests were done in triplicate to minimize the test error.

RESULTS AND DISCUSSION

The antibacterial activity of the extracts (Ethanolic and Aqueous) at different concentrations was determined by measuring the zone of inhibition. The results are given in the table 1.

Table 1
Antibacterial Activity of the heartwood Extracts of Acacia catechu Willd.

<table>
<thead>
<tr>
<th>Extract</th>
<th>conc. (µg)</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
<th>B4</th>
<th>B5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanolic</td>
<td>100</td>
<td>12</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>19</td>
<td>9</td>
<td>14</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>24</td>
<td>11</td>
<td>19</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Aqueous</td>
<td>100</td>
<td>10</td>
<td>7</td>
<td>8</td>
<td>8.5</td>
<td>no zone</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>16</td>
<td>8</td>
<td>11</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>20</td>
<td>10</td>
<td>16</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>30mcg/disc</td>
<td>24</td>
<td>26</td>
<td>22</td>
<td>23</td>
<td>22</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5mcg/disc</td>
<td>22</td>
<td>25</td>
<td>24</td>
<td>25</td>
<td>21</td>
</tr>
</tbody>
</table>

B1= Staphylococcus aureus B2 =Bacillus subtilis B3= Escherichia coli B4= Klebsiella pneumoniae B5= Pseudomonas aeruginosa.

*Each value represents the mean of three determinants.

Both the extracts at different concentration exhibited antibacterial activity against all bacterial strains tested. Ethanolic extract exhibited comparably a high degree of activity than the aqueous extract.

Graph 1

Antibacterial Activity of the Heartwood Extracts of Acacia catechu Willd.
(EE- ethanolic extract AE – aqueous extract.)

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The ethanolic extract was more effective against *Staphylococcus aureus* with a zone of inhibition of 24 mm diameter (at conc 200 µg.) and was least effective against *Pseudomonas aeruginosa* and *Bacillus subtilis* with zone of inhibition of 10mm (at conc. 200 µg.) and 11mm (at conc. 200 µg.) respectively. Among the other bacterial species studied *E.coli* showed a zone of inhibition of 19mm diameter (at conc. 200 µg.) and *Klebsiella pneumoniae* showed inhibition zone of 16mm diameter (at conc. 200 µg.).

**CONCLUSION**

From the results, it can be concluded that the *Acacia catechu* heart wood extracts has got antibacterial activity after extensive investigation. Further work will emphasize the isolation and characterization of active principle responsible for antibacterial activity of heartwood extracts of *Acacia catechu* Willd.

**ACKNOWLEDGEMENTS**

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