Antibacterial activity of Crotalaria pallida Aiton. (Fabaceae)

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ABSTRACT

Crotalaria pallida Aiton. Fabaceae has been used for the treatment of various diseases like. The aim of the present study is to assess antibacterial activity of methanolic leaves extract of Crotalaria pallida Aiton. Escherichia coli and Klebsilla pneumoniae showed maximum susceptibility 19±1 and 18.66±0.57 at 25 mg/ml concentration respectively while Pseudomonas aeruginosa, Bacillus sp. and Staphylococcus aureus showed less susceptibility 11±1, 11.33±1.5 and 15±1 at 25 mg/ml concentration respectively. Overall results have proved that C. pallida leaves posses significant antibacterial activity.

1. Introduction

Medicinal plants have played a pivotal role in the primary healthcare and formed the basis of traditional systems of medicines. Plants have been bestowed us with food, spices, flavours, fragrances, medicines, etc. Plant are being used to treat many diseases or ailments viz. infectious diseases, inflammatory disorders, skin diseases, etc. since ancient time [1-3]. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties [4]. Natural products of higher plants may give a new source of antimicrobial agents. There are many research groups that are now engaged in medicinal plants research [5-7]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated the search for new antimicrobial substance from other sources. Screening of medicinal plants for antimicrobial activities and phytochemical is important for finding potential new compounds for therapeutic uses. Therefore, current scientific investigation dwells in identifying new leads from the plant sources possessing significant antibacterial activity.

Crotalaria is one of the largest genera in tropical Africa. The genus includes 690 species that are mainly situated in Africa and Madagascar [8]. Species have also been found in India, United States of America (USA) and China. Crotalaria pallida Aiton. belongs to the family fabaceae. This is an erect shrub, annual or short-lived perennial herb of 1.5 m or more tall. The stout stem is hairy and has longitudinal grooves. Leaves are trifoliate with a 2-8.5 cm long petiole, leaflets 3-13 x 2-5 cm and elliptical to obovate. Flowers are yellow, often reddish-brown veined and borne on 15-40 cm long racemes, each with 20-30 flowers. Fruits are 3-5 x 0.6-0.8 cm, 30-40 seeded that are heart-shaped, 3 x 2 mm, shiny, mottled ochre and dark grey-green or brown. The plant is grown as a ground cover and a green manure crop, especially in the inter-rows of rubber trees and coconut palms. Flowers are eaten as a vegetable in Cambodia, where the seeds are roasted and grounded for use as a sort of coffee beverage. The roots are sometimes chewed with betel nuts in Vietnam. In traditional medicine, the plant is used to treat urinary problems and fever, a poultice of the roots is applied to swelling of joints and an extract of the leaves is taken to expel intestinal worms [9].

2. Materials methods

2.1 Plant material

The leaves of C. pallida Aiton were procured from natural vicinity of Pollachi town, Coimbatore city, India during the month of April 2013. The leaves of this plant were separated, shade dried, powdered and stored in air tight container at room temperature. The plant was identified and authenticated (410) by Botanical Survey of India, TNAU campus, Coimbatore, Tamilnadu, India, as Crotalaria pallida Aiton.

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2.2 Preparation of plant extract

About 30g of powdered sample of C. pallida Aiton was dissolved in 300ml of methanol and kept in an orbital shaker for overnight. The extract was filtered using Whatman filter No.1 to obtain a particle free extract. The solvent extract was evaporated to dryness after extraction under reduced pressure using rotary evaporator. The obtained methanol extract was stored at 4ºC for further analysis. All the chemicals and solvents used for experimental analysis were of analytical grade.

2.3 Bacterial strains used

About five human pathogenic bacterial strains were used. The Gram-negative Pseudomonas aeruginosa, Escherichia coli and Klebsilla pneumoniae and Gram-positive bacteria Staphylococcus aureus and Bacillus sp. were used for this study.

2.4 Agar well diffusion method

Antibacterial activity was screened by agar well diffusion method [10, 11]. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 12 hours-old broth culture of respective bacteria. Using the sterile cork borer, the well (6mm) was made into the each Petri-plate. Different concentrations of methanolic leaves extract (in vivo) of C. pallida Aiton, (3.125-25 mg/ml) were used to assess the activity in dose dependent manner. The methanol showed no zone of inhibition and acts as a negative control and was added into the wells by using sterile micropipettes. Simultaneously the standard antibiotic (as positive control) was tested against the pathogens. Kanamycin is used as a positive control against bacterial pathogens. Then the plates were incubated at 37°C for 24 - 48 hours. After the incubation period, the diameter of the inhibition zones of each well was measured. And the values were noted. Triplicates were maintained in each extract and the average values were calculated for the eventual antibacterial activity.

3. Results

Results of antibacterial activities by agar well diffusion method were presented in Table 1. The antibacterial activity was tested on the basis of the magnitude of zones of inhibition (in 6mm). The activity of Crotalaria pallida has also been compared with the broad spectrum commercially available antibiotic (Kanamycin). All bacteria were found to be resistant towards commercially used antibiotic. The detailed analysis of the antibacterial activity of the methanolic leaf extract showed dose dependent activity and the activity was shown at an amount of 25 mg/ml well. While less activity was shown when 3.125 mg/well amount of extract was used. Escherichia coli and Klebsilla pneumoniae showed maximum susceptibility 19±1 and 18.66±0.57 at 25 mg/ml concentration respectively while Pseudomonas aeruginosa, Bacillus sp. and Staphylococcus aureus showed less susceptibility 11±1, 11.33±1.5 and 15±1 at 25 mg/ml concentration respectively (Figure 1). Gram negative bacteria were more susceptible towards this extract than tested Gram positive ones (Figure 2).

Table 1: Antimicrobial activity

<table>
<thead>
<tr>
<th>Concentration</th>
<th>3.125mg/ml</th>
<th>6.25mg/ml</th>
<th>12.5mg/ml</th>
<th>25mg/ml</th>
<th>Kanamycin 30µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>12±1</td>
<td>13±1</td>
<td>14±1</td>
<td>19±1</td>
<td>20.3±0.57</td>
</tr>
<tr>
<td>Klebsilla pneumoniae</td>
<td>14±1</td>
<td>16±1</td>
<td>17.33±0.57</td>
<td>18.66±0.57</td>
<td>20.3±0.57</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
<td>-</td>
<td>9±1</td>
<td>11±1</td>
<td>21.3±0.57</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>8±1</td>
<td>9±1</td>
<td>10±1</td>
<td>11.33±1.5</td>
<td>21.3±0.57</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>10±1</td>
<td>12±1</td>
<td>13.66±0.57</td>
<td>15±1</td>
<td>21.3±0.57</td>
</tr>
</tbody>
</table>

n=3; mean±SD

Zone of inhibition showed by E. coli

Zone of inhibition showed by K. pneumonia
4. Discussion

Nowadays, research is going in around the world to exploit the traditional medicinal plants for therapeutic value scientifically. The qualities like low toxicity, inexpensive and potent pharmacological activities made medicinal plants very useful to mankind. For the past two decades, there has been an increasing interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents [12].

The antibacterial activity of the methanolic leaf extract of *C. pallida*
showed dose dependent activity and maximum activity was shown at an amount of 25 mg/ml. While less activity was shown at 3.125 mg/ml concentration. n-hexane fraction of whole plant of \textit{Cassia senna} leaves shows a moderate antibacterial activity for two gram-positive bacteria like \textit{Bacillus cereus} (12mm) & \textit{Staphylococcus aureus} (14mm) & two gram-negative strains \textit{Escherichia coli} (18mm) and \textit{Vibrio mimicus} (16mm) where \textit{Pseudomonas aeruginosa} (10mm) possess less effect in contrast to standard Kanamycin [13]. The maximum antibacterial activity of \textit{Crotalaria juncea} flower was seen in \textit{Crotalaria juncea} petroleum ether fractioned ethanol extract against \textit{K. pneumonia} (18mm) and \textit{S. aureus} (18mm) at 500µg/disc concentration [14].

The highest antibacterial activity of ethanol extract of \textit{Crotalaria pallida} plant was observed on \textit{Xanthomonas axonopodis pv. Malvacearum} (16 ± 2), \textit{E. coli} (14 ± 2) and \textit{Clavibacter michiganensis} sub spp. \textit{Michiganensis} (13 ± 2) at 1mg/ml concentration [15].

The maximum antibacterial activity of \textit{Crotalaria burhia} root was seen in \textit{C. burhia} methanol extract against \textit{Bacillus subtilis} (18.7±0.28) at 1000µg/ml concentration and 8.1±0.26 at 100µg/ml concentration, \textit{E. coli} (15.6±0.18) at 1000µg/ml concentration and 8.6±0.24 at 100µg/ml concentration, \textit{Staphylococcus aureus} (15.4±0.18) at 1000µg/ml concentration and 7.4±0.08 at 100µg/ml concentration, \textit{Pseudomonas aeruginosa} (18.3±0.08) at 1000µg/ml concentration and 8.0±1.38 at 100µg/ml concentration [16].

In methanolic root extract of \textit{Caesalpinia pulcherrima} the maximum zone of inhibition (27 mm) was observed in 225 µg/mL concentration against \textit{Klebsiella pneumonia}, while the minimum zone of inhibition (18 mm) was observed in 75 µg/mL concentration against \textit{Staphylococcus epidermidis} [17].

5. Conclusion

The results of antibacterial study have provided scientific justification for the use of \textit{Crotalaria pallida} extract as antimicrobial agent. The extract of this medicinal plant possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

References


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