Original Article

Antibacterial Activity of *Plumbago zeylanica* leaf extracts

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Abstract

Emergence of more and more multidrug resistant pathogens was reported to be one of the leading causes of death world-wide. Many infectious microorganisms are resistant to synthetic drugs; hence an alternative therapy is very much needed. Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of immense therapeutic value. Over the past few decades there has been much interest in natural materials as sources of new antibacterial activity. At the present study antibacterial activity of methanolic and chloroform extracts from the *Plumbago zeylanica* were carried out against five different organism of *Streptococcus aureus*, *Staphylococcus aureus*, *Bacillus*, *Pseudomonas aeruginosa*, *E.coli* using disc diffusion method. Both the extracts showed antibacterial activity against most tested bacteria. The methanolic extracts were more active against the entire tested organism.

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Key words: *Plumbago zeylanica*, Antibacterial activity, Methanol, Chloroform.

1. Introduction

Plant materials remain an important resource to combat serious diseases in the world. The pharmacological investigations of plants were carried out to find novel drugs or templates for the development of new therapeutic agents (Iwu et al., 1999). The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries (Al-Bari et al., 2006). Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. India is a land of rich biodiversity. The total number of lower and higher plants in India is about 45,000 species. The plants are potential source of medicines since ancient times (Cragg et al., 2001).

Plants have many antimicrobial properties as secondary metabolites such as alkaloids, phenolic compounds, etc. The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections (Vijaya and Ananthan, 1997; Dilhuydy and Patients, 2003). Plants are the cheapest and safer alternative sources of antimicrobials (Pretorius and Watt, 2001; Sharif and Banik, 2006; Doughari et al., 2007). Yet a scientific study on plant to determine their antimicrobial material is...
comparatively new. Numerous surveys on antimicrobial activity of medicinal plants had been made in United States and in many countries throughout the world. Such study had demonstrated the wide occurrences of active compounds in higher plants (Hughes, 1952).

2. Materials and Methods

2.1. Plant material

Plumbago zeylanica leaves were collected, washed with fresh water and dried under shade at room temperature. The leaves were powdered and stored in sterile containers. The 50 g of dried powdered sample was dissolved in 150 ml of methanol and chloroform. Both the preparations were kept in shaker for 3 days. Then the solvents were filtered through filter paper to remove free extractable substances. The filtrate was concentrated by drying at room temperature for several days.

2.2. Test organisms

The strains used for the present study were Streptococcus aureus (Check), Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli. The culture was maintained in control condition and used for the test.

2.3. Preparation of the disc

The agar disc diffusion technique involved placing sterile paper disc (Whatman No 1 filter paper) of 5 mm diameter impregnated with different crude extracts and dried in a hot air oven at 60°C on agar plates seeded with the test organism. Three types of discs were used for antimicrobial screening: samples discs, standard discs and blank discs. Then the sample disc was prepared by applying sample solution of the desired concentration on the sterile filter paper discs (5 mm in diameter) with the help of a micropipette under the aseptic condition. Similarly blank discs and standard discs were prepared to serve as negative control and positive control respectively. In this investigation kanamycin (30 µg/disc) standard disc was used as reference and methanol/Chloroform was used as blank. These discs were kept for few minutes in aseptic condition under UV light for complete sterilization.

2.4. Anti-bacterial activity assay

Sample impregnated discs, standard discs and negative control discs were placed gently on the solidified agar plates, freshly seeded with the test organisms, with the help of a sterile forceps to assure complete contact with medium surface. The special arrangement of the discs was such that the discs were not closer than 15 mm to the edge of the plate and far enough apart to prevent overlapping the zones of inhibition. The antibiotics kanamycin and blank (methanol or chloroform) disc were used as positive and negative controls respectively. The whole set-up was incubated at 37°C for 24-48 hrs after which diameter of zones of inhibition was measured.

3. Results and Discussion

Plants have been a source of medicine in pharmacopoecia. Herbs have been utilized to treat acute and chronic disorders for thousands of year. Herbs have recently attracted attention as health beneficial foods (physiologically functional foods) and as source of materials for drug development. The disc diffusion method is used to detect the antimicrobial activity of plant extract. The solidified Muller Hinton agar plates were swapped with the test organism and the standard sample and blank discs were impregnated. After the incubation the zone was measured. The antimicrobial activity of plant extracts was detected by the indication of zone around the disc. The antimicrobial activity of methanol and chloroform extract showed positive results against the entire organism. The methanol extract inhibit Streptococcus aureus, Staphylococcus aureus, Bacillus subtilis and Escherichia coli at 10 µl concentration indicated by the zone of inhibition around the disc in the culture plate except that the plate containing Pseudomonas aeruginosa (Table 1). But in 20 µl concentration the plant extract showed positive result against all the five tested samples. Chloroform leaf extract showed antibacterial activity against Streptococcus aureus, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli. Its inhibition was moderate and lower. The obtained result indicates both methanol (10µl) and chloroform extracts has antimicrobial activity against organism especially in methanol extract than chloroform extract.

The results show that the methanol extract of Plumbago zeylanica showed more inhibitory effect than the other plant extracts. This tends to show that the active ingredients of the plant parts are better extracted with methanol than Chloroform. The methanol extracts contain alkaloids, coumarains and tannins (Okemo, 1996). Coumarins and tannins have antibacterial properties (Hedberg et al., 1983), also Eloff (1998) and Cowan (1999) found that methanol was more efficient than of chloroform in extracting phytochemicals from plant materials.

The results of the present study reveals the fact that the organic solvent extracts (chloroform and methanolic extracts) exhibited greater antimicrobial activity because the antimicrobial principles were either polar or non-polar and they were extracted only through the organic solvent medium (Krishna et al., 1997; Natarajan et al., 2003). The present study justifies the claimed uses of Plumbago zeylanica leaf in the traditional system of medicine to treat various infections diseases caused by the microorganisms.
Table 1. Zone of inhibitory activity of different plant extracts against bacteria

<table>
<thead>
<tr>
<th>Organism</th>
<th>Concentration of the extract</th>
<th>Methanol Mean ± SD</th>
<th>Chloroform Mean ± SD</th>
<th>Kanamycin (30 mcg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>11.33 ± 1.53</td>
<td>10.03 ± 0.25</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.67 ± 0.58</td>
<td>16.90 ± 0.36</td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus aureus</em></td>
<td>10</td>
<td>12.43 ± 0.51</td>
<td>8.97 ± 0.25</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>22.43 ± 1.40</td>
<td>16.40 ± 0.53</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>10</td>
<td>18.17 ± 1.04</td>
<td>8.40 ± 0.78</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>28.33 ± 0.58</td>
<td>16.10 ± 0.26</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>10</td>
<td>9.00 ± 1.00</td>
<td>8.97 ± 0.35</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>16.33 ± 1.15</td>
<td>15.67 ± 0.81</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>10</td>
<td>13.17 ± 0.76</td>
<td>9.07 ± 0.21</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>23.00 ± 0.50</td>
<td>18.07 ± 0.31</td>
<td></td>
</tr>
</tbody>
</table>

4. Conclusion

The results suggested that the different solvent extracts under study showed antibacterial activity. The antibacterial action of various extracts of *Plumbago zeylanica* leaves may indicate their potential as antibacterial herbal remedies. Further work is needed to locate the active principle from the various extracts and their phytopharmaceutical studies. Research into the effects of local medicinal plants is expected to boost the use of these plants in the therapy against disease caused by the test bacterial species and other microorganisms. It is possible that better therapy for many microbial diseases can be found in the leaves extracts. The Preliminary results of this investigation indicates that *Plumbago zeylanica* leaves have high potential of antimicrobial activity.

Reference


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