Efficacy of Couroupita guianensis Against Selected Human Pathogens


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Abstract: The antimicrobial activity of the plant extract of Couroupita guianensis, was assessed against various human pathogenic bacteria. The extract was prepared with two solvents (dichloromethane and acetone). The acetone extract of Couroupita guianensis shows activity of 25 mm against Staphylococcus sp. and 28 mm in dichloromethane extract. The bioactive compound of Couroupita guianensis that possess antibacterial activity was also assessed. The compound is found to be benzene dicarboxylic acid (BDCA) which possesses antimicrobial activity. Medicinal plant based antimicrobial has enormous therapeutic potential as they can serve the purpose with lesser side effects and boon for development of chemotherapy.

Key words: Couroupita guianensis • Drug resistance • Medicinal plant • TLC • GC-MS

INTRODUCTION

Infectious diseases are an important hazardous all over the world both in developing and developed countries. Several synthetic antibiotics are employed in the treatment of infectious and communicable diseases [1].

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore care must be taken to reduce this problem, e.g., develop research to better understand the genetic mechanism of resistance and continue studies to develop new drugs either synthetic or natural. The ultimate goal is to provide appropriate and efficient antimicrobial drugs for the patient [2].

Plants are an essential part of human society since the civilization started. Medicinal plants are the boon of nature to cure a number of ailments of human beings. In many parts of the world medicinal plants are used against bacterial, viral and fungal infections. Evaluation of plants bearing efficiency in healing various diseases is growing in recent years. Innumerable biologically active compounds of plants are found to possess antibacterial properties [3].

Medicinal plants contain accumulated natural products biologically active and ingredients which have various effects. Some of these active ingredients accumulate in certain part of the plant. It is only those portions of the plants contain active ingredient are used in therapeutic purposes. The use of plant extracts with known antimicrobial properties can be greater significance in the treatment of various microbial infections [4-7].

Couroupita guianensis is a tree possesses antibiotic, antifungal, antiseptic and analgesic qualities. The trees are used to cure colds and stomach aches. Juice made from the leaves is used to cure skin diseases. Shamans of South America have even used tree parts for treating Malaria. The inside of the fruit and disinfect wounds and young leaves cure tooth ache.

The present investigation was mainly focused on the evaluation the efficacy of the medicinal plant extract against drug resistant clinical isolates.

MATERIALS AND METHODS

Collection of Clinical Isolates: Bacterial cultures were collected from various laboratories such as Bose laboratory, Rose lab and Vijay Lab, Madurai.

Collection of Plant Materials: Fresh leaves part of the medicinal plant Couroupita guianensis, were collected from in and around Sivakasi. The plant materials were collected during the month of November, 2010. Sivakasi is located at 9.28' North latitude and 77.48' East longitude. This city is located 100.07 meter above sea level. Sivakasi belongs to Virudhunagar District of Tamil Nadu State, India.
Selection of Solvent: The non-polar solvent dichloromethane and the polar solvent acetone were taken for this study.

Preparation of Extract: 50g of powdered plant material was weighed and soaked in 500 ml of dichloromethane and acetone respectively. The flasks were completely covered with aluminum foil and shaken for every 30 minutes for 6 hours and then allow standing for another 48 hours. The solution was subsequently shaken and filtered using Whatman No.1 filter paper. The filtrate was allowed to evaporate at room temperature. The extracts were stored at 4°C in a refrigerated before use.

The 100mg of residue which obtained from the each plant extracts, with the help of dichloromethane and acetone was mixed with 1 ml of respective solvent and vortex the mixture completely.

Preparation and Standardization of Inoculums: Colonies from pure growth of each test organism were transferred to 5 ml of Mueller Hinton Broth. The broth was incubated at 37°C for 24 hours. The turbidity of the culture was inoculated within 20 minutes.

Well Diffusion Technique: Screening of antibacterial activity was performed by well diffusion technique. The Mueller Hinton agar plates were seeded with 0.1 ml of the standardized inoculum of each test organism. The inoculum was spread evenly over plate with sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37°C for 20 minutes. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the MHA and 50 and 100 µl of each leaf extract was introduced in the well. Respective solvent was used as control. The inoculated plates were incubated at 37°C for 24 hours and zone of inhibition was measured to the nearest millimeter (mm) [8].

Partial Purification of Bioactive Compound (Couroupita Guinanensis) by Thin Layer Chromatography: The crude leaf extracts of Couroupita guinanensis was subjected for the identification of bioactive compounds with the help of Thin Layer Chromatography (TLC). TLC was performed on a silica gel plate. An aliquot of the leaf extracts was spotted on the silica gel plate and eluted with solvent gradient system consisting of chloroform/methanol (10:1; v/v). The spot produced by the active compounds was determined by using the UV detector and then visualize the plate by spraying with spraying solution (The spraying solution consists of 1% potassium ferric cyanide in water and 1% ferric chloride in water).

Extraction of Bioactive Compound (Couroupita Guinanensis) from Slica Gel: The spot detected by the UV detector was marked as C1, C2 and C3….C9. These compounds were separated from the silica gel plates with the help of a sterile scrapper. Each compound was scrapped carefully and collected to a sterile eppendorf tubes. The tubes along with the compound was added with the solvent, dichloromethane vortex the mixture carefully and allow them to stand still for 30 minutes. The tubes were then centrifuged at 10000 rpm for 15 minutes. The supernatant was collected from them. Again the solvent was added and vortex the mixture. The tubes were left undisturbed for 30 minutes. The tubes were subjected for centrifugation at 10,000 rpm for 15 minutes. The procedure is repeated for several times, until the compound get separated completely from the silica gel. The supernatant was collected and they were air dried at room temperature. The compound was stored in the refrigerator at 4°C. The compound obtained from the silica gel was subjected for the determination of antibacterial activity.

Antibacterial Activity of the Bioactive Compounds (Couroupita Guinanensis): The antibacterial activities of the bioactive compounds were tested with the pathogenic clinical isolates namely E. coli, Klebsiella sp, Pseudomonas sp, Bacillus sp and Staphylococcus sp. It was determined by means of well diffusion method. 50 µl of each sample (C1, C2 and C3….C9) was loaded into the respective wells. Incubate at 37°C for 24 hours.

Identification of Bioactive Compounds (Couroupita Guinanensis) with Help of GC – MS: GC – MS analysis was performed using a Varian CP – gas chromatography. The injected port was heated at 220°C. The injection was performed in split less mode. The carrier gas was helium C- 60 at a constant flow of 1 ml/minute. The over temperature was set at 40°C, then increasing 2°C per minute to 220°C and held for 30 minutes. Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds analyzed under same condition.

RESULTS

Antibiotic Susceptibility Test for the Clinical Isolates: Antibiogarm of five clinical isolates namely, E. coli, Klebsiella sp, Pseudomonas sp, Bacillus sp and Staphylococcus sp for different classes of antibiotics was performed. The culture plates after the period of incubation was examined for the presence or absence of
Table 1: Antibiogram of Clinical isolates

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>Penicillin (mm)</th>
<th>Erythromycin (mm)</th>
<th>Colistin (mm)</th>
<th>Chloramphenicol (mm)</th>
<th>Polymyxin-B (mm)</th>
<th>Bacitracin (mm)</th>
<th>Imipenem (mm)</th>
<th>Vancomycin (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>R</td>
<td>R</td>
<td>19</td>
<td>R</td>
<td>19</td>
<td>R</td>
<td>21</td>
<td>R</td>
</tr>
<tr>
<td>Pseudomonas sp</td>
<td>R</td>
<td>R</td>
<td>12</td>
<td>19</td>
<td>19</td>
<td>R</td>
<td>7</td>
<td>R</td>
</tr>
<tr>
<td>Klebsiella sp</td>
<td>R</td>
<td>9</td>
<td>30</td>
<td>27</td>
<td>18</td>
<td>R</td>
<td>18</td>
<td>R</td>
</tr>
<tr>
<td>S. aureus</td>
<td>R</td>
<td>25</td>
<td>R</td>
<td>30</td>
<td>15</td>
<td>9</td>
<td>33</td>
<td>16</td>
</tr>
<tr>
<td>Bacillus sp</td>
<td>R</td>
<td>22</td>
<td>R</td>
<td>28</td>
<td>18</td>
<td>R</td>
<td>24</td>
<td>R</td>
</tr>
</tbody>
</table>

Table 2: Antibacterial activity of Couroupita guianensis

<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>Acetone</th>
<th>DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50µl(mm)</td>
<td>100 µl(mm)</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>NZ</td>
<td>NZ</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>22</td>
<td>27</td>
</tr>
<tr>
<td>Pseudomonas sp</td>
<td>NZ</td>
<td>NZ</td>
</tr>
<tr>
<td>Klebsiella sp</td>
<td>NZ</td>
<td>NZ</td>
</tr>
<tr>
<td>S. aureus</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>E. coli</td>
<td>NZ</td>
<td>NZ</td>
</tr>
</tbody>
</table>

Fig. 1: Antibacterial activity of *Couroupita guianensis*

zone of inhibition. It is measured to the nearest millimeter to determine whether they are resistant intermediate as sensitive and they are depicted in Table 1. *E. coli* shows resistance to the antibiotics such as Penicillin, Erythromycin, Chloramphenicol, Bacitracin and Vancomycin. Where as *Pseudomonas* sp shows resistance to Penicillin, Erythromycin, Bacitracin and Vancomycin. *Staphylococci* sp shows sensitivity to the entire antibiotic reported except Penicillin, Colistin and *Klebsiella* sp show resistance to most of the antibiotics Penicillin, Bacitracin and Vancomycin.

**Antibacterial Activity for the Medicinal Plant Extract:**

The leaf extract obtained from the medicinal plant *Couroupita guianensis* was added at a concentration of 100mg/ml. It shows antibacterial activity against the common clinical pathogens such as *E. coli*, *Klebsiella*, *Pseudomonas*, *Bacillus* sp and *Staphylococcus* sp. As illustrated in Table 2, by using acetone as the solvent, 100 µl extract of *Couroupita guianensis* shows activity against the *Bacillus sp* and *Staphylococcus sp* with the zone of inhibition, of 27mm and 25mm respectively. And 50 µl extract of *Couroupita guianensis* shows activity against the *Bacillus sp* and *Staphylococcus sp* with the zone of inhibition of 22mm and 19mm respectively. Increase of dichloromethane as the solvent, 100 µl extract of *Couroupita guianensis* shows activity only against the *Staphylococcus sp* with the zone of inhibition of 28mm. And 50 µl extract of *Couroupita guianensis* shows activity against the *Staphylococcus sp* with the zone of inhibition of 24mm (Fig. 1).

**Partial Purification of Compound by TLC:** The bioactive compounds present in the *Couroupita guianensis* were tentatively detected with the help of Thin Layer Chromatography. After spraying with the solution composed of 1% Potassium ferric cyanide and 1% ferric chloride. Nine spots were detected when visualized under UV detector. All the compounds were separated from the silica plate to determine the antibacterial activity (Fig. 2).
The clinical isolates such as *E. coli, Pseudomonas, Klebsiella, Staphylococcus* sp and *Bacillus* sp are the opportunistic pathogens which are involved in the outbreak of various communicable disease and nosocomial infections such as burns, wound infection and urinary tract infection etc.,

Even though pharmacological industries have produced a number of new antibiotics, in the last three decades, resistant to these drugs by micro organisms have been increased. To control the use of antibiotics, we develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drugs either natural or synthetic. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient [2].

In the present study five clinical isolates namely, *E. coli, Klebsiella* sp, *Pseudomonas* sp, *Bacillus* sp and *Staphylococcus* sp were tested against different classes of antibiotics. *E. coli* shows resistance to the antibiotics such as Penicillin G, Erythromycin, Chloramphenicol, Bacitracin and Vancomycin and sensitive to Colistin (19 mm), Polymyxin (19 mm) and Imipenem (21 mm), where as *Pseudomonas* sp shows resistance to Penicillin, Erythromycin, Bacitracin, Imipenem and vancomycin and sensitive to Colistin (12 mm) and Polymyxin (19 mm).

*Staphylococci* sp shows sensitivity to the entire antibiotic reported except Penicillin G, colistin and Bacitracin and *Klebsiella* sp show resistance to most of the antibiotics and sensitive to Colistin, Chloromphenicol, Imipenem Polymyxin and Erythromycin with the zone of inhibition of 30 mm, 27 mm and 18 mm respectively.

The antibacterial activity of the medicinal plant using the two solvents dicholoromethane and acetone was accessed against the clinical isolates. The 100 µl concentration of acetone extract of *Couroupita guianensis* shows greatest activity against the *Bacillus* sp (27 mm) and *Staphylococcus* sp (19 mm) and DCM shows activity against *Staphylococcus* sp amounted to 24 and 28 mm for 50 µl and 100 µl extract concentration, respectively [9].

In the present study it is recorded that, the purification of the crude extract was monitored by thin layer chromatography. The bioactive compound of *Couroupita guianensis* obtained from the silica gel plates were assessed for antibacterial activity. The compound

**Discussion**

Antibacterial Activity of Bioactive Compounds of *Couroupita guianensis*: All the nine bioactive compounds (C1, C2 …C9) are treated for antibacterial activity against the test organism *Bacillus* sp and *Staphylococcus* sp. The bioactive compound namely C5 show greater activity with the zone of inhibition of 25 mm for *Bacillus* sp and *Staphylococcus* sp respectively. The other compounds show no activity.

Identification of Bio Active Compounds by GC-MS Analysis: The partially purified bio active compounds were characterized with the help of GC-Ms analysis. Based on this analysis the responsible compounds for antimicrobial activity were found to be Benzene Benzoate. The chromatogram and Mass spectrum with similar known compounds was shown in the Figure. 3. In this analysis various peaks were observed at the retention time of 8-23 minutes. The peaks were compared with the existing data available from the library was searched and the maximum similarity was showed with BDCA compounds.
C5 alone shows activity against *Bacillus* sp (26 mm) and *Staphylococcus* sp. (26 mm) respectively. The partially purified compounds which possess antibacterial activity were identified by means of GC-MS analysis.

Benzyl benzoate, the simplest aromatic compounds. It occurs naturally in both plants and resins. They are used as a preservative for food drug and personal products. Sodium benzoate, sodium salt of benzoic acid is used as the one of the principal antimicrobial preservative in food. Benzyl benzoate is active against gram positive bacteria (*Bacillus* sp and *Staphylococcus* sp.) but not against gram negative bacteria (*E. coli*, *Klebsiella* sp and *Pseudomonas* sp).

**CONCLUSION**

The antimicrobial activity of the different plant extracts such as, *Couropita guianensis*, was assessed against various human pathogenic bacteria. Plant based antimicrobial have enormous therapeutic potential as they can serve the purpose with lesser side effects and boon for development of chemotherapy.

**ACKNOWLEDGEMENT**

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**REFERENCES**