In-vitro evaluation of cisplatin nanoparticles encompass natural polymer

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ABSTRACT

Opportunistic bacterial infections are common in the various parts of the human body. In recent years bacterial species have shown resistance against several synthetic drugs. This study measured the antibacterial activity of bacterial strains against five common pathogenic bacteria related strains. Cup plate method and two-fold serial dilution method were used to evaluate by antibacterial activity by the help of different bacterial related strains. The results revealed that Cisplatin (CIP) using natural as a polymer showed a minimum inhibitory concentration (MIC) at 250 mg/ml to 500 mg/ml of the broth against all bacterial strains. CIP using natural as a polymer was prepared different doses of 1000 μg/ml and 2000 μg/ml and measured zone of inhibition dose dementedly reduced when compared to standard. The CIP using natural as a polymer exhibited intense antibacterial activity against five different species of bacteria, and this may be attributed to various active components. Our research work has been indicated Nanoparticles containing CIP using natural as a polymer formulated for the enhanced anticancer activity through an antimicrobial mechanism.

INTRODUCTION

Multidrug resistance is the most common worldwide issue which arises in managing infectious disease caused by pathogens [1]. The main cause behind multidrug resistance is irrational to use of antibiotics not only in human health care but also in agriculture and veterinary medicines. Acinetobacter baumannii, Streptococcus pneumoniae, Klebsiella pneumoniae, methicillin-resistant S. aureus, extensively drug-resistant Mycobacterium tuberculosis are some of the most commonly seen drug-resistant pathogens [2]. Nosocomial infections which are associated with pneumonia, surgical site infections and some bloodstream infections are mainly because of Methicillin-Resistant S. aureus (MRSA) [3, 4]. These problems lead to the discovery of novel agents which are having broad-spectrum therapeutic potency. Recent literature background revealed that metal nanoparticles play a potential role as antibacterial agents. But the usage of hazardous material and having economically less feasibility made its application very limited [5, 6]. Metal nanoparticles can be synthesized by physical and chemical methods like chemical reduction, ion sputtering and sol-gel etc. but green synthesis is most preferred over other techniques because of stability of synthesized nanomaterials and reproducibility [7, 8]. The green method involves a synthesis of nanoparticles from algae, bacteria, acti...
nomycetes, bacteria, plants and other substances. Of the above methods, the faster method is plant-mediated synthesis [9]. Murrava koenigii (curry leaves) is a small aromatic tree and belongs to the family Rutaceae. In the Indian medicinal system, the leaves of this tree have many uses. On rats, the leaves have shown antihyperglycemic effect [10]. It also showed the antioxidant property and anti-inflammatory properties by hydroalcoholic extract of M. koenigii [11, 12]. Alkaloids such as 9-formyl-3-methylcarbazole, 9-carbethoxy-3-methylcarbazole, and 3-methylcarbazole are observed under phytochemical characterization [13]. Curry leaves have antioxidant protein which ameliorates the effect of DNA damage to erythrocytes due to oxidative stress [14, 15].

The antioxidant and antimutagenic potential of curry leaves has been proved [16, 17]. For the synthesis of silver nanoparticles, the leaves of M. koenigii were widely used. In this study, we assessed the antibacterial activity of M. koenigii against Gram-positive and Gram-negative MDR bacteria.

MATERIALS & METHODS

Cup Plate Method

Saboraud’s Dextrose agar plates were prepared, and 0.1ml of organism spread the plate, then put four wells for one plate by the help of borer then pour the Nanoparticles containing CIP into two wells (1000 µg/ml and 2000 µg/ml) other two wells add dimethyl sulphoxide as control and positive control as ciprofloxacin for throughout the experiment. After pouring the plate was frig for 10 mins and bacterial plates are kept 48 hours at 37°C inside the incubator. The Zone of inhibition was recorded bacteria [18].

TWO FOLD SERIAL DILUTION METHOD

The drug at doses ranging from 1000 µg/ml to 31.25 µg/ml broth was tested for their antibacterial activity against several fungal species using two-fold serial dilution technique—the 24 hours of bacterial culture containing 10^5 to 10^6 c.f.u. Per ml used seeded broth incubation temperature at 37°C, and results were recorded after 48 hours of incubation. Ciprofloxacin was used as the positive control, and the whole study was carried out in triplicate the lowest concentration of the test drug that caused complete inhibition of growth of the microorganism was taken as the minimum inhibitory concentration of the drug. It was confirmed by plating, and results are recorded in Table 1 [19].

Disk Diffusion

Mueller-Hinton Agar Medium

Mueller-Hinton agar medium is the best medium for TiO2 e susceptibility testing for non-fastidious bacteria susceptibility test. It has so many reasons which include

1. Batch-to-batch reproducibility which is acceptable for susceptibility testing
2. Satisfactory growth of non-fastidious pathogens has been observed
3. Very low in sulphonamide, tetracycline and trimethoprim inhibitors.
4. Has a lot of data regarding susceptibility tests which are performed in this medium.

Though Mueller-Hinton agar medium is a reliable medium for susceptibility testing, the results in some batches occasionally have shown significant variation. The zones obtained in disk diffusion method will be quite more extensive if the batch of the medium does not meet adequate growth of test microorganism.

Mueller-Hinton medium formulation which will meet the acceptance limits described in, NCCLS document M62-A7- Protocols for evaluation of Dehydrated Mueller-Hinton agar should be used [20].

Preparation of Mueller-Hinton Agar

1. The commercially available dehydrated base should be used for the preparation of Mueller-Hinton medium as per the manufacturer’s instruction, Autoclave it and allow it to heat at 45-50°C water bath
3. Pour the cold medium into either glass or plastic and flat-bottomed Petri plates on a level, horizontal surface to get the uniform depth of 4 mm. which equals to 60-70 ml of medium for 150 mm diameter plates and 25 to 30 ml of plates for 100 mm diameter plates.
4. Cool the agar medium to room temperature & if it is used on the same day, it can be stored in the refrigerator at 2-8C.
5. Plates should be used within a week after preparation. Precautions should be taken to prevent drying of the agar like the wrapping of plastic etc.
6. Each batch of plates will be the representative, and it should be examined for sterility by incubating it at 30-35°C for 24 hours or even longer. Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish & sterilized in a hot air oven.
Table 1: Effect of CIP Gum ghatti Nanoparticles on selected bacterial strains (Two-fold serial dilution)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Organisms</th>
<th>Extracts MIC Values μg/ml</th>
<th>Standard drug MIC Values μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>E. coli</td>
<td>500</td>
<td>3.625</td>
</tr>
<tr>
<td>2.</td>
<td>S. aureus</td>
<td>500</td>
<td>3.625</td>
</tr>
<tr>
<td>3.</td>
<td>B. subtilus</td>
<td>500</td>
<td>3.625</td>
</tr>
<tr>
<td>4.</td>
<td>B. coagulants</td>
<td>500</td>
<td>1.8625</td>
</tr>
<tr>
<td>5.</td>
<td>B. megaterium</td>
<td>250</td>
<td>1.8625</td>
</tr>
</tbody>
</table>

Standard drug – Ciprofl oxacin
Extract – CIP Gum ghatti Nanoparticles

Table 2: Effect of CIP Gum ghatti Nanoparticles on selected bacterial strains (Cup Plate method)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Organism</th>
<th>Concentrations extract</th>
<th>Zone of inhibition (cm)</th>
<th>Standard drug Zone of inhibition values (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Well 1</td>
<td>Well 2</td>
<td>Well 1</td>
</tr>
<tr>
<td>1.</td>
<td>E. coli</td>
<td>1000 μg/ml</td>
<td>2.2 1.1</td>
<td>2.4 1.4</td>
</tr>
<tr>
<td>2.</td>
<td>S. aureus</td>
<td>1000 μg/ml</td>
<td>2.3 1.3</td>
<td>2.4 1.4</td>
</tr>
<tr>
<td>3.</td>
<td>B. subtilus</td>
<td>1000 μg/ml</td>
<td>2.2 1.7</td>
<td>2.1 1.1</td>
</tr>
<tr>
<td>4.</td>
<td>B. coagulants</td>
<td>1000 μg/ml</td>
<td>2.5 1.7</td>
<td>1.0 2.3</td>
</tr>
<tr>
<td>5.</td>
<td>B. megaterium</td>
<td>1000 μg/ml</td>
<td>2.1 1.5</td>
<td>1.5 1.2</td>
</tr>
</tbody>
</table>

Standard drug – Ciprofl oxacin
Extract – CIP Gum ghatti Nanoparticles
Table 3: Antimicrobial studies of CIP Nanoparticles

<table>
<thead>
<tr>
<th>Organism</th>
<th>Samples (Zone of inhibition mm)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>150</td>
</tr>
<tr>
<td>E. coli</td>
<td>02</td>
<td>03</td>
</tr>
<tr>
<td>S. aureus</td>
<td>06</td>
<td>08</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>05</td>
<td>07</td>
</tr>
<tr>
<td>B. coagulants</td>
<td>06</td>
<td>08</td>
</tr>
<tr>
<td>B. megaterium</td>
<td>04</td>
<td>06</td>
</tr>
<tr>
<td>Shigella</td>
<td>05</td>
<td>06</td>
</tr>
<tr>
<td>S. typhi</td>
<td>06</td>
<td>07</td>
</tr>
</tbody>
</table>

The loop used for delivering the antibiotics is made of 20 gauge wire & has a diameter of 2 mm. It gives 0.005 ml of antibiotics to each disc [21].

Turbidity Standard

- A BaSO₄ turbidity standard of 0.5 or its optical equivalent such as latex particle suspension should be used to standardize the inoculum density for susceptibility test. 0.5 standard of BaSO₄ can be prepared in the following steps:
  - Add 0.5 ml of 0.048 mol/L BaCl₂ (1.175% w/v BaCl₂ · 2H₂O) to 99.5 ml of 0.18 mol/L H₂SO₄ (1% v/v) by constantly stirring to get a suspension.
  - By using U.V. spectrophotometer, the exact density of the turbidity should be verified, and it should be 0.008 to 0.10 at 625 nm for 0.5 standards.
  - Transfer Barium Sulfate suspension to 6 ml aliquots into screw-cap tubes of the same size which are used in growing or diluting the bacterial inoculum.
  - Seal the tubes tightly and store it in the dark at room temperature.
  - Vigorously agitate the barium sulphate turbidity standard on vortex mechanical mixer before every use and inspected for turbid appearance. Replace it, if the large particles appear. But latex particle suspensions should not be mixed by vortex mixer but by inverting it gently [22].
  - Replace or verify the densities of barium standard sulfate standards monthly. For our study, different species of bacteria can be used for research such as E. coli, S. aureus, B. subtilis, B. coagulants, Shigella, S. typhi [23].

RESULTS AND DISCUSSION

The two-fold serial dilution method results revealed that Nanoparticles containing CIP showed a minimum inhibitory concentration (MIC) at 250 mg/ml to 500 mg/ml of the broth against all bacterial strains (Figure 1).

Two different dose levels used for cup plate method but 1000 mg/ml have shown lesser activity when compared to 2000 mg/ml of Nanoparticles containing CIP have more antifungal action. In our study revealed that the 1000 mg/ml of Nanoparticles containing CIP had shown more antibacterial activity against S when compared to each other species of bacterial at the dose level of 1000 mg/ml. Overnight cultures (E.coli & S. aureus) were swabbed amid into Muller Hinton agar (MHA) plates on the way to determine the Zone of inhibition. Pre-cleaned pathogens were used on the road to take a look at the mode of action of the drug. The wells (20mm) were created on the plates & 10µl of samples were adscititious. The plates were incubated at 37°C for 24hrs. The Zone of inhibition was calculated against the management (Tables 2 and 3).

Antibacterial activity of CIP Gum ghatti Nanoparticles CIP - Gum ghatti Nanoparticles shows the more significant Zone of inhibition against Staphylococcus aureus when compared to E.coli.

CONCLUSION

On the other hand, there are multiple mechanisms involved in the antibacterial activity; it may be investigated. Further, it may be due to enhancement antimicrobial activity Nanoparticles containing CIP. Since it is a Nanoparticles containing CIP, further anticancer studies to be carried out, and it may be subjected to other systemic bacterial infections.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

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REFERENCES
