An assessment of antibacterial activity of ZnO nanoparticles, Catechin, and EDTA on standard strain of pseudomonas aeruginosa

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Abstract

Introduction:
Pseudomonas aeruginosa is a key opportunistic pathogen causing severe acute and chronic nosocomial infections in immunocompromised or catheterized patients. It is prevalent in burn wound infections and is generally multi-drug resistant. This study evaluated antimicrobial activity of ZnO nanoparticles, catechin and EDTA on the most common pathogen bacteria, Pseudomonas aeruginosa, by standard microdilution test depending on NCCLS.

Material and Methods:
Zinc acetate dehydrate and ethilen glycol (EG) were dissolved in 50 ml distilled water with a ratio of 10/1 (Zn/EG). The solution was heated at 80 °C until a little gel of zinc acetate was obtained. Then, the gel was dried in the oven for 12h. The dried gel was calcinated at 300 °C for 24h. The size and type of these nanoparticles were characterized by scanning electron microscopy (SEM) and X-Ray: Diffraction (XRD). Pseudomonas aeruginosa strain (ATCC 27853) were cultured on nutrient agar medium (NA) for 24h at 37 °C. The microbial suspension (1×10⁶ cells/ml) was prepared. Minimum Inhibitory Concentration (MIC) test for ZnO, EDTA and Catechin were evaluated by microdilution test.

Results:
Evaluation of morphology and measurement of the size of the ZnO showed that nanoparticles were spherical with a diameter of 30-90 nm. MIC of ZnO, EDTA and Catechin on pseudomonas aeruginosa was 0.0003, 24.92 and 7.24 µg/ml, respectively.

Conclusion:
In this study, ZnO nanoparticles were synthesized using the chemical method. It was revealed that it has an optimal antimicrobial activity in low concentration as compared with Catechin and EDTA. It is recommended that catechin and ZnO nanoparticles are good candidates for eliminating some contaminations including Pseudomonas aeruginosa in medical industrial.

Keywords: Pseudomonas aeruginosa, Nanoparticles, Catechin, Anti-Bacterial Agents

Introduction:
Nowadays, nosocomial infections constitute a major challenge for the medical community and particularly the immunocompromised patient. Pseudomonas aeruginosa is among the principal microbial agents involved in nosocomial infection and burn victims, and a cause of mortality for patients with cystic fibrosis. P. aeruginosa is a motile organism measuring 0.6-2 microns and grows readily on a variety of culture
media. It is a major cause of pulmonary infection in patients with cystic fibrosis and those using ventilators. Moreover, it is responsible for cutaneous infections in burn victims as well as septicemia in immunocompromised patients or those with intravenous catheters. Local and disseminated infection following surgery and urinary tract infections in patients with urinary catheters are other clinical pictures of the bacterium. It is abundant in nature and is commonly isolated from moist hospital environments (1-3).

The microbe is able to bond to organic surfaces and create a biofilm, thus giving rise to a primary contaminating source. Considering the increasing rate of drug resistance, which is the result of gene mutations, countering the infection requires ever-increasing doses of antibiotics and antiseptics with their consequent complications. Since high doses of chemical agents entail adverse effects and confer resistance as well, it is crucial to identify new antimicrobial agents. Li et al (2008) demonstrated potent antimicrobial activity for titanium dioxide and zinc oxide nanoparticles, exerted through photocatalytic mechanisms, thus suggesting an application for them in microbial control and water treatment (4). Dastjerdi & Montazer (2010) used titanium oxide, zinc and copper oxide nanoparticles as well as titanium dioxide nanotubes and nanocomposites to modify and improve antimicrobial properties of textiles (5).

In the present study, we use catechin (a major component of green tea), ethylenediaminetetraacetic acid (a substance with the ability to disintegrate bacterial membrane) and colloid solution of zinc oxide nanoparticle to inhibit the growth of $P.\ aeruginosa$ – an important nosocomial pathogen with increasing drug resistance.

Material and Methods:

Bacterial culture:
Standard $P.\ aeruginosa$ strain ATCC 27853 was cultured on nutrient agar medium for 18-24 hours at 35°C to yield a monoclonal.

Preparation of microbial suspension:
After 18-24 hours of bacterial culture, a single colony was added to sterile physiology serum to yield a suspension with 0.5 McFarland turbidity. The resulting suspension was then diluted with sterile physiology serum with a 1/100 ratio to yield a suspension with bacterial concentration of $1 \times 10^6$ bacteria per mL.

Preparation of different concentrations of dipotassic EDTA:
A water stock of dipotassic EDTA powder was prepared and different dilutions (Table 1) were prepared and filtered for the microdilution test.

Preparation of different concentrations of catechin:
A water stock of catechin powder (purchased from Sigma Co.) was prepared and different dilutions (Table 1) were filtered for the microdilution test.

Preparation of different concentrations of broad-spectrum antibiotic imipenem:
Considering the good solubility of imipenem in sterile distilled water, we prepared a water stock and different dilutions for the test (Table 1).

Preparation of different concentration of ZnO nanoparticle:
5 g zinc acetate was placed in a flask with 50 mL deionized distilled water at 80°C until it reached one fifth of its original volume. It was then incubated in oven at 100 ± 5°C for 12 hours to dry gradually. Subsequently, it was preserved at 300 ± 20°C for 24 hours until crystals were formed (6).

In this study, we used scanning electron microscopy to evaluate the nanoparticles.
For this purpose, the samples were covered with a fine layer of gold or carbon and studied by scanning electron microscopy. In order to achieve a better understanding of the crystal structure of ZnO particles, we implemented x-ray diffraction, which is used for studying compounds with a crystal structure. Serial dilutions (Table 1) of the colloid solution of ZnO in distilled water were prepared and sterilized with 0.22 μm syringe filter.

**Determination of minimum inhibitory concentration of EDTA, ZnO nanoparticle, catechin and imipenem on P. aeruginosa growth by microdilution test:**

After preparation of different dilutions of the study agents, microdilution test was completed according to the standard protocol (7). For this purpose, 10 μL of diluted microbial suspension with 100 μL of Mueller-Hilton Broth culture medium was placed in each well of a 96-well plate. Different dilutions of the study agents were then added to it. Each test was performed in triplicate. The 96-well plates were incubated at 35°C for 24 hours. Subsequently, 10 μL was acquired from each well and inoculated onto nutrient agar for confirmation and determination of colony counts. The plates were then incubated for 24 hours at 35°C, after which the colonies were counted.

**Results:**

X-ray diffraction and electron microscopy confirmed the structure of ZnO nanoparticle and determined its dimensions. The results of scanning electron microscopy (SEM) revealed a spherical ZnO nanoparticle, measuring 30-90 nm (Figure 1). X-ray diffraction indicated a hexagonal morphology for the synthesized ZnO nanoparticles (Diagram 1).

After microdilution, we determined the minimum inhibitory concentration (MIC) through acquiring the supernatant fluid from the wells, inoculating it into the solid nutrient culture medium and subsequently counting the number of colonies. In this way, we found the MIC of catechin inhibiting *P. aeruginosa* growth to be 7.24 μg/mL, while it was 24.92 μg/mL for EDTA and 0.43 μg/mL for imipenem. Thus, it may be stated that catechin inhibits pseudomonas growth at one third the concentration of EDTA. The ZnO nanoparticle inhibits growth at a far less concentration – 0.0003 μg/mL (Table 2).

![Figure 1: Electron microscopy image of ZnO nanoparticle](image-url)
Table 1: Concentration of study agents tested on the standard strain of *P. aeruginosa* (ATCC27853)

<table>
<thead>
<tr>
<th>Test Agent</th>
<th>Concentrations</th>
</tr>
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<tbody>
<tr>
<td>EDTA (mg/mL)</td>
<td>0.00243, 0.00323, 0.00404, 0.00809, 0.0202, 0.0404, 0.0606, 0.080, 0.10, 0.12, 0.14, 0.16</td>
</tr>
<tr>
<td>ZnO (mL)</td>
<td>1, 3, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90</td>
</tr>
<tr>
<td>Catechin (mg/mL)</td>
<td>0.01, 0.012, 0.015, 0.018, 0.02</td>
</tr>
<tr>
<td>Imipenem (mg/mL)</td>
<td>0.008, 0.010, 0.0125, 0.016, 0.025</td>
</tr>
</tbody>
</table>

Table 2: Minimum inhibitory concentration (MIC) values of study agents tested on the standard strain of *P. aeruginosa* (ATCC27853)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Minimum Inhibitory Concentration (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>7.24</td>
</tr>
<tr>
<td>EDTA</td>
<td>24.92</td>
</tr>
<tr>
<td>ZnO</td>
<td>0.0003</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.43</td>
</tr>
</tbody>
</table>

**Discussion:**

Considering the infectivity of *Pseudomonas aeruginosa* for burn victims, immunocompromised patient and those with cystic fibrosis, we conducted the present study to compare catechin, zinc oxide, and EDTA with imipenem as the control antibiotic. The latter is one of the most potent antibiotics used against pseudomonas and, due to its wide coverage, is extensively administered to critically ill patients as well as cases of multiple infection and resistant microorganism (8). Nevertheless, similar to other beta lactams, imipenem may potentially entail cerebrospinal lesions and disorders such as convulsion (9), and therefore it is crucial to identify safer alternatives with comparable antimicrobial potencies. Ethylenediaminetetraacetic acid (EDTA) is a metal chelating agent which affects the permeability of the outer membrane of planktonic cells. It chelates doubly charged cations in the outer membrane, thus separating the lipopolysaccharide from the cell and increasing membrane permeability (10). Catechin is a component of green tea with antimicrobial properties. The phenol, saponin, and flavonoid compounds present in plants confer antimicrobial activity on them. These compounds impact the permeability of plasma membrane and its structural enzymes. In 2005, Sattari et al...
studies the antibacterial activity of eucalyptus extract on *P. aeruginosa* to conclude that the extract inhibits bacterial growth at concentrations lower than the MIC (11). Tea leaves contain compounds such as catechin, thiamine and caffeine which play a role in its antimicrobial activity. Catechin is a polyphenolic compound of the tea, accounting for 30% of dry tea weight (12). The variations in catechin content reflect the type of the plant as well as its harvest season. Catechin influences a number of cell-related procedures, including cell cycle and signaling, arachidonic acid metabolism, cell proliferation and apoptosis, as well as suppression of tumor cells (13-15). In addition to antioxidant activity, catechin has antiviral and antifungal properties. A study in 1971 demonstrated the antiviral activity of catechin against tobacco mosaic virus (16). Hidetoshi et al (2004) indicated that catechin kills microorganisms through production of hydrogen peroxide (17).

In the present study, we also investigated the antimicrobial property of ZnO oxide in inhibiting *P. aeruginosa* growth. ZnO has long been a constituent of cosmetic and hygienic products. In 1995, magnesium and calcium oxide nanoparticles were shown to have antibacterial activity. They were even found to be effective against spores which are highly resistant to temperature and pressure (18-20). Atmaca et al found that the element zinc may inhibit the growth of *Staphylococcus aureus* and *Staph. epidermidis*. Steven et al used a photocatalytic method to study zinc oxide impact on fungi and bacteria; they observed that ZnO kills *Candida albicans* after 120 minutes (21,22). Numerous factors affect the antimicrobial properties of nanoparticles, including concentration, size, and surface area. The smaller the ZnO nanoparticles, the greater their antimicrobial activity (6,23).

**Conclusion:** Since ZnO nanoparticle managed to inhibit *P. aeruginosa* at a concentration less than imipenem, it may be concluded that the method of particle synthesis and the antibacterial activity of the particle have been acceptable. Our findings indicate that the herbal compound catechin inhibits *P. aeruginosa* at a lower concentration compared to EDTA, while ZnO suppressed *P. aeruginosa* at a far less concentration compared to catechin. Considering the history and favorability of zinc and tea in the society and their acceptable antibacterial activity, they may be used for therapeutic purposes and coverage of medical equipment and instruments after the completion of comprehensive studies in animal models.

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**References:**