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Evaluation of Antimicrobial Effects of Zinc Oxide Nanoparticles and extract of *Solanum nigrum* on *Pseudomonas aeruginosa* isolated from clinical specimens

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ABSTRACT

Background and aims: The purpose of this study was to evaluate the antimicrobial effects of zinc oxide nanoparticale and extract of *Solanum nigrum* on *Pseudomonas aeruginosa* bacteria isolated from clinical specimens.
Methods: Zinc oxide was purchased from the market. 20g of the dried and pounded leaves of *S. nigrum* was used and its extract was prepared in rotary device. 12 isolates of P. aeruginosa were isolated from referred patients to hospital Zabol. Finally, 12 isolates were used to growth inhibitory activity assay. Minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of Zinc oxide and extract plant against *P.aeruginosa* were evaluated using micro broth dilution method.
Results: The highest inhibitory concentration for *P. aeruginosa* is 1500 µg/ml, with four bacterial strains being inoculated. Also, the results showed that the highest drainage concentration was 3000 µg/ml, which two strains were inhibited in this concentration and the lowest trap concentration was 93 µg/ml. The lowest inhibitory concentration of extract plant was 0.62 mg / ml, with only one strain being inhibited in this concentration. The highest inhibitory concentration for *P. aeruginosa* is 40 mg / ml, with four bacterial strains being inoculated.
Conclusion: The results showed that zinc oxide and extract of *Solanum nigrum* have a good antimicrobial activity on the bacterium and increases the concentration of antimicrobial activity.

Keywords: Zinc oxide nanoparticles, *Solanum nigrum*, Antimicrobial Activity, *Pseudomonas aeruginosa*.

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INTRODUCTION

Pseudomonas aeruginosa is a gram-negative, aerobic and gram-negative bacterium that is abundant in water, soil, plant, and animals.[1] This bacterium lives on the skin, perforation and upper respiratory system of humans as a natural flora. More than 40% of healthy people have the bacterium in their intestine, and this percentage increases in hospitalized patients based on the length of hospitalization.[2] In the second half of the last century, *P. aeruginosa* was considered an important hospital pathogen.[3] *P. aeruginosa* is the most common cause of human pathogenesis in the genus Pseudomonas. This bacterium is a common cause of nosocomial infections, which in fact has high resistance to most common antibiotics and therefore the treatment of infections caused by it is associated with many problems.[4] This bacterium causes serious infections such as septicemia, pneumonia, endocarditis, otitis and keratitis. The bacterium lives in a wide range of environments, where human activity is located.[5] *P. aeruginosa* has inherent resistance to a wide range of antimicrobial and antiseptic agents, such as ammonium compounds, hexachlorophene, soaps and iodized solutions.[6]

The belief that nanotechnology is another era of science and is a combination of engineering and biology, chemistry, medicine, and physics has been accepted by the general public. These features have facilitated the use of Nano materials, so that they are common in all aspects of life such as electrical systems, fight against germs and diagnosis and treatment of diseases.[7]

Zinc is one of the essential and non-toxic elements for humans. Zinc oxide does not have toxic properties for human cells. The safety of this substance has been announced by the Food and Drug Administration.[8] One of the characteristics of zinc oxide nanoparticles is their antimicrobial property within the normal pH range (pH = 7) and in the absence of light.[9] The zinc oxide nanoparticles have selective toxicity and can be an antimicrobial component with an ideal potential that can be used as an alternative to some antibiotics, and even affect spores that are resistant to temperature and pressure.[10]

Solanum nigrum, commonly known as black nightshade is a member of the Solanaceae family. Locally, it is known as "Kambai. *S. nigrum* is a traditionally used important medicinal plant with multiple therapeutic properties such as antiproliferative, antiseizure, anti-inflammatory, antipoison, antioxidant and antipyretic.[11] Some traditional uses of the plant include the treatment of pain, inflammation and fever.[12,13] In India, its roots are boiled with a little sugar to increase fertility in women. Its root juice is also used to treat asthma and whooping cough. Leaf paste is used to treat rabies and for wound healing. In Algeria, whole plant decoction is traditionally used to treat burns and dermal infections.[11]

The purpose of this study was to evaluate the antimicrobial effects of zinc oxide nanoparticale and extract of *S. nigrum* against *P. aeruginosa* isolated from clinical specimens.

METHODS

Isolation of *P. aeruginosa*

Different strains of P. aeruginosa used in this study were collected and isolated from patients admitted to Amir al-Momenin hospital in Zabol, Iran. To identify the genus *P. aeruginosa*, hot curing, catalase, oxidase and confirmation tests of sugars were used.

Nanoparticles of zinc oxide with a size of 10-30 nm were commercially purchased from the Iranian nanomaterials pioneer's company (IRANIAN NANOMATERIALS PLONEERS COMPANY).

Preparation of plant extract

The leaf of *S. nigrum* was collected from Zabol, southeastern Iran and dried at room temperature. Samples were crushed and kept in a glass container until extraction procedure was performed in the laboratory. For preparing extracts, plants were properly dried and pulverized into a coarse powder. Forty gram of each sample of grinded powder was separately soaked in 50 ml of 95% ethanol for 20 h (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper). Then, the filtrates were evaporated in a rotary evaporator. At last, 0.97 g of dried extracts were obtained and then stored at 40ºC in air tight screw cap tube.

Determination of 12 strains' sensitivity of *P. aeruginosa* to zinc oxide and plant extract Broth microdilution method was used to determine the minimum bacterial concentration (MIC) and minimum bacterial concentration (MBC). All the tests were performed in Mueller-Hinton broth supplement-ed with Tween 80 at a final concentration of 0.5% (v/v). Serial doubling dilutions of the extract were prepared in a 96-well micro titer plate. To each well, 10 µl of indicator solution and 10 µl of Mueller-Hinton broth were added. Finally, 10 µl of the bacterial suspension (106 CFU/ml) was added to each well to achieve a concentration of 104 CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria were not dehydrated. Plates were pre-pared in triplicates and then placed in an incubator at 37°C for 18–24 hours. Afterwards, the color change was assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of the three values was calculated providing the MIC values for the tested extract. MIC was defined as the lowest concentration of the extract at which the micro-organism does not demonstrate any visible growth. The microorganism growth was indicated by turbidity. MBC was defined as the lowest concentration of the extracts at which the incubated microorganism was completely killed.

Statistical analysis

One-way ANOVA was used to determine the significant difference between different treatments and then Duncan multiple range tests ($P<0.05$) was performed. All the statistical analyses were done using the Statistical Package for Social Sciences (SPSS) for Windows (version 18.0).

RESULTS

The results of this study showed that the MIC of zinc nanoparticle was 46 µg/ml, with only one strain inhibited in this concentration. The highest MIC of zinc nanoparticle against *P. aeruginosa* was 1500 µg/ml, with four bacterial strains inoculated (Table1).

The results also showed that the highest MBC was 3000 µg/ml, where two strains were inhibited in this concentration and the lowest MBC concentration was 93 µg/ml (Table1).

The MIC (minimum inhibitory concentration) of extract plant against *P. aeruginosa* was 0.62 mg/ml, with only one strain inhibited in this concentration. The highest MIC of extract plant against *P. aeruginosa* was 40 mg/ml, with four bacterial strains being inoculated.

Table 1: Results of MIC and MBC of zinc oxide nanoparticles and plant extract against *P. aeruginosa*

Bacterial code	MIC Of zno	MBC Of zno	MIC Extract plant	MBC Extract plant
1	187	375	0.62	1.25
2	375	750	20	40
3	46	93	1.25	2.5
4	1500	1500	5	10
5	375	750	1.25	2.5
6	750	750	5	10
7	375	375	2.5	5
8	1500	3000	40	40
9	1500	3000	20	40
10	375	750	5	10
11	93	187	2.5	1.25
12	1500	1500	10	20

Discussion

P. aeruginosa is an opportunistic bacterium that causes systemic and malignant skin infections in humans. It is the most strong antimicrobial and antibiotic agent. Therefore, fighting and eliminating infections caused by this bacterium is really important. The problem is that in some cases the bacterium leads to failure of the treatment. Due to comprehensive efforts to control infections, *P. aeruginosa* and other resistant bacteria, especially in burn infections, the identification of nanoparticles as anti-odometric agents is crucial. In this study, the antibacterial properties of zinc oxide nanoparticles and extract plant were investigated. Matthews et al., in a research on the use of antibacterial nanoparticles in treatment of medicine, and can completely prevent the growth of a high proportion of gram-positive and gram-negative bacteria.[14] Ag-NPs have been also demonstrated as an effective biocide against drug- resistant strains.[15]

Rajaei et al. studied the inhibitory activity of zinc oxide nanoparticles in *P. aeruginosa* strains isolated from burn wounds, and their results showed that *P. aeruginosa* was sensitive to the antibiotics piperacillin, tobramycin, and gentamicin. Zinc oxide nanoparticles of 5 nm in at 25, 50, and 100 mg/ml and a size of 100 nm at a concentration of 100 mg/ml had antibacterial activity against *P. aeruginosa* isolated from burn wounds.[16]

Nabi Pour et al. showed that silver nanoparticle and zinc oxide nanoparticles at 0.5% concentration had bactericidal properties and were able to eliminate approximately 100% of *Staphylococcus aureus* and *P. aeruginosa*.[17]

Atmaca et al. discovered that oxidation nanoparticles could inhibit the growth of these microorganisms by studying the anti-bacterial activity of azathiroid nanoparticles against *P. aeruginosa*, *S. aureus*, and *S. epidermidis*.[18]

Jafari et al. studied the anti-bacterial silver nanoparticles synthesized by wet chemical method in combined and single plants on *P. aeruginosa*, Bacillus subtilis, S. gallinarum, *E.* coli, and *S. aureus*. Results showed that *E. coli*, *S. gallinarum*, and *P. aeruginosa* were more susceptible to *S.*

aureus strains, and *B. subtilis* is more susceptible to the combination of two nanoparticles, and the combination of zinc and silver nanoparticles also increased bacterial activity.[19]

Sa'adat et al. analyzed the antibacterial activity of nanoparticle, in comparison with imipenem against *P. aeruginosa*. Nanoparticles inhibited the growth of *P. aeruginosa* in a concentration lower than that of imipenem, and had desirable antibacterial properties.[20]

Zveki et al. evaluated the antibacterial activity of zinc oxide nanoparticles against *S. aureus*, *E. coli*, *P. aeruginosa*, and Saccharomyces cerevisiae. In the presence of zinc oxide nanoparticles, the growth of *S. aureus*, *P. aeruginosa* and *S. cerevisiae* bacteria was 90-100 and 100% inhibited, while the antibacterial effect of nanoparticles on *E. coli* requires more research.[9]

The study of Rajathi D Modilal et al. showed that the plant extracts exhibited remarkable activity against the studied organisms isolated (*E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus*, and *S. pyrogens*) with zone of inhibition ranging from 5 to 25 mm. In Table, it is evident that for the four concentrations taken for each extract, the highest concentration (100 mg/ml) caused the maximum zone of inhibition for the five bacterial species. Ethanolic extract of *S.* nigrum caused maximum zone of inhibition for *P. aeruginosa* followed by the *S. pyrogens*. Aqueous extract of *S. nigrum* causes maximum zone of inhibition for *S.* aureus followed by *P. aeruginosa* and *S. pyrogens*. Petroleum ether extract of *S. nigrum* caused maximum zone of inhibition for S. pyrogens followed by S. aureus.[21]

The study of Bashir Dar, evaluating the antimicrobial potential of aqueous and methanol extracts of *S. nigrum*, a traditionally used medicinal plant with various therapeutic properties, the highest antibacterial activity was exhibited by aqueous extract of *E. coli* (16 ± 0.23 mm) followed by *S. aureus* (15 ± 0.15 mm) at the concentration of 100 mg/ml of the plant extract. Methanol extract showed highest antibacterial activity against *S. aureus* and *P. aeruginosa* with 14 ± 0.11 mm and 14 ± 0.26 mm zones of inhibition at the same concentration (100 mg/ml), respectively. The highest antifungal potential was exhibited by the methanol extract against S. cerevisiae (26 ± 0.27 mm) and Candida albicans (22 ± 0.13 mm), while the aqueous extract exhibited the highest antifungal potential against *S. cerevisiae* (23 ± 0.14 mm) followed by *C. albicans* (21 ± 0.10 mm) and Aspergillus fumigatus (16 ± 0.11 mm) at 100 mg/ml.[22]

Zubair et al. carried out a study to evaluate the antimicrobial activity of methanol extract and different fractions (n-butanol, ethyl acetate, chloroform and n-hexane) of *S. nigrum* leaves. The antimicrobial activity was determined by the disc diffusion method and minimum inhibitory concentration (MIC) against a panel of microorganisms (four bacterial strains, i.e. *Pasteurella multocida*, *E. coli*, *B. subtilis* and *S. aureus* and three fungal strains, i.e Aspergillus flavus, *A. niger* and Rhizoctonia solani). The results indicated that the leaf extract and fractions of *S. nigrum* were mildly potent antibacterial agents, while antifungal activity of *S. nigrum* leaves extract/fractions was poor.[23]

CONCLUSIONS

It should be noted that in recent years, several reports about resistance of Pseudomonas to chemical fungicide have been released. Based on the obtained data in this study, the results indicate that the zinc oxide nanoparticles and extract of *S. nigrum* partials plays an important role in inhibiting the Pseudomonas growth.

CONFLICT OF INTEREST

All authors disclose any financial and the authors declare that there are not any potential conflicts of interest.

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