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Short Communication

Antibacterial Activity of Silver Nanoparticles Produced by Plantago Ovata Seed Extract Against Pseudomonas Aeruginosa

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ABSTRACT

Objective: Development of resistance against many of the commonly used antibiotics is an impetus for further efforts to search for new antimicrobial agents. The aim of the study was determined as antibacterial activity of silver nanoparticles produced by *Plantago ovata* seed extract against *Pseudomonas aeruginosa*. **Methods:** All 30 strains of *P. aeruginosa* were isolated from isolates of the urinary tract infection of Hospital and the minimum inhibitory concentrations were distinguished by microdulition method. **Results:** The silver nanoparticles revealed Gaussian distributions with average diameter of 13 nm with some deviations. The result of plant extraction showed that the most MIC value was 100 ppm concentration, and 9 strains of pseudomonas were inhibited. **Conclusion:** Ag nanoparticles prepared by the effective cost reduction method described here which is greatly promising as antimicrobial agents. Applications of Ag nanoparticles based on these findings may lead to valuable discoveries in various fields such as medical devices and antimicrobial systems.

1.INTRODUCTION

Pseudomonas aeruginosa is a non-fermentative, aerobic, Gram-negative rod that normally lives in moist environments. It has minimal nutrition requirements while being able to use several organic compounds for growth. *Pseudomonas aeruginosa* can colonize in human body sites, with the preference for moist areas, such as the perineum, axilla, ear, nasal mucosa and throat; as well as stools. The prevalence of colonization by *P. aeruginosa* in healthy subjects is usually low, but higher colonization rates can be observed in following hospitalization, especially amongst subjects treated with broad-spectrum antimicrobial agents specifically for extended spectrum cephalosporin. In a recent surveillance study on nosocomial bloodstream isolates which carried out in the Americas, P. aeruginosa was found to be the third most common pathogen (1). *P. aeruginosa* can also cause peritonitis in patients on chronic ambulatory peritoneal dialysis (2). Such problems and needs have led to resurgence in the use of silver-based antiseptics that may be linked to a broad-spectrum activity and considerably lower propensity to induce microbial resistance compared with those of antibiotics (3, 4). Accordingly, an environmental process for the synthesis of silver nanoparticles is important. Plant extract solutions and bio-organisms have been in

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spot light for their extreme ability to synthesis nanoparticles, including silver and gold nanoparticles. The study aim was determined as antibacterial activity of silver nanoparticles produced by *Plantago ovata* seed extract against *Pseudomonas aeruginosa*.

2.MATERIALS AND METHODS

2.1. Bacteria strains

Various strains of *Pseudomonas aeruginosa* for this study were taken from positive cultures of hospitalized patients in zahedan hospitals. Cultures were grown on nutrient agar. For identifying the kinds of pseudomonas form tests of gram stain, catalase, oxidize, glucose tests, OF (oxidation fermentation), TS (triple sugar iron) were used (5).

2.2. Plant materials and MIC determination of silver nanoparticles

The seeds of *Plantago ovata* were collected in the region of Iran. The seeds were dried at room temperature and transferred into glass containers and preserved until extraction procedure was performed in the laboratory and Silver nitrate ($AgNO_3$) was used as the source for silver synthesis. The biosynthesis of silver nanoparticles using the extract of *P. ovata* was preliminary confirmed by the color change of the solution from yellow to brown and the MIC (Minimum Inhibitory Concentration) was determined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The broth micro-dilution method was used to determine MIC. Briefly, serial doubling dilutions of the silver nanoparticles produced in the plant *P. ovata* seed extract were prepared in a 96-well micro-titer plate ranged from 12.5ppm to 200ppm. To each well, 10 µl of indicator solution and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^6 CFU/ml) was added to each well to achieve the concentration of 10^4 CFU/ml. The plates were wrapped loosely within cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37ºC for 18-24 hours. The color change was then evaluated visually. The microorganism growth was indicated by turbidity.

3. RESULT

The silver nanoparticles revealed Gaussian distributions with average diameter of 13 nm with some deviations. The result of plant extraction showed that the most MIC value was 100 ppm concentration, and 9 strains of pseudomonas were inhibited.

4. DISCUSSION

Silver is said to be a universal antimicrobial substance for centuries. Though, silver ions or salts have limited usefulness as an antimicrobial agent: Such as, the interfering effects of salts and antimicrobial mechanism of continuous release of enough concentration of Ag ions from the metal form. This kind of limitation can be overcome by using silver nanoparticles. However, to use silver against microorganisms, it is essential to prepare it with environmental friendly and cost- effective methods. In the other hand, it is also important to enhance the antimicrobial impact of silver ions (6). The result of plant extraction indicated that the most MIC was 100 ppm concentration, and 6 strains of pseudomonas were inhibited. In the study of Soo-Hwan, the result showed that Ag-NPs have potent antibacterial activities against S. aureus and E. coli cells (7). In the study of Shameli, the result show that in the aqueous phase systems, the antibacterial activity of Ag-NPs at 3 and 6 h stirring times in S.aureus is higher than that of the Ag+ ions. Similarly, the antibacterial activity of Ag-NPs in S. typhimurium is generally higher than that of the Ag+ ions (8). The nanoparticles of silver showed high antimicrobial and bactericidal activity against gram positive bacteria such as *Escherichia Coli*, *Pseudimonas aureginosa* and *staphylococcus aureus* which is a highly methicillin resistant strain(9). The mechanism of inhibitory action of silver ions on microorganisms is partially known. It is believed that DNA loses its replication ability and cellular proteins become inactivated on Ag+ treatment (10). In addition, it was also shown that Ag+ binds to functional groups of proteins, leads in protein denaturation (11).

Table 1 : MIC of Nano silver product in plant extract on drug resistant bacteria strains

Bacterial cod	MIC(ppm)	Bacterial cod	MIC(ppm)	Bacterial cod	MIC(ppm)
1	12.5	11	12.5	21	100
2	12.5	12	25	22	100
3	12.5	13	25	23	100
4	12.5	14	100	24	50
5	6.25	15	100	25	25
6	6.25	16	50	26	50
7	6.25	17	100	27	25
8	6.25	18	100	28	100
9	6.25	19	100	29	12.5
10	12.5	20	50	30	12.5

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