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Antimicrobial Effects of *Ferula persica* Gum Extract and Gold Nanoparticles on *Pseudomonas aeruginosa*

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Abstract

Background: In the treatment of bacterial infections, drug resistance is a global problem; *Pseudomonas aeruginosa* is no exception. This bacterium is among the important causes of nosocomial infections, especially burn wound infections, and it is resistant to most applicable antibiotics.

Objectives: The present study aimed to evaluate the anti-bacterial effects of *Ferula persica* gum extract and gold nanoparticles on *P. aeruginosa* strains isolated from burn wound infections in Isfahan in 2012.

Materials and Methods: In this experimental study, 150 *P. aeruginosa* strains carrying the *bla*SPM-1 gene were isolated from burn wound infections and were confirmed by biochemical tests. The anti-microbial activities of *Ferula persica* gum extract and gold nanoparticles were evaluated by agar well-diffusion assay and microdilution antimicrobial susceptibility tests. The results were compared to ciprofloxacin.

Results: The highest mean zone of inhibition (18 mm) was observed in a concentration of 250 mg/mL of *F. persica* extract, which was equal to that of ciprofloxacin. In the gold nanoparticles, the highest mean growth inhibition zone (10.18 mm) was observed in the dilution of 50 ppm. The highest zone of inhibition of nanoparticle-extract synergy was observed in the dilution of 125 mg/mL extract + 25 ppm nanoparticle (9.89 mm). The MIC and MBC of the extract were 69.25 $\pm$ 42.36 mg/mL and 102.25 $\pm$ 16.76 mg/mL, respectively. The MIC and MBC of the nanoparticle-extract mixture were 50.78 $\pm$ 19.26 mg/mL and 54.11 $\pm$ 19.72 mg/mL, respectively.

Conclusions: The results of the present study showed that the extract of *F. persica* gum and the gold nanoparticles had anti-*Pseudomonas aeruginosa* effects, which were more pronounced when they were used separately.

Keywords: Gold Nanoparticle, Anti-Infective Agents, *Pseudomonas aeruginosa*, *Ferula persica*

1. Background

Pseudomonas aeruginosa, a ubiquitous gram-negative bacterium, is an important causative agent of opportunistic and severe acute nosocomial infections in humans. It particularly affects immunocompromised patients or those admitted to the intensive care unit (ICU). *P. aeruginosa* is the primary pathogenic agent in burn wound infections and ventilator associated pneumonia, both of which are associated with a considerably high (> 30%) mortality rate (1). The bacterium is also responsible for a variety of nosocomial infections, such as urinary tract or wound infections, endocarditis, and bacteremia (2).

P. aeruginosa is the most frequently encountered source of chronic or acute burn wound infection by gram-negative bacteria in the US. The bacterium has a propensity for suitable warm and moist wound environments, therefore presenting a major challenge for patients with burn wounds (3). Because of the adaptability of *P. aeruginosa* and its exceptionally good capacity to evade the activity of antimicrobial drugs, this bacterium is one of the most feared pathogens. Today, the increased prevalence of nosocomial infections caused by extreme drug-resistant and multidrug-resistant *P. aeruginosa* strains considerably limits the selection of proper antibiotics and therefore increases the morbidity and mortality of patients (1, 4, 5).

In previous decades, reports have warned about the occurrence of outbreaks caused by extensively drug-resistant and multidrug-resistant *P. aeruginosa* isolates within the hospital environment. Recent studies have provided evidence of the existence of extensively drug-resistant and multidrug-resistant clones that are transmitted rapidly in several hospitals worldwide (1, 6). The extensive and indiscriminate use of antibiotics is considered the major cause of the development of drug resistance (7).

Based on the literature, most drugs are not effective against the infections caused by *P. aeruginosa*. Thus, in this situation, some alternative drugs, such as herbal remedies, may be helpful. *Ferula persica* is a member of the genus *Ferula*, which can be found throughout central Asia, especially in Iran. In folk medicine, the roots of *F. persica* are used in

the treatment of diabetes (8). Moreover, the anti-microbial activity of *Ferula* extract has been also reported (9). Furthermore, nanoparticles have distinctive physicochemical properties (10), such as antimicrobial activity (11).

2. Objectives

The aim of the present study is to determine the antimicrobial effects of *Ferula persica* extract and gold nanoparticles on the growth of *P. aeruginosa* carrying *bla*SPM-1 gene in a culture media.

3. Materials and Methods

3.1. Bacterial Isolation and Antibiogram

In this experimental study, specimens of *P. aeruginosa* carrying the *bla*SPM-1 gene were collected from 150 patients with burn wound infection in Isfahan city, central Iran during 2012. Biochemical tests were used to confirm that the isolates were gram-negative bacilli, citrate positive, TSI Alk/Alk, non-fermentative, motile, H_2S negative, oxidase positive, urease negative, and catalase positive. The confirmed isolates were kept frozen at -70°C for PCR (12). The drug resistance pattern of the isolates was evaluated by using the Kirby-Bauer disk diffusion method (13).

3.2. Polymerase Chain Reaction (PCR)

The amplification of the *bla*SPM-1 gene then confirmed that the isolates had the metallo-beta-lactamase gene.

3.2.1. DNA Extraction

DNA was extracted from the isolates by the boiling method, as previously described (14).

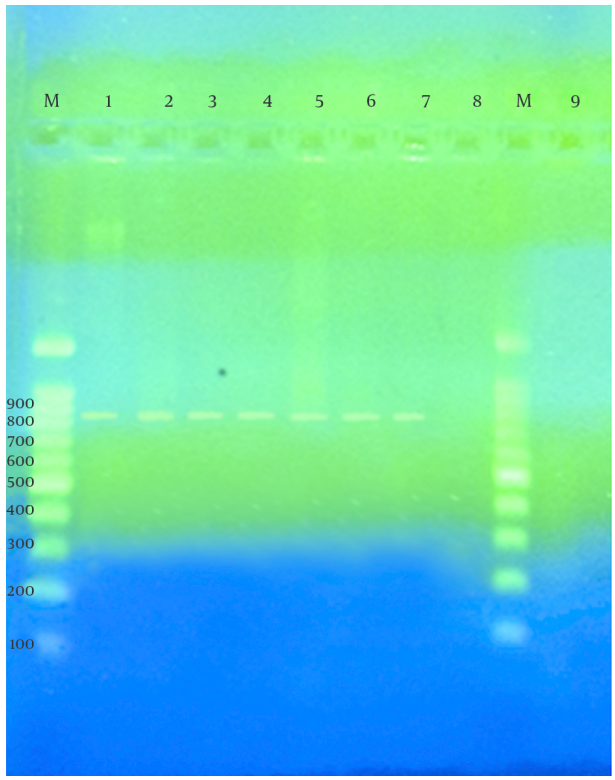
3.2.2. PCR

The isolates underwent PCR amplification of the gene using pairs of a specific primer (Fermentas) (blaSPM-1 F: 5′GCGTTTTGTTTGTTGCTC3′; *bla*SPM-1 R; 5′TTGGGGATGTGAGACTAC3′) (15). Gel electrophoresis was performed on the PCR products, and the bands were studied using a transilluminator device (Figure 1). The PCR products (831 bp) were then sequenced and analyzed.

3.3. Preparation of Ferula Persica Extract

Ferula persica gum was purchased from an oil-seed producing center in Isfahan. The gum was washed and dried in air at room temperature. It was then pulverized and extracted using chloroform by maceration for 72 hours. The chloroform extract of *F. persica* was dried by oven, and the powder of the extract was produced (16). To evaluate the antimicrobial activity in the extract, one gram of the dried extract was dissolved in four ml of 5% dimethyl sulfoxide (DMSO).

Figure 1. Gel Electrophoresis of PCR Amplification of blaSPM-1 Gene (831 bp)



lane M, DNA ladder 100 bp; lane 1-6, *P. aeruginosa* carrying *bla*SPM-1 gene; lan 7, positive control; lan 8, negative control.

3.4. Gold Nanoparticle

Spherical gold nanoparticles (Pars Azma, Iran) were purchased in a colloidal suspension (10 nm in diameter, 100 ppm). The following serial dilutions were prepared from the nanoparticles for antimicrobial testing: 50, 25, 12.5, and 6.125 ppm.

3.5. Agar Well Diffusion Assay

An agar well-diffusion assay was used for the antimicrobial testing of the gold nanoparticles and the *F. persica* extract. The growth inhibition zone of *P. aeruginosa* on a Muller-Hinton agar medium was measured after 24 hours of incubating the gold nanoparticles and *F. persica* gum extract separately (100 mL each). In the next step, the synergistic effects of the two tested materials were determined. Sterile deionized water and chloramphenicol were used as negative and positive controls, respectively (17).

3.6. Microdilution Antimicrobial Susceptibility Test

To conduct the broth microdilution, 100 μL of Muller-Hilton broth was added to each well of a 96-well microplate. In the next step, the serial dilution of the gold

nanoparticles and *F. persica* extract was prepared in the wells. To determine the MIC, a suspension equivalent to the McFarland Turbidity Standard No. 0.5 from *P. aeruginosa* was prepared and added to the wells (10 μL per well). The microplates were then incubated at 37 °C for 24 hours. After the incubation period, the wells were checked for growth inhibition by determining the existence of turbidity and MIC in the gold nanoparticles and *F. persica* extract.

3.7. Data Analysis

SPSS v.15 software was used to analyze the data using the Kruskal-Wallis and Mann-Whitney tests.

4. Results

4.1. PCR

The gel electrophoresis of the PCR product showed the 831 bp *bla*SPM-1 gene, which was confirmed by the sequence analysis (Figure 1).

4.2. Effect of F. persica Gum Extract on P. aeruginosa Growth

Based on the observations, no growth inhibition zone in dilutions higher than 62.5 mg/mL was recorded. Increasing the concentration (125 and 250 mg/mL) increased the antimicrobial effect of the extract (Table 1). The mean *P. aeruginosa* growth inhibition zone in the 250 mg/mL concentration of the *F. persica* gum extract was significantly higher than in 250 mg/mL ($P < 0.001$). The mean growth inhibition zone of the ciprofloxacin was also higher than that of 125 mg/mL of the *F. persica* gum extract ($P > 0.001$). However, the difference between ciprofloxacin and 250 mg/mL *F. persica* gum extract was not significant ($P = 0.370$).

4.3. Effect of Gold Nanoparticles on P. aeruginosa Growth

To evaluate the antimicrobial effects of the gold nanoparticles on *P. aeruginosa*, serial dilutions of 50, 25, 12.5, 6.25 ppm were prepared. Although the results showed no growth inhibition zone in 6.25 ppm, some degrees of growth inhibition were shown in the higher concentrations (Table 2). The mean growth inhibition zone was observed to be significantly greater in 50 ppm than in 25 ppm ($P < 0.001$) and 12.5 ppm ($P > 0.001$) of the gold nanoparticles.

4.4. Synergistic Effect of Gold Nanoparticles With F. persica Gum

A stock solution containing 50 μL from 250 mg/mL of the extract and 50 μL from 50 ppm of the gold nanoparticles was prepared and then evaluated for its effect on the growth of *P. aeruginosa*.

The mixture of 62.5 mg/mL *F. persica* gum extract plus 12.5 ppm of gold nanoparticles showed no growth inhibition zone. In addition, the concentration of 62.5 mg/mL *F. persica* gum extract plus 12.5 ppm gold nanoparticles showed some degree of growth inhibition. However, it was lower than that of ciprofloxacin (Table 3). Ciprofloxacin showed significantly higher growth inhibition on *P. aeruginosa* compared with the mixture of 125 mg/mL extract plus 25 ppm gold nanoparticle ($P < 0.001$).

4.5. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

In order to determine the MBC and MIC of the experimented materials, the microdilution method was used. Serial dilutions of the *F. persica* extract, the gold nanoparticles, and the mixture of both were prepared and tested. The mean MIC and MBC of the *F. persica* extract, the gold nanoparticles, the mixture of the extract, and the ciprofloxacin are shown in Table 4.

The results showed that the difference between the mean MIC of extract/nanoparticle with ciprofloxacin was not significant ($P = 0.965$). However, it was significantly higher in the extract compared to the ciprofloxacin ($P = 0.018$). The mean MIC was also significantly higher in the extract compared to the extract-nanoparticle mixture ($P = 0.003$). Furthermore, the difference observed between the mean MBC of the extract and the ciprofloxacin was not statistically significant ($P = 0.290$). However, it was significantly lower in the nanoparticle-extract synergy compared to the ciprofloxacin ($P < 0.001$) and the *F. persica* extract ($P < 0.001$)

For the gold nanoparticles, the mean MIC was lower (9.73) compared to extract-nanoparticle mixture (10.51), but it was not significant ($P = 0.112$). The mean MIC of the ciprofloxacin was observed to be significantly higher compared to the gold nanoparticles ($P < 0.001$) and the nanoparticle-extract mixture ($P < 0.001$). Moreover, the mean MBC of the nanoparticle-extract mixture was significantly lower than that of the ciprofloxacin ($P < 0.001$) and the gold nanoparticles ($P < 0.001$). Furthermore, it was observed to be significantly higher in the nanoparticles than in the nanoparticle-extract mixture ($P < 0.001$) (Table 5).

5. Discussion

In the history of humankind, infectious diseases have been major causes of mortality worldwide. A primary

Table 1. Mean Growth Inhibition Zone of *P. aeruginosa* on Muller Hinton Agar, When Treated With *F. persica* Gum Extract And Ciprofloxacin (N = 150)

	Min	Max	Mean	St. Dev
***F. persica* gum extract, mg/mL**				
250	15.00	18.00	16.71	1.54
125	0.00	12.00	11.01	5.22
Ciprofloxacin	15.00	18.00	17.08	1.57

Table 2. Mean *P. aeruginosa* Growth Inhibition Zone When Treated With Gold Nanoparticles and Ciprofloxacin (N = 150)

	Min	Max	Mean	St. Dev
Gold nanoparticle, ppm				
50	0.00	16.00	10.18	5.92
25	0.00	12.00	7.26	4.89
12.5	0.00	9.00	1.25	3.93
Ciprofloxacin	15.00	18.00	17.08	1.57

Table 3. Mean *P. aeruginosa* Growth Inhibition Zone When Treated With the Mixture of Gold Nanoparticles With *F. persica* Gum Extract and Ciprofloxacin (N = 150)

	Min	Max	Mean	St. Dev
***F. persica* gum extract, mg/mL + Gold nanoparticle, ppm**				
125 + 25	0.00	11.00	9.89	4.36
62.5 + 12.5	0.00	0.00	0.00	0.000
Ciprofloxacin	15.00	18.00	17.08	1.57

Table 4. Mean MIC and MBC of *F. persica* Extract, Mixture of Extract and Nanoparticles and Ciprofloxacin

	MIC	MBC
***F. persica* gum extract**	69.25 ± 42.36	102.25 ± 16.76
Extract-Nanoparticle	50.78 ± 19.26	54.11 ± 19.72
Ciprofloxacin	49.36 ± 72.61	99.36 ± 37.41

Table 5. Mean MIC and MBC of Gold Nanoparticles, Mixture of the Extract and Nanoparticles and Ciprofloxacin

	MIC	MBC
Gold nanoparticles	9.73 ± 5.84	16.57 ± 11.49
Extract-Nanoparticle	10.51 ± 4.36	11.25 ± 5.20
Ciprofloxacin	49.36 ± 72.61	99.36 ± 37.41

cause of nearly 9% to 10% of nosocomial infections is *Pseudomonas aeruginosa*. The extreme resistance of *P. aeruginosa* to antibiotics makes it an outstanding pathogen (18, 19). In the US, among gram-negative bacteria, *P. aeruginosa* is the most frequently encountered source of chronic and acute burn wound infections. Because the bacterium has a propensity for suitable warm and moist wound environments, it presents a major challenge for patients with burn wounds (3).

Because the options for multidrug resistance and extreme drug resistance *P. aeruginosa* are limited, new treatment strategies and drugs are necessary. Thus, alterna-

tive drugs such as herbal remedies may be helpful in drug refractory infections, especially *P. aeroginosa*. *Ferula persica* is a member of the genus *Ferula*, which can be found throughout central Asia, especially in Iran. In folk medicine, *F. persica* root is used for the treatment of diabetes (8). The present study evaluated the potential antimicrobial effects of *F. persica* gum extract, gold nanoparticles, and the synergy of both on metallo-beta-lactamase producing *P. aeruginosa* strains isolated from burn wound infections.

The results of the present study indicated that higher concentrations of gold nanoparticle possessed degrees of antimicrobial effects on *P. aeruginosa*. However, the effects were considerably lower than the effects of ciprofloxacin. Furthermore, high concentrations of *F. persica* gum extract showed antimicrobial activity that was similar to that of ciprofloxacin. In the extract-nanoparticle mixture, the mean MIC of the synergy of both agents was higher than that of ciprofloxacin. However, the MBC mean and growth inhibition zones were considerably lower compared to ciprofloxacin. The results showed that, the *F. persica* gum extract and gold nanoparticles separately had more anti-*Pseudomonas* activity than the synergy of the nanoparticle-extract mixture did. Sewage is also reported as a rich source of phage that can be studied against *Pseudomonas aeruginosa* (20).

In a study conducted by Geethalakshmi and Sarada (21), the antimicrobial activity of gold and silver nanoparticles against *Staphylococcus aureus*, *Streptococcus faecalis*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus subtilis*, *Yersinia enterocolitica*, *Klebsiella pneumoniae*, and *Candida albicans* was evaluated using the Kirby-Bauer method. In their study, both the synthesized gold and silver nanoparticles exhibited great antimicrobial activity against *Y. enterocolitica*, *P. vulgaris*,*S.faecalis*, *S. aureus*, and *E. coli*. Their reported antibacterial effect was well noted in Gram-negative bacteria than Gram-positive ones. Similar to the results of the present study, the gold nanoparticles showed lower antimicrobial activity against *P. aeruginosa* compared to chloramphenicol/nystatin, which supports our findings regarding the gold nanoparticles (21).

Iranshahi et al. (2012) reported that umbelliprenin extracted from *F. persica* root could inhibit the production of red pigment in *Serratia marcescens*. They also found that neither the chloroform extract nor the isolated umbelliprenin fraction had antimicrobial activity against *S. marcescens*. On the contrary, the bacteria showed depigmentation zones on culture media (16).

Pavlovic et al. (9) studied the antimicrobial and cytotoxic activity of *Ferula heuffelii* griseb extracts. They reported that the MeOH and $CHCl_3$ extracts of the root of *F. heuffelii* possessed moderate antimicrobial property, mostly against gram-positive rather than gram-negative bacteria. They observed the strongest antibacterial activity against *S. aureus*, with the MIC of 12.5 μg/mL in both extracts. *Micrococcus luteus* had the MIC of 50 (MeOH) and 12.5 μg/mL ($CHCl_3$). In *P. aeruginosa*, the two extracts showed MIC > 100, indicating poor anti-*Pseudomonas* activity, which was similar to our findings (9). In the present study, the mean MIC of 69.25 $\pm$ 42.36 mg/mL and the MBC of 102.25 $\pm$ 16.76 mg/mL were observed for *F. persica* gum extract against *P. aeruginosa*. These results indicate that *F. persica* gum extract has moderate antimicrobial activity, and the antimicrobial activity of gold nanoparticles is lower than that of the extract.

5.1. Conclusion

Based on the results of the present study, *Ferula persica* gum extract, gold nanoparticles, and the synergy of both have anti-*Pseudomonas aeruginosa* activity, which is more pronounced when they are used separately, but not in synergy.

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Footnote

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