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Original Article

Green Medicine: A Novel Preparation Method for Green Synthesizing of Iron Nanoparticles Derived from *Beta Vulgaris* Extract

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Abstract

This study aimed to synthesize new iron nanoparticles (FeNPs) using *Beta Vulgaris* (beet) extract as a reducing agent and test its bioactivity against *Pseudomonas aeruginosa*. In total, five grams of beet were ground and dissolved in 50 ml of distilled water and filtered with filter paper. The filtrate was then isolated. Different concentrations, including 25%, 50%, 100%, and 150% of the isolated filtrated substances were prepared from the stock solution. FeNPs were prepared from 0.5 moles of iron nitrate salt ($Fe(NO_3)3.9H_2O$ which was mixed with the aqueous solution of beet extract. Moreover, two aqueous solutions were mixed thoroughly with continuous stirring at 60°C. The FeNPs were isolated, separated, identified, and characterized using different physicochemical techniques (i.e., X-Ray Diffraction, Ultraviolet-visible Spectroscopy, and Atomic Force Microscope). Subsequently, the bioactivity of the NPs against *P. aeruginosa* was tested. The Vitek antibiotic test for *P. aeruginosa* showed resistant activity against Piperacillin/Tazobactam, Cefazolin, Ceftazidime, Cefepime, Imipenem, Cefepime, Ceftazidime, Cefazolin, and Piperacillin/Tazobactam; in addition, it revealed high sensitivity toward Tobramycin, Levofloxacin, Trimethoprim, Gentamicin, Nitrofurantoin, and Ciprofloxacin. The FeNPs at 50% concentration showed the best inhibition activity against *P. aeruginosa*. In the current study, novel FeNPs were synthesized which showed activity toward *P. aeruginosa* that could be used to replace certain antibiotics as a green medicine.

Keywords: Antibacterial, *Beta Vulgaris*, Extract, Iraq, Iron Nanoparticles

1. Introduction

Beet (*Beta Vulgaris*) is a plant known as a garden beet or beetroot. The utmost importance of this plant is its usage as the main source for table sugar production (1). Beets are considered a kind of vegetables that are simply available in most areas of the world. Chemical studies and investigations show that this plant contains different bioactive organic compounds and complexes (betalains), such as terpenoids, alkaloids, reducing sugar, saponins, and tannins (2, 3).

In 2018, Nahla, Wisam (4) found that the extract of beet contains very important compounds (phenolic) which could be used as an antioxidant. This result is consistent with the findings of a study conducted by Kumar and Brooks (5). They also investigated the antimicrobial activity of the beet extract and the possibility of using it in food industries. Clifford, Howatson (6) discovered that beet extract can be used in wound healing, and this application was confirmed Domínguez, Cuenca (7). Recently, Mirmiran, Houshialsadat (8) studied the anti-inflammatory, anti-diabetic, and anticancer activity of the beet extract.

Therefore, all of these properties of beet extract enable it to be used widely in different meals as a functional ingredient (9, 10).

Bindhu and Umadevi (11) synthesized a silver nanoparticles (AgNPs) complex with beetroot; moreover, they characterized the AgNPs using different physicochemical techniques, such as X-Ray Diffraction, Ultraviolet-Visible Spectroscopy (UV-Vis), and Transmission Electron Microscopy. They found that these NPs are useful to be used as anti-hazardous toxic materials. However, Mehdizadeh, Ghasemi (12) synthesized the AgNps from the aqueous solution of beet extract, and they investigated the activity of the NPs against *Salmonella typhymurium*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*; moreover, their findings were promising against Gram-negative bacteria. Filipović, Ušjak (13) synthesized selenium nanoparticles (SeNPs) with beet extract and evaluated its antimicrobial activity against many strains, such as ATCC 10231, ATCC 9027, ATCC 9341, ATCC 6538, ATCC 29212, and NCIMB 9111. Their finding revealed that the new SeNPs exhibited antimicrobial and anticancer activity.

This study aimed to synthesize new FeNPs with the solution of beet extract using green synthesis; moreover, it attempted to characterize the new NPs using various physicochemical techniques and test their bioactivity against *Pseudomonas.*

2. Materials and Methods

2.1. Preparation of the Aqueous Solution of the Beet Extract

The beet fruit was collected from the local markets, washed, and cleaned thoroughly. Iron nitrate salt (Fe(NO3)3.9H2O was obtained from Sigma Aldrich Chemicals. The water used throughout this experiment was distilled water. All glass wares were properly washed with distilled water and dried in the oven before use. Subsequently, 5 g of beet were ground and dissolved in 50 ml of distilled water, and filtered with filter paper. The filtrate was isolated and different concentrations (25%, 50%, 100%, and 150%) were prepared from the stock solution (by dilution the stock solution). The beet solution preparation was carried out according to Udonkang, Inyang (14).

2.2. Preparation of Iron Nanoparticles

0.5M aqueous solution of (Fe(NO3)3.9H2O was mixed with 100 ml of the aqueous solution of beet extract, and the two aqueous solutions were then mixed thoroughly in a ratio of 1:2 (V/V) with continuous stirring at 60°C. Immediately, the FeNPs were noticed and clarified visually. They were then isolated, separated, and washed with distilled water and ethanol (50%). Afterward, the NPs were identified and characterized using different physicochemical techniques.

2.3. X-Ray Diffraction

The spectra of X-ray diffraction was obtained using X-Ray Diffractometer, and the size of the crystals was predicted using the equation below (15):

$$d = 0.89\,\lambda\,(B\cos\theta)B \ldots\ldots\ldots\ldots\ldots (1)$$

Where 0.89 is the Scherrer constant, λ signifies the Cu-Kα radiation (λ =1.54056 Å), B indicates the peak width at the half height at 2θ, and θ B represents the Bragg angle.

2.4. Atomic Force Microscopy Analysis of FeNPs

NPs are often measured using atomic force microscopy (AFM) or other scanning probe microscopy methods. In the current study, deionized water was used to dilute the FeNPs (only 20 µL) utilizing AppNan (USA), and the process was carried according to Klapetek, Valtr (16) as follows:

In order to simulate the full process of NPs deposition, measurement, analysis, and data modeling were performed in several successive steps: 1) Modeling of a rough surface, 2) Simulation of particle deposition on the surface, 3) Creation of virtual AFM images by tip-sample convolution, and 4) Nanoparticle statistical analysis using virtual AFM images and data processing software.

In order to simulate the effects of both surface roughness and NPs clustering, there was a need for varying the following parameters in steps 1-3: 1)

Surface roughness (parametrized using the root mean square roughness and autocorrelation length), 2) Number of particles and their size (parametrized using the surface coverage and the particle radius), 3) AFM tip shape (parametrized using the tip radius and the apex ratio).

The resulting NPs statistical properties were then compared to values used in step 2 (particle deposition). The algorithms used for data modeling are described in more detail in the next two sections. All data modeling and processing algorithms were implemented in Gwyddion open source software http://gwyddion.net and are available for the public in the present version of the software.

2.5. Spectroscopic Analysis: Ultraviolet-Visible Spectroscopy

The ultraviolet (UV) spectra of the FeNPs were determined following the procedure carried out by Justin, Philip (17). UV-Vis absorbance value was obtained between 200 and 800 nm using UV 3600 Shimadzu UV-Vis spectrophotometer.

2.6. Antibiotic test and Antibacterial Activity Measurements

The antimicrobial test was detected using the Irby Bauer disc diffusion method and utilizing Mueller Hinton agar. This was suggested by the clinical lab according to their standard. The suspension of the bacterial was inoculated for 24 h at 37°C, and the resistance of Entero Cloacae was measured to the FeNPs at different concentrations (1, 2, 3, and 4 mg/mL) according to Arakha, Pal (18).

3. Results and Discussion

Previously published studies showed the antimicrobial activity of FeNPs (18).However, the mechanism behind FeNPs antimicrobial activity is a matter of intensive investigation. A previously published record has shown that FeNPs have antimicrobial activity against *E. coli*, and the activity increases with an increase in the concentration of FeNPs . On the other hand,Borcherding, Baltrusaitis (19) has shown that FeNPs have no antimicrobial activity. The previously conducted studies have been extended to use different antimicrobial and biophysical investigations, draw a concluding remark against these contrasting statements, and introduce a novel method in FeNPs preparation.

The mechanism of the bactericidal effect of FeNPs against bacteria is not well documented. It is assumed that the antibacterial activity of metal nanoparticles, such as Ag and FeNPs, are probably derived through the electrostatic attraction between negatively charged cell membrane of microorganisms and positively charged nanoparticles. The inhibitory effect of FeNPs on microorganisms tested is affected via two possible mechanisms, including the electrostatic attraction between the negatively charged cell membrane of the microorganisms and the positively charged Fe, as well as the formation of ‘pits’ in the cell wall of bacteria related to Fe concentration (11). In the previously published records, it is well documented that the AgNPs have some antimicrobial activity against *E. coli*, *S. aureus*, and *Salmonella typhi*. They reported that the effect was dose-dependent and more pronounced against Gram-negative organisms, compared to Gram-positive ones.

In the present study, the antibacterial assay of FeNPs was performed on *Pseudomonas aeruginosa* (Gram-negative), which is commonly found in water. Antimicrobial sensitivity of *P. aeruginosa* was determined by the disk diffusion technique (also known as Kirby-Bauer method, one of the oldest approaches to antimicrobial susceptibility testing in which Mueller-Hinton agar plates are used, and it employs the direct colony suspension method to make a suspension of the organism in saline to the density of a McFarland 0.5 turbidity standard) in compliance with the National Committee for Clinical and Laboratory Standards (2013) criteria or the European Committee on Antimicrobial Susceptibility Testing. The isolates whose antibiogram yielded intermediate results were considered resistant. The following available

antimicrobial discs (Newprov®, Curitiba, Brazil) (Table 1) were used to evaluate the antimicrobial sensitivity (11).

Table 2 tabulates the *Pseudomonas* Vitek (antibiotic tests results, the test was carried out according to Saegeman, Huynen (20).

Table 1. Antimicrobial sensitivity and the concentrations

No.	Compound	Concentration
1.	Cloxacillin	5 µg
2.	Ampicillin	25 µg
3.	Imipenem	10 µg
4.	EDTA	980 µg
5.	Suphamethoxazole	23.75
6.	Trimethoprim	1.25
7.	Rifampicin	5 µg
8.	Nitrofurantoin	100 µg
9.	Vancomycin	30 µg
10.	Amikacin	10 µg
11.	Ciprofloxacin	10 µg
12.	Gentamycin	10 µg

Table 2. Results of Vitek antibiotic test for *Pseudomonas aeruginosa*

Susceptibility Information	Analysis Time: 10.95 hours		Status: Final		
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Piperacillin/Tazobactam	>=128	R	Amikacin	4	S
Cefazolin	>=64	R	Gentamicin	4	S
Ceftazidime	32	R	Ciprofloxacin	>=0.25	S
Cefepime	32	R	Levofloxacin	2	S
Imipenem	2	S	Tigecycline	>=8	R

+= Deduced drug *= AES modified **= User modified
R= Resistance, S= Sensitive

The antibiotic sensitivity test data for *P. aeruginosa* in table 2 shows resistant activity against Piperacillin/Tazobactam, Cefazolin, Ceftazidime, Cefepime, Imipenem, Cefepime, Ceftazidime, Cefazolin, and Piperacillin/Tazobactam. Furthermore, it reveals high sensitivity toward Tobramycin, Levofloxacin, Trimethoprim, Gentamicin, Nitrofurantoin, and Ciprofloxacin. Figure 1 illustrates the UV-Vis spectra for the FeNPs, while figure 2 shows the AFM for FeNPs.

The results for both Figures (1 and 2) were as expected, and the size of FeNPs ranged from 5 to 40 nm.

After comparing the bioactivity of the iron salt, extract, and FeNPs, it was noticed that the FeNPs were more active toward the sensitive antimicrobial complexes. The inhibition zones for the three compounds are shown in figure 3.

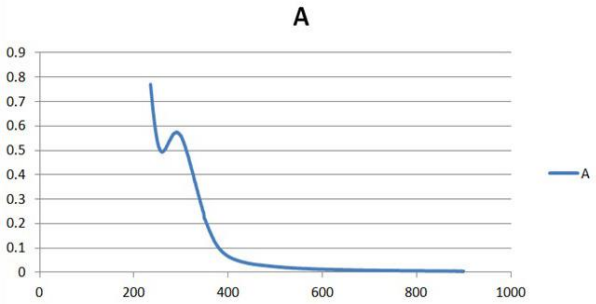

Figure 1. UV-Vis spectra for FeNPs

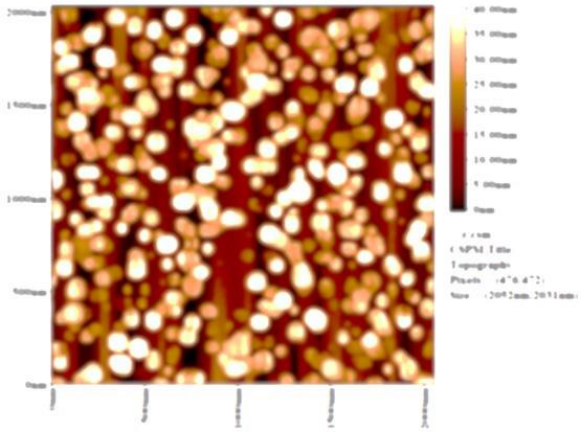

Figure 2. AFM Scan for FeNPs

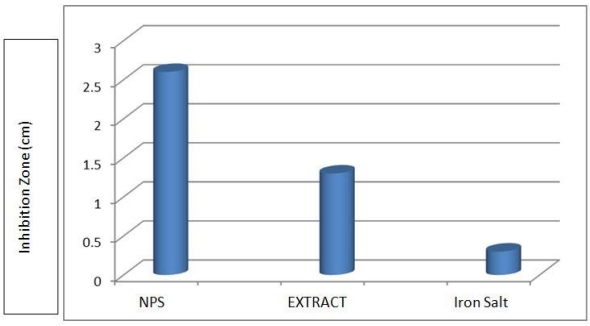

Figure 3. Inhibition zone comparison among the three compounds

The results indicate that NPs show bioactivity against *P. aeruginosa* more than the beet extract and iron salt. These results are in line with the findings of a study conducted by Borcherding, Baltrusaitis (19) who concluded that the FeNPs could be applied not only to inhibit anti-microbial peptides but also to replace some antibiotics that were considered sensitive towards *P. aeruginosa*.

Different concentrations were used to inhibit *P. aeruginosa*, and it was noticed that the best concentration to be used is 50% since increasing the concentration over 50% will give the same effect toward *P. aeruginosa*; additionally, it will lose more raw materials. These results are consistent with the findings of a study performed by Armijo, Wawrzyniec (21).

In conclusion, the results of the current study revealed the following:

- Novel applications for beet.
- A novel method for FeNPs preparation using *Beta Vulgaris*.
- The use of FeNPs to inhibit *P. aeruginosa*.

The use of FeNPs to replace some antibiotics to inhibit *P. aeruginosa*.

Authors' Contribution

Study concept and design: M. K. M
Acquisition of data: S. H. A.
Analysis and interpretation of data: R. A. J. A.
Drafting of the manuscript: M. K. M
Critical revision of the manuscript for important intellectual content: S. H. A.
Statistical analysis: R. A. J. A.
Administrative, technical, and material support: M. K. M

Conflict of Interest

The authors declare that they have no conflict of interest.

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