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Original Research Article

Antibacterial Effect of Iron Nanoparticles Synthesized from Rosemary Leaves Extract (*Rosmarinus officinalis* L)

Zena Hassan Jazar[1*]

[1]Biology Department, College of Science, Mustansiriyah University, Baghdad, Iraq

***Corresponding Author:** Zena Hassan Jazar
Biology Department, College of Science, Mustansiriyah University, Baghdad, Iraq

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Abstract: Industries that used nanoparticles has wide applications, among them are; in the production of cosmetics and pharmaceutical industries, therapies, and much more. Plants, algae, or microorganisms can be used in the synthesis nanoparticles, and combustion byproducts can also be used to make nanoparticles. In this study, silver nanoparticles (NPs-Fe) were synthesized from rosemary leaf extracts (*Rosmarinus officinalis* L) in order to create an antibacterial compounds for use as a fruit preservative. By using UV-VIS spectroscopy and phytochemical analysis to analyze NPs-Fe qualitatively and quantitatively, which are revealed an absorption in the 389-418nm regions, which matches the resonance of their surface plasmons. The size and morphology of the NPs-Fe were also determined using scanning electronic microscopy, which revealed a spherical shape with a diameter of 23nm, while the bioactivity exam to shows inhibitory effect against several pathogenic bacteria.

Keywords: Silver nanoparticle, spectroscopy, aqueous extract, rosemary.

INTRODUCTION

A minuscule particle with at least one dimension less than 100 nanometers is known as a nanoparticle [1]. They differ from bulk materials in that they have distinctive optical, thermal, electrical, chemical, and physical properties [2]. As a result, they are used in a wide range of industries, including heavy industry, consumer goods, chemistry, environment, energy, agriculture, information technology, and communication [3].

The production of nontoxic, environmentally benign metal nanoparticles is a continuous goal of nanobiotechnology [4] The solvent medium, a reducing agent, and a nontoxic nanoparticle stabilizer are the three basic needs for the synthesis of a metal nanoparticle..Due to their abundance in active chemicals, plants are a useful source of reducing agents for the synthesis of nanoparticles [5]. The solvent medium, a reducing agent, and a nontoxic nanoparticle stabilizer are the three basic needs for the synthesis of a metal nanoparticle [6], due to their abundance in active chemicals; plants are a useful source of reducing agents for the synthesis of nanoparticles [7].

The current was carried to prepare natural extract derived from Rosemary Leaves (*Rosmarinus officinalis* L) and synthesize iron nanoparticles (FeNPs) and test the antibacterial activity of the new nanoparticles (NPs)

EXPERIMENTAL SECTION

Materials and Procedures

Leaf Extract

For this investigation, fresh, green rosemary leaves were grown in the greenhouse at the college of science. After being cleaned, cut, and cooked for 30 minutes in 0.1L of double-distilled water were 10 grams of healthy leaves. The broth was filtered, brought to room temperature, and then kept at 4°C [8].

FeNPs Biosynthesis

According to procedure [8], the rosemary leaves' aqueous extract was combined with iron salts to create the nanoparticles. 25ml of the extract was added to 250ml of DI, and the mixture was heated to 80°C while being constantly stirred. The extract was stirred while 0.01g of ferrous sulphate $Fe_2(SO_4)_3$ was added, and instantly the color changed to black. The solution was then allowed to cool while the pH was again calculated to be 8.74. Until further inspection, the colloidal solution for the nanoparticles was kept at 4°C in the refrigerator.

Characterization of the Prepared Nanoparticles

Analyzing the nanoparticles as-prepared involved examining how the reaction mixture's color changed. Every 30 minutes, the absorbance of the reaction mixture was measured over the wavelength range of 200-700nm. The zeta potential analysis was used to determine the average size of the nanoparticles as-prepared. A red laser with a wavelength of 633nm was used to measure the hydrodynamic size and zeta potential of the produced FeNPs at a scattering angle of 173°. The manufactured particles' average size was utilized to determine.

Characterization Nanoparticles

The following tools were used at the Nanoscale Research Center (University of Technology, Baghdad, Iraq) to determine the characteristics of iron nanoparticles:
Using a UV-Visible spectrometer, describe some UV-Visible properties. In order to analyze the optical (meterteehsp 8001) in the 300–800nm range, ultraviolet radiation was used [9].

Electron microscope for scanning (SEM) An electrical voltage of 30 KV and a wavelength of 3nm were used using a scanning electron microscope (TESCAN-VEGA).

X-ray (Shimadza maxima-a)

With an electric voltage of 40KV and a current of 30MA and a scanning range between (100.000-20.000) degrees, X-Ray Diffract (XRD700) was used to identify the crystal phases and calculate the size of the crystals. Using Cu tubes with a copper wavelength of 1.54A, the XRD patterns were captured within 0.12 seconds of the scan speed [9].

Scanning Electron Microscope (SEM)

An electrical voltage of 30KV and a wavelength of 3nm were used using a scanning electron microscope (TESCAN-VEGA) [8].

Analysis using GC and GC/MS Two fused silica capillaries (30, 0.32, and 032mm film thickness) and one polar stationary phase StabilaxR (60m0.25mm0.25m film thickness) were employed in the gas chromatography (GC) analysis, which was done using a gas chromatograph linked to mass spectrometer columns. The column temperature program was 50°C for 3 minutes, followed by a 2°C/min climb to 250°C and a 15-minute hold at that temperature. Split mode injection was carried out at 250°C.

A carrier gas (He) flow rate of 1.2mL/min was employed. Mi70eV in one. Finding the components was successful; the sample was injected into crolitre. The relative retention indices on HP5MSTM and StabilwaxR columns, which were calculated using a homologous series of C5-C28 n-alkanes injected in the GC and GC/MS under the same conditions as the oils, and by comparing their mass spectral fragmentation patterns with those in the data bank (Wiley library) and the literature, were used to determine the ionization mode [10].

Preserving and Perpetuating Isolates

The biology department of the college of science of Mustansiriyah University donated the bacterial isolates, which included *Klebsiella spp., Staphylococcus epidermidis, and Candida albicans.* The disk diffusion method was used to assess the antibacterial activity of the produced Fe-NPs against each of the bacteria indicated, were maintained in slanted culture media (Slants) from the clays fed at 4°C, temperature, and the perpetuation process continued every month through the renewal of cultivation in new media to guarantee their survival during the research period. Use the nutrient broth medium with glycerol at a ratio of 15% to preserve the bacterial isolates for a long time. Distribute the medium in small bottles at a rate of (5ml) each and sterilize it with an autoclave. Then, let the medium cool down at room temperature. Then, inoculate the medium with pure colonies of bacteria growing on the clogsed by the conveyor (Loop). Store the bottles at (-20°C) [11].

Use a sterile culture diffuser (Spreader) to evenly distribute a sterile micropipette (0.1ml) of the previously prepared bacterial suspension in the spine on the surface of the culture medium. The plates were set in the air of the room for 20 to 30 minutes to allow the culture to absorb and the medium to dry. Following this, six antibiotic tablets were placed on the surface of the culture media using sterile forceps and light pressure. The dishes were incubated in the air for 24 hours at a temperature of 37m, according to the criteria outlined in, the findings were determined by measuring the

antibiotics' regions of inhibition, and the bacteria were classified as either sensitive (Sensitive) or resistant (Resistant) (as described by Elcey *et al.*, 2014 [12].

Agar-Well Diffusion Technique

Injection of a swab from a bacteria culture containing (1.5 108cells/ml) into the surface of the blisters for comparison with a solution of constant turbidity, then the dishes were left to dry at room temperature. A pit with a diameter of (5mm) was made in the culture media by the cork punch, and then different concentrations of the original concentration were prepared using sterile distilled water as follows 2mg/ml. Then an amount (0.1) of the above concentrations was added to each pit in sequence, and the control pit was created by adding sterile distilled water. The plates were incubated at (37°C) for a period of (24) hours. The efficacy of the extract was determined by measuring the diameter of the inhibition zone around each pit.

RESULTS AND DISCUSSION

Due to the interaction of the nanoparticles with particular wavelengths, UV-vis spectroscopy was utilized as the main technique to verify the synthesis of the particles. An initial color change from yellow to brown served as first confirmation that iron ions were synthesized into nanoparticles in the presence of rosemary plant extract. The existence of nanoparticles in the extract was confirmed by the surface plasmon resonance (SPR) signal at 400 nm (Figure 1). SPR is caused by the free electrons in the nanoparticles resonating at specific wavelengths. The SPR peak can be lowered by reducing the particle size, which links the absorption to the particle size [13, 14].There was a difference in the compounds produced for the nanoparticles prepared from rosemary extract compared to the aqueous extract of rosemary, as it was discovered that the results of the GC-Mass examination of iron nanoparticles showed the following compounds which gave the highest%:
12.46 3-PHENYL, 11.13 Neoisolongifolene, and 8-oxo- 4,6-DEUTEROPHENYLPYRAZOLE, 13.66 (E,Z)-1,1'-Biphenyl-2-yl, 1,1'-Biphenyl-1,3-butadiene.

11 chemicals were identified by the GC-Mass analysis of rosemary extract, including:
10.12 4-(trimethylsilyl)dibenzofuran, 25.74 1,8-cineole, 15.77 camphor, and 14.48 -nitrophthalic acid.

Table 1: GC_MS results of iron particles for (rosemary) extract

No	RT (min)	Area%	Name	Quality	CAS Number
1	4.232	7.69	1-(3-Isopropylidene-5,5-dimethyl-bicyclo[2.1.0]pent-2-yl)-ethanone	41	000000-00-0
2	10.132	7.01	Camphor	98	000076-22-2
3	10.656	3.75	endo-Borneol	94	000507-70-0
4	12.97	12.46	3-PHENYL-4-DEUTEROPHENYLPYRAZOLE	53	024798-21-8
5	14.35	10.44	Dodecamethylcyclohexasiloxane	58	000540-97-6
6	17.494	6.63	2-(5-acetyl-2-furyl)-1,4-naphthoquinone	90	099113-72-1
7	17.982	5.85	Dodecamethylpentasiloxane	46	000141-63-9
8	19.648	7.44	(+) spathulenol	98	077171-55-2
9	19.772	6.28	Alloaromadendrene	91	025246-27-9
10	22.6	11.13	Neoisolongifolene, 8-oxo-	44	000000-00-0
11	24.058	3.48	Isopropyl myristate	50	000110-27-0
12	28.51	4.21	2-methyl-5,12-dithianaphtho[2,3-b]quinoxaline	38	106161-11-9
13	31.851	13.66	(E,Z)-1-(1,1'-Biphenyl-2-yl)-1-(4-chlorophenyl)-1,3-butadiene	38	000000-00-0

Table 2: GC_MS results of Rosemary extract

No	RT (min)	Area%	Name	Quality	CAS No.
1	4.237	4.74	N-ethyl-1,3-dithioisoindoline	80	035373-06-9
2	7.433	25.74	1,8-Cineole	99	000470-82-6
3	10.132	15.77	Camphor	98	000076-22-2
4	10.65	9.64	BORNEOL L	95	000464-45-9
5	11.263	3.21	m-Mentha-1,8-diene,	94	000499-03-6
6	12.965	10.12	4-(trimethylsilyl)dibenzofuran	59	017943-24-7
7	17.489	6.97	3,4,8-trimethyl-9-oxy-1-trimethylsilyloxybicyclo[4.3.1]non-1-ene	86	121944-74-9
8	17.982	3.74	1,1,1,3,5,7,9,9,9-Nonamethylpentasiloxane	52	084409-41-6
9	34.705	14.48	m-Nitrophthalic acid	74	000603-11-2
10	36.095	3.04	1-(2-trimethylsiloxy-1,1-dideuteriovinyl)-4-trimethylsiloxy-benzene	52	126210-55-7
11	39.157	2.57	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	41	018030-67-6

UV-Vis

The iron oxide displayed a distinctive peak at 220 nm as it was being produced, which is consistent with results from other investigations [10]. As seen in Figure 1, the spectrum also included several low intensity peaks that might be attributed to the n-* electronic * transitions for the active groups in the extract's active compounds.

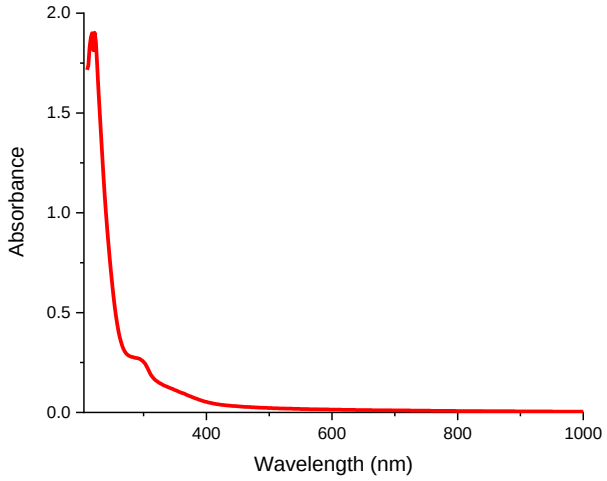

Figure 1: UV-Vis of FeOAR

UV-Vis

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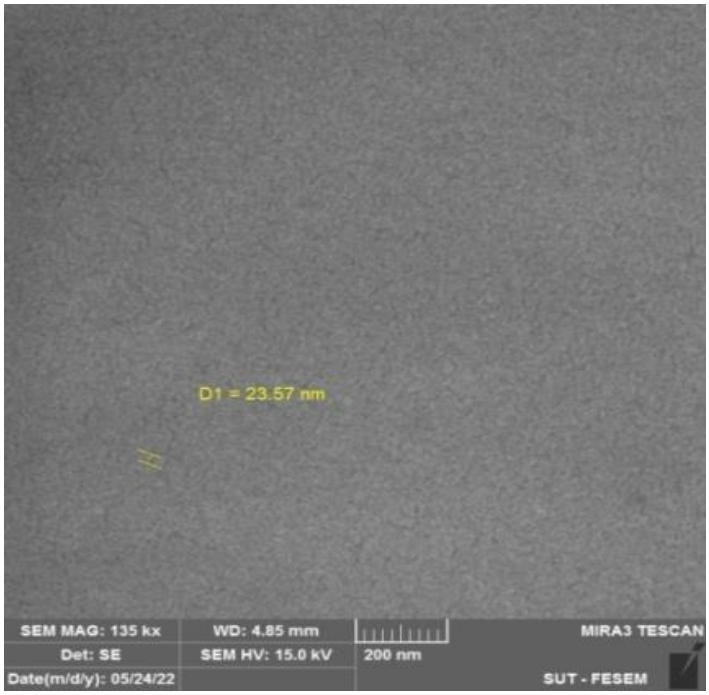

Figure 2: SEM of FeOAR

XRD

The XRD analysis revealed a broad peak with a center angle of 23.54 degrees, but in addition, we see a low intensity peak at 36.14 degrees, as well as another peak at 42 and 61 degrees, which demonstrate the development of iron oxide nanoparticles with few crystalline characteristics.

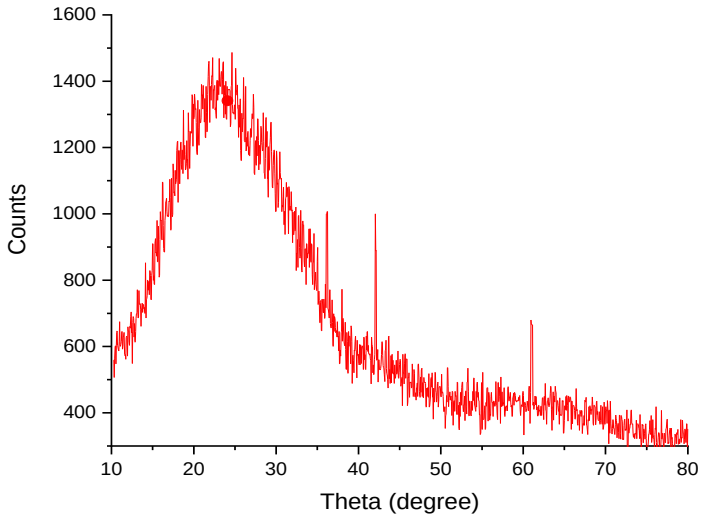

Figure 3: XRD of FeOAR

The antibacterial efficacy of a particle depends on its small size and round shape. Our results agree with many previous studies reporting high antimicrobial activities Table 3: Antibacterial activity of the biosynthesized IronNPs prepared with aqueous *R. officinalis* leaf extract against species tested by disc diffusion assay [15]. The results showed that iron nanoparticles gave the best inhibition of the bacteria under study compared to the results of the aqueous extract of rosemary as follows: *S. aureus* [20mm], *S. epidermidis* [19mm]. These findings match with Liu *et al.*, 2020 [16], and Shabatina *et al.*, 2020 [17].

Table 3: Antimicrobial activities of Iron NPs

Strain	Water Extract	IronNPs
S.aureus	11mm	20mm
S. epidermidis	8mm	19mm
K. pneumoniae	13mm	17mm
Candida albicans	12mm	17mm

CONCLUSIONS

- New FeNPs were synthesized and characterized successfully.
- The novel NPs were synthesized using the extract of Rosmarinus Officinalis L leaves.
- The novel FeNPs are more antimicrobial bioactivity than the extract of the leaves.
- The novel FeNPs are promising materials as anticancer (recommended for further work).

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Conflict: No conflict of interest.

Author Contributions

Zena Hassan Jazar (ZHJ) conducted the experiment, Sundus Hameed Ahmed (SHA) and Alyaa Muhsin Yousif (AMY) wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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