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Original Research Article

Studying the Antibacterial and Insecticidal Properties of Rosemary Extract by Iron Nanoparticles Prepared by Using Ultrasound

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Abstract: The green plant-mediated synthesis of iron nanoparticles (GPS-Fe) nanoparticles (NPs) has become more and more popular. In this study, fresh rosemary leaf aqueous extracts that decreased aqueous silver nitrate were used to demonstrate the synthesis of FeNPs. This procedure allowed for the synthesis of NPs that were examined using a different of analytical techniques, including transmission electron microscopy (SEM), energy-dispersive X-ray spectroscopy, FTIR, ultraviolet-visible (UV-Vis) spectrophotometry, and studies on dynamic light scattering. According to ocular observation and UV-Vis spectra, fresh leaf extract treatment changed the color of aqueous ferric nitrate to a bluish hue. Additionally, the TEM analysis revealed that the synthesized NPs were evenly dispersed and had average sizes less than 65 nm. Meanwhile, the bioactivity test shows that the NPs had an inhibitory effect on a number of pathogenic bacteria and caused a high rate of mortality in three stages of house fly larvae.

Keywords: Antibacterial, Insecticidal Properties, Rosemary Extract, FeNPs.

INTRODUCTION

One modern field that aims to create materials with diameters between 1 and 100 nm is nanotechnology. The oxides of metals such as titanium dioxide (TiO_2), silver oxide (AgO), iron oxide (FeO), gold oxide (AuO), zinc oxide (ZnO), and copper oxide (CuO), were found widespread application sectors with the advent of nano-scale production [1-3]. Iron oxide nanoparticles (IONPs) are used in biomedicine for a variety of purposes, including magnetic resonance imaging [4], protein and DNA isolation [5, 6], the systems of drug delivery [7], cytotoxicity testing, and anti-microbial research [8–10].

Metal oxide nanoparticles are produced primarily through physical, chemical, and green synthesis techniques. Physical techniques demand expensive Materials, equipment, and soaring temperatures. Hazardous compounds like sodium borohydride and hydrazine hydrate can negatively impact both the environment and living organisms when used as reducing agents in chemical operations [11–13]. The use of green synthesis is recommended as a viable alternative to chemical and physical processes as a result of these limitations. In general, plants, algae, bacteria, and fungi were chosen in the manufacturing of green nanoparticles. The best of them are plant extracts because they expedite the process and lower the risk of further contamination. A substantial and rich resource of bioactive substances such protein, enzymes, amino acids, polysaccharides, polyphenols, and fatty acids, that can play cytotoxic role in quite a few types of cancers are medicinal plant extracts. However, these bioactive substances have the ability to lessen the effect of metal ions (M^+) which secure the NPs to their desired shapes, and sizes [14, 15].

The chemical texture and structure of rosemary extracts was extensively studied [16] and is frequently related to geographic races or ecotypes based on prominent components. Understanding the variation in the oil's chemical composition and figuring out which components contribute the most to bioactivity are crucial when using marketable resources of the rosemary oil for the creation of insecticides. Relying on these toxicologic characteristic, “quality” of

rosemary extract (particularly the oil) for insecticidal reason may be very different from other uses' definitions of "quality" (flavorings, fragrances, medicinal uses).

MATERIAL AND METHOD

The Synthesis of IronNPs

Rosemary Extract is used to Make Rosemary-FeNPs.

The leaves of rosemary (Rosmarinusofficinalis L.), which were harvested locally from a botanical garden at Mustansiriyah University's college of science, were properly cleaned in deionized water, dried, and then finely crushed with a clean knife. Then, 20 g of crushed leaves were cooked in 200 ml of water for 1 hour at 60 °C, vacuum-filtered, and stored at 4 °C for later use.FeSO4 was employed as a Fe precursor to create iron nanoparticles, which A bluish colloid was seen after mixing approximately 5 mL of the aqueous extracts with 50 mL of FeSO4 (1.0 mM); this indicated the formation of FeNPs. The mixtures were then exposed to ultrasonication for 15 min, then by stirring for 15 min (400rpm). After that, they were separation (Figures 1 and 2), It remained stable for several weeks at room temperature in glass conical flasks sealed with foil.

Analysis of Nanoparticles

The features of FeNPs were ascertained at the Nanoscale Research Center (University of Technology, Baghdad, Iraq) using the following instruments:

Atomic force microscopy (AFM), Transmission electron microscopy (SEM), and energy dispersive X-ray spectroscopy (XRD) were used to characterize nanoparticles. Using a BiocromBiowave UV-vis spectrophotometer operating at room temperature in the 200–800 nm range, the rate of nanoparticle formation under various reaction conditions was assessed.

Analysis using GC and GC/MS Two fused silica capillaries (30m0.32mm032m film thickness) and one polar stationary phase StabilwaxR (60m0.25 mm 0.25m film thickness) were employed in the gas chromatography (GC) analysis, which was done using a gas chromatograph linked to mass spectrometer columns. For three minutes, the column temperature program was 50 °C. 250 °C was reached and maintained for 15 minutes at a rate of 2 °C/min. At 250 °C, injection was done in split mode (1/20). 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. Using GC and GC/MS under the same conditions as the oils, and by comparing their mass spectrum data, as explained in [10].

Vermin Bioassay

The house fly (Muscadomistica), which is free of insecticides and pathogenic organisms, was used as the source for the insect colony. This animal home belongs to the biology department in the college of science at Al-Mustansiriyah University. The colony was kept at or below 28 2 °C (starting from 1s larval stage reaching to 3rd instar stage). Healthy first, second, and third instar larvae were finished environment, with three replications of each test. The Muscadomistica larvae that had been collected were put in a cup with 10 g of nutritive material (this media consist of 200 g of fish food and 10 g of yeast extract, and also 100 ml of distilled water). Each cup received 2 mL of each concentration of Iron NPs, which were then covered with muslin fabric and left at RT (room temperature) for 24 hours. Adult medical care: percentage of mortality was calculated by applying Abbott's formula 20:

$$Corrected\ control\ Perent = \frac{X - Y}{X}\ x\ 100$$

X: alive in the check%
Y: alive in the treatment%

CONCLUSION AND DIALOGUE

In the current study, iron nanoparticles were created. Iron ions were reduced with rosemary, and the color changes are clearly visible in figure 1. Due to the stimulation of surface plasmon vibrations in iron nanoparticles, it was observed that iron nanoparticles exhibit yellowish-darker shading in an aqueous arrangement. This was further supported by UV-Vis spectroscopy (Figure 2) displays the optical absorbance spectrum for a solution containing an extract of the rosemary plant for the wavelength range (200-800nm). The figure shows that the absorbance is highest in the ultraviolet spectrum up to a wavelength of 306 nm, then rapidly decreases until a wavelength of 500 nm, indicating that the material is not optically transparent until that wavelength.

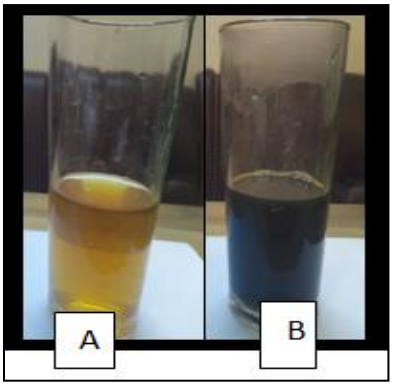

Figure 1: A: Rosemary extract and B: Rosemary mediated Iron nanoparticles

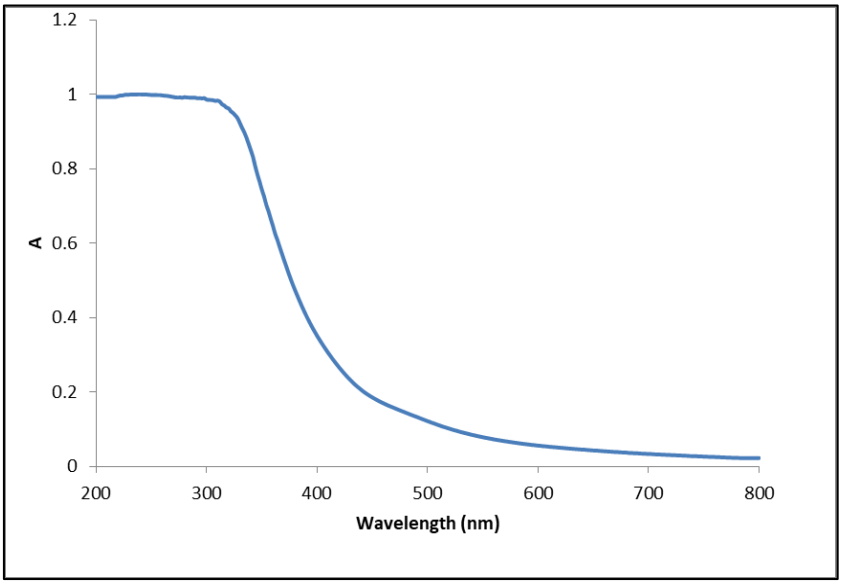

Figure 2: UV-Vis Spectroscopy of Rosemary oleoresin mediated Iron Nanoparticles

Figure 3 shows the results of a SEM analysis of a rosemary extract solution that was deposited on glass using the drop-casting method and prepared using the melting method at concentrations of (0.1 M |) and (30 °C). We can see that the solution contains semi-spherical particles that combine with one another when there are cracks and a few cracks. Its particles are 65 nm in size.

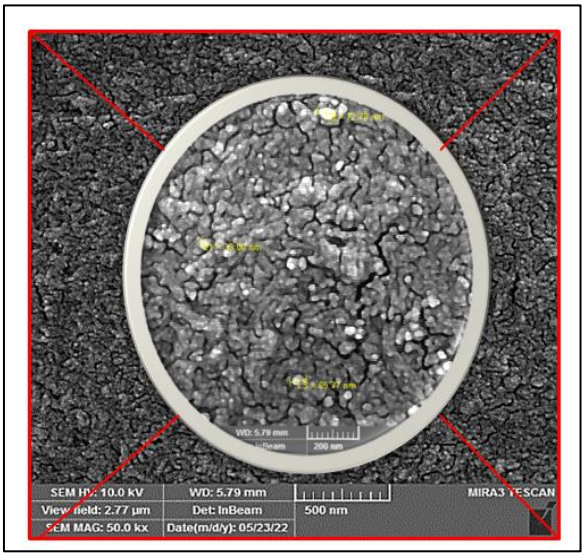

Figure 3: Spectrum analysis of FeNPs synthesized using rosemary extract

We can see one peak at angle 24.69 in the X-ray diffraction spectrum of the rosemary material that was dropped onto the glass in figure 4. This peak can be calculated using the sheerer equation in the table above.

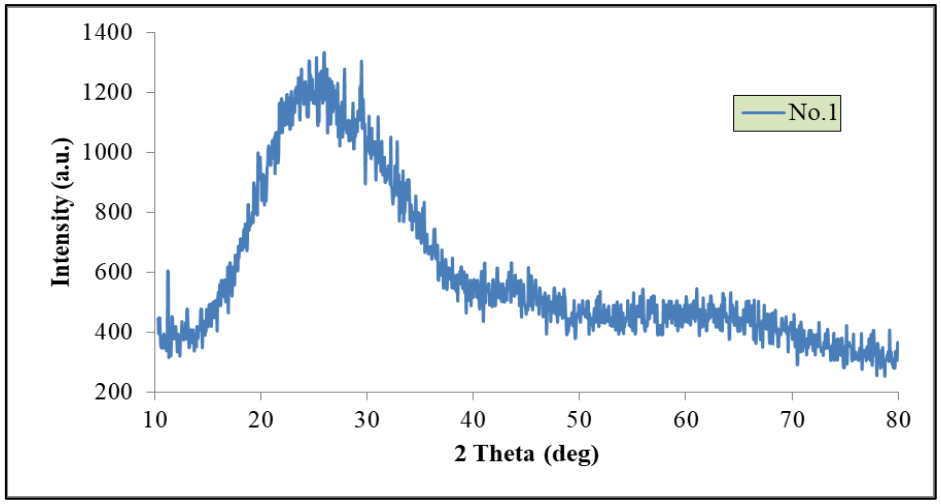

Figure 4: XRD spectrum analysis of FeNPs synthesized using rosemary extract

In addition to the gauzy distribution of the average grain size of rosemary plants coated on glass by drop-casting with a thickness of 2 micrometers, Figure 5 also depicts a two-dimensional afm image.

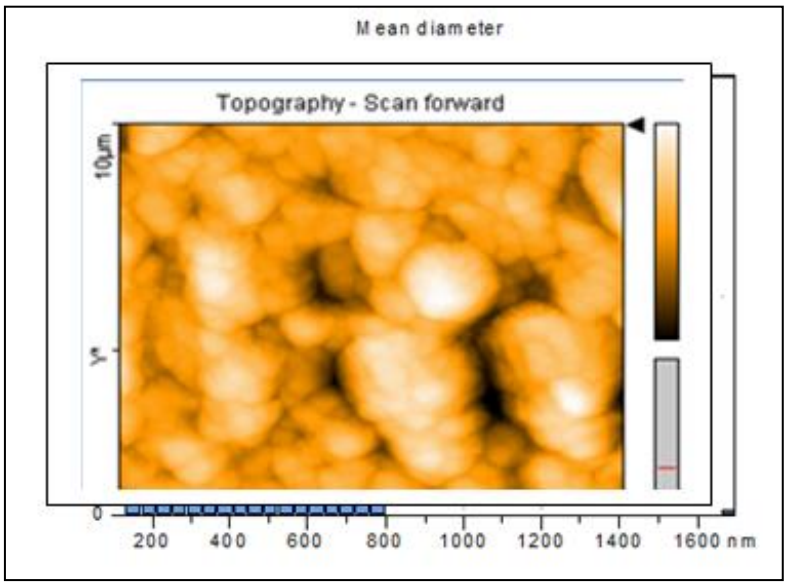

Figure 5: AFM spectrum analysis of FeNPs synthesized using rosemary extract

The compounds created for the nanoparticles made from rosemary extract varied from those produced for those made from the aqueous extract of rosemary, as shown by the findings of the GC-Mass analysis of iron nanoparticles, which revealed the following chemicals gave the highest%:

13.66 (E,Z)-1,1'-Biphenyl-2-yl, 1,1'-Biphenyl-1,3-butadiene, 12.46 3-PHENYL, 11.13 Neoisolongifolene, and 8-oxo-4,6-DEUTEROPHENYLPYRAZOLE.

The GC-Mass analysis of rosemary extract revealed 11 compounds, including: 15.77 camphor, 10.12 4-(trimethylsilyl) dibenzofuran, 25.74 1,8-cineole, and 14.48 -nitrophthalic acid.

Table 3 illustrates the larvicidal analysis, in which the larval deaths were scored after 24 hours. The results demonstrate that, depending on the concentration of each dose, there was a significant mortality observed throughout the growth of (Muscadomistica) larvae. First stage mortality was highest, followed by second and third larval and adult stages, respectively. Although barely detectable mortality was seen in vials of the first larval stage treated with water (0.3%), as shown in Table (2, 3, 4, 5). The death rate is inversely correlated with the concentration of synthetic iron, indicating the critical importance of concentration in larvicidal and adult action. For each concentration we discovered, three replicates of the control group were used in each test showed that house flies exposed to ultrasonication produced

the highest level of death across all phases. The house fly is covered in metal nanoparticles. The ability of the Fe NPs to create Reactive Oxygen Species comes into the most likely mechanism behind the death of via oral route or through rupturing of the cuticle membrane, according to [13-15] (ROS), Due to their toxic tension, these particles cause oxidative stress, which damages eggs and prevents them from moving on to their subsequent developmental phases. By using magnetite nanoparticles that exhibit bioactivity against Drosophila melanogaster, a comparable process was discovered [16-18]. In addition to the extract itself, other factors that affect the yield and properties of FeNPs when they are produced include the synthesis process temperature, the acidity the reaction (pH), the metal salt purity, and others. In this study, the researchers developed a novel green synthesis method to create FeNPs using rosmary leaf extract; this method had never been employed previously, FeNPs exhibits effective larvicidal effects against the house fly domistica. These findings will pave the way for numerous novel biomedical applications.

Table A: shows the iron particle results for the (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 B: GC-Mass result of Rosemary extract

No	RT (min)	Area%	Name	Quality	CAS Number
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

Table 1: Percentage of mortality of house fly larvae treated with the extract of rosmary

24 hr	**Control**	200**ppm**	4**00ppm**	6**00ppm**
1st larva	0.3	6.6	10	20
2nd larva	0	3.3	6.6	10
3rd larva	0	0	3	3
Adult	0	13.3	16.6	20

Table 2: Percentages of hous fly killing by $FeNo_3$

24 hr	Control	200ppm	400ppm	600ppm
1st larva	0.3	33.3	36.6	40
2nd larva	0	10	26.6	30
3rd larva	0	3.3	13.3	26.6
Adult	0	10	23.3	36.6

Table 3: Percentages of killing insects by nanoextraction before altrasound

24 hr	Control	200ppm	400ppm	600ppm
1st larva	0.3	86.6	93	100
2nd larva	0	50	60	73.3
3rd larva	0	46	50	60
Adult	0	70	76,6	90

Table 4: Percentages of insect killing by nanoextracts after altrasound

24hr	Control	200ppm	400ppm	600ppm
1st larva	0.3	96.6	100	100
2nd larva	0	60	73.3	83.3
3rd larva	0	60	66	73.3
Adult	0	76.6	86,6	100

The antibacterial activity of a NPrely on its shape and size. The obtained results in the current work agreed with several earlier studies [19, 20], the collected data of antimicrobial activities are listed in Table 5. 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 [25mm], S. epidermidis [22mm].

Table 5: Antimicrobial activity of the Extract and FeNPs

Strain	Water Extract	Iron Nps
S.aureus	14mm	25mm
S. epidermidis	7mm	22mm
K. pneumoniae	14mm	16mm
Candida albicans	11mm	16mm

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